Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) Study
Official Protocol and Manual of Procedures
August 20, 2009

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PROTOCOL

*F*olic *A*cid for *V*ascular *O*utcome *R*eduction *I*n *T*ransplantation
(FAVORIT)

A Randomized, Controlled Clinical Trial of the Effect of a High Dose Combination of Folic Acid, Vitamin B6 and Vitamin B12, on Arteriosclerotic Cardiovascular Disease Outcomes in Chronic, Stable Renal Transplant Recipients

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This document is a summary of the key elements of the FAVORIT study. For details, it is essential to consult the Manual of Operations. This document can be obtained from:

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SUMMARY

This multicenter, randomized, double-blind controlled clinical trial has been designed to determine whether total homocysteine (tHcy)-lowering treatment with a standard multivitamin augmented by a high dose combination of folic acid, vitamin B12, and vitamin B6, versus treatment with an identical multivitamin containing no folic acid, and Estimated Average Requirement (EAR) amounts of vitamin B6 and vitamin B12., reduces the pooled rate of recurrent and de novo cardiovascular disease [CVD] outcomes (i.e., pooled occurrence of non-fatal and fatal arteriosclerotic outcomes, including coronary heart, cerebrovascular, and peripheral vascular disease events = primary outcome), among clinically stable renal transplant recipients (RTRs) who have mild to moderately elevated tHcy levels. The basic eligibility criteria are age 35 to 75 years old, functioning renal allograft for ≥ 6-months with serum creatinine based glomerular filtration rate (GFR) ≥ 30 mL/min for men and ≥25 mL/min for women, and a screening random tHcy level ≥ 11 μmol/L for women, or ≥ 12 μmol/L for men. Patients will be stratified by clinic, and randomly assigned to treatment with a standard multivitamin containing a high dose combination of folic acid, vitamin B6, and vitamin B12, or an identical multivitamin containing no folic acid, and Estimated Average Requirement (EAR) amounts of vitamin B6 and vitamin B12. All patients will receive standard clinical management for traditional CVD risk factor reduction. The study is designed to recruit 4000 patients (2000 in each group; 30%-35% in each group will have diabetes) over a 2-year period for ***83 to 87% power to detect a 19.0 to 20.0 % treatment effect during 5-years of follow-up.*** Preceded by a careful chart review, study eligibility is further determined in conjunction with a routine renal transplant clinic visit, with the addition of random tHcy and creatinine determinations. Appropriately processed and stored EDTA plasma and serum aliquots will be shipped to the central lab for tHcy and creatinine analysis each week. Only women with a random tHcy level ≥ 11 μmol/L, or men with a random tHcy level ≥ 12 μmol/L, as well as a serum creatinine based GFR ≥ 30 mL/min for men and ≥25 mL/min for women, will be eligible to be randomized. All data required for randomization will be made available to the clinical sites within ≤ 2-3 weeks of a potential participant's screening visit. The baseline/randomization examination requires: informed consent; medical history & detailed current medication review; intake of folic acid, vitamin B12, and vitamin B6 from supplements; basic physical activity data collection; random blood collection for tHcy, folate, vitamin B12, pyridoxal 5'-phosphate (PLP), lipid profile, creatinine, & glucose determinations. Patients will be stratified by clinic, and randomly assigned to receive a daily multivitamin devoid of folic acid, vitamin B12, or vitamin B6, or a multivitamin containing, in addition to other standard multivitamins, a high dose of folic acid, vitamin B6, and vitamin B12. ***Follow-up clinic visits each 12-months for evaluation*** will include general medical histories focusing on hospitalizations, emergency room , and physician's office visits; full medication inventories; intake of folic acid, vitamin B12, and vitamin B6 from supplements; pill counts; and blood tests. In addition, questionnaires regarding hospitalizations, & intake of folic acid, vitamin B12, and vitamin B6 from supplements, will be administered ***at 6-month intervals after each of these clinic visits, during telephone follow-up. Follow-up continues until death or a common end date of a minimum of 4.5- years after the last participant is randomized***. ***Follow-up for events is expected to continue through July 31, 2011 or until death. Study exit visits are planned to be conducted between August 1, 2011 and October 31, 2011.*** For the primary analysis of the primary pooled CVD endpoint, participants with allograft failure requiring initiation/re-initiation of chronic maintenance dialysis will be censored at 3-months post-dialysis. A secondary analysis of the same endpoint will be performed without censoring. Data analysis will be performed on the basis of original randomization (intention to treat) using the log-rank test of difference in survival-without-endpoint curves.

I. STUDY HYPOTHESES

Patients with chronic renal disease occupy the highest risk stratum for subsequent arteriosclerotic cardiovascular disease (CVD) events (1). The excess risk of CVD in chronic renal disease is due in part to a higher prevalence of established arteriosclerotic risk factors, including older age, hypertension, diabetes, dyslipidemia, and physical inactivity (1). However, unique renal insufficiency/"uremia"-related risk factors likely also contribute to this excess CVD risk (1). Prominent among these unique risk factors in the chronic renal disease population are elevated levels of the putatively atherothrombotic sulfur amino acid homocysteine (2). Homozygous genetic disorders (i.e., the "homocystinurias" [3-5]) resulting in marked hyperhomocysteinemia (total homocysteine levels of 100 to 500 μmol/L) are clearly associated with precocious atherothrombotic events (6), and total homocysteine (tHcy)–lowering treatment appears to reduce the incidence of such outcomes among these patients (6,7). In addition, pooled data from prospective observational studies suggest that mild to moderate hyperhomocysteinemia (tHcy levels of 12 to 99 μmol/L[8]) may also be a significant risk factor for arteriosclerotic CVD among general populations of men and women (9). However, randomized, controlled clinical trial data confirming these reported associations are unavailable (10). Moreover, the impact of cereal grain flour fortification with folic acid (10,11) on plasma tHcy levels within the general population may obfuscate the results from any such trials conducted in the United States. Chronic renal disease patients, ***including renal transplant recipients***, have an excess prevalence of mild to moderate hyperhomocysteinemia, which has been independently linked to their development of CVD outcomes in recent prospective observational studies (11-15).

Hypothesis: Lowering tHcy levels in patients with chronic renal disease will reduce their excess incidence of arteriosclerotic CVD outcomes.

Renal transplant recipients comprise a unique subpopulation for testing this tenable hypothesis within the overall chronic renal disease population, given:

A) the high rate of de novo and recurrent cardiovascular disease outcomes in these patients (1);

B) their excess prevalence of hyperhomocysteinemia in the era of folic acid fortified cereal grain flour, which *contrasts with all other* potential target populations with normal renal function (16);

C) the ability to safely and successfully "normalize" their tHcy levels with combined folic acid, vitamin B12, and vitamin B6 treatment (17,18), which differs dramatically from patients with true end-stage renal disease (19)

D) that renal transplant recipients (RTR) are a highly motivated group of patients (20) treated almost exclusively in large medical centers, which is conducive to overall recruitment into clinical trials, while minimizing sampling bias, and greatly enhancing follow-up for endpoint ascertainment; centralized care & follow-up of RTR stands in stark contrast to the diffuse care of patients with chronic renal insufficiency who have not yet reached end-stage renal disease (21)

E) overall "conditions" in the renal transplant population (renal impairment, mild-to-moderate hyperhomocysteinemia which can be normalized by B-vitamin supplements, and excess CVD outcomes) are representative of the larger population of patients with chronic renal insufficiency who have not yet reached end-stage renal disease (2,17,18).

<u>We are performing a randomized, controlled trial to test the following primary hypothesis:</u>

(I) Treatment with a high dose combination of folic acid, vitamin B6, and vitamin B12 will reduce the rate of pooled arteriosclerotic CVD outcomes (i.e., pooled occurrence of non-fatal and fatal arteriosclerotic outcomes, including coronary heart, cerebrovascular, and

peripheral vascular disease events = primary outcome), relative to treatment with an identical multivitamin containing no folic acid, and Estimated Average Requirement (EAR) amounts of vitamin B6 and vitamin B12, among chronic, stable renal transplant recipients

We will also examine the following *secondary hypotheses* identified *a priori*:

(I) **Treatment with a high dose combination of folic acid, vitamin B6, and vitamin B12 will reduce the rate of total mortality relative to treatment with an identical multivitamin containing no folic acid, and Estimated Average Requirement (EAR) amounts of vitamin B6 and vitamin B12, among chronic, stable renal transplant recipients**

(II) **Treatment with a high dose combination of folic acid, vitamin B6, and vitamin B12 *among chronic, stable renal transplant recipients with baseline diabetes, specifically*, will reduce their rate of pooled arteriosclerotic CVD outcomes (i.e., pooled occurrence of non-fatal and fatal arteriosclerotic outcomes, including coronary heart, cerebrovascular, and peripheral vascular disease events = primary outcome), relative to treatment with an identical multivitamin containing no folic acid, and Estimated Average Requirement (EAR) amounts of vitamin B6 and vitamin B12**

(III) **Treatment with a high dose combination of folic acid, vitamin B6, and vitamin B12 will reduce the rate of decline in (creatinine-based estimates of) renal function, or the rate of graft failure requiring initiation of chronic dialysis, relative to treatment with an identical multivitamin containing no folic acid, and Estimated Average Requirement (EAR) amounts of vitamin B6 and vitamin B12, among chronic, stable renal transplant recipients**

II. BACKGROUND AND SIGNIFICANCE

II.A. Chronic Renal Transplantation: A "Model" for Chronic Renal Insufficiency.

The overall chronic renal disease population comprises four subpopulations encompassing various stages, treatment modalities, and treatment settings. These include chronic renal insufficiency, defined as a reduction in glomerular filtration rate (GFR) due to chronic renal disease, generally manifest as an elevated serum creatinine; end-stage renal disease (ESRD) treated by hemodialysis, ESRD treated by peritoneal dialysis, and renal transplant recipients [RTRs] (1). RTRs were considered to have chronic renal disease because, typically, GFR is reduced and declines progressively over time. Although the most common cause of progressive renal function decline in RTRs is chronic rejection, a number of non-immunologic factors have been shown to be associated with chronic rejection, and commonly, such factors are identical to those associated with progressive renal disease in native kidneys (1).

II. B. Arteriosclerotic Cardiovascular Disease in Renal Transplant Recipients.

Table 1. Arteriosclerotic cardiovascular disease incidence after renal transplantation (from ref.22)

	Post-transplant incidence*		Expected**
	Patients without CVD pre-transplantation (%)	All Patients (%)	CVD Incidence (%)
1. Angina	6.4	10.3	2.3
2. Myocardial infarction	6.4	7.8	1.3
3. Transient ischemic attacks	3.7	4.5	-
4. Thrombotic strokes	3.3	3.7	0.6
5. Peripheral vascular disease	2.6	3.0	-
6. Coronary heart disease (1&2)	11.0	15.1	3.4
7. Cerebrovascular disease (3&4)	6.0	7.3	-
8. "Total " CVD (1&2&3&4&5)	15.8	21.3	4.7

* based upon a mean of 46 ± 36 months of follow-up; **Framingham data (23)

Arteriosclerotic cardiovascular disease (CVD) is the most common cause of death after renal transplantation (1,22), as well as a major source of morbidity (1,22). Renal transplant recipients (RTRs) experience at least twofold increases in arteriosclerotic CVD mortality (1,22), and fourfold increases in pooled non-fatal and fatal CVD incidence (1,22) see Table 1 above), relative to population-based estimates. Established arteriosclerotic risk factors such as age, sex, cigarette smoking, diabetes, hypertension, and dyslipidemia do not account adequately for this excess risk (1,22). Furthermore, management of dyslipidemia and hypertension in this patient population may be complicated by immunosuppressive medication interactions, and residual renal insufficiency, or renal vascular disease (24). Accordingly, there is a compelling need to identify and safely manage other putative CVD risk factors contributing to the excess occurrence of CVD among RTRs. Given these considerations, elevated plasma tHcy is an excellent candidate risk factor because tHcy-lowering can be achieved safely, relatively rapidly, and inexpensively, with B-vitamin intervention.

II.C. Determinants of Homocysteine Levels, and the Prevalence and Etiology of Hyperhomocysteinemia.

II.C. 1. Determinants of Homocysteine Levels, and the Prevalence and Etiology of Hyperhomocysteinemia in General Populations:

Approximately 70-80% of circulating plasma/serum tHcy is bound to large proteins (e.g., albumin) (8), the remainder consisting of a "free" acid-soluble fraction, i.e. reduced Hcy (<1%), homocysteine disulfide, and the predominant non protein-bound forms, homocysteine-mixed disulfides (8). Folate, pyridoxal 5'-phosphate (PLP or "active" vitamin B6), and vitamin B12, are the main vitamin co-factors / substrates for homocysteine metabolism. Vitamin B-12 and folate play critical roles in the remethylation of homocysteine to methionine (25). Betaine (trimethylglycine) is another substrate that participates in the remethylation of homocysteine to methionine via a B12 / folate-independent reaction (25). Vitamin B6 (as PLP), conversely, has a minor role in the remethylation pathway, but is crucial for the irreversible transsulfuration of homocysteine to cystathionine, as well as the subsequent hydrolysis of cystathionine to cysteine and alpha-ketobutyrate (25). Consistent with this underlying biochemistry, population-based data indicate that intake and plasma status of folate, vitamin B6, and vitamin B12 are important

determinants of tHcy levels (26). Mild, subclinical inherited defects in the key remethylation or transsulfuration pathway enzymes, alone or via interactions with B-vitamin status, may also influence tHcy levels in general populations (25,27). Selhub and Miller (25) have hypothesized that two distinct forms of hyperhomocysteinemia can result when normal S-adenosylmethionine (SAM)-regulated partitioning of homocysteine between the remethylation and transsulfuration pathways is disrupted. Impairment of the remethylation pathway due primarily (on a population basis) to inadequate status of folate or vitamin B12 results in hyperhomocysteinemia under fasting conditions. Conversely, impairment of the transsulfuration pathway is associated with normal or only very mildly elevated tHcy levels under fasting conditions, but substantial elevations following a methionine load. Both animal model findings (28), and clinical observations from humans (25) support this hypothesis. Indeed, a randomized, placebo-controlled 2x2 factorial designed tHcy-lowering intervention study recently demonstrated that B6 treatment independently reduced the 2-hour post-methionine load increase in tHcy levels among stable renal transplant recipients (17).

Creatinine (29) and albumin (30) are two additional, independent determinants of tHcy levels in general populations, unrelated to B-vitamin status. The generation of s-adenosylhomocysteine from s-adenosylmethionine is coupled to creatine-creatinine synthesis (31), which likely accounts for the direct association observed between creatinine and fasting tHcy levels in persons with normative renal function (29). As noted earlier, 70-80% of serum/plasma tHcy is protein-bound, most likely to albumin (8) which may account for the direct relationship between albumin and tHcy levels found in the general population (30).

Severe cases of hyperhomocysteinemia, as in homocystinuria, may be due to rare homozygous defects in genes encoding for enzymes involved in either homocysteine remethylation or transsulfuration. The classic form of such a disorder is that caused by homozygosity for a defective gene encoding for cystathionine beta synthase (CBS), a condition in which fasting plasma total homocysteine concentrations can be as high as 400-500 μmol/L (3,7). Homozygous defects of other genes that lead to similar elevations in plasma homocysteine concentration include those encoding for methylenetetrahydrofolate reductase (MTHFR) (4), or for any of the enzymes which participate in the synthesis of methylated vitamin B12 (5). (also see Table 2.)

II.C. 2. Determinants of Homocysteine Levels, and the Prevalence and Etiology of Hyperhomocysteinemia in Chronic Renal Transplantation/Chronic Renal Insufficiency.

Nine independent studies (32) reported between 1981 and 1999, provided data on free or tHcy levels in stable RTR. We recently provided controlled findings describing an increased prevalence of fasting hyperhomocysteinemia in RTR (33). In addition, our study provided the initial documentation of an apparent excess prevalence of post-methionine load hyperhomocysteinemia (matched odds ratio 6.9), and combined fasting and post-methionine load hyperhomocysteinemia (matched odds ratio 18.0) in renal transplant recipients, relative to age and sex-matched population-based controls with normative renal function (also see Table 3).

In an early (i.e., pre-cyclosporine/tacrolimus era) study of n=27 stable renal transplant recipients, Wilcken and colleagues (34) reported a significant association between creatinine and cysteine-homocysteine mixed disulfide within a range of serum creatinine, consistent with mild to moderately impaired renal function (i.e., serum creatinine of ~ 100-500 μmol/L). Consistent with these data, we found that renal function may be a particularly crucial determinant of tHcy levels in renal transplant recipients, both under fasting conditions (35), and post-methionine loading (33). Although Arnadottir and colleagues (36) have suggested that cyclosporine use exerts an "independent" influence on fasting tHcy levels in these patients, both matched analyses, and multivariable regression modeling of data from a total of over 500 RTR (35,37,38), have revealed that cyclosporine use is not an independent

determinant of tHcy levels, after appropriate adjustment for renal function indices (in particular), age, and sex. Recently, we performed an additional analysis which further validates these previously published findings (39). We measured fasting plasma tHcy, folate, pyridoxal 5'-phosphate (PLP), and B12 concentrations, in addition to serum creatinine and albumin concentrations, in 86 chronic, stable renal transplant recipients, and 238 patients with chronic renal insufficiency. The two patient groups had serum creatinine levels encompassing equivalent total ranges (i.e., renal transplant recipients = 53.0 to 371.3 μmol/L [0.6 to 4.2 mg/dL]; CRI = 61.9 to 362.4μmol/L [0.7 to 4.1 mg/dL]), with identical geometric means (renal transplant recipients = chronic renal insufficiency = 150.3 μmol/L [1.7 mg/dL]). Geometric mean tHcy levels did not differ between the groups in either unadjusted analyses (renal transplant recipients = 15.0 μmol/L; chronic renal insufficiency = 14.9 μmol/L, P = 0.899), or by general linear modeling with analysis of covariance adjusted for the major determinants of tHcy levels, i.e., age, sex, B-vitamin status, albumin, and creatinine (renal transplant recipients = 15.6 μmol/L; chronic renal insufficiency = 14.6 μmol/L, P= 0.173). As anticipated, renal function, gauged as a simple creatinine measurement, was the major independent determinant of plasma tHcy concentrations, accounting for ~ 80% to 90% of the total variability in tHcy predicted by the full model (i.e., full model R^2) containing, in addition to creatinine, the seven other potential explanatory variables. Finally, although unadjusted correlations between fasting plasma tHcy and folate levels among RTRs have been reported (34,36,37), multivariable modeling (35) has revealed that the independent strength of this association is minor, relative to a simple creatinine-based estimate of renal function.

It has been convincingly demonstrated that normal urinary excretion of homocysteine is trivial (40), and plasma elimination of homocysteine in ESRD is grossly retarded (40). However, a consensus statement from The Second International Conference on Homocysteine Metabolism (Nijmegen, The Netherlands, April, 1998; Drs. AG Bostom and CD van Steuhower, co-authors) concluded that at present, the ultimate etiology of the mild hyperhomocysteinemia so consistently noted in renal insufficiency (2,39) (including renal transplantation [2,32,39]) and ESRD (2), remains unexplained. Despite in vitro studies demonstrating renal tubular metabolism of homocysteine (41,42), and rat model evidence of significant in vivo renal homocysteine metabolism (43,44), non-significant *mean* human renal arteriovenous differences for (total and non-protein bound) homocysteine were recently reported (45).These findings (45) have rekindled a search for "uremia-induced" extrarenal (46), presumptively, hepatic defects in homocysteine metabolism. It should be noted, however, that mild decrements in glomerular filtration rate (GFR), *encompassing clearly non-uremic ranges of GFR*, determined either by direct measurement (47-49), or using a sensitive surrogate like cystatin C (50-52), are strongly and independently associated with (linear) increases in fasting tHcy levels.

Table 2. Range of Plasma Total Homocysteine Levels in Homocystinuria, End-Stage Renal Disease, Chronic Renal Insufficiency /Chronic Renal Transplantation, and the General Population (from ref. 32)

Group	10^{th} to 90^{th} Percentile Range of Plasma Total Homocysteine (μmol/L)
Homocystinuria	50-300
End-Stage Renal Disease	12-39
Chronic Renal Insufficiency*	9-25
Chronic Renal Transplantation*	9-25
Normal Renal Function, Population-Based Controls**	6-12

*chronic renal insufficiency patients receiving no immunosuppressive drugs, and chronic renal transplant recipients on standard immunosuppressive therapy, with equivalent renal function
**current era of folic acid fortified cereal grain flour

Table 3. Prevalence of Mild Fasting and/or Post-Methionine Load Hyperhomocysteinemia: Chronic, Stable Renal Transplant Recipients Versus Matched Population-Based Controls (from ref. 33)

	Renal Transplant Recipients	Age and Sex-Matched (2:1) Controls*
N	29	58
Total homocysteine levels:		
Fasting > 14.2 μmol/L, %	59**	9
Post-load increase > 26.1 μmol/L, %	32**	9
Fasting > 14.2 μmol/L *and* Post-load increase > 26.1 μmol/L, %	32**	2

*free of clinical renal disease, and with serum creatinine ≤ 1.5 mg/dL
** P< 0.001, from matched chi-square

II.D. Homocysteine and Arteriosclerosis: Epidemiological Evidence from Prospective Studies.

II.D.1. The Natural History of Cystathionine Beta Synthase Deficiency.
Through their painstaking characterization of the natural history of children and young adults with cystathionine beta synthase deficiency, Mudd and colleagues (6), first highlighted the potential link between marked hyperhomocysteinemia (i.e., equivalent to total homocysteine levels of 100 to 450 μmol/L by current assays), and premature atherothrombotic sequelae. In addition, these investigators provided the initial evidence that treatments designed to lower the markedly elevated total homocysteine levels observed in cystathionine beta synthase deficiency, appeared to reduce atherothrombotic event rates in this patient population.

II.D.2. Prospective Data from General Populations.
A consistent, but not unequivocal body of evidence has emerged during the past decade from prospective cohort studies of adult populations suggesting that even mild elevations in total homocysteine levels might confer an increased risk for cardiovascular disease outcomes (9). Ueland, Refsum, Beresford, and Vollset (9) recently performed a meta-analysis of 14 prospective studies of the relationship between baseline total homocysteine levels and (primarily) coronary heart disease outcomes in population-based cohorts, reported through the end of 1999. Most of the studies evaluated by these authors characterized the association between total homocysteine levels and risk of (primarily) coronary heart disease, upon adjustment for age, smoking, blood pressure, and serum cholesterol. Several studies further adjusted for body mass index, diabetes, and physical activity. For all studies, the authors calculated or estimated the risk per 5 μmol/L change in total homocysteine concentration. Nine of the 14 studies provided information specific to men and six provided information specific to women. Within the 4 studies that reported results for each sex separately, the pooled relative risk estimates for men, and the pooled relative risk estimates women, did not differ. Accordingly, the results for men and women from these studies were combined. Each of the 14 studies contributed one relative risk estimate, and pooling these, the aggregate relative risk estimate (from a total of 2786 cases) per 5 μmol/L change in total homocysteine concentration was 1.20 (95% CI = 1.14-1.25).

II.D.3. Prospective Data from Populations with Chronic Renal Disease.
Intractable survivorship effects resulting from the excess yearly mortality in ESRD (1), and the failure to establish whether or not arteriosclerotic outcomes antedated the development of ESRD, renders hazardous any inference about tHcy-CVD associations suggested by retrospective studies of patients treated by either maintenance dialysis or renal transplantation. The potential relationship between

hyperhomocysteinemia and arteriosclerotic outcomes in both chronic dialysis or RTR populations requires more rigorous validation via prospective observational studies, and ultimately, clinical tHcy-lowering intervention trials. Reported findings from each of the three published prospective studies of pre-dialysis, or dialysis-dependent ESRD populations (12-14), as well as the pooled analysis of these data (2), have revealed a linear trend for increased CVD risk, i.e., per μmol/L increase or across quantiles of tHcy. The prospective data reported by Ducloux et al (15) from chronic RTR, have also demonstrated a continuous relationship between tHcy levels and CVD risk, although, as expected, the greatest relative risk, was confined to the uppermost distribution. Specifically, Ducloux et al (15) have reported that among N= 207 RTR [age: mean ± SD = 48 ± 14 years old] operationally defined as "chronic and stable" (i.e., transplant duration > 6-months; no evidence of acute rejection; serum creatinine concentration < 4.5 mg/d), there were a total of 30 new CVD events (cumulative incidence = 14.5%), after a mean follow-up of 19.7 ± 4.4 months. Using multivariable-adjusted (i.e., for age, sex, prior CVD, creatinine, cyclosporine use, smoking, hypertension, diabetes, dyslipidemia, and tHcy) proportional hazards modeling, only age, serum creatinine, and fasting tHcy levels were independently predictive of CVD events during follow-up. *Each one μmol/L increase in tHcy was associated with a 6-7% increase in the risk for developing CVD* (i.e., multivariable-adjusted hazards ratio = 1.06 to 1.07, 95% confidence interval = 1.04-1.09; $P<0.001$).

II.E. Homocysteine and Arteriosclerosis: Experimental Evidence.

The pathologic mechanisms by which homocysteine promotes arteriosclerosis remain unclear. Experimental data (2,32) support a range of possibilities, including endothelial cell injury, enhanced low density lipoprotein oxidation, increased thromboxane-mediated platelet aggregation, inhibition of cell surface thrombomodulin expression and protein C activation, enhancement of lipoprotein (a)-fibrin binding, and promotion of smooth muscle cell proliferation. The in vivo relevance of findings from such experimental studies, however, has been seriously questioned (53) due to their lack of specificity to Hcy versus other much more abundant plasma thiols, including cysteine, and the use of grossly supraphysiologic concentrations or non-physiologic forms (i.e., D-L as opposed to L) of reduced Hcy. The data of Mansoor and colleagues (54) provide the background appropriate for adequate understanding of the specific criticism regarding grossly supraphysiologic concentrations. These investigators assessed concentrations of reduced Hcy across the widest possible spectrum of tHcy concentrations. Their data revealed that at tHcy concentrations of up to 100 μmol/L, levels of reduced Hcy accounted for only 1% or less (i.e., < 1 μmol/L) of plasma tHcy. When tHcy exceeded 100 μmol/L, reduced Hcy began to rise exponentially, likely due to saturation of plasma protein-binding sites (54). However, the highest reduced Hcy value these authors documented was in a subject with homozygous homocystinuria who had a tHcy > 350 μmol/L, but a reduced Hcy of < 100 μmol/L (54). When juxtaposed to the concentrations of reduced Hcy used in experimental studies (2,32), i.e., 1000 to 10,000 μmol/L, the findings of Mansoor and colleagues (54) illustrate the very dubious clinical relevance of these published data. In contrast, physiologic models of mild, dietary-induced hyperhomocysteinemia (i.e., tHcy ≤ 15 μmol/L) causing subclinical or frank atherothrombotic sequelae have recently been described in minipigs (55) and cynomolgus monkeys (56). Follow-up investigations employing these models may elucidate the in vivo relevance of the putative pathologic mechanisms outlined above.

II.F. Treatment of Hyperhomocysteinemia.

II.F.1. The Cystathionine Beta Synthase Deficiency "Experience".

The severe hyperhomocysteinemia (i.e., tHcy levels of 100-400 μmol/L; see Table 2) found in homozygous cystathionine beta synthase (CBS) deficiency has been treated with methionine restriction

and supraphysiologic doses of vitamin B-6, vitamin B-12, folate, and betaine (3,6,7). Such treatment lowers Hcy levels, and more importantly, appears to reduce the incidence of atherothrombotic events and mortality in these patients (3,6,7). Management of this severe form of hyperhomocysteinemia is the paradigm for treatment of the more common mild to intermediate forms of hyperhomocysteinemia. With the exception of methionine restriction, all the major therapeutic approaches to lowering Hcy attempted in homocystinuria, have been applied to populations with moderate hyperhomocysteinemia, including patients with chronic renal disease.

II.F.2. Treatment of ESRD Patients:

In dialysis-dependent/"dialysis-imminent" ESRD patients, folic acid, or reduced folate-based B-vitamin regimens, including folic acid/reduced folates at doses of 5-60 mg/day, B12 at up to 1 mg/day, and B6 at up to 100 mg/day, may lower fasting tHcy levels by up to 50%, but over 90% of treated subjects continue to exhibit mild to moderate hyperhomocysteinemia (2,57,58). *Moreover, in ESRD patients receiving standard of care daily multivitamins which contain 1 mg/day of folic acid, carefully controlled analyses have revealed that there is little or no further tHcy-lowering benefit of adding even grossly pharmacological doses of folic acid to this baseline supplementation regimen (59).*

II.F.3. Treatment of Renal Transplant Recipients:

Open label findings from RTR with much milder decrements in renal function (32) have suggested that these patients, in contrast, *are much less refractory* to high dose folic acid-based tHcy-lowering supplementation. We performed a block randomized, placebo-controlled two by two factorial study of n=29 clinically stable RTR (17) demonstrating that, in contrast to what we observed in their ESRD counterparts undergoing maintenance dialysis, the mild hyperhomocysteinemia in the RTRs proved very amenable to high dose combination B-vitamin therapy (folic acid 5.0 mg/day, vitamin B6 50 mg/day, and vitamin B12 0.4 mg/day). Treated patients experienced mean reductions of fasting and post-methionine load tHcy levels of ~ 25% after only 6-weeks, with 75% achieving "normalization" of their tHcy levels (17). In a subsequent investigation (18), we found that a standard US multivitamin dose of folic acid (i.e., 0.4 mg/day) provided clearly suboptimal tHcy-lowering efficacy relative to a supraphysiological dose (2.4 mg/day), in chronic, stable RTR. We have also demonstrated (19) that in comparison to renal transplant recipients with equivalent baseline tHcy levels, the mild hyperhomocysteinemia of ESRD patients undergoing maintenance hemodialysis is much more refractory to tHcy-lowering B-vitamin treatment regimens featuring *even greater* supraphysiological amounts of folic acid, or the reduced folate, L-5-methyltetrahydrofolate. Specifically, we compared the relative responsiveness of (n=10) RTR and (n=39) hemodialysis [HD] patients with equivalent baseline total homocysteine (tHcy) levels [i.e., RTR range= 14.2- 23.6 μmol/L; HD range= 14.4- 24.9 μmol/L] to 12-weeks of tHcy-lowering treatment. The RTR received 2.4 mg/day of FA, 50 mg/day of vitamin B6, and 0.4 mg/day of vitamin B12, while the HD patients received 15 mg/day of FA or an equimolar amount (17 mg/day) of the reduced folate, L-5-methyltetrahydrofolate, in addition to 50 mg/day of vitamin B6, and 1.0 mg/day of vitamin B12. The mean percent (%) reductions (± 95% confidence interval) in tHcy were: RTR=28.1% (16.2-40.0%); HD=12.1% (6.6-17.7%), P=0.027 for comparison of between groups differences by analysis of covariance adjusted for baseline tHcy levels. Moreover, 5/10 (50.0%) of the RTR versus only 2/39 (5.1%) of the HD patients had final on-treatment tHcy levels < 12 μmol/L, P=0.002 for comparison of between groups differences by Fisher's exact test. We concluded (19) that relative to RTR with comparable baseline tHcy levels, the mild hyperhomocysteinemia of maintenance HD patients is much more refractory to tHcy-lowering B-vitamin treatment regimens featuring supraphysiological amounts of folic acid, or the reduced folate, L-5-methyltetrahydrofolate. Accordingly, RTR are a preferable target population for controlled clinical trials testing the hypothesis

that tHcy-lowering B-vitamin intervention may reduce arteriosclerotic CVD event rates in patients with chronic renal disease (60).

II.G. Clinical Trials Testing the "Homocysteine Hypothesis" Against A Background of Folic Acid Fortification of Cereal Grain Flour.

The US Food and Drug Administration (FDA) published a regulation in early 1996 (61) that all enriched flour breads, rice, pasta, cornmeal, and other cereal grain products would be required to contain 140 μg of folic acid per 100 g by January 1998. A similar enriched flour fortification initiative has been under way in Canada (62). The goal of these fortification policies was to increase intake of folate by women of childbearing age to reduce the risk of neural tube defects. Cereal grain flour products fortified with 140 μg folic acid per 100g flour began appearing voluntarily in the United States after March, 1996 (10). We have demonstrated the profound effect that this fortification policy has had on the prevalence of both low folate status (i.e., a > 90% decline in the prevalence of plasma folate levels < 3 ng/mL), and mild hyperhomocysteinemia (a decline of ~ 50% in the prevalence of fasting plasma total homocysteine levels > 13 μmol/L) among chronic non-users of vitamin supplements in the population-based Framingham Offspring Study (10). The powerful impact of fortification in another region of the US was subsequently highlighted by crude time trend analyses of serum folate status in the enormous Kaiser Permanente Health Maintenance Organization database (63). Moreover, data just made available from the National Health and Nutrition Examination Survey (NHANES) III and NHANES 1999 (64) have confirmed the dramatic impact of this fortification policy on serum and red cell folate status among a representative sample of the entire US population.

Large randomized, controlled trials of total homocysteine lowering for the potential reduction of cardiovascular disease outcomes are ongoing in the United States and Canada (The Vitamin Intervention for Stroke Prevention [VISP], Women's Antioxidant Cardiovascular Disease [WACS], and Heart Outcomes Prevention Evaluation [HOPE-2] studies; see ref. 65 and Table 4). The dramatic impact of implemented policies to fortify cereal grain flour products with folic acid may reduce the statistical power of these trials. All three trials assume the active treatment groups will achieve the same mean total homocysteine lowering treatment effects reported in the absence of the background effect of folic acid fortified cereal grain flour. We re-examined those assumptions using data from total homocysteine lowering treatment efficacy studies conducted in populations with cardiovascular disease in the United States and Canada, exposed to folic acid fortified cereal grain flour products (66,67). These data reveal that VISP, HOPE-2, and WACS will likely achieve only about 20-25% of their projected mean total homocysteine lowering treatment effects (reductions of 1.0-1.5, versus 4.0-6.0 μmol/L). As a result, all three trials would be substantially underpowered to test their specific total homocysteine lowering hypotheses identified *a priori* (see Tables 4. & 5.). In contrast, renal transplant recipients exhibit a persistent excess prevalence of hyperhomocysteinemia in the era of fortification (16), while remaining very responsive to supraphysiological dose folic acid-based supplementation, achieving mean reductions in their total homocysteine levels of 5.0-6.0 μmol/L (18). Thus, unlike other high cardiovascular disease risk populations with normal renal function who are impacted profoundly by fortification efforts, renal transplant recipients are uniquely suited for a controlled trial of the "homocysteine hypothesis" (60,68).

Table 4. Design Features of Three North American Trials of Total Homocysteine Lowering for Cardiovascular Disease Outcome Prevention (from ref. 65)

Study [Reference(s)]	Start Date	Study Population	Main Outcome(s)	Treatment Regimen (per day oral doses)	Sample Size
Vitamins in Stroke Prevention [VISP; refs. 3,14]	1998	Patients with non-disabling stroke	Recurrent stroke	Folic acid 2.5 mg + B6 25 mg + B12 0.4 mg, vs. Folic acid 0.02 mg + B6 0.2 mg + B12 0.06 mg	3600*
Women's Antioxidant Cardiovascular Disease Study [WACS; ref. 3]	1998	Patients with (primarily) coronary artery disease, or multiple CVD risk factors	Pooled arteriosclerotic CVD outcomes	Folic acid 2.5 mg + B6 50 mg + B12 1 mg, vs. placebo	5449**
Heart Outcomes Prevention Evaluation [HOPE-2; ref. 3]	1999	Patients with (primarily) coronary artery disease, or diabetes and at least one other CVD risk factor	Pooled arteriosclerotic CVD outcomes	Folic acid 5 mg + B6 50 mg + B12 1 mg, vs. placebo	5000*

*Projected; recruitment ongoing; ** Recruitment completed short of projected goal of 8000

Table 5. Potential impact of fortification on mean plasma total homocysteine lowering effects and statistical power in VISP, WACS, HOPE-2, and renal transplant recipient (FAVORIT) trial (from ref. 68)

Trial	Difference in mean total homocysteine (μmol/L), treated vs. placebo group	Corresponding percent reduction in primary outcome rate (%)[c]	Corresponding Power[d,e]
VISP	- 5.0[a]	25.0%	≥ 95.0%
	-1.5[b]	7.5%	≤ 20.0%
WACS	- 4.0[a]	20.0%	≥ 95.0%
	-1.0[b]	5.0%	≤ 20.0%
HOPE-2	- 4.0[a]	20.0%	≥ 95.0%
	-1.0[b]	5.0%	≤ 20.0%
FAVORIT	- 5.5 [b]	27.5%	≥ 95.0%

[a]Pre-fortification era projection; [b]Likely effect post-fortification based on references 8,9,17;

[c]Assumes each μmol/L decrease in mean plasma tHcy results in a 5% reduction in the CVD outcome rate of interest;

[d]Based on sample sizes listed in Table 1, placebo group event rates standardized to 20.0% for each trial, and a two-tailed alpha of 0.05;

[e]Based on total renal transplant recipient trial population of 4000, 2000 receiving active treatment, and 2000 receiving placebo treatment

III. EXPERIMENTAL DESIGN AND METHODS

III.A. Study Population.

III.A.1.Eligibility.

III.A.1.1. Definition of Chronic, Stable Post-Transplant Renal Function.

The primary fundamental eligibility criterion is that patients evidence chronic, clinically stable renal function post-transplantation. Kidney-pancreas transplant recipients with stable renal graft function will also be eligible for study participation, as will recipients of bone marrow transplants. Recipients of any other organ transplants, such as liver, heart or lung, are not eligible. Stable renal function will be ascertained by careful chart review establishing that the patient's current graft has been functioning for at least six-months post-transplantation, patients are not in the midst of treatments for acute rejection, and a Cockcroft-Gault serum creatinine based estimates (69) of GFR are ≥ 30 mL/min for men and ≥25 mL/min for women. At pre-randomization "eligibility" visits (i.e., routine renal transplant clinic visits), random serum will be obtained, aliquoted, stored at -80 degrees C, and sent on dry ice by overnight courier (in weekly batches) for serum creatinine determinations to be made by the central laboratory, to confirm that the individual patient's current creatinine based estimates of GFR are ≥ 30 mL/min for men and ≥25 mL/min for women. Within 2-weeks, the central lab will provide the results of these creatinine analyses to the Data Center for transmission to the clinical site.

III.A.1.2. Definition and Determination of Mildly Elevated Total Homocysteine (tHcy) Level.

The second fundamental eligibility criterion is a random plasma tHcy level ≥12.0 μmol/L for men, or ≥11.0 μmol/L for women. Plasma tHcy will be determined by a modification of the method of Araki and Sako (70). The method is reliable and accurate (see protocol appendix). In advance (~2-3 months) of their regularly scheduled clinic visits, potentially eligible patients will be contacted. Those currently using vitamin supplements will be asked to abstain from multivitamin, B-complex, or specific individual vitamin supplements containing folic acid, vitamin B6, or vitamin B12, for at least 4-weeks prior to their examination. Informed consent for this specific purpose (i.e., abstention from usual vitamin use) will be obtained. As indicated above for serum creatinine, during these pre-randomization "eligibility" visits (i.e., routine renal transplant clinic visits), random EDTA plasma will obtained, aliquoted, stored at -80 degrees C, and sent on dry ice by overnight courier (in weekly batches) for plasma tHcy determinations to be made by the central laboratory, to confirm that the individual patient's tHcy levels are ≥ 11.0 μmo/L for women or ≥ 12.0 μmol/L for men. Within 2-weeks, the central lab will provide the results of these tHcy analyses to the Data Center for transmission to the clinical site.

III.A.1.3. Additional Inclusion Criteria.

In addition, inclusion into the trial requires the following:

1. Age 35 to 75 years at time of randomization
2. Cognitive function adequate for patient to give accurate information
3. Geographically accessible for follow-up
4. Informed consent
5. Adequate transportation facilities

III.A.1.4. Exclusion Criteria.

Presence of:

1. Cancer, end-stage congestive heart failure, liver, or pulmonary disease, progressive HIV or other chronic wasting illness, which in the opinion of the study physician, would limit the life expectancy of the patient to less than two

years or prevent evaluation of recurrent or de novo CVD; Other conditions that prevent reliable participation in the study, such as refractory depression, severe cognitive impairment, or alcoholism or other substance abuse
2. Pregnant or lactating women or women of childbearing potential not practicing birth control.
3. Participation in another clinical trial specifically involving CVD risk factor management
4. Inability to be randomized within 120 days of screening.
5. Less than 3-months post acute myocardial infarction, or stroke, or less than 3-months post coronary artery, renal artery or lower extremity artery PTCA, or lower extremity amputation
6. Less than 6-months post coronary artery bypass graft surgery, abdominal aortic aneurysm repair surgery, or carotid endarterectomy.

III.B. Recruitment and Follow-Up Schedule.

III.B.1. Screening and Recruitment.
Initially, twenty large academic renal transplant centers across North America will be participating in the proposed study. Additional transplant centers will join the study in 2005. By database review, potentially eligible patients under the care of each transplant center will be contacted for consent to be screened for the trial during their next regularly scheduled clinic visit. They will be informed that this screening process will entail abstaining from multivitamin, B-complex, or specific individual vitamin supplements containing folic acid, vitamin B6, or vitamin B12, for at least 4-weeks prior to their examination, and having two to six additional tubes of random blood drawn. The clinical evaluation must be sufficient to establish eligibility and rule out any exclusion criteria. Each center must also determine that the patient is able to be randomized within 120 days of screening. After the 120 day timeframe the patient would have to be rescreened. Distance from the clinic and extended vacations must be carefully reviewed. The local team, consisting of the nephrologist or transplant surgeon, principal investigator, and the study coordinator, is responsible for verifying that the patient meets all the eligibility criteria except the tHcy and creatinine levels/creatinine-based GFR estimates (to be available within 2-3 weeks). The patient coordinator completes the study Recruitment Log daily, continuing until recruitment is completed. [The Manual of Procedures provides additional details relevant to the enrollment and randomization methods; treatment; procurement and dispensing of study vitamins.]

III.B.1.1. Simple Screening Visit.
This screening method is used for participants who, if eligible, will return to the transplant clinic within 120 days of screening for the clinic randomization visit. Completing the informed consent process is the first activity. The clinical evaluation must be sufficient to establish eligibility and rule out any exclusion criteria. Only two tubes of random blood are drawn. A sample of participants will provide one additional tube of blood for blind replicate quality control assessments.

III.B.1.2. Screening/Baseline Combination Visit.
For eligible participants who will be randomized over the telephone, much of the baseline data collection coincides with screening to become a screening/baseline combination visit. Completing the informed consent process is the first activity. The clinical evaluation must be sufficient to establish eligibility and rule out any exclusion criteria. Six tubes of random blood are drawn, and a clean catch urine specimen is collected. A sample of participants will provide one additional tube of blood and/or a

urine specimen for blind replicate quality control assessments. Blood pressure, height, weight, relevant medical history, regular medication use, and personal identifying information are obtained.

III.B.2. Enrollment and Randomization Methods.

The study nephrologist/transplant surgeon assesses the patient's eligibility and suitability and gives approval prior to enrollment in the screening/eligibility phase. Thereafter, this physician and/or the nurse coordinator discuss with the patient and family member(s) the nature, importance, potential benefits and risks, and duration of the study. The patient is informed that he/she may or may not qualify for the next phase of the study, the randomized clinical trial, depending on the results of the blood tests. The patient is informed that he or she is at liberty to refuse participation or to withdraw at any time. If the patient agrees, properly witnessed informed consent is obtained, and the patient is scheduled for a randomization clinic visit or telephone contact within 3-4 weeks. The Central Lab screening data are forwarded to the Data Center which in turn (typically within 48-hours) notifies the clinical center that a patient has met the tHcy & creatinine eligibility criteria, and the patient can be randomized pending confirmation of ongoing eligibility at the time of the actual randomization visit or telephone contact . Interested persons who do not meet laboratory eligibility criteria may be "re-screened" at their next scheduled routine clinic visit.

Eligible subjects will be randomized in a double blind manner to one of two treatment groups: multivitamins containing a high dose combination of folic acid, vitamin B6, and vitamin B12, or an identical multivitamin containing no folic acid, and Estimated Average Requirement (EAR) amounts of vitamin B6 and vitamin B12. The permuted block method for the random allocation will be used. At least three different block sizes will be used, with the order of blocks determined randomly. Patients will be stratified by clinical center, only. To implement this procedure, a sequence of treatment assignments will be computer generated for each stratum. These assignments will be merged with the sequence of post-randomization participant ID number. To reduce the possibility of randomization error and improve security, a microcomputer based randomization procedure is proposed. The data management system provided to each clinic will include the clinic's sequence of vitamin supply assignments in an encrypted file. After entering the required participant identifying and pre-randomization information the software will confirm the participant's eligibility and permanently assign the medication supply number from the clinic's sequence. Only then would the vitamin number to be used be displayed to the clinic staff. Using this approach, the vitamin supplies need not be assigned sequentially, eliminating any possibility of clinic staff manipulating the order of treatment assignments.

III.B.3. Baseline and Follow-up Evaluations.

III.B.3.1. Overview. (See also Table 6)

Prior to obtaining screening bloods at a routine clinic examination, the clinic database and individual patient clinic records/charts will have been thoroughly reviewed by the site PI and nurse coordinator to assure that potential participants meet basic eligibility criteria, and to establish the absence of obvious exclusion criteria. Potential participants will be then be contacted by telephone and informed that the screening process will entail abstaining from multivitamin, B-complex, or specific individual vitamin supplements containing folic acid, vitamin B6, or vitamin B12, for at least 4-weeks prior to their scheduled clinic examination, and having additional tubes of random blood drawn. Patients who have provided informed consent for the study at the screening exam, and are subsequently confirmed eligible for the study by their laboratory tests (i.e., for tHcy and creatinine/estimated GFR), will be randomized at either a study renal transplant clinic visit or over the telephone pending confirmation of continued eligibility.

III.B.3.1.1. Clinic Randomization Visit.
Participants who completed the Simple Screening Visit need to return to the renal transplant clinic for a study visit if they are lab-eligible to have final eligibility confirmed and be randomized. Entry examination at time of randomization will include an updated history focused on *relevant* (i.e., specific to CVD and/or renal disease, diabetes, or the major exclusion criteria, namely any condition that would limit the life expectancy of the patient to less than two years or prevent evaluation of recurrent or de novo CVD, and pregnancy) intercurrent hospitalizations, emergency room, or physician's office visits; intake of folic acid (in particular), vitamin B12, and vitamin B6. Blood pressure, height, weight, relevant medical history, regular medication use, brief, focused queries regarding physical activity, cereal consumption, and dietary supplement use, and personal identifying information are obtained. Four tubes of random blood are drawn, and a clean catch urine specimen is collected. A sample of participants will provide one additional tube of blood and/or a urine specimen for blind replicate quality control assessments.

III.B.3.1.2. Telephone Randomization Contact.
Participants who completed the Screening/Baseline Combination Visit and meet laboratory eligibility criteria will be contacted by telephone to determine final eligibility for the study. Eligibility will be assessed for all non-laboratory criteria as of the time of the telephone contact, so participants will be queried about *relevant* (i.e., specific to CVD and/or renal disease, diabetes, all intercurrent hospitalizations, emergency room, or physician's office visits; intake of folic acid (in particular), vitamin B12, and vitamin B6; age; transplant history; major illnesses or conditions that would limit participation in the study; and if applicable, child-bearing potential.

III.B.3.1.3. Follow-up Evaluations.
After randomization, patients return periodically for (yearly) clinic visits or are contacted by phone (intercurrent 6-months; see Table 6.). Yearly clinic follow-up largely mirrors the clinic randomization visit. A sample of participants will provide one additional blood collection tube or urine specimen for blind replicate quality control assessments. Telephone follow-up includes history (focused on intercurrent *hospitalizations*), determination of study vitamin compliance (i.e., pill counts by patient), and assessment of intake of folic acid (in particular), vitamin B12, and vitamin B6 from non-study vitamin supplement capsules/tablets. Also, during each follow-up telephone and clinic visit, study participants will be asked to report *possible* adverse reactions to the vitamins. These limited queries will focus on pruritus, urticaria, and gastrointestinal disturbances. Telephone interviews and clinic visits are alternated every ***six*** months ***until the end of follow-up*** , or until the occurrence of death. Participants who develop dialysis-dependent ESRD will be followed until their **first** primary outcome occurs, after which mortality surveillance sufficient to distinguish CVD from non-CVD death continues until the end of the follow-up period for that participant. The scheduled date of the visit or phone interview is determined by the date of randomization, not by the date of the previous contact. All interviews and clinic exams are to be made within ten days of the scheduled date. If the follow-up schedule must be changed due to illness, geographic relocation or extended vacation, procedures are followed to document the change in schedule. If a patient misses an appointment s/he or her/his family will be contacted by the patient coordinator by phone or mail to inquire about a possible recurrent or de novo CVD event, return to chronic dialysis, or death.

Table 6. Visit Schedule. Required exams and procedures by month on study. RC= regular renal transplant clinic visit; SC= study renal transplant clinic visit* (*coordinated with RC visits to as great an extent as possible; "SC/RC"); P= telephone "visit"; tHcy= total homocysteine; PLP= pyridoxal 5'-phosphate

Procedure	RC Simple Screening	RC Screening/ Baseline	SC Base-Line/ Randomization 0	P Randomization	Follow-up P	Follow-up SC/ RC
(Prior) Chart Review	X	X				
Random tHcy & creatinine	X	X				
Random bloods for tHcy, folate/B12, PLP, Lipid profile, creatinine, glucose, fructosamine, & archiving		X	X			X
Clinic Exam & Medical History		X	X			X
Medical History (abbreviated)					X	
Medication Inventory		X	X			X
Focused Adverse Reactions Survey					X	X
Focused Surveys: a) Intake of supplemental folic acid, vit. B6, & vit. B12 b) Physical Activity		X X	X X			X X
Final Eligibility Check			X	X		
Pill count						X

III.B.3.2. Central Laboratories Blood and Urine Studies.

All study bloods will be drawn random with recording of time since last consumption of any liquids or solids other than water. For screening eligibility, venous blood (7 ml in EDTA; 7 mL without anticoagulant) from samples properly collected, handled, and separated, will be aliquoted into EDTA plasma, serum, and buffy coat aliquots, and cryopreserved (in the –80 degree C freezers provided by the study) at each clinical site. Screening EDTA and serum aliquots will be regularly (~weekly) shipped on dry ice by overnight courier for central determination of tHcy and creatinine levels at the Vitamin Bioavailability (i.e., tHcy), and Nutrition Evaluation Laboratories (creatinine), respectively, within The Jean Mayer USDA Human Nutrition Research Center on Aging in Boston, MA. For the baseline and yearly follow-up visits thereafter, venous blood (20 ml in EDTA, i.e., two 10 mL tubes; 10 mL without anticoagulant; 7 mL citrate) will be drawn. For ALL study visits, the EDTA and citrate containing tubes will be immediately placed on wet ice and centrifuged (in a refrigerated centrifuge) within 3 hours at 2800 rpm for 15 minutes at 4 degrees C. The EDTA and citrate plasma will be separated, immediately refrigerated, and aliquoted in special screw capped cryopreservation tubes. The whole blood without anticoagulant will be allowed to clot for 15-20 minutes, and the serum separated, immediately refrigerated, and aliquoted in special screw capped cryopreservation tubes. For patients randomized into

the intervention phase, specific visits will also include obtaining and saving buffy coat aliquots from the EDTA tube, once the plasma has been aliquoted. In addition, spot urine samples will be obtained at the randomization, and each of the follow-up visits, immediately refrigerated, and then aliquoted into 1.5 mL aliquots for long-term storage at – 80 degrees C. All specimens requiring centrifugation should be placed in the centrifuge within 3 hours of collection. All processing should be completed and the aliquots placed in the –80 degree C freezer within 4 hours of specimen collection. EDTA plasma, serum, citrate plasma, buffy coat, and urine aliquots cryopreserved at -80 degrees C, will be utilized as follows:

1) for tHcy (EDTA plasma, 0.5 mL aliquot)
2) for folate, PLP, and vitamin B12 (EDTA plasma, 0.5 mL aliquot)
3) for creatinine & glucose (serum 0.5 mL aliquot)
4) for total cholesterol, HDL-cholesterol, direct-LDL, and triglycerides (serum, 1.0 mL aliquot)
5) for fructosamine (serum, 0.5 mL aliquot)
6) for albumin/creatinine ratio* (urine, 1.0 mL aliquot) *(pending ancillary study support)
7) for additional ancillary studies/archiving (multiple 0.5 mL aliquots of EDTA and citrate plasma, buffy coat, serum, & 1.5 mL aliquots of urine)

III.B.3.3. CVD Risk Factor Surveillance.

All randomized patients are continued on their usual post-transplant medical/surgical and traditional CVD risk factor management. The risk factors predisposing toward recurrent or de novo clinical arteriosclerotic CVD are well recognized and include cigarette smoking, both diastolic and systolic hypertension, sedentary lifestyle/obesity, diabetes mellitus, elevated serum LDL cholesterol and/or reduced HDL-cholesterol. These risk factors will be assessed and recorded, but only pre-determined "alert" values for blood pressure and body weight/body mass index, as well as *any* ongoing cigarette smoking, will be reported to the patient's primary care physician, for further specific evaluation and treatment of individual patients. There are formal American Society of Nephrology guidelines for managing cardiovascular disease risk factors in renal transplant recipients. These guidelines will be copied and distributed to each site Principal Investigator. While plasma and serum will be obtained at each clinic visit for the purposes of the study, these specimens will be aliquoted, stored, banked, and batch-analyzed, and will *not* be available for clinical purposes. To reduce participant burden, study clinic physicians and nurse coordinators will synchronize usual clinical care blood draws with study blood draws to the greatest extent possible. Follow-up of these specific clinical test results will be via standard mechanisms in place at each center.

CVD risk factor surveillance will include:

1. *Hypertension*. At each visit, blood pressure will be measured twice, 10 to 15 minutes apart, with the patient sitting, and use of antihypertensive medications recorded.
2. *Obesity*. The patient's body weight and height will be recorded at each clinic visit.
3. *Dyslipidemia*. Sera will be stored from each clinic visit for analysis of total cholesterol, HDL cholesterol, LDL-cholesterol, and triglycerides, and use of lipid lowering medications recorded.
4. *Diabetes mellitus*. Sera will be stored from each clinic visit for analysis of fructosamine and glucose, and use of insulin preparations and/or oral anti-diabetic agents recorded.
5. *Cigarette smoking*. Patients will be questioned about cigarette smoking at each clinic visit, and the average number of cigarettes smoked per day, recorded.
6. *Sedentary lifestyle*. Responses to brief, focused queries will be obtained from each participant at the randomization/baseline visit, and yearly until the end of the study.

III.B.3.4. Assessment of Supplemental Intake of Folic Acid, and Vitamins B6 and B12.
Supplemental intake of folic acid, vitamin B6, and vitamin B12, will be assessed at each of the annual clinic visits (i.e., from vitamin capsules/tablets, as well as heavily fortified cereals & liquid/powdered supplements), and the semi-annual telephone interviews (from vitamin capsules/tablets, only) to obtain data on any changing levels of non-study vitamin supplementation.

III.C. Intervention.

III.C.1. Treatment Protocol.
All patients are continued on usual post-transplant general medical and CVD risk factor management as determined by their treating physician. *Half the randomized patients will receive multivitamins containing high doses of folic acid, vitamins B6 & B12, and the other half will receive* an *identical multivitamin containing no folic acid, and Estimated Average Requirement (EAR) amounts of vitamin B6 and vitamin B12.* ***To address any concerns about randomizing study participants to a treatment tablet devoid of folic acid,*** we analyzed whole food intake (i.e., *exclusive* of dietary supplements) of total folate (expressed as Dietary Folate Equivalents [DFEs], see below) in n=46 RTRs (35) using the Willett Food Frequency Questionnaire (71). Based on the recent Institute of Medicine [IOM] Report (72), in order to account for the increased bioavailability of folic acid (i.e., "synthetic" folate, vs. naturally occurring food folate), each food must be partitioned into the component that is synthetic folate (i.e., "added" to whole foods), and that which is naturally occurring. The amount that is synthetic must be multiplied by a factor of 1.7 to account for its increased bioavailability. This "corrected" synthetic value must then be added to the naturally-occurring amount in order to express the total amount of folate as "Dietary Folate Equivalents" [DFEs], in each food. Based upon recently published data (73), we determined the amount of synthetic folate (i.e., folic acid) the RTRs surveyed obtained from specific fortified breakfast cereal products (35), ***as well as an estimate of the synthetic folate they obtained as a result of "generic" fortification of cereal grain flour as per the Food and Drug Administration mandate (10,73).*** If any patient becomes pregnant during the study they will be encouraged to take standard prenatal vitamin supplements regardless of study treatment arm.

Table 7. Whole food, non-supplement intake of dietary folate equivalents in n=46 chronic, stable renal transplant recipients (RTR), updated to reflect impact of flour fortification using latest Framingham Study estimates

Estimated Average Requirement* (i.e., recommended population 50th percentile)	RTR 50th percentile	Lowest single RTR value
320 mcg	646 mcg	346 mcg

*Institute of Medicine recommendation

In summary, *the absolute lowest* daily whole food intakes of DFEs among the surveyed RTRs was above the estimated average requirements for this micronutrient (72).

The composition of the two multivitamins (see Table 8 below) has been adapted from Nephro-Vite, a supplement developed specifically for end-stage renal disease. The composition of the vitamins for the two vitamin treatment arms are listed in Table 8). Patients will be instructed to take one tablet a day for the length of the study. Study tablets will be re-supplied each 12-months in lots of 400.

Table 8. – "Active and "Placebo" Vitamin Formulation Contents

COMPONENT	**"Active" Formulation**	**"Placebo" Formulation**
Vitamin B6 (Pyridoxine HCl)*	**50 mg**	*1.4 mg*
Folic acid *; **	**5.0 mg**	**0.0 mg****
Vitamin B12*	**1.0 mg**	*2.0 mcg*
Vitamin B1 (Thiamine HNO3)*	**1.5 mg**	**1.5 mg**
Vitamin B2 (Riboflavin)*	**1.5 mg**	**1.5 mg**
Vitamin C (Ascorbic Acid)*	*60 mg*	*60 mg*
d-Biotin***	**300 mcg**	**300 mcg**
Niacinamide*	**20 mg**	**20 mg**
Pantothenic Acid (Calcium Pantothenate)***	**10 mg**	**10 mg**

***All values, (i.e., placebo or "active" formulations), at or above the Estimated Average Requirement (EAR)**
**Fortification of all enriched cereal grain flour provides an average of 340 mcg/d folate to non-supplement users
*** All values, (i.e., placebo or "active" formulations), at or above the Average Intake (AI), since EAR is unknown

III.C.2. Procurement and Dispensing of Study Vitamins.
The vitamin supplements will be supplied by Pamlab, L.L.C. (Covington, LA), being manufactured, stored, and distributed by Anabolic Laboratories. However, Pamlab, L.L.C. is designated as the Vitamin Distribution Center (VDC). The responsibilities of the VDC include: procurement of the necessary materials, coding and labeling of the packaged vitamins, and storage and distribution of the finished products, with appropriate quality controls at each stage. Because the study is double-blind, the vitamins will be dispensed with identical appearing tablets, bottles, closures, and external seals. Matching of the two dosages will be pre-tested but is not likely to be a problem because both tablets will contain active ingredients, although weight and specific gravity of the tablets could differ.

III.D. Trial Conduct.

III.D.1. Determination of Endpoints.

III.D.1.1. Overview of Composite Primary Endpoint.
The primary end point is recurrent or de novo arteriosclerotic cardiovascular disease (CVD), defined as the occurrence of non-fatal or fatal arteriosclerotic outcomes, including coronary heart, cerebrovascular, and peripheral vascular disease events. ***These outcomes will include: CVD death or nonfatal major arteriosclerotic events, specifically: myocardial infarction, resuscitated sudden death, coronary artery***

revascularization, stroke, and requirement for an invasive procedure for peripheral or renovascular disease (i.e., angioplasty/stenting, endarterectomy, aneurysm repair, or lower extremity amputation for an arteriosclerotic complication). All relevant medical history and records will be obtained, and any potential arteriosclerotic CVD outcomes will be validated according to standardized definitions of the outcomes, with putative endpoints reviewed on an ongoing basis by the Clinical Endpoints Center.

III.D.1.2. Adjudicated Endpoints.

Events to be adjudicated are as follows:
Death
Myocardial Infarction
Stroke
Resuscitated Sudden Death

Patients will be followed for the above events from the time of randomization until the date patient follow-up ends, or death, whichever occurs first. The primary outcome will be the first occurrence of FAVORIT-defined "pooled CVD", with (statistical) censoring at 3-months post-dialysis (in addition to censoring at end of study follow-up period, or death). For the primary pooled CVD endpoint, renal transplant recipients who become dialysis-dependent prior to experiencing a primary outcome, will be followed until their first primary outcome occurs, after which mortality surveillance continues until the end of the follow-up period for that participant. Clinical sites will be notified as early as possible when such primary outcomes have been adjudicated, at which point only mortality surveillance sufficient to determine CVD vs. non-CVD death will be required. Renal transplant recipients who become dialysis-dependent after experiencing a primary outcome will undergo only mortality surveillance sufficient to determine CVD vs. non-CVD death, until the end of the study. Again, the Data Center and Endpoints Core will work assiduously to inform the clinic about adjudicated (non-fatal) primary outcomes (in the "pre-dialysis" observation period), as early as possible.

III.D.1.2.1. Fatal Events: Cardiovascular Versus Non-Cardiovascular.
Death will be classified in two categories, cardiovascular or non-cardiovascular. All deaths will be assumed cardiovascular in nature unless a non-cardiovascular cause can be clearly shown.
Death will be classified in the following categories:

I. Arteriosclerotic Coronary Heart Disease

Acute Myocardial Infarction
Sudden Death
Non-Sudden Death
- Unwitnessed Death
- (Coronary) Procedural Death

II. Arteriosclerotic vascular disease, excluding coronary disease

Cerebrovascular disease, i.e., stroke
Aortic, mesenteric, renal vascular, or peripheral vascular disease
Procedural: death occurring during a hospitalization for or after a vascular procedure (i.e., carotid endarterectomy, abdominal aortic aneurysm repair), when the circumstances surrounding the death can be linked to a vascular procedure.

III. *Other Cardiovascular Disease (Non-Arteriosclerotic)*

Pulmonary Embolism
Endocarditis
Valvular Disease
Procedural
Other

IV. Non-Cardiovascular

Infectious
Malignancy
Pulmonary
Gastrointestinal
Accidental
Suicide
Diabetes
Renal
Other

V. Unknown

III.D.1.3. Unadjudicated Procedural Endpoints.

1. Coronary artery disease (i.e., undergoing coronary artery revascularization, either bypass surgery or angioplasty)
2. Lower extremity arterial disease (i.e., undergoing lower extremity arterial angioplasty or bypass surgery, or for severe disease [rest pain and/or gangrene], lower extremity amputation above the ankle).
3. Extracranial carotid arterial disease (i.e., undergoing carotid endarterectomy [CEA], or angioplasty).
4. Abdominal aortic aneurysm (i.e., undergoing abdominal aortic aneurysm [AAA] repair).
5. Renovascular disease (i.e., requiring renal artery revascularization, either bypass surgery or angioplasty)

III.D.1.4. Reporting of Death.

In the event of the death of a study patient all possible efforts will be made to obtain relevant records from the hospital or the patient's primary care physician, including death certificates, to determine cause of death. An Outcomes Documentation form is completed and entered into the data entry system as soon as possible. If the patient was admitted to a hospital during his/her final illness, a Hospitalization form is also completed. Since there may be considerable delay in obtaining the records needed to complete some of these forms, all deaths are to be reported to the DCC within three days of the date that the clinical center is notified of the event, regardless of availability of records. Mortality classification forms will be completed for all participants who die during the course of the study.

III.D.1.5. Graft Failure.

Graft failure will be defined as return to dialysis, and will be considered as a secondary outcome. Retransplantation, for the purposes of censoring, will **not** be considered a graft failure. Participants who develop dialysis-dependent graft failure will be followed until their first primary outcome occurs, after which mortality surveillance continues until the end of the follow-up period for that individual. Participants who have had a primary outcome prior to the initiation (re-initiation) of dialysis will have

mortality follow-up sufficient to distinguish CVD from non-CVD mortality until the end of the follow-up period for that participant. For the purpose of the primary analysis of the primary endpoint, participants with graft failure will contribute person-time until the first primary outcome or until three months *after* the resumption of dialysis, whichever is *earlier*. A secondary analysis of the primary endpoint will be done without censoring at three months post-graft failure. (see also earlier section **III.D.1.2.**)

III.D.2. Evaluation of Adverse Reactions.
Although we have found absolutely no placebo-controlled evidence of adverse reactions to doses (comparable to or greater than those for the proposed trial) of folic acid, vitamins B6 and B12 given to either maintenance dialysis or renal transplant patients, during follow-up telephone and clinic visits, study participants will be asked to report possible adverse reactions to the vitamins. These limited queries will focus on pruritus, urticaria, and gastrointestinal disturbances. Symptoms suggesting severe allergy are grounds for discontinuation of treatment. Mild gastrointestinal distress, presumably representing placebo effect, will be discussed with the patient. At each contact the patient will be asked about these possible adverse study vitamin reactions, and responses will be recorded on the appropriate forms. Patients required to discontinue study vitamin treatment or refusing to take it will not be removed from the study. Investigators will attempt to re-institute study vitamins in any patients who discontinue them. *Given the lack of serious side effects of multivitamins, emergency unblinding should rarely be required. In most cases, adverse events can be managed without knowledge of treatment assignment discontinuing study vitamins if appropriate. Clinical centers will be provided with the home phone numbers of two DCC staff members who will keep treatment assignment lists at home, in case unblinding is necessary.* Because the plasma tHcy and B-vitamin level results could unblind the investigators, these will be restricted to the DCC, and Data and Safety Monitoring Board.

III.D.3. Concomitant Medications and Vitamin Supplements.
Patients will be encouraged to use only the study multivitamins and not to take additional multivitamins. A special study brochure of instructions will be developed and distributed to patients. Use of all medications, and vitamin capsules/tablets, will be queried and recorded at each yearly clinic visit. Clinic visits will also include focused assessment of heavily fortified cereals & liquid/powdered supplements. During telephone follow-up, only vitamin/capsule use will be queried and recorded.

III.D.4. Masking.
Tablets for the high dose folic acid, vitamin B6, vitamin B12, and placebo folic acid, EAR dose vitamin B6, and EAR dose vitamin B12 arms, will be identical in size, shape, color, odor, and markings, consistent with the double-blind design of the protocol. The bottles, closures, seals, labels (except the code numbers) and markings for all packaging of study drugs will also be identical for the two kinds of tablets.

III.D.5. Monitoring Compliance.
At standard intervals (i.e., yearly clinic visits), plasma will be drawn for blind analysis of folate, vitamin B12 and PLP levels to assess compliance. Results will be available only to the Data Coordinating Center. In addition, at each clinic visit, the patient will bring study containers dispensed at the previous visit, and the patient coordinator will count the tablets remaining. Returned containers will be handled by the patient coordinator using the method to be provided by the VDC. Tablet counts and their variance from prediction will be recorded on the appropriate form. Visit adherence reports will also be monitored for each clinical center and overall, and reports will be distributed to the PIs, Operations Center, the Executive Committee, and the Data and Safety Monitoring Board. Steps will be taken within the trial structure to assist a clinic with lagging performance. Reasons for missed visits and phone calls will be

recorded. Non-compliance with study vitamins, operationally defined as < 75% compliant by pill count, will be tracked site by site, and routinely reported to the Executive Committee. Study coordinators will encourage non-compliant patients to improve their compliance by focusing, for example, on synchronizing study vitamin intake with their usual immunosuppressive drug intake (drugs which renal transplant recipients are typically religious about taking). Because statistical analysis will be based upon intention to treat, non-compliant patients will be encouraged to adhere to protocol but will not be removed from the study, because in all probability, this will occur equally in both groups.

III.D.6. Data Collection Instruments.
Examples of data forms for the pre-screening chart review/phone contact, screening visit, randomization visit, and follow-up telephone and clinic visit examinations are listed below, and actual samples are available on the secure FAVORIT website (http://www.cscc.unc.edu/favorit/) :

1. Pre-Screening Form (PRS)
2. Screening Form (SCR)
3. Screening Phlebotomy Forms (SPC and SPP)
4. Randomization Visit Forms (REL, RPC, MSR, PUF, PHC, and PHP)
5. Follow-Up Contact Form (FUP)
6. Vitamin Distribution Log (VDL)
7. Hospitalization Report Form (HOS)
8. Outcomes Documentation Form (OUT)

III.D.7. Data Management.
A PC-based distributed data management system (DMS) will be used for this study. The data management system will be implemented using Visual FoxPro Version 6.0 and installed on computers in each Clinical Center. This system will provide all of the capabilities required for research data management, including: data transfer, data entry, data validation, database updating, database closure, data retrieval, data inventory, security and confidentiality, and archiving, and in addition will support randomization.

Each Clinical Center will be responsible for entering and transferring the data it collects. The DMS will be installed on at least one computer per site, with the capacity for multiple desktop or laptop computers per site for data collection. The clinical center staff will use the DMS to enter screening data and eligibility data, run an algorithm to determine eligibility, and for each eligible patient, the DMS will issue a random treatment assignment. Follow-up data will also be entered at the clinical sites into the DMS.

One primary computer at each Clinical Center will have the capability to randomize participants and will host the main integrated database system. Centers with multiple computers for data collection will consolidate the data on these systems into the main database on the designated primary computer. The main database system will be used to generate reports for the Clinical Center and will be used to produce data transfer files for transfer to the Data Coordinating Center (DCC) at the Collaborative Studies Coordinating Center (CSCC) at the University of North Carolina.

III.D.7.1 Data Entry and Validation.
Direct data entry, where data initially are entered on the screen without having completed a paper form first, will be available at each center. Direct data entry eliminates the time-consuming and error prone process of keying from paper forms. Paper versions of each data collection instrument will be available as backup in situations in which the computer systems are inaccessible for any reason. In addition, if

there are forms that are routinely collected on paper for convenience or another reason, then the data on these forms will subsequently be keyed at the Clinical Centers using the distributed data entry system. The data entry system will display data entry screens that closely resemble the paper data collection forms. The system will be menu driven, with context-sensitive help available at any time.

Each data field will be edited during entry. Values that fail a validation routine will cause a message to be displayed. The person entering the data will then have three options:

- To correct the value, in which case the new value will be validated as was the previous entry;
- To flag the value as questionable, in which case the system will generate a printed data query form to document the question, and for use in recording a resolution; or
- To confirm the value as known to be correct, overriding the validation routine.

The data entry system will flag each data value with a "status character" documenting the current validation status of the item (empty, skipped, questionable, clean, confirmed, etc.).

The DMS provided to the Clinical Centers will include the ability for each center to generate locally a variety of summary reports concerning the data completeness, outstanding questionable values, etc. It has been our experience that most study coordinators find such a capability valuable in permitting them to monitor the quality of their center's performance. This facilitates timely identification and resolution of problems in data collection and processing.

III.D.7.2 Data Transfer and Processing.
Data from the main database at each Clinical Center will be transferred electronically to the DCC on a regular schedule. The transfer files will be encrypted when created by the clinical center data management system. Upon receipt, data files are logged in and imported to the study's database that consolidates data from all of the sites and the central laboratories. On a regular basis, data will be retrieved from the consolidated database and SAS® datasets will be prepared for statistical analysis purposes.

III.D.7.3 Central Laboratory Data Management.
The central laboratories will prepare data files from their local data management systems in a standardized format and transfer these to the DCC on a regular schedule. Upon receipt at the DCC, these data files will be processed analogous to the clinical center data files.

III.D.7.4 Data Security.
The DMS will require entry of a valid user ID and password for use. Sensitive files will be encrypted. Regular back-ups that are stored apart from the DMS will be required.

All data transferred to the DCC will be stored, processed and analyzed within the CSCC office suite. At the CSCC, all access to office space containing data is controlled through locked doors. Visitors are screened by SCC staff and cannot move about without a CSCC escort. All office space is locked after working hours. Access to computer data is controlled by passwords released only to those CSCC personnel who use the files. In addition, critical data files are encrypted.

III.D.7.5 Data Reporting.

On a monthly basis, the DCC will prepare a study data report that provides clinic-specific and overall summaries of patients screened and randomized by month. Timeliness and completeness of follow-up contacts will also be reported. In addition, the DCC will routinely generate reports for the clinical sites and laboratories concerning data quality (missing or overdue forms, outstanding data queries, etc), and facilitate the timely review, correction and resolution of data quality issues at the clinical sites.

III.D.7.6 Public release of data.

If the study is required by NIH to release a version of data for public use or if the executive committee approves release of the data to an ancillary study, all personal identifiers will be removed. All federal recommendations for insuring anonymity of the data will be implemented. The CSCC has experience with distributing data files both to study investigators and to the public, and has in place a multilevel check to ensure that confidential identifiers are not released. This check is performed by different staff members at the time a data distribution is requested, again by the programmer preparing the file to be distributed, by the head of the programming staff who reviews all such programming, by the study manager, and by the Coordinating Center PI.

III.D.8. Study Communications and Monitoring.

The Operations Center will maintain current contact information on the study web page for all study staff from the clinical sites, central laboratories, Data Coordinating Center, and Operations Center. Methods of study communications will include e-mail, web-postings, telephone, fax, and regular mail.

III.D.8.1 Technical Support.

The clinics will have a liaison at the DCC who can be called for an immediate answer to an operational or data management question or for help in obtaining clarification of a particular situation. For questions of a clinical nature, the clinics have a liaison at the Operations Center who directs the question to the appropriate committee chairperson. For each clinic, a primary study coordinator is identified, and a principal transplant nephrologist or surgeon investigator is identified who has the overall responsibility for the recruitment of patients and management of the study at the center.

III.D.8.2 Site Visits.

Site visits will be made to individual participating centers by a clinic monitor from the DCC or Operations Center to observe patients during clinic visits and to compare data sent to the DCC to that in the hospital records to verify adherence to protocol. In addition, if recruitment falls below a certain level, appropriate personnel designated by the Steering Committee, such as a team consisting of a transplant nephrologist or surgeon from a highly productive center, can be sent to advise on recruitment strategies. Monthly status reports of data quality and participant follow-up are prepared and circulated. These are reviewed to determine which clinics may need to be visited.

III.D.9. Statistical Power Calculations and Analysis Plan.

III.D.9.1. Power Calculations.

The primary comparison will test the null hypothesis that there is no difference between treatment groups in overall event rates, versus the alternative that the rates differ, using a *two sided* test with alpha=.05. All randomized patients will be included in the primary analysis, according to the group to which they are randomized, whether or not they actually receive study treatment (i.e., an intention to treat analysis.) The power calculations are based on looking at whether or not each subject has a CVD

outcome in the course of the study, rather than on the time to such an outcome, using the conventional normal approximation to the binomial distribution. *This is a conservative approach since the planned primary analysis using the log-rank test to compare the survival curves provides slightly greater power*. Subjects within a stratum/treatment group combination are all assumed to have the same risk of experiencing a CVD outcome. We have assumed that there are two primary strata--subjects with a history of diabetes, including those with successful pancreas transplants (35%; see discussion below) and subjects without a prior history (65%). Those with a history of diabetes are assumed to be at higher risk of experiencing a CVD outcome in the course of the study than those without a prior history. However, in order to calculate power we needed to make some assumptions about how many subjects would leave their assigned treatment group and the effect on disease risk for these subjects. We have assumed that subjects who drop out of the group assigned to the multivitamin devoid of folic acid, and with EAR amounts of vitamins B6 & B12, have the same risk as those who remain in this group. That is, the multivitamin devoid of folic acid, and with EAR amounts of vitamins B6 & B12, has no effect on risk of CVD. Subjects assigned to this multivitamin group who purchase and consume the B-vitamins of interest, and subjects assigned to the group given a multivitamin containing high doses of folic acid, vitamins B6 & B12, who do not adhere to this treatment but purchase and consume B vitamins over the counter, are assumed to receive a lower dose of these vitamins than the subjects who adhere to treatment with the multivitamin containing high doses of folic acid, vitamins B6 & B12. These subjects experience just some fraction of the benefit provided by the multivitamins containing high doses of folic acid, vitamins B6 & B12. Subjects who drop out of the treatment group receiving multivitamins containing high doses of folic acid, vitamins B6 & B12 and do not consume any multivitamin supplements are assumed to have the same risk of disease as subjects assigned to the multivitamin devoid of folic acid, vitamins B6 & B12, who adhere to this treatment.

The power depends on the underlying rate of the event(s) of interest. The background section reviews the available published information (6-10). However, for the calculations described herein, we updated this previously published information (6-10) with specific estimates for CVD incidence rates more germane to FAVORIT, as kindly provided by the United States Renal Data System, USRDS (Drs. Shuling Li and Charles Herzog, unpublished information; for details, see Protocol Appendix 1). In brief, as of January 1, 1995, a cohort of 12, 358 renal transplant recipients with the following criteria was established: at least 6-months of stable graft function as of January 1, 1995; Cockcroft-Gault estimated creatinine clearance of at least 30 mL/min within 6-months forward in time after January 1, 1995; and age between 35 to 75 years old on January 1, 1995. Using a start date of January 1, 1995, five-year incidence densities for the pooled (first) occurrence of any of the following non-fatal or fatal CVD events were calculated: myocardial infarction; stroke (atherothrombotic or hemorrhagic); abdominal or thoracic aortic aneurysm repair; revascularization for coronary artery, carotid arterial, renal arterial, or lower extremity arterial disease; lower extremity amputation above the ankle. Censoring events included: 30-days after return to dialysis (*i.e., 10% of the sample over 5-years*); loss to follow-up; death; or the end of December 31, 1999. The demographics of this cohort are characterized in Table 9.

Table 9. Demographics Distribution (N=12,358)

Mean Age (years)	49
Gender	
Male (%)	61.22
Female (%)	38.78
Race	
White (%)	74.83
Black (%)	19.89
Native America (%)	1.27
Asian (%)	3.11
Others (%)	0.91
Primary Diagnosis	
Diabetes (%)	23.40
Hypertension (%)	16.09
Others (%)	60.51

The CVD incidence rates of this USRDS sample cohort, stratified by presence or absence of diabetes (i.e., diabetes as the putative cause of ESRD, only), are presented in Table 10.

Table 10. Combined events including acute MI, stroke, aneurysm, amputation, revascularization, and CVD death.

	Follow-up Time				
Primary Diagnosis	1-year	2-year	3-year	4-year	5-year
Non-DM (N=9466)	308 (3.25%)	584 (6.17%)	864 (9.13%)	1106 (11.68%)	1285 (13.57%)
DM (N=2892)	339 (11.72%)	599 (20.71%)	778 (26.9%)	913 (31.57%)	1018 (35.2%)
Overall (N=12,358)	647(5.24%)	1183(9.57%)	1642(13.29%)	2019(16.34%)	2303(18.64%)

Random sample surveys of the twenty proposed FAVORIT centers (see Table 13) revealed that ~**35% of those renal transplant recipients meeting basic eligibility criteria** (i.e., with respect to age, time since renal transplantation, and current creatinine clearance) **were diabetic** (i.e., currently undergoing treatment with insulin or oral anti-diabetic medications). Power calculations used the USRDS diabetic stratum- specific CVD rate (in Table 2, above), applied to a projected FAVORIT population whose prevalence of diabetes at randomization would be 35%. Based on the tHcy screening eligibility criterion, our published treatment data (18) and the prospective data of Ducloux et al (15), the active treatment will reduce tHcy levels by a mean of ~6 μmol/L, which *could* translate into a reduction in the CVD event rate of ~35-40%. However, we have made the following conservative final estimates, based on the germane USRDS sample event rates (above) for both the pooled CVD outcome of interest, and the development of dialysis-dependent ESRD: ***With a sample size of 4000, and with 5% of each treatment group assumed to take no vitamins, and 5% of each group assumed to instead take a standard over the counter vitamin preparation, power is calculated to be 83.0% to detect a 19% treatment effect, and 87% to detect a 20% treatment effect, i.e., either a 19.0% or 20.0% reduction in their pooled CVD***

event rate, for those assigned to the multivitamin containing high doses of folic acid, vitamins B6 & B12.

III.D.9.2. Analysis Plan.

The results of high dose folic acid, vitamin B6, & vitamin B12 combined with standard multivitamin supplementation and usual chronic post-transplant medical management and CVD risk reduction, will be compared to standard multivitamin supplementation devoid of folic acid, but containing EAR amounts of vitamins B6 and B12, and usual chronic post-transplant medical management and CVD risk reduction, taking into account both stratification and randomization strategies. This will be done by comparing the treatment groups with respect to the distribution of time from randomization to first event. A log rank test will be used for this comparison, the main test of the primary hypothesis of the study. For this main test, patients will be censored three-months after allograft failure requiring initiation/re-initiation of chronic maintenance dialysis. In a secondary analysis of the primary hypothesis, the analysis will be by intention-to-treat, with no censoring after allograft failure.

The Kaplan-Meier method will be used to estimate unadjusted treatment-specific survival curves and to test for differences at the various times. Proportional hazards models will also be used, if the assumptions are satisfied, to adjust for other variables, such as initial (and or serial /"time-dependent") blood pressure, cigarette smoking, diabetes (diabetic recipients of successful pancreas transplants will be classified as diabetic since they carry the accumulated effects of prior years of diabetes), levels of lipids/lipoproteins, and creatinine-based renal function. The same analysis methods will be applied to secondary outcomes (i.e., total mortality; pooled CVD outcomes within the diabetic stratum; and development of dialysis-dependent ESRD). Analysis will also be done by sex, age group, race and by tHcy levels at the randomization visit. Treatment group comparisons, for interim and final analyses, will be for primary events, and secondary events (i.e., death; development of ESRD). Planned sample sizes for the study are based only on the analysis of differences between treatment groups in the time to primary event both because that relates to the main hypothesis of interest and because it is not expected that the treatment will affect rates of noncardiovascular disease death. However, differences in any of the three types of analysis found to be significant by our interim 'stopping rule" will be reported to the Monitoring Board for consideration.

III.D.9.3. Stopping Rules.

The Data and Safety Monitoring Board (DSMB) will examine the data at several points in time to determine whether the study should be stopped. To assist them, comparison of endpoint probability curves will be made at periodic intervals. As quoted by Halperin et al. (74) from Canner, (75) "..decision making in clinical trials is complicated and often protracted..", thus, "..no single statistical rule or procedure can take the place of the well-reasoned consideration of all aspects of the data by a group of concerned, competent, and experienced persons with a wide range of scientific backgrounds and points of view." The investigators agree with Halperin et al that, "statistical analyses of the accumulating data play an important but not dominant role". The particular statistical techniques chosen to determine the advisability of early stopping are stochastically curtailed tests. The trial is terminated if, at semi-annual examination, it appears that conditional on current data, either it is likely the null hypothesis will be (I) rejected at the end of the study, or (II) it is highly likely that the null hypothesis will not be rejected. In monitoring for efficacy or harmful effects, a number of methods for the repeated analysis of accumulating data have been proposed and used. When considering the stopping of a trial in which efficacy of the experimental treatment is claimed, the method used for monitoring the trial should be conservative: the trial should be stopped only if the treatment is clearly

superior. The O'Brien-Fleming' type boundary provides such a conservative approach (76). When the number and/or timing of interim evaluations cannot be fixed in advance, a Lan-DeMets (77) type spending rule which approximates the O'Brien-Fleming boundary is often used (76). The DSMB has proposed two interim looks: at approximately one-third and at two-thirds of expected numbers of events. In order to provide the flexibility to adjust to DSMB requests for changes in this schedule, we will use a Lan-DeMets boundary (78). In addition, conditional power as proposed by Halperin (74) will be used in the decision to stop the trial if the difference between treatments is small. This method computes the conditional probability of rejecting the null hypothesis given a specific alternative and the data at the time of the analysis. If this probability is too small, one may choose to discontinue the trial. Computationally, we will use a generalization of the method of Lan and Wittes (78).

III.E. Study Timetable.

Table 11.

Months	Planning, including final protocol, operations manual, and training	Recruitment	Follow-Up	Phase-Out and Analysis
Months 0-6 (August 01-Jan 02)	X			
Months 7-12 (Feb 02-July 02)		X		
Months 13-18 (August 02-Jan 03)		X	X	
Months 19-24 (Feb 03-July 03)		X	X	
Months 25-30 (August 03-Jan 04)		X	X	
Months 31-36 (Feb 04-July 04)		X	X	
Months 37-48 (August 04-July 05)		X	X	
Months 49-60 (August 05-July 06)		X	X	
Months 61-66 (August 06-Jan 07)		X	X	
Months 67-123 (Feb 07-Oct 11)			X	
Months 124-127 (Nov 11-Feb 12)			X*	X

*Time period for Clinics to compile final hospitalization data and to complete all data checks.

III.F. Statement on the Use of Human Subjects in FAVORIT.

III.F.1. Basic Rationale.

Pooled observational studies suggest that mild to moderate hyperhomocysteinemia (tHcy levels of 12 to 99 μmol/L[8]) may be a significant risk factor for arteriosclerotic CVD among general populations of men and women (9). However, randomized, controlled clinical trial data confirming these reported associations are unavailable. Moreover, the impact of cereal grain flour fortification with folic acid (10,16) on plasma tHcy levels within the general population may obfuscate the results from any such trials conducted in the United States. Chronic renal disease patients have an excess prevalence of mild to moderate hyperhomocysteinemia, which has been independently linked to their development of CVD outcomes in recent prospective observational studies (11-15). Renal transplant recipients comprise a unique subpopulation for testing this tenable hypothesis within the overall chronic renal disease population, given: the high rate of de novo and recurrent cardiovascular disease outcomes in these patients (1,15,22); their excess prevalence of hyperhomocysteinemia in the era of folic acid fortified cereal grain flour, which *contrasts with all other* potential target populations with normal renal function (16); the ability to safely and successfully "normalize" their tHcy levels with combined folic acid, vitamin B12, and vitamin B6 treatment (17,18), which differs dramatically from patients with true end-stage renal disease (2,19,32); that renal transplant recipients (RTR) are a highly motivated group of patients (20) treated almost exclusively in large medical centers, which is conducive to overall recruitment into clinical trials, while minimizing sampling bias, and greatly enhancing follow-up for endpoint ascertainment; centralized care & follow-up of RTR stands in stark contrast to the diffuse care of patients with chronic renal insufficiency who have not yet reached end-stage renal disease (21); overall "conditions" in the renal transplant population (renal impairment, mild-to-moderate hyperhomocysteinemia which can be normalized by B-vitamin supplements, and excess CVD outcomes) are representative of the larger population of patients with chronic renal insufficiency who have not yet reached end-stage renal disease (1,2,32).

III.F.2. Study Population.

The subject population consists of renal transplant recipients, at least 6-months post-transplantation. Specific inclusion and exclusion criteria are described in the Research Design and Methods section. *The goal of the study is 4000 randomized patients, ranging in age from 35 to 75. Patients are not excluded on the basis of sex or race. Women and minority groups will be recruited actively in order to achieve balanced representation. Published data are inconclusive on significant differences of clinical or public health importance in intervention between men and women; and there are essentially no data on differences between racial/ethnic subgroups.* We have adopted strategies for selecting centers, and for patient recruitment that should assure recruitment of representative numbers of the relevant subgroups. We asked potential centers to provide information about the gender and racial/ethnic distribution of renal transplant recipients at their centers. Several of the centers have clinical populations with a proportion of non-white patients substantially in excess of the overall US population (University of Alabama-Birmingham [African American]; UCLA [Hispanic]; Hennepin County Medical Center and London Health Sciences Center [Native American]; Oregon Health Sciences Center [Asian]). *Our most recent survey data from the participating sites are consistent with the USRDS sample data in Table 14 below, with respect to the prevalence of men and women (i.e., we expect our study population to be* 40% *female). Moreover, given the ethnic composition of the centers noted above, we expect our overall study population to include at least the 25% non-whites observed in the relevant USRDS sample (see Table 12, below). All of the centers have participated in other clinical trials involving renal transplant recipients and their expertise in recruiting and retaining these patients will be utilized in the training of personnel during the final protocol development and field testing phase. Specific methods for*

recruitment and retention in minority populations will be covered and detailed in the Operations Manual, which will include race and gender information; will be monitored to determine if there are differences between these subgroups in the proportion of randomizations to the number eligible. Up-to-date reporting will be stressed to ensure early identification of problems so that appropriate measures can be taken.

Table 12. Demographics of USRDS Sample Meeting Basic FAVORIT Eligibility Criteria

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	White, not of Hispanic Origin	Other or Unknown	Total
Percents of USRDS* Total (N=12,358)	1.3%	3.1%	19.9%	74.8%	0.9	100%

*USRDS sample meeting basic FAVORIT eligibility criteria: 61.2% men; 38.8% women

III.F.3. Data to be Collected.
Medical history, demographic data, and data from specific tests and stored specimens (as detailed earlier) are collected on patients who are screened and likely to be eligible. Additional and follow-up data are collected only on patients with random tHcy levels ≥ 11 or 12 μmol/L and estimated GFRs ≥ 30 mL/min for men and ≥25 mL/min for women, who are randomized. Sources of the data include the patient himself, the patient's family, and the patient's medical records. The vast majority of the data collected are specifically for research purposes, and not for patient care. Those data collected specifically for study purposes and reimbursable by the study include: blood drawn at screening, randomization, and every 12 months for determination of tHcy, creatinine, lipids, and glucose. Potential DNA analyses to be performed will relate ***only*** to possible genetic causes of CVD or renal disease, and/or abnormalities of homocysteine metabolism.

III.F.4. Recruitment and Consent Procedures.
Following an extensive chart review, potentially eligible patients are recruited from the renal transplant centers where they receive their routine follow-up care. They sign an informed consent form for the entire study that allows for screening bloods to be drawn at a regular clinic visit, and complete the process to affirm that they meet study eligibility criteria. When the Central Laboratory notifies the center that specific patients have met the tHcy and estimated GFR eligibility criteria, these patients are eligible to participate in the intervention phase. At randomization, these patients are informed again of the purpose of the intervention phase of the study, the treatment alternative, the random manner of assignment to treatment, the need to be available for telephone follow-up and return clinic visits at regular intervals for questionnaires and study phlebotomy, and of their options to accept or refuse entry into the study.

III.F.5. Potential Risks.
Butterworth and Tamura (79) found no evidence of adverse effects when folic acid was administered at up to 15-60 mg/day. Bendich and Cohen (80) have reported that vitamin B6 at doses of up 200 mg/day for two to twenty years was not associated with peripheral neuropathy, or other deleterious effects.

Chronic (2 to 15+ years) oral or parenteral vitamin B12 at 1 mg/day has been given safely to elderly patients with pernicious anemia (81), and children with homocystinuria and methyl malonic aciduria due to inborn errors of metabolism (82). Concerns about masking (while not treating) cobalamin deficiency with supraphysiologic doses of folic acid given without concomitant vitamin B12 (81) will not be relevant to the proposed study as the 5.0 mg folic acid dose is always given in a tablet also containing 0.4 mg of vitamin B12 (81,83). Published data from short term placebo-controlled interventions in both maintenance dialysis (2,57,58) and renal transplant recipient patient populations (17,18) are in accord with these earlier findings. We believe the risks of the vitamin therapy proposed are so minimal as to be inconsequential. This is a particular advantage both for safety and cost effectiveness.

III.F.6. Protecting Against Potential Risks.

III.F.6.1. Protecting Against Potential Risks to Personnel.
Biological hazards associated with these investigations relate to personnel exposure to blood and blood-borne pathogens in clinical specimens. Accordingly, the following guidelines and standards will be adhered to in addressing health and safety concerns for laboratory and clinical research personnel. "Universal Blood and Body Fluid Precautions," as established by Centers for Disease Control, will be utilized to include personnel training, specimen handling, use of protective barriers, and waste management. Diagnostic laboratory specimens submitted to the Tufts USDA HNRCA Vitamin Bioavailability Laboratory will be packaged, labeled, and transported in a manner consistent with the National Committee on Laboratory Standards". In accordance with the Occupational Safety and Health Administration's final rule on transmission of blood-borne pathogens, a written 'exposure control plan' will be established that identifies personnel at risk for occupational exposure to blood and other potentially infectious materials and training and information on specific "engineering controls" to protect them against exposure will be provided, including hepatitis B vaccination and post-exposure evaluation and follow-up in the event of an exposure".

III.F.6.2. Protecting Against Potential Risks to Patients.
Confidentiality of patient computer data is protected by the use of passwords, data encryption and secure, limited access storage. The DCC has programs, policies and facilities in use at the present time to ensure the security and confidentiality of the data it manages.

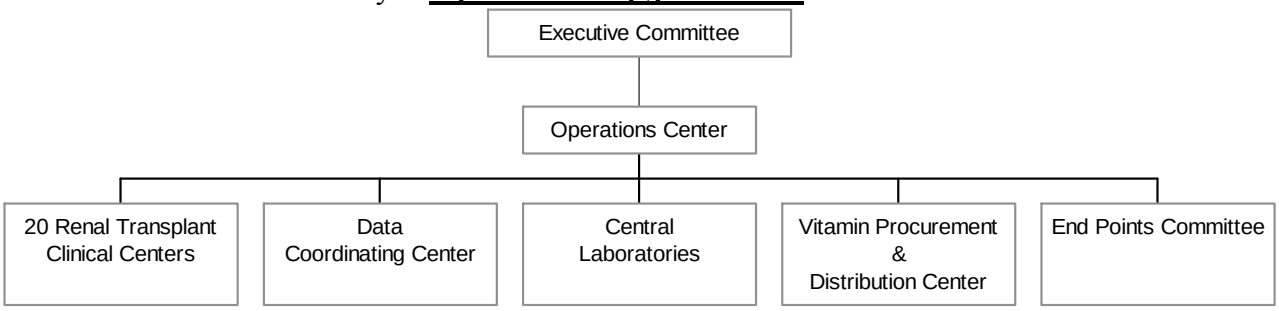
The DCC, and the NIH-appointed Data and Safety Monitoring Board play key roles in detecting any hazards the study may pose for its participants. Data are routinely collected and regularly monitored to watch for unusual mortality or morbidity associated with study-related procedures in each clinic. Timely reports will be made to the Monitoring Board. In addition, the SCC is responsible for calling the Board's attention to significant interim developments. Results for the different clinics are compared to identify the sources and causes of any remarkable deviations from the average performance. The Monitoring Board is responsible for advising early termination of the trial in the event that unexpectedly large treatment differences provide overwhelming evidence in favor of one intervention before the scheduled end of the trial. Early termination may also be considered if it becomes clear that the study is unlikely to be able to demonstrate a significant treatment difference. It will be left to the Monitoring Board to weigh the evidence and advise on early termination.

III.F.7. Risks versus Benefits.
There is little risk but potentially great benefit if the administration of high dose vitamin supplement proves to be a potent intervention for reducing the excess risk of arteriosclerotic CVD events in chronic, stable renal transplant recipients.

IV. TRIAL AND COMMITTEE ORGANIZATION

IV.A. Trial Organization.

Basic infrastructure of the study is ***depicted in the figure below:***



IV.A.1. Clinical Centers.

Listed below are the names of clinical centers willing to participate in the trial and their principal investigators, subject to final approval by the Executive Committee. Table 15 has a compilation of random chart review survey results completed in August/September 2001 to provide a hard estimate of the number of patients at each of these sites meeting basic FAVORIT eligibility criteria with respect to age, time since transplantation, and current creatinine clearance.

30 Primary Centers

University of Wisconsin- Madison	John Pirsch, MD
University of Alabama-Birmingham	Clifton Kew, MD
University of California-San Francisco	Deborah Adey, MD
Ohio State University	Todd Pesavento, MD
Cedars-Sinai Health System	Alice Peng,MD
University of Toronto	Edward Cole, MD
University of California-Los Angeles	Gabriel Danovitch, MD
Oregon Health Sciences University	Douglas Norman, MD
Medical College of Wisconsin	Barbara Bresnahan, MD
University of Maryland	Matthew Weir, MD
Washington University (St. Louis)	Matthew Koch, MD
University of Indiana	Muhammad Yaqub, MD
University of Michigan	Akinlolu Ojo, MD
Rhode Island Hospital/Lifespan	Andrew Bostom, MD
University of Iowa	Lawrence Hunsicker, MD
Albany Medical Center	David Conti, MD
Duke University	Stephen Smith, MD
Hennepin County Medical Center	Bertram Kasiske, MD
SUNY Health Science Center-Brooklyn	Mariana Markell, MD
London Health Sciences Center	Andrew House, MD
Northwestern University	Lorenzo Gallon, MD
University of Minnesota	Arthur Matas, MD
Mayo Clinic	Fernando Cosio, MD
Brigham and Women's Hospital	Ajay Singh, MD
Maine Medical Center	John Vella, MD
Banner Good Samaritan Transplant Services	Alfredo Fabrega, MD

Universidade Federal de Sao Paulo	Alvaro Pacheco-Silva, MD, PhD
Southern Illinois University	Tim O'Connor, MD
Drexel University	M.S. Anil Kumar, MD
East Carolina University	Paul Bolin, Jr., MD

Back-up Centers

Columbia Presbyterian	Mark Hardy, MD, and David Cohen, MD
University Hospitals of Cleveland	Donald Hricik, MD
Rush Presbyterian (Chicago)	Janis Orlowski, MD
Emory University	Carlos Zayas, MD

Table 13. Listing of renal transplantation centers agreeing to participate in proposed trial, with approximate numbers of eligible patients at each site.

Center	Approximate # patients meeting basic eligibility criteria & regularly attending center clinic	Approximate # of ***diabetic*** patients meeting basic eligibility criteria & regularly attending center clinic
University of Wisconsin-Madison	2340	1170
University of California-San Francisco	2380	770
University of Alabama-Birmingham	2014	836
Ohio State	1377	357
University of Pittsburgh *	1368	540
University of Toronto	910	260
University of California-Los Angeles	636	172
Oregon Health Sciences University	1075	301
Medical College of Wisconsin	648	207
University of Maryland	1806	903
Washington University-St. Louis	900	330
University of Indiana	770	210
University of Michigan	1244	526
Rhode Island Hospital/Lifespan	468	163
Albany Medical Center	483	224
University of Iowa	666	204
Duke University	570	278
SUNY-Downstate	264	92
London Health Sciences Center	482	176
Hennepin County Medical Center	664	160
TOTALS	20,365	7879

Lastly, 4-additional transplantation centers, (i.e., Columbia Presbyterian Medical Center; Rush Presbyterian; Emory University; and Municipal Hospitals of Cleveland) have agreed to be considered "back-up" centers in the event that one of the 20-selected centers cannot continue to participate.

IV.A.2. Operations Center.
Dr. Andrew G. Bostom, the Principal Investigator is the Project Director of the Operations Center. He has the overall responsibility for the study and chairs the Executive Committee. While the committee structure described below advises the Executive Committee in many areas related to the scientific conduct of the study, the Operations Center generally is the coordinating center for all the clinical and administrative activities of the trial. The Executive Committee will oversee the Operations Center on issues related to study design, progress and presentation of results, and statistical analyses. The organization of the Operations Center at Rhode Island Hospital includes the study Principal Investigator, epidemiological, administrative, and secretarial support. Dr. Bostom, as Principal Investigator and Chairman of the Executive Committee, provides medical /scientific, and epidemiological leadership. Joyce L. McKenney, MPH manages the Operations center, provides epidemiological leadership, and oversees the daily activities of the project. Persons with clinical, epidemiological, and nutritional/biochemical expertise have been recruited to be consultants to the project.
To forestall and minimize problems, one of the main responsibilities of the Operations Center is in adequate study-wide communication among clinical centers, the central laboratory, the VDC, study committees, the Data Coordinating Center, and the funding agency. This requires regular contact by e-mail, telephone, fax, mail and/or visit with all participating individuals.

The Operations Center interacts with the other investigators at semi-annual meetings. All contact with the funding institute and all administrative matters will be handled through this center. The members of the Center plus representatives from the DCC will attend the meetings of the Data and Safety Monitoring Board, *but only designated DCC representatives will participate in the confidential portion of the meeting related to interim efficacy analyses.* Site visits to the participating centers will be organized through the Operations Center. Investigators from this center will also serve on various subcommittees. The Operations Center will be linked electronically with all other centers.

We propose that this study will be a consortium between the funding agency, Rhode Island Hospital, and the collaborating organizations. The Financial Officer for the study is the Chief Financial Officer of Rhode Island Hospital who is responsible for receipt and dispersal of funds and prepares a statement of receipts and dispersals. The Fiscal Manager within the operations center is responsible for daily financial management of budgets and supervises the overall management of consortiums and total funding.

IV.A.3. Data Coordinating Center.
The Data Coordinating Center (DCC) provides epidemiological, biostatistical, data management, study management, and general scientific support for all components of the study. Technical support for the installation, use, and maintenance of local data collection equipment and software is provided by in-house staff. Dr. Lloyd Chambless heads the study's Statistical Coordinating Center, part of the Department of Biostatistics, School of Public Health, University of North Carolina at Chapel Hill.
The DCC staff participates in the activities of the Executive Committee and all subcommittees, providing technical assistance in study design, data collection, processing and analysis, training and certification, quality assurance, evaluation, and study implementation. For example, the DCC supports the End Point Committee in monitoring the status of each study end point preparing documentation of events to be verified and creating a final diagnosis file. The DCC's responsibility for the centralized management of the study includes the provision and tracking of training and certification, monitoring protocol adherence in the clinical centers, quality control in the central laboratory, and data management, including the development of a computerized data collection system, on-site and centralized data processing and data analysis. The SCC has over a 20 year record of successful collaboration with clinical centers, laboratories and other sponsoring agencies to meet the needs of the

projects for which it has been responsible. As the coordinating center for a number of multi-center medical studies, it has provided statistical, data management quality assurance, and study management services. The organization includes professional personnel from biostatistics, epidemiology, computer science/data management medicine, pharmacy, and nutrition. The professional personnel are supported by staff with training and experience in all of these fields as well as in study management office management and communications. DCC staff have been authors on over 200 peer-reviewed publications as well as several hundred presentations at scientific meetings. The DCC will perform the following functions in the support of the study:

1. Develop the study forms and the Manual of Operations with the guidance of the Steering Committee and assistance of the Operations Center.
2. Set up the distributed data entry system.
3. Train and certify clinical and laboratory personnel in the use of the data collection forms and the operation of the microcomputer system for data transmission and management support systems.
4. Receive data from all centers and the Central Laboratory and edit the data for errors.
5. Analyze the data.
6. Generate regular recruitment and quality control reports for the Steering Committee and, in addition to these, endpoint reports for the Data and Safety Monitoring Board.
7. Serve on study committees.
8. Assist in the preparation and authorship of papers, including providing statistical support.
9. Provide and facilitate communication support among the study units. In this regard, it is extremely important that the coordinators in each of the clinical centers, the Central Laboratory, and the Project Manager at the Operations Center develop a close rapport in their communications. An electronic mail media will be employed for the transmission of routine messages and for resolution of data problems. Telephone conference calls are also to be used for addressing larger problems.

IV.A.4. Central Laboratories.

The core laboratories for the study will be the adjacent Vitamin Bioavailability (VBL) and Nutrition Evaluation (NEL) Laboratories at the Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA) in Boston, Massachusetts. The principal investigator of the tHcy central laboratory (VBL) is Jacob Selhub, PhD. Gayle Perrone, MBA is a co-investigator at HNRCA who directs the Nutrition Evaluation Laboratory (NEL). Paul Jacques, ScD is a co-investigator at HNRCA who heads the Nutritional Epidemiology Program. *Random blood samples will be collected at all visits*. For screening, samples will be obtained for determination of tHcy, creatinine, and glucose, and collection of red blood cells and buffy coat specimens. For baseline and periodically throughout follow-up, samples will be collected for tHcy determinations, as well as for determination of folate, vitamin B12, and pyridoxal 5'-phosphate levels, creatinine, lipids/lipoproteins, glucose, and fructosamine. The sampling scheme for the performance of these assays from banked randomization and follow-up specimens (i.e., in the entire cohort, or within the context of specific "nested" designs) will be finalized by the Executive Committee. In addition, spot urine aliquots from baseline and follow-up visits will also be banked, and their analysis (for eg., for albumin/creatinine ratio) will be deferred pending ancillary study funding. Similarly, cells/buffy coat from the baseline and follow-up visits will also be banked for deferred analysis pending ancillary study support. Plasma (EDTA and citrate), serum, and cells/buffy coat will be aliquoted into cryopreservation tubes, frozen, and stored in the –80 degree freezers provided to each site. These specimens will be shipped to Boston in batches. While the protocol and timetable for shipment will be finalized during the 6-month protocol development and field testing phase, screening values for tHcy

and creatinine will be made available to the Data Center for transmission to the clinical centers within 2-3 weeks of specimen collection. The central laboratories will perform the following functions in support of the study: receive plasma samples, analyze (at the VBL) for tHcy, folate, B12, and PLP, as well as (at the NEL) creatinine, lipids/lipoproteins, glucose, and fructosamine, and store aliquots (both labs).

IV.A.5. Vitamin Distribution Center.
Pamlab, L.L.C. will serve as the Vitamin Distribution Center (VDC). The distribution of the vitamin supplements will be managed by the VDC. The vitamin supplements will be manufactured, bottled, labeled, and stored by Anabolic Laboratories, under the direction of Pamlab, L.L.C. The timetable for procurement and preparation of the vitamin supplies will be finalized during the protocol development phase to allow for the distribution of the prepared vitamin products in time for the planned start-up of randomization.

IV.B. Committee Organization.

IV.B.1. Executive Committee.
This is the policy and decision-making committee for the study, providing clinical and scientific direction at the operational level. Upon it sits the Principal Investigator for the Operations Center, the two key nephrologist consultants, the PIs for the Statistical Coordinating Center, and the Central Laboratory, a representative from the funding agency, and representative nephrologists/transplant surgeons from the clinical sites. This committee shares with the Principal Investigator the responsibility for overseeing performance in the study. Its membership consists of key individuals:

Andrew G. Bostom, MD, MS (Chairman) Principal Investigator	Operations Center, Rhode Island Hospital
Andrew S. Levey, MD	Nephrologist, New England Medical Center
Lawrence Hunsicker, MD	Transplant Nephrologist, University of Iowa Medical Center
Myra A. Carpenter, PhD	Epidemiologist, Data Coordinating Center UNC-Chapel Hill
Paul Jacques, ScD	Nutritional Epidemiologist, Jean Mayer USDA Human Nutrition Research Center
Marc Pfeffer, MD, PhD	Cardiologist, Director of Clinical Endpoints Core, Brigham and Women's Hospital
John W. Kusek, PhD	Project Officer, NIDDK/KUHD

The Executive Committee will meet by conference call at least bimonthly to deal with interim business (between Steering Committee meetings) to discuss the day-to-day and logistical needs of the study. Other individuals such as representatives from the Vitamin Procurement and Distribution Center, consultants, investigators from the participating centers and staff from the Operations and Statistical Coordinating Centers may be invited to attend Executive Committee meetings as ad hoc liaison.

The major responsibilities of the Executive Committee are in reviewing overall progress of the study with particular emphasis on the following activities:

(1) review and approval of any proposed revisions to the protocol or operations manual

(2) review of preliminary, non-confidential data

(3) review of reports from the Data Coordinating Center on performance of each participating institution, specifically:
   a) the rate of patient entry into the study as a whole and from each participating institution
   b) the timeliness and accuracy of data submission, including on-study data, treatment and information, follow-up reports
   c) completeness of baseline and follow-up data

   These reports will not include any data related to treatment efficacy, and will be presented in such a way as to preserve masking.

(4) identification and implementation of solutions to problems which arise, specifically:

   a) the consideration of on-site visits to those institutions with deficiencies
   b) consideration of additional institutions if one drops out or is dropped

(5) proposals to reflect additional information on patients, i.e., ancillary studies

(6) overseeing subcommittees listed below

IV.B.2. Steering Committee.
This committee consists of personnel from the Operations Center, the Data Coordinating Center, & Central Laboratories, the principal investigator from each of the participating clinical centers, as well as representatives from the patient coordinators, the funding institute, Vitamin Distribution Center, and consultants. The Steering Committee meets at least once a year throughout the study, including the period for final analysis and writing activities that follow the conclusion of patient follow-up.

To reduce costs and minimize travel time, attempts are made to schedule the meetings around professional meetings which investigators are already planning to attend, e.g., American Society of Nephrology, American Society of Transplant Physicians, etc. These meetings bring together investigators and clinical coordinators from the various participating centers for discussion regarding progress of the trial, possible changes in the protocol or methodology, new developments in the field, revitalization of interest, and other matters of concern to participants in the study. These meetings also provide an opportunity for staff training and education.

Various study design and planning committees assist the Executive Committee in such tasks as writing, revising and implementing the Manual of Operations, in standardizing diagnostic or therapeutic methodology, in monitoring the accumulation of patients, and in carrying out editorial work on abstracts, presentations and manuscripts. Much of the work of the subcommittees is handled through regular phone and e-mail/mail communications and the annual Steering Committee Meeting, but

conference calls and special meeting are called as needed. These committees, which report to the Executive Committee, are open to all investigators from the participating clinical centers and include representatives from the Operations and Data Coordinating Centers. The membership roster will be completed during the Protocol and development and field testing phase.

IV.B.3. Endpoints Verification Committee.
This committee, headed by Dr. Marc Pfeffer of Brigham & Women's Hospital Cardiovascular Division, has responsibility for the development and validation of the system for end point review and verification. Throughout the entire study, this committee advises the Executive Committee on any modifications or enhancements to the system. This committee has the responsibility for classification of whether or not a patient has reached a verified non-fatal myocardial infarction (MI) or coronary heart disease death end point, as well as for classification of whether or not a patient has reached a verified end point of non-fatal or fatal stroke. The role of this committee is ongoing throughout the entire study period. This committee analyzes the data biannually during the study to review the documentary evidence (without identity of treatment) and decision by the End Point Verification system on each patient evaluated by the system as suspect of having had an MI, coronary heart disease death, non-fatal stroke, or stroke death. Such central evaluation, with the subject's identity and intervention group assignment blinded, helps to assure unbiased classification of reported events and to eliminate problems of variable interpretation of event definition.

IV.B.4. Data and Safety Monitoring Board.
This committee will be set up by the funding institute, independent of the study investigators, to monitor the study results for evidence of adverse or beneficial treatment effects throughout the study period. The Monitoring Committee will remain "blinded" to outcome characteristics of the study for as long as possible. While the committee may have access to any information that is deemed necessary to make an appropriate determination, highly sensitive information in relation to the outcome of the study will be requested on a "need know" basis as it may arise during the course of the committee's deliberations. The committee's concerns will be directed to patient accrual, appropriate follow-up, compliance, data acquisition, undue complications, and whether the study as it is currently being conducted will be able to answer the hypothesis it addresses. The membership and frequency of meeting are at the discretion of the funding institute but will presumably consist of at least 5 members including a biostatistician, 3 clinical investigators, and a scientist from the funding institute. It is expected that this committee will meet one to two times per year and will report to the funding institute on scientific and administrative issues. For example, the Board has the responsibility for recommending early termination in case of unanticipated toxicity or greater than expected benefit. The responsibility for subject safety is particularly important, since the individual investigators are unaware of the group assignments.

IV.B.5. Additional Committees/Subcommittees.

IV.B.5.1. Recruitment Committee.
The responsibilities of this committee are to develop materials and presentations to assist investigators in recruitment of cases, and materials to encourage patient and physician interest and participation in the study. Throughout the study recruitment period, this committee advises the Executive Committee on suggested strategies to decrease deficiencies in patient accrual and where necessary, works with specific centers with particular problems.

IV.B.5.2. Quality Control Committee.
This committee will be responsible for assuring high quality data by monitoring clinic and center performance and initiating corrective action when needed. Internal quality control reports provided by the DCC will be reviewed. A system for sending blinded replicate samples to the central lab will be developed by this committee and implemented by the DCC with the results monitored by this committee.

IV.B.5.3. Publications Committee.
This committee, will formulate publication policy for this collaborative research and review all abstracts, papers and scientific presentations which utilize study data. The Publications Committee will be responsible for identifying topics for publication as well as making writing group assignments. The subcommittee will review and recommend approval or disapproval of all scientific abstracts and papers or presentations using unpublished study data, as well as every paper using published data that purports to represent official study views or policy. Another major responsibility of the Publications Committee is in the development of plans for the dissemination of trial findings and incorporation of the findings into medical care policy. This will involve not only reports in medical journals but consideration of continuing education courses, conferences and seminars and special efforts such as press conferences, editorials, physician newsletters and presentations at local medical association meetings.

IV.B.5.4. Ancillary Studies Committee.
It is anticipated that both intramural and extramural investigators will wish to capitalize on the potential for collaborative ancillary investigations afforded by the implementation of the main study. The Ancillary Studies Committee, headed by, will formally review and recommend approval or disapproval of all proposed ancillary studies, considering both their impact on the conduct of the main study, and their scientific merit.

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Chapter 10. Endpoints

10.1 Overview

The primary endpoint of the study is comprised of recurrent or de novo cardiovascular disease (CVD) outcomes. It is the pooled occurrence of non-fatal and fatal arteriosclerotic outcomes, including coronary heart, cerebrovascular, and peripheral vascular disease events. The CEC will receive and adjudicate death, myocardial infarction (MI), unstable angina (USA)*, stroke and resuscitated sudden death (RSD). Specific procedural events will be classified based on clinical site reporting.

* The CEC will treat USA events as MI events. USA events are being reviewed in order to make the determination whether the event met the criteria for a MI event.

10.2 Definition of Primary and Secondary Endpoints

The primary and secondary endpoints are pooled from the following specific outcomes:

- CVD death
- Myocardial Infarction
- Stroke
- Resuscitated Sudden Death
- Coronary artery disease (CAD) determined by participant undergoing coronary artery revascularization, either bypass surgery or angioplasty*
- Lower extremity arterial disease determined by participant undergoing lower extremity arterial angioplasty or bypass surgery, or lower extremity amputation above the ankle for severe disease (rest pain and/or gangrene)*
- Extracranial carotid arterial disease determined by participant undergoing carotid endarterectomy (CEA), or angioplasty*
- Renovascular disease determined by participant requiring renal artery revascularization, either bypass surgery or angioplasty*

*These events will not require review and classification by the Clinical Endpoints Center. If any of these events occur, they are reported on the Hospitalization and Outcomes Documentation forms.

10.2.1 Definition of Primary Endpoint

The primary endpoint is defined as the first occurrence of one of the above-listed recurrent or de novo arteriosclerotic outcomes during the primary endpoint follow-up period. The primary endpoint follow-up period is defined as follows:

- For randomized participants who become dialysis-dependent prior to the end of the study, statistical censoring will occur at 3-months (90 days) after the (re-)initiation of dialysis.
- For all other randomized participants, the primary endpoint follow-up period is until the end of the study.

10.2.2 Definition of Secondary Endpoint

Secondary endpoints consist of all cause mortality, graft failure, individual and relevant combinations of the components of the primary endpoint, and the number of these endpoints that occur during the study follow-up period (all participants will be followed for endpoints until death or the conclusion of study follow-up with the exception of participants who return to dialysis dependence and are followed only until the first primary endpoint.)

NOTE: Refer to Section III.D.1 – Section III.D.1.5 in the FAVORIT study protocol for more information on study endpoints.

10.3 Clinical Endpoints Center (CEC)

10.3.1 Introduction

Given the wide range of FAVORIT clinical sites, there will be differences in how participants are treated and how certain diagnoses are made. For example, from clinic to clinic, there will be subtle differences in how the diagnosis of a myocardial infarction is made and what is done to treat each event. There are also apt to be differences in how death is classified from clinic to clinic.

In order to eliminate these expected site-to-site differences, all reported death, myocardial infarction, stroke, and resuscitated sudden death events will be reviewed and classified by one group (the CEC) who will use standardized criteria to adjudicate each event. At the end of the trial, when reports are generated on the study data and a comparison is made between the number of events in one treatment group versus the number of events in another treatment group, investigators can be confident the events reviewed by the CEC all met the same criteria and are supported by source documentation.

10.3.2 Role and Responsibilities

The Clinical Endpoints Center at Brigham and Women's Hospital serves as an independent committee responsible for defining, reviewing and classifying FAVORIT endpoints. Every death, myocardial infarction, unstable angina, stroke, and resuscitated sudden death event reported by each site will eventually be sent to the CEC. The CEC will then review each event and determine whether it meets the pre-specified endpoint criteria. In the case of death, the CEC will classify each death according to pre-specified 'death classifications'.

10.4 Clinical Site Procedures for Data Collection

The clinical sites have a crucial role in identifying and facilitating the classification of FAVORIT endpoints. In addition to providing information about potential events on case

report forms ((CRF), Informant Interview, Hospitalization and Outcomes Documentation Forms), sites provide essential source documents for use in the CEC review process. **Without the contributions from the sites, the CEC will not have the necessary information to review and classify endpoints in a standardized manner. The CEC therefore is only as good as the information provided by the sites.** Each site contributes to the classification process by promptly reporting potential endpoints on the Informant Interview, Hospitalization and/or Outcomes Documentation forms and submitting the appropriate source documentation with each event (see section 10.4.2 and 10.4.3, below, for source documents required for each event). In certain instances, the CEC may have questions after the site has reported the event and sent in the necessary source documents. The sites are essential in providing additional information that may be requested by the CEC.

It is important to note that if the CEC receives an event and determines that it does not meet the pre-specified criteria for this event, it does not mean that the event did not occur. Rather, it means the event did not meet the FAVORIT criteria for a study endpoint.

10.4.1 Assignment of Event Packet ID (EPID)

For every hospitalization that reports a death or FAVORIT potential endpoint event requiring CEC review (i.e., triggers an Outcomes Documentation Form) an Event Packet ID will be assigned. The same EPID number will be entered in both the HOS and the OUT. The HOS allows study coordinators to report up to three of the same kind of events (e.g., 1 or 2 or 3 MI's), but the OUT allows for only one of each kind of event. Therefore multiple OUT forms must be used when reporting more than one of the same types of event. In this case, the same EPID number is used on the HOS and on all of it's associated OUT forms.

If the participant was transferred to a second hospital a second HOS form is completed for the transfer hospitalization. Both HOS forms will be assigned the same EPID if either triggered an OUT. See HOS QxQ's for more details.

If the participant dies out-of-hospital an Informant Interview Form (INF) is completed and an EPID number is assigned. Likewise, an OUT is completed and the same EPID number is used.

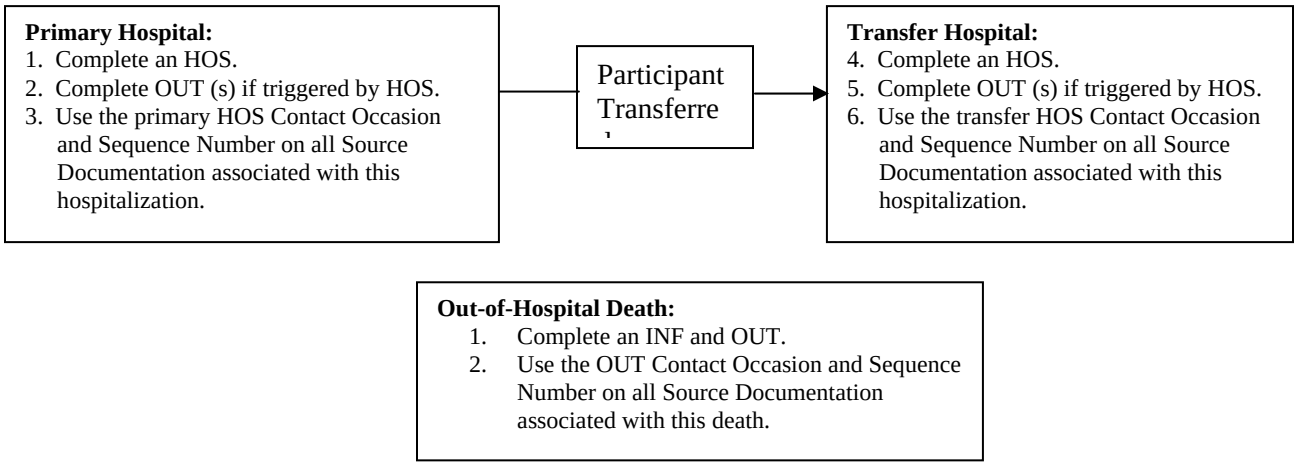
EPID labels will be provided to the clinics by the DCC along with an EPID Log Sheet. The Study Coordinators record the participant's FAVORIT ID, all forms, contact occasions and sequence numbers associated with each EPID on the EPID Log Sheet (see table 10.1).

10.4.2 Source Documentation

In addition to the entry of FAVORIT case report forms, supporting source documentation is required to be submitted. **The CEC relies heavily on source documentation in order to determine whether the criteria were met for a particular event.** For events to be reviewed by the CEC (death, myocardial infarction, unstable angina, stroke, resuscitated sudden death, and suspected peri-operative/post-CABG MI), source documentation must be filed locally **and** submitted to the DCC. For procedural events, source documentation must be filed locally in the participant's study binder/file.

All source documentation submitted to the DCC must include the EPID, participant ID number, contact occasion and sequence number corresponding to the associated HOS or OUT entered into the DMS. See below for which contact occasion and sequence number to include.

Contact Occasion and Sequence Numbers for Source Documentation:



An Endpoint Source Documentation Cover Sheet will accompany all source documentation being sent to the DCC. Use a separate cover sheet for each EPID number. See table 10.2.

In addition, **all documentation sent to the DCC must have confidential identifiers masked.** Information to mask, or black-out, includes participant name, social security number, physician and other individual names, medical record numbers, and other identifiers deemed confidential by local regulations.

Masked and labeled supporting documentation and cover sheet can be mailed to:

Barbara Brown
FAVORIT DCC
CSCC-UNC Biostatistics Dept.
137 E. Franklin Street, Suite 203, CB# 8030
Chapel Hill, NC 27514-4145

or faxed to Barbara Brown at 919 962-3265.

The following table provides an overview of the supporting source documentation that is required for each event type:

Suspected Event	Site Source Documentation
MI and USA	- Lab reports, if drawn with ULN values. Only need peak values. - If CK is the only marker being reported, site is required to submit all CK values with serial changes. - If Troponin reported in ranges, all reference ranges must be reported. - If the event being reported occurs within the setting of a coronary revascularization, lab reports should be provided for labs drawn both immediately before the procedure and for all labs drawn within 24 hours following the procedure. - Discharge Summary/ Physician Narrative, as described on Outcomes Documentation Form. *Note: ECGs are not required*
Urgent Coronary Revascularization (PCI or CABG)	- See above. - In addition, if there were new Q waves or other wall-motion abnormalities identified peri-operatively, require documentation of at least two comparative ECGs documenting new Q waves or echo report documenting wall-motion abnormality.
Stroke	- Discharge Summary/ Physician Narrative, as described on Outcomes Documentation Form. - Neurology consult note, if performed and available; otherwise a Physician Narrative. - Imaging reports, if performed and available; otherwise a Physician Narrative.
RSD	- Discharge Summary/ Physician Narrative*, as described on Outcomes Documentation Form. * Should include confirmation of cardiac arrest, loss of consciousness, and what resuscitative measures were used.
Death	- Discharge Summary/Physician Narrative, as described on Outcomes Documentation Form. - Autopsy report, copy of report or principal findings is required if performed. - If MI was suspected prior to death, please provide marker results and any ECG findings. - Death certificate, if no data available* - US Renal Data System (USRDS) form, if no data available* * Should be provided if no other documentation is obtainable; otherwise, the DCC will request on a case-by- case basis per the CEC Physician Reviewers

10.4.2.1 Physician Narrative

As mentioned in the above table, a Physician Narrative is often requested. A physician narrative should be sent to the DCC when medical records that can be obtained for an event do not adequately provide a clear and accurate description of the event being reported. NOTE: If each event being reported is well supported by source documentation, it is not necessary to provide a Physician Narrative.

These Narratives are most often provided when:

- Discharge Summary cannot be obtained or does not address the event being reported (i.e., if the participant was hospitalized for 2 months and the stroke being reported was not a major component of the hospitalization and was only briefly mentioned or if a participant died in the hospital after a long hospitalization but the details leading up to the death were not well documented); or,

- Death is reported that occurs outside of a hospital or in an emergency room (i.e.,

when details of the participant's condition leading up to the death may be scarce).

For non-fatal events being reported in a Physician Narrative, the Narrative should be a clear and accurate description of key details to each event being reported in the hospitalization. For example, if two MI's and a Stroke are being described in a Physician Narrative, there should be three distinct summaries of the three events being reported including key details relevant to each event: description of the clinical course in the hospital, symptoms, marker elevations, ECG changes, neurological deficits, etc. Please keep in mind however, if there is adequate source documentation for the three events except the neurology consult note cannot be obtained, it is only necessary to provide details about the neurologists findings as the other events are well documented elsewhere in the medical record.

Considerations for completing a physician narrative for other hospitalization events:

- Is the hospital discharge summary available? If so, does it include dates of admission and discharge, and all admitting and discharge diagnoses?
- Does the documentation provided provide a full description of clinical symptoms and physical findings?
- Are reports being provided that document the relevant tests that were performed and principal findings?

For death events being reported in a Physician Narrative, the key information to provide are reasons that support the local FAVORIT investigator's death classification, details of the participant's condition prior to death, and a general description of the circumstances surrounding the death. In addition, if an autopsy was performed but the report is not able to be obtained, the Physician Narrative can be substituted to provide the key autopsy findings.

Considerations for completing a physician narrative for a death:

- Did the death occur shortly after an important medical event? If so, did that non-fatal event contribute to, or directly cause the participant's death?
- Was the participant expected to die? Describe the participant's condition in detail, prior to death (i.e., if the participant had been failing or had a poor prognosis prior to death, please explain).
- If the participant had been stable or out of the hospital, support your cause of death and describe the circumstances by which the participant was found to have died.
- Key autopsy findings, if performed, when report unobtainable.

10.4.3 Clinical Site Procedures for Events to Undergo CEC Review

Events that are reviewed by the CEC are:

- Death
- Myocardial Infarction (MI)
  - Non-Procedural MI
  - Post-PCI MI
  - Post-CABG MI

- Unstable Angina (USA)
- Stroke
- Resuscitated Sudden Death (RSD)

See Figure 1 for information regarding the procedure to follow when reporting FAVORIT events and the specific source documents required for each event. Events should be reported (i.e., HOS and/or OUT completed and appropriate source documentation submitted) as soon as they come to the attention of the clinical site. Do **not** wait until a scheduled study contact. Refer to Chapter 14, section 14.2 for instructions for assigning the proper contact occasion and sequence number for data collection between scheduled contacts.

10.4.3.1 Death

The HOS, OUT, and INF (if the participant died outside of the hospital) are designed to capture the key information required for classification of death events. In order for the study to have an accurate and knowledgeable classification of each death that occurs during the FAVORIT trial, it is important to provide clear, thorough, and accurate information about the participant's status prior to the death, the events leading up to death, and the circumstances of the participant's death.

Since there may be considerable delay in obtaining the records needed to complete some of the forms, **all deaths are to be reported to the DCC within one day of the date that the clinical center is notified of the event, regardless of availability of records. This report should be made by fax using a Death or Serious Adverse Event FAX Notification (DSA) form (See Chapter 9).**

The principal categories listed below are listed in the death section of the OUT. When death occurs, the investigator should review this list and determine the most applicable classification of death. The same categories below will be used by the CEC, after reviewing case- report forms and source data provided by the site, to classify death. For some analyses, death will be further classified in two broad categories: Cardiovascular or Non-Cardiovascular. The classifications provided by the CEC will be used by the study in the final analysis.

- Cardiovascular
    - Atherosclerotic Coronary Heart Disease
    - Atherosclerotic vascular disease, excluding coronary disease
    - Other Cardiovascular Disease (Non-Atherosclerotic)
- Non-Cardiovascular

- Unknown

10.4.3.2 Myocardial Infarction and Unstable Angina Events

The HOS and OUT are designed to capture the key information required for classification of myocardial infarction events. Positive cardiac markers are required to meet the criteria for a FAVORIT non-procedural MI. Sites should report all peak values of cardiac markers, including the respective upper limit of normal (ULN) values, for each event in order for it to

be reviewed by the CEC. Either positive markers or new Q waves are required to meet criteria for a FAVORIT post- PCI or post-CABG MI. Sites should report all peak cardiac markers and/or provide ECG documentation when there is objective evidence of either for a post-procedural MI.

Although not a study endpoint, sites are also asked to report all unstable angina events. The purpose for this is so the CEC can screen for possible events that meet the criteria for myocardial infarction. In the opinion of the study investigator, if the participant is felt to have had an episode of unstable angina, consisting of myocardial ischemic pain where cardiac enzymes and/or ECGs were done to rule out a myocardial infarction, this should be reported. The CEC will review the data from the unstable angina event to determine whether the FAVORIT criteria for myocardial infarction were met.

NOTE: Sites should be sure that all lab reports and all ECGs that may be sent in are labeled with the date and time.

In the HOS, additional questions are asked when an urgent coronary revascularization occurs. These questions query whether a possible post-procedural MI occurred in association with the coronary revascularization *and it otherwise is not being reported by the site as a post-procedural MI*. Answer these questions to the best of your knowledge and following the instructions on the form. In some instances you may be asked to complete an OUT form and send in the appropriate source documentation if a post-procedural MI is suspected.

10.4.3.3 Stroke

The HOS and OUT are designed to capture the key information required for the review and classification of stroke events. In general, strokes are considered to be neurological deficits not resolving within 24 hours. Transient ischemic attacks (TIAs) should <u>not</u> be reported.

10.4.3.4 Resuscitated Sudden Death (RSD)

The HOS and OUT are designed to capture the key information required for classification of this event. In general, the participant must be successfully resuscitated after a cardiac arrest with loss of consciousness. Events where the participant is brought to the Emergency Room, suffers a cardiac arrest, and subsequently dies in the Emergency Room would not be considered 'resuscitated'. If there is any uncertainty as to whether an event would be considered resuscitated, the event should be reported. The CEC will determine whether the FAVORIT criteria are met for this event.

10.4.3.5 Procedural Events

The following are procedural events that will contribute to the pooled primary endpoints:

- CAD determined by participant undergoing coronary artery revascularization, either bypass surgery or angioplasty
- Lower extremity arterial disease determined by participant undergoing lower extremity arterial angioplasty or bypass surgery, or lower extremity amputation above the ankle for severe disease (rest pain and/or gangrene),
- Extracranial carotid arterial disease determined by participant undergoing CEA or

angioplasty
- Renovascular disease determined by participant requiring renal artery revascularization, either bypass surgery or angioplasty

10.4.3.5.1 Procedural Events Supporting Source Documentation

Procedural Events not associated with an MI: For procedural events that will contribute to the pooled primary endpoint, sites must insure that the appropriate supporting documentation for each of these events is stored in the participant's FAVORIT file at the clinical site. For example, if a site reports that a participant underwent lower extremity amputation, thus meeting the criteria for Lower Extremity Arterial Disease, the site should keep a copy of the operating report and discharge summary in the study file.

Urgent Post-Procedural PCI or CABG Events associated with an MI: If a post-PCI or post-CABG MI was performed urgently and is being reported within the setting of a MI, lab reports should be provided for labs drawn both immediately before the procedure and for all labs drawn with 24 hours of the procedure. If there were Q waves or other wall-motion abnormalities identified peri-operatively source documents of at least two comparative ECG's documenting new Q waves or an echo report documenting wall-motion abnormalities should be sent to the DCC.

10.4.4 How long to monitor for and report on potential endpoints

All <u>non</u>-dialysis dependent participants: Participants will be followed for potential endpoints from the time of randomization until the date participant follow-up ends, or death, whichever occurs first.

Participants who become dialysis dependent: Participants who become dialysis-dependent prior to experiencing a primary endpoint, will be followed indefinitely until their first primary outcome occurs. Clinical sites will be notified as early as possible when a primary outcome has been classified, at which point only mortality surveillance sufficient to determine CVD vs. non-CVD death will be required.

Renal transplant recipients who become dialysis-dependent after experiencing a primary outcome will undergo only mortality surveillance sufficient to determine CVD vs. non-CVD death until the end of the study. The DCC, working with the CEC, will notify each site as early as possible when non-fatal endpoint follow-up can cease on participants who become dialysis-dependent. This will be a continuous process throughout the course of the study.

Figure 1: Overview of Reporting Procedure

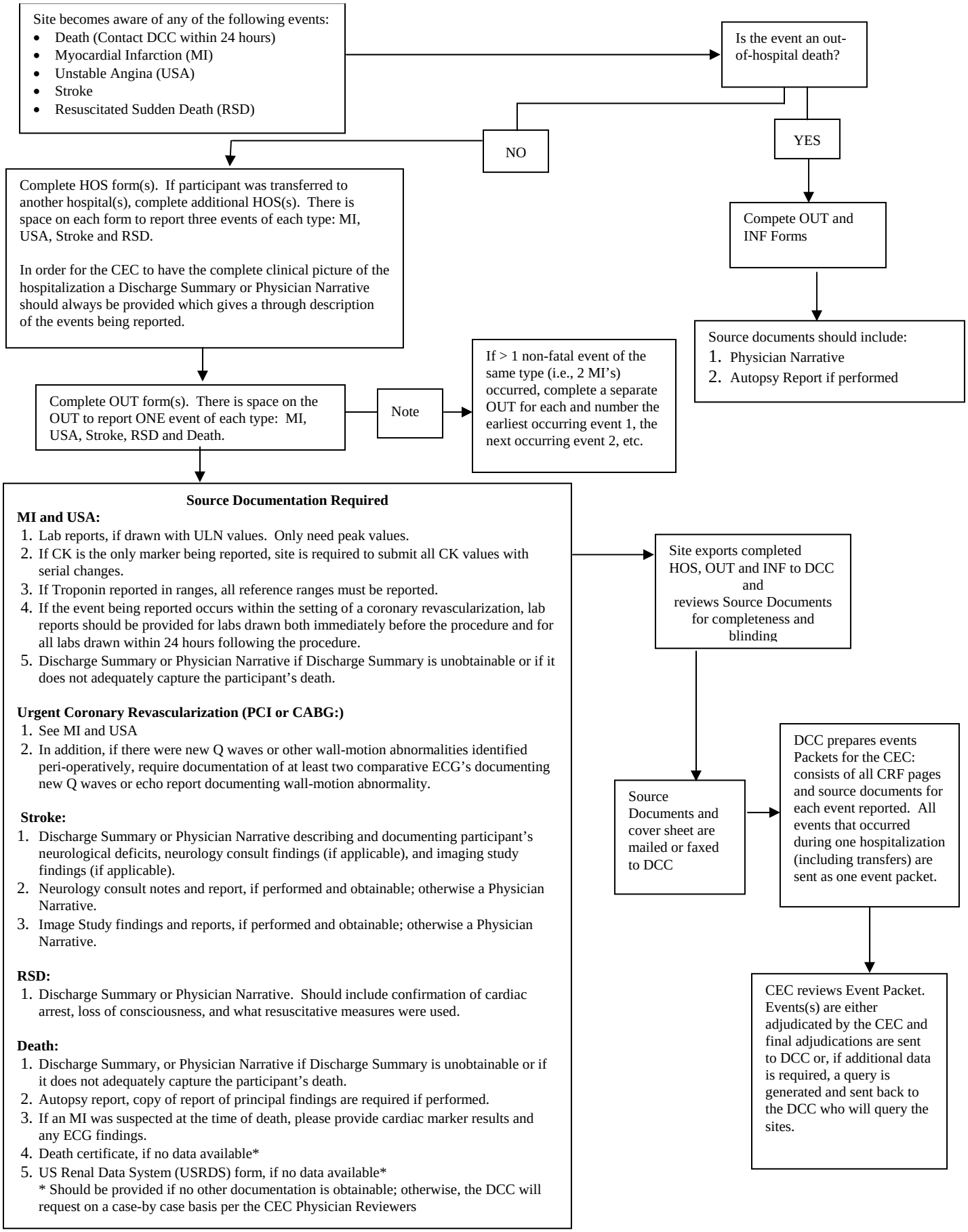


Table 10.1: EPID Log Sheet

EPID#	PARTICIPANT ID#	FORM	CONTACT OCCASION	SEQUENCE NUMBER
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5
		HOS OUT INF	01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	00 01 02 03 04 0 5

Table 10.2: Endpoint Source Documentation Cover Sheet

FAVORIT
Folic Acid for Vascular Outcome Reduction in Transplantation

ENDPOINT SOURCE DOCUMENTATION COVER SHEET

FORM CODE: ESD VERSION: A 04/11/03

TO: Barbara Brown
FAVORIT DCC

FROM:

FAX: 919-962-3265

DATE:

PHONE: 919-962-3092

Total # of pages, including cover page:

Use this cover sheet when faxing/mailing source documentations for events to the Data Coordinating Center. Use the preprinted labels for the Participant ID and EPID. Record the Contact Occasion and the Sequence Number from either the Hospitalization (HOS) or Outcomes Documentation (OUT) Form. Record the **number of pages** being faxed/mailed for each of the source documentations.

Participant ID: | | | | | | | | **EPID:** | | | | | | | |

A. Primary Hospital

HOS Contact Occasion:_____

HOS Sequence Number:_____

Hospital Admission Date:_____

Source Documentation	Total # of pages
Discharge/Death Summary	
Physician Narrative	
Autopsy Report	
Lab Report	
Neurology Report	
Imaging studies Report	
ECG/Echo Report	
Other	

B. Transfer Hospital

HOS Contact Occasion:_____

HOS Sequence Number:_____

Hospital Admission Date:_____

Source Documentation	Total # of pages
Discharge/Death Summary	
Physician Narrative	
Autopsy Report	
Lab Report	
Neurology Report	
Imaging studies Report	
ECG/Echo Report	
Other	

C. Out of Hospital Death

OUT Contact Occasion:_____ **OUT Sequence Number:_____**

Source Documentation	Total # of pages
Death Summary	
Physician Narrative	
Autopsy Report	
Other	

Chapter 11. Quality Assurance and Control

11.1 Overview

The integrity and ultimate credibility of the study depend on such factors as ensuring adherence to the protocol, completion of follow-up information on all participants enrolled, and using quality control measures to establish and maintain high standards for data quality.

Procedures designed to enhance adherence to the protocol begin with the training and certification of clinic personnel before participants are enrolled. Once enrollment begins these same measures will continue along with monitoring clinics, operating suitable quality control systems, checking the quality of data collection and the timeliness with which data are received from the clinics and Central Laboratory.

11.2 Quality Assurance and Control

Quality assurance is considered here as relating to activities to assure quality of data which take place prior to collection of data, while quality control relates more to efforts during the study to monitor the quality of data at identified points in the collection and processing stages. This chapter will focus on quality control, whereas quality assurance is the essence of the entire Manual of Procedures (MOP) and includes the following activities:

- Detailed procedures. A clear description of the study design, training, certification, and the various data collection activities provides the blueprint for the study. The MOP is a written reference for study coordinators. Procedures for handling the routine, as well as the unexpected, are given.
- Training and updating training. Training is the transfer of the study plans in the Manual of Procedures to the study coordinators and other staff members. Special materials for this purpose will be developed for FAVORIT and will be the basis for initial training and for continuing education during the study.
- Certification. Criteria to examine the adequacy of an individual's training will be established. Individuals meeting these criteria will then be qualified to execute a procedure. The Data Coordinating Center (DCC) will monitor the study to ensure that staff performs only those functions for which they are certified.

For quality control purposes, FAVORIT data collection and transmission will be monitored by observation and by quantitative assessment using both specific quality control procedures (e.g., repeated samples for laboratory assays) and statistical analysis of study data. Monitoring will be performed by the DCC via periodic site visits. A summary of selected aspects of FAVORIT quality control follows:

- Protocol adherence. Periodic monitoring visits will be made to each site to review adherence to FAVORIT procedures and audit a sample of the data collected. Feedback and general recommendations for improvements will be provided. These visits also provide an opportunity for clinical staff to discuss questions, concerns, and suggestions with the DCC.

- Quantitative monitoring. Random repeat measurements will be used to evaluate laboratory blood samples. Duplicate blood samples will be obtained in tandem with approximately 5% of the specimens collected for the study. These samples will be processed, shipped and stored in the same way as regular samples. The laboratory will be blinded to the identity of the duplicate samples and will run assays on them in the same way as for other samples. The DCC will then produce statistical analyses of the repeatability of the results.

- Reporting results. Two aspects of reporting quality control monitoring should be emphasized. First, the results must be timely. When remedial action is required, reporting must be prompt so that a return to an acceptable level of performance is not delayed. Second, the reporting format must be easily understood.

- Action on results. With conscientious and trained staff, quality control reports provide an opportunity to praise a job well done. On the other hand, a poor performance is the basis for remedial action. Depending upon past performance, the amount of error, and, taking due account of personal circumstance, the appropriate action may be a simple discussion to encourage a better performance. Re-training may also be appropriate at times.

11.3 Training and Certification of Clinical Center Staff

Each new coordinator will attend central training at the DCC soon after starting with the FAVORIT study. Continued investment in quality data during the study will be made by periodic refresher training sessions which review the protocol and update personnel on any changes which may have occurred.

Certification of study personnel is an essential aspect of effective quality assurance in FAVORIT. After attending a central training or being trained at the local clinic by another centrally-trained coordinator, and passing the lab questionnaire, the coordinator is considered certified. Areas of training essential for certification include specimen collection and processing, endpoints ascertainment and learning the data management system. The DCC will monitor the study data collection to ensure that staff performs only those functions for which they are certified . To protect the quality of the study results, data will not be collected by non-certified personnel.

In order to maintain proper collection of data despite potential for personnel changes over the long term follow-up period, the DCC and the Operations Center are jointly responsible for establishing and providing the requisite minimum criteria and training and ensuring continued adherence to standards.

11.4 Clinic Monitoring

There are two major concepts in the FAVORIT approach to clinic monitoring.

Firstly, clinic staff are encouraged to contact the DCC for an immediate answer to an operational question or for help in obtaining clarification of a particular situation. For questions of a clinical nature, the clinic staff has a liaison at the Operations Center who directs the question to the appropriate committee chairperson. For each clinic, a primary Study Coordinator is identified. Each site has a Principal Investigator who has the overall responsibility for the recruitment of participants and management of the study at the clinic.

Secondly, site visits are made to individual participating centers by a clinic monitor from the DCC or the Operations Center. Monitors will compare data sent to the DCC to that in the clinic and hospital records, and verify adherence to protocol. In addition, if recruitment falls below a certain level, appropriate personnel designated by the Executive Committee, such as a team consisting of a transplant nephrologist or surgeon and coordinator from a highly productive center, will be sent to advise on recruitment strategies. There may also be times when a particular clinic demonstrates a need for assistance in following protocol, filling out forms, or documenting events beyond what can be done through telephone conversations

11.4 Data Quality Monitoring

The DCC helps reduce the frequency of errors as much as possible through discussion of study procedures before recruitment begins, and review of the protocol during the study as necessary. Data checks (queries) from data collection forms are sent to clinics on a monthly basis to ensure correct data. Monthly data management reports of data quality and participant follow-up are prepared and circulated by the DCC. These reports are reviewed to determine which clinics may need to be visited.

11.4.1 Data Check Reports

Data checks are compiled and distributed monthly for use in investigating and correcting specific problem items in collected data. The reports list problems - by participant - regarding missing, unexpected, and inconsistent data. Follow-up is provided by the DCC to help sites resolve identified problems.

11.4.2 Data Management Reports

Data management reports are compiled and distributed monthly for use in identifying general problem areas in data collection. The standard reports include data on:

- Screening, randomization and follow-up contacts
  - Timeliness of blood sample processing
  - Completeness of forms
  - Timeliness of randomization and follow-up contacts

- Participant Withdrawal Rate
  - Number of participants who have withdrawn from the study

- Participant Vitamin Adherence Rate
  - Number of participants whose adherence is less than 75%

11.5 Laboratory Quality Monitoring

11.5.1 Internal Central Laboratory QC

Specimens are assayed in duplicate within the same assay run for each procedure.

Specimens are re-assayed when quality control material for an analytical run is outside the acceptable range, when the analytical result is outside the reference range and/or when the analytical result is outside the assay performance range (i.e., outside the linear range, off the standard curve, etc.).

Each assay has a standard operations procedure (SOP) for calibration. The policies, where applicable, established by the Clinical Laboratory Improvement Act (CLIA) and the State of Massachusetts, are followed for calibration and linearity studies.

Each assay has a SOP for verification procedures. The Central Laboratory participates in the College of American Pathology's (CAP) Excel and Survey proficiency program for procedures where obtainable. For analytical procedures that are not part of the CAP Excel proficiency program, the laboratory uses either other commercial proficiency program or reference materials such as those from the National Institute of Standards and Technology (NIST). When commercial material is not available, the laboratory will participate in independent Round Robin programs with outside laboratories.

The Central Laboratory runs at least two quality control levels for each procedure. Where available, it uses commercial materials which supply assayed normal and abnormal levels. For laboratory methods in which no commercial materials are readily available, in-house pools established at a low and high level are used. The ranges for QC materials are determined by assaying the material repeatedly in separate runs, determining the mean of all the control measurements, adding 2 standard deviations to the mean to get the upper end of the QC range and subtracting 2 standard deviations to get the lower cut-off of the QC range.

It is expected that the established ranges from commercial material will coincide with the published values. If this is not the case, procedures are in place to address this problem.

The general quality control policy in the Central Laboratory is to assay the QC material exactly according to the procedure used for "real" samples and to scatter them throughout the run, (i.e., beginning, middle and end). The data from the controls are reviewed daily through application of the Westgard Rules. The analytical run is repeated when the control values fall outside the acceptable ranges established for each set of quality control specific for each procedure. Daily logbooks are kept for each day's accepted quality control data. Monthly Levey-Jennings plots and/or Statistical Table Reports are generated from the daily QC data logs. These reports are reviewed for trends and to identify causative factors for outlying data points.

Remedial actions, specific for each procedure, are in place for calibration and/or quality control failing to meet acceptable criteria.

Alternate methods for performing a test or storing specimens should a system fail, are in place for each procedure. In most cases for research projects, the laboratory will repeat the test using the "SAVE" aliquot, (i.e., backup aliquot) provided one exists once the system is up and running.

A subject's results are entered into the Central Laboratory's Information System (LIS - Lab Web, Psyche System, Inc., Welleslay, MA) or a Project Data base system, either via automatic data transfers from instrument to subject's electronic record, or by manual data entry directly into the subjects electronic record. Reports are made after each data entry to ensure correct input of values. LabWeb reports for the Principal Investigator are downloaded directly from LabWeb into Excel files. The NEL manager reviews the final reports before electronically sending the data set in an Excel file to the principal Investigator. Reports for projects that utilize data bases that are separate from LabWeb, are defined by each project and the procedures are documented on file.

11.5.2 External Laboratory QC: Blind Replicate Matching

Blind Replicate Matching (BRM) for the FAVORIT study is a method of quality control to check the precision of methods used by a laboratory analyzing blood and urine samples. This is a '**blind**' check as the lab does not know which samples are being used to test the quality of its work. Essentially, two '**replicate**' tubes from the same person are labeled with two different IDs so that the lab does not know they are from the same person. The DCC will '**match**' these two IDs from the one participant to compare the results from the analyses.

The study goal is to collect BRM QC on approximately 5% of all vial types for all clinic visits. This means a BRM will be scheduled at approximately every 20 participant clinic visits for each clinic visit type (i.e., screening, randomization, and each annual visit).

Each site will be notified periodically by the DCC to collect extra BRM specimens from participants during clinic visits. When notified, the site will collect, process, and ship or store the replicate tubes as well as the regularly collected tubes for that specific visit type.

To minimize participant burden, no more than one BRM blood tube is drawn per participant visit. Therefore, a complete set of BRM tubes for a contact occasion will be comprised of extra specimens collected from multiple participants. However, the same participant can donate an extra tube at different contact occasions (i.e., screening, randomization, annual follow-ups). This extra tube must be the last tube drawn from the participant, and should never require an additional 'stick'. If blood flow is insufficient to fill the BRM tube, a different participant should be selected for collection of the BRM tube.

The link between a participant's ID and the blind replicate specimen label ID is made through the Blood Collection and Processing Forms (SPP and PHP). These forms contain an indicator of whether the sample is a blind replicate duplicate. This is the only way in which quality control duplicates are distinguished from regular samples. In all other respects they are indistinguishable. The Q x Q's for the SPP and PHP provide detailed instruction for completing the BRM section of these forms.

Chapter 12. Training and Certification

12.1 Overview

Certification of study personnel is an essential aspect of effective quality assurance in FAVORIT. In order to maintain proper collection of data despite potential for personnel changes over the long-term follow-up period, the Data Coordinating Center (DCC) and the Operations Center are jointly responsible for establishing and providing the requisite minimum criteria and training and ensuring continued adherence to standards.

The complexity of the design requires that Study Coordinators be instructed and certified on specific tasks. To protect the quality of the study results, data are to be collected by certified personnel only.

12.2 Study Coordinators

Study Coordinators are responsible for providing the thread of continuity from participant recruitment, evaluation and treatment through follow-up, endpoint determination and ultimately trial closeout. Coordinators routinely initiate recruitment, conduct interviews and administer questionnaires. Coordinators serve as the liaison with the Operations Center, the DCC, the Central Laboratory, and the clinical site. They familiarize their physicians with study procedures and implement operational modifications. The Study Coordinator is ultimately responsible for accurate collection of data at the clinic and its transfer to the DCC. The Coordinator is also responsible for overseeing the collection, processing, and shipment of blood samples to the Central Laboratory. Therefore, an in-depth knowledge of all aspects of the protocol is required. As such, Study Coordinators attend a training session initially held before recruitment into FAVORIT commences.

In general, certification requires proficiency in all aspects of collection of data on paper forms and the computerized data management system (DMS) to ensure the accuracy and integrity of the collaborative database. The Study Coordinator must demonstrate proficiency in the use of the DMS to be certified to enter data and randomize participants on the DMS.

12.3 Training Procedures

Every Study Coordinator must be trained by the DCC and Operations Center. If a clinic has more than one Study Coordinator, then the DCC and the Operations Center must train each Study Coordinator.

Once trained, a Study Coordinator can subsequently train additional personnel (auxiliary, back-up) who may perform any or all of blood processing, data entry, overall data collection and cardiovascular endpoint data collection, provided each person passes certification

requirements specific to the activity. In most clinics it is expected that the Study Coordinator will perform all duties relevant to screening and randomization.

Training of the initial group of Study Coordinators was accomplished by participation in a central training session held by the DCC and Operations Center.

12.4 Certification Requirements

12.4.1 Blood Collection and Processing Certification

Initial certification will be based on attendance at FAVORIT central training, and:

- A complete reading of the Manual of Procedures.

- Demonstration of an acceptable level of proficiency based on a laboratory quiz of specimen collection and processing provided by the Central Laboratory. Clinics utilizing other lab personnel to collect, process and/or ship specimens will be required to give the lab personnel the quiz and submit it to the Central Laboratory for evaluation. Phlebotomists who perform the venipuncture **only** are not required to take the laboratory quiz, but are required to receive instructions in FAVORIT protocol on the order of draw and handling of the vacutainer tubes.

12.4.2 Endpoints Ascertainment Certification

Initial certification will be based on attendance at FAVORIT central training.

12.4.3 Data Management System (DMS) Certification

Initial certification is based on attendance at the central training session.

12.5 Additional Personnel

Once training and certification requirements for the Study Coordinators are met, additional personnel may be trained by the Study Coordinator or by the DCC and Operations Center to perform tasks related to the FAVORIT study under certain conditions.

Local laboratory personnel may perform blood processing activities. In this case, the Study Coordinator is still responsible for study protocol and must be certified in blood collection and processing. Lab technicians must be FAVORIT-certified and their initials are entered on the phlebotomy form as the person processing the blood.

Data entry personnel may enter data into the DMS from paper forms that have been completed by certified personnel, after training by a DMS-certified Study Coordinator or training during a DCC monitoring visit. These technicians are certified for entering data into the DMS only.

12.6 Coordinator Turnover

If the primary Study Coordinator leaves the study, the certified and auxiliary back-up personnel can continue to function in their roles. A back-up Coordinator who has completed all certifications may be designated as the new Study Coordinator with no interruption of study activities. Or, the fully certified back-up Coordinator may continue all study activities until a new primary Coordinator is trained and certified. The new primary Study Coordinator must be trained by the DCC/Operations Center.

Randomizations must cease if the new Coordinator has not been trained and certified by attending a central training or an on-site training session and completing his/her certifications. Effort will be made to complete such training within 30 days of loss of the primary Study Coordinator.

For clinics with auxiliary personnel, it is recommended that the new Coordinator certify as quickly as possible in the areas the previous Coordinator handled exclusively. When the clinic has someone certified for every aspect of the study (combination of new, auxiliary, and back-up), then the clinic can continue study activities.

New Coordinators should strive to complete all certifications within two months or less of receiving training in order for the center to maintain its basic funding and continue to participate in the study.

12.7 Recertification of Coordinators

The need for recertification on any of the study certification requirements may be triggered by monitoring visits, or upon recommendation of the Data and Safety Monitoring Board or upon the Executive Committee's review of the data. This recertification may be study-wide or clinic-specific.

CHAPTER 13
FAVORIT Trial Data Management System
Users Guide

Version 2.1
April 25, 2006

Prepared by the
Collaborative Studies Coordinating Center

TABLE OF CONTENTS **PAGE**

This Users Guide contains instructions pertinent to the operation of version 2.1 of the FAVORIT Trial Data Management System [DMS]. If your DMS is a later version than this guide, please refer to the update memos and any available addenda to this guide for complete instructions. In the event of significant changes to the DMS, a complete updated Users Guide will accompany the update.

Once the FAVORIT Trial DMS has been installed on a computer, please do not move the system or reconfigure the hardware (e.g. replace the C: drive) without contacting the Coordinating Center first. Doing so could result in the loss of research data. We have provided a label containing this warning. The label should be placed on any computer running the system.

13.1 Overview of Data Collection

In the course of performing a study, data for a number of participants must be collected at various times for later analysis. These data items are organized into groups of logically related information called forms or form types. Each form is then assigned a brief mnemonic code for easy reference, i.e. "VDL" for Vitamin Distribution Log, “REL” for Randomization Eligibility, etc.

It is sometimes necessary to change the content of a form during the course of a study. To allow for such changes, we assign a version letter to each form. The initial version is "A", and subsequent versions follow alphabetically. Thus, "VDLA" refers to ”Vitamin Distribution Log, Version A."

Since each form must be collected one or more times for each study participant, extra information is included to uniquely identify each recorded instance, or record, of a form. These identifiers, or key fields, include Subject ID (ID) and Contact Number (Cont. #). The ID is a unique code assigned to the participant. The Contact Number specifies the contact at which the form was collected. If more than one record is collected for a participant at a given Contact, a unique Sequence number must be assigned to each record.

We refer to all data items on a form as questions and assign a question number to each item. Typical question numbers may include both letters and numbers, e.g. 1, 2, 3a, 3b, etc.

Data items may be initially collected on paper forms and subsequently entered (or keyed) into an electronic database for statistical analysis. Or data may be recorded directly on the screen without being transcribed from a paper form.

A database consists of tables of data, arranged into fields and records. Each table (form) can store many records (instances of a form), each containing a set of values for every field (question) in the table.

Each table in the database must have a unique name for identification, as must each field in a table. We assign each table's name to be the form and version of the source of its data. We

assign each field's name to be the name of its table and the question number of the source of its data. Hence, the table containing data for form VDL, version A, is named VDLA and contains fields named VDLA1, VDLA2, VDLA3, etc.

Each record in a table is uniquely identified by its set of key fields. Thus, no two records in a table may have the same set of key field values (ID, Form, Contact Number).

13.2 DMS Functions

The FAVORIT Data Management System [DMS] is a set of programs which manage data collected in the FAVORIT Clinical Centers. The DMS uses the FoxPro data base management system for screen display, data editing and storage.

The DMS provides several major functions:

- Data Entry: Allows data to be keyed, edited and updated.
- Data Transfer: Allows data to be sent to the FAVORIT Coordinating Center for inclusion in a consolidated database. Also allows data keyed on secondary pcs (usually laptops) to be moved to the primary pc (often a desktop pc but in Favorit, a laptop).
- Randomization: Determines patient eligibility and, if eligible, assigns a bottle code to the patient.
- Reports: Provides counts of records entered by form type, export counts, form prints, etc.

13.2.1 User Interface Standards

The DMS uses a combination of menus, function key commands and mouse clicks to control its actions.

13.2.1.1 Keyboard and Mouse

The DMS uses the keyboard in a conventional way:

The typewriter keys are used to type numbers, letters and symbols.

The cursor control arrow keys highlight menu bar options. Once the cursor is on a menu option, the ENTER key either performs the action or brings up a submenu. The left and right arrow keys move within a field. The down arrow key and TAB move the cursor to the next field. The up arrow key and SHIFT+TAB move the cursor to the previous field. The Home and End keys move to the beginning and end of a field respectively. PAGE UP and PAGE DOWN move to the previous and next screens in a form. CTRL+PAGE UP and CTRL+PAGE DOWN move to the previous and next record.

Pressing ALT moves the cursor to the menu bar. Pressing ALT again or pressing ESC returns the cursor to the data entry window.

Most menu options have shortcut keys which are a combination of the ALT key and a letter, usually the highlighted letter. In this guide these are written as ALT+*letter*, for example ALT+E. To use the shortcut hold down the ALT key and simultaneously press the letter. Specific shortcut keys will be described when the menus are discussed.

Most submenu options (the lists displayed after you choose a menu item) have shortcut keys which are usually a combination of the CTRL key and a letter. To initiate an action with a shortcut key hold down the CTRL key and simultaneously press the letter.

F1 (function key 1) is the help key.

F2 (function key 2) is the field duplication key.

F3 (function key 3) is the list display key.

Menu items can be selected using the mouse. To select an item, move the pointer to the item and press the left mouse button once.

13.2.1.2 Menus

Most screens in the DMS have horizontal menu bars on the first line. These menus list the options available from the screen. To move the cursor to the menu, tap the ALT key. Once the cursor is on the menu bar, there are two ways to select an option:

Use the left and right arrow keys to move the highlighted bar to the desired option and press ENTER, or type the highlighted letter of the desired option.

Some menu options have further choices which are displayed in a pull-down list when the option is selected. Use the up and down arrow keys to move the bar to the desired option or type the highlighted letter.

Shortcut keys have been defined for some menu and submenu options. To use a shortcut key, press the key combination while the cursor is in the data entry section of the screen. The cursor does not have to be on the menu. Shortcut keys for menu options are ALT and the highlighted letter of the options. Most shortcut keys for submenu options are a combination of the CTRL key and a letter. The shortcut keys are displayed on the pulldown lists. Exceptions to these rules are the help key (F1), the field duplication key (F2), the list display key (F3) and the movement keys (PAGE UP, PAGE DOWN, CTRL+PAGE UP, CTRL+PAGE DOWN).

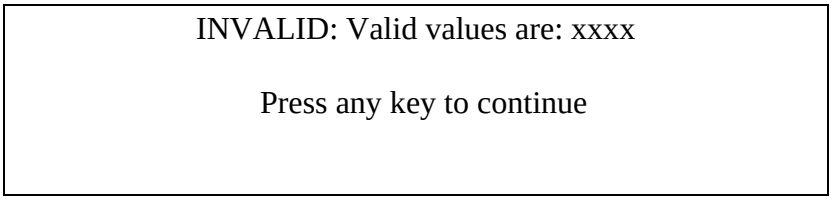
Under some conditions menu options are unavailable. For example if a user does not have delete privileges, the Delete option is not available. Unavailable options are not highlighted and cannot be selected.

13.2.1.3 Lists

Some fields, for example the form field on the ID screen, can be selected from master lists. When the cursor is on the form field, a list of all available form types can be displayed using F3. Use the arrow, page up and page down keys to move the highlighted bar through the list. To select an item, place the highlighted bar on the item and press ENTER. The item under the bar will be put in the field.

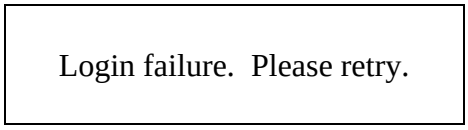
13.2.2 Information and Warning Messages

Messages from the DMS are of two types. The first displays a message and tells you to press a key to continue:



The message remains on screen until you press a key.

The other type of message is used when no user action is required:



It will disappear from the screen after a few seconds. However, you can make it disappear instantly by pressing a key.

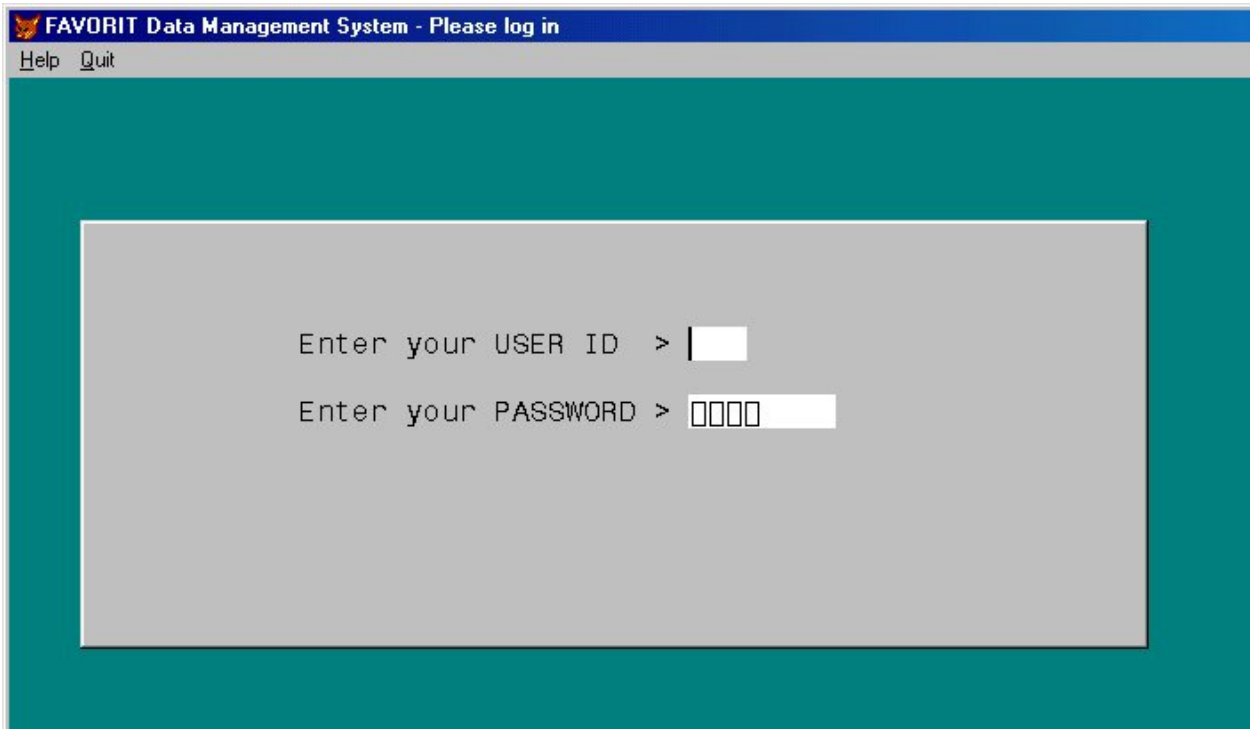
13.3 Starting the DMS

On the primary laptop, you must have the FAVORIT Memory key inserted into the USB port in order to run the DMS. Then, double click the 'FAVDMS' icon on the Windows desktop.

If you forget to insert the key before you start up the DMS, you will get the message "Please insert the disk named SECURE_DMS." Then at that time you can insert the key.

13.3.1 Desktop, User IDs and Passwords

The password screen is the first DMS screen displayed:



At the top of the screen the menu bar lists two options:

(1) Help presents a list from which you can select a DMS topic.

(2) Quit terminates the DMS.

To use DMS functions you must enter a valid ID and password at the Login screen. These are assigned using the System Administration utility described later. IDs are three characters long. Passwords are at least three and at most eight characters long and are not shown on the screen. If you enter an invalid ID or password, an error message is displayed.

The cursor returns to the ID field for re-entry. After 3 failures, the DMS exits automatically.

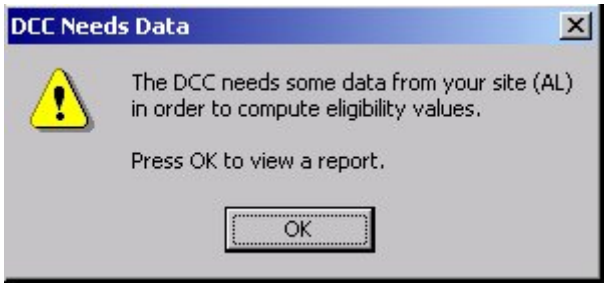
To exit the DMS from this screen, select Quit from the menu.

13.3.2 Timeout

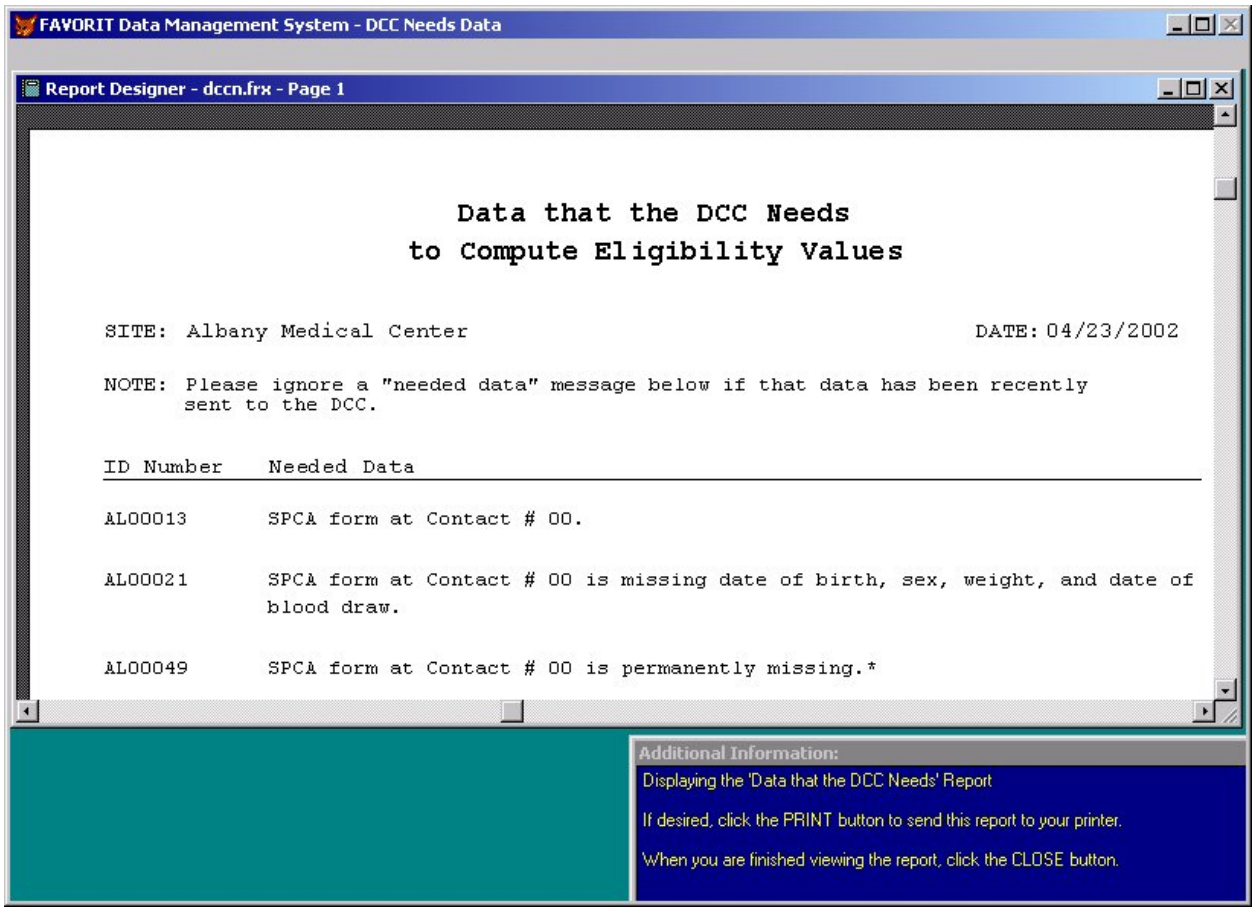
If the DMS is started and left unattended, a security problem could occur because an unauthorized person might view confidential data. If a screen saver is installed with password protection, using a reasonable timeout setting (under 10 minutes) then this potential problem can be avoided.

13.4 Data Entry

After you enter a valid ID and password, one of two screens will be displayed. If the coordinating center is missing some data value which are required for the determination of eligibility values the following screen will be displayed:



When you press OK, the Data that the DCC Needs report is shown:



You can view or print the report. See the section Import Eligibility Values for more information on this process.

If the coordinating center is not waiting on any data value which are required for the determination of eligibility values, the DMS Main Menu is displayed:

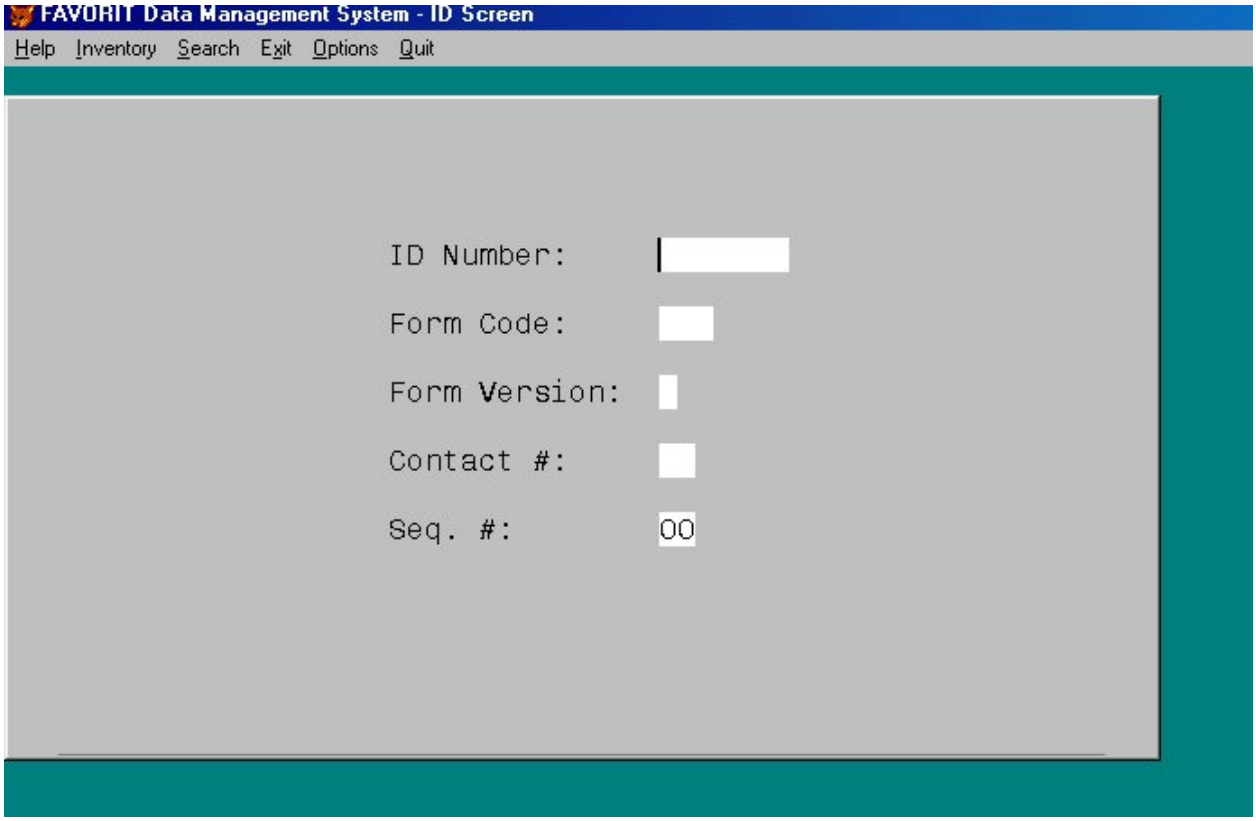
- Select Data Entry to add and modify participant data.
- Select Utilities to run support programs such as the password program and the export and import programs.
- Select Reports to run report programs such as the Missing Fields Report.
- Exit returns to the Password screen.
- Quit exits the DMS.

The highlighted letters of each option indicate which letter, in combination with the ALT key, comprises the shortcut. The shortcut key for Help is F1.

Press ALT+D to start the Data Entry System.

13.4.1 ID Screen

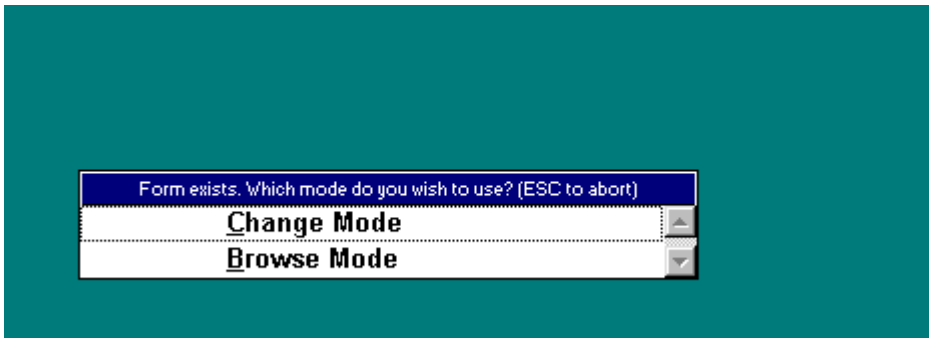
The ID screen is the first screen shown:



The data entry mode (e.g. add, change or browse) is determined by which key fields you enter, which records exist in the database, and some form-specific rules.

If you enter all key fields and a record with the specified keys does not exist in the database, the mode is Add. An empty record is displayed for data entry. If you do not have Add privileges and are trying to add a record, you are informed that you cannot add records and are returned to the ID screen.

If you enter all key fields and the specified record does exist in the database, you are notified that the record exists and are asked to choose Change or Browse mode.



If you enter only some of the fields on the ID screen and select 'Search' from the top menu bar on the ID screen, the system searches for a record which matches those keys, ignoring the keys which you left blank. If a match is found, you are notified that a matching record was found and are asked to choose Change or Browse mode.

The following table summarizes the mode the data entry system assumes when certain fields are entered on the ID screen:

Fields Entered	Found in DB?	Mode
All Fields	No	Add
All Fields	Yes	Choice of Change or Browse
Some Fields and hit 'Search'	Yes	Choice of Change or Browse
Some Fields and hit 'Search'	No	Disallowed, return to ID Screen

To summarize, to add a new record for a participant you must complete the ID, form and Contact Number fields. To change records you can enter all or some of the fields on the ID screen.

As you enter the ID, it is checked (or edited) by the DMS for validity. If it is not a valid FAVORIT ID, an error message is displayed and the cursor remains in the field. If it is valid, the cursor moves to the form field.

FORM can be entered in one of two ways. You can type a form abbreviation into the field, in which case the default or current version is automatically chosen and displayed in the version field. Or you can press the F3 key to display a list of all valid form types. Then use the down-arrow key to enter the scrolling list of forms, position the highlighted bar on the desired form, and press ENTER. The form and version are plugged into their respective fields. Note that this second method is the only way to choose a version other than the default.

Next, the cursor enters the Contact Number field. Enter the Contact Number at which the record was collected. Edits check that you have entered a valid Contact for the form type.

You can leave the ID screen by one of two methods. If you have entered values for all the fields, the requested record is automatically displayed when the last field is filled. Or you can choose a menu option. The menu options are:

Search:	display a record which most closely matches those fields entered.
Inventory:	display counts of records in the DMS
Exit:	leave the ID screen and return to the Menu Menu
Quit:	leave the DMS.

.

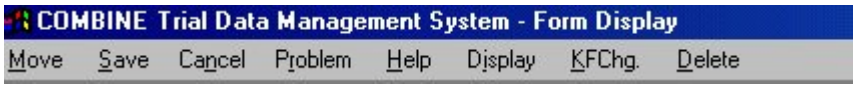
13.4.2 Add / Change Menu

When a record is displayed in Add or Change mode, the top portion of the screen shows the key fields for the record. The cursor is on the first data field of the record. A menu bar fills the first line of the screen. The Add and Change menus are identical with three exceptions: Permanently Missing applies only in Add mode; Delete and Key Field Change apply only in Change mode.

Some options on the menus may not be highlighted. This means that they are not available. For example if you do not have delete privileges, Delete is not highlighted. If you do not have change privileges, Save is not highlighted.

Add Menu:

Browse/Change Menu:



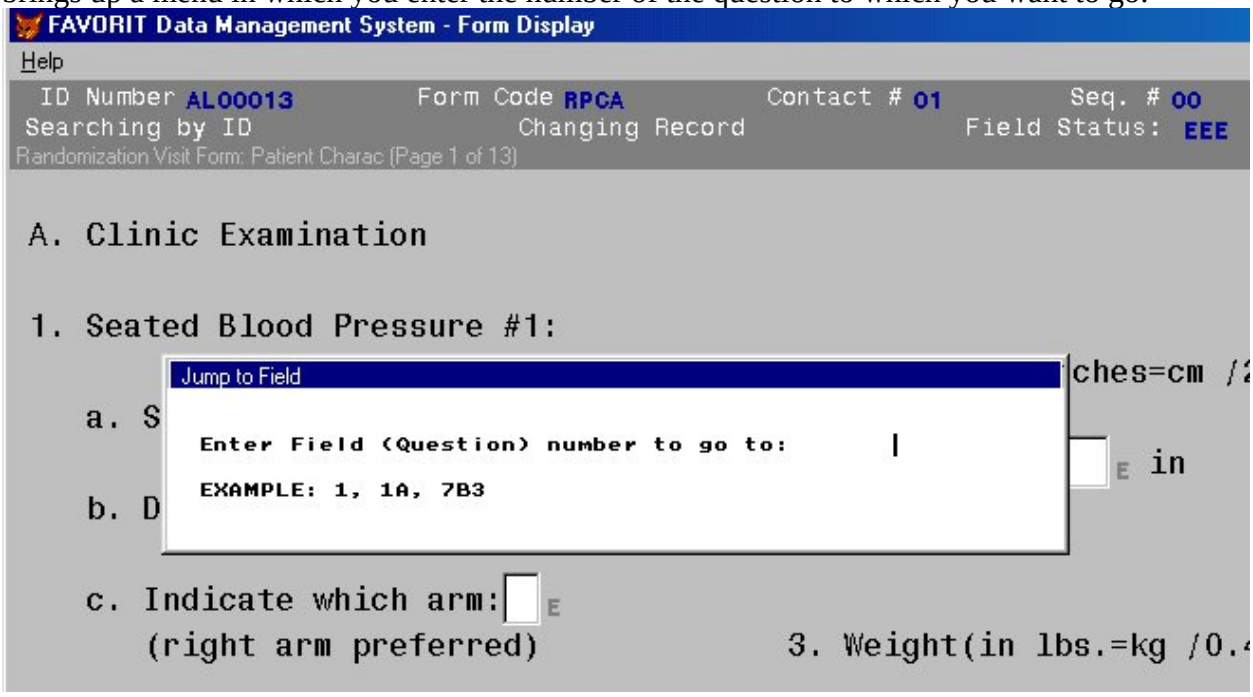
13.4.3 Field, Screen and Form Movement

In Add mode you will usually enter fields in sequence. However in both Change and Add modes you can move through fields, screens and records using the menus or shortcut keys. The Move option of the menu bar lists the available options:

Next Field	TAB, Down Arrow
Prev Field	BACKTAB, Up Arrow
Next Screen	PGDN
Prev Screen	PGUP
Next Form	CTRL+PGDN
Prev Form	CTRL+PGUP
Next Line	CTRL+RIGHTARROW
Prev Line	CTRL+LEFTARROW
Jump to Field	CTRL+J
Switch Paths	CTRL+W

Most of the options are self-explanatory. TAB (and RIGHT ARROW) and BACKTAB (and LEFT ARROW) move to the next and previous field respectively. PAGE UP and PAGE DOWN move to the next and previous screen of a form. CTRL+PAGE UP and CTRL+PAGE DOWN move to the next and previous records in the current search order. If you go past the last record in the current search order, the first record will be shown again. CTRL+RIGHTARROW and CTRL+LEFTARROW move to the next and previous line of a multi-line form. If the form is not a multi-line form these options are dimmed.

Jump to Field allows you to move to a specific question on the form. Selecting this option brings up a menu in which you enter the number of the question to which you want to go:



If you enter an invalid number the message 'Field not found' will be displayed in the window.

Enter another field or press ESC to return to the data screen.

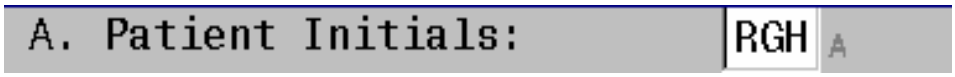
Jump to Field allows you to go to skipped fields, permitting you to view screens which may have been skipped entirely. However you cannot enter values in these skipped fields. Jump to field will not let you bypass a must enter (mandatory) field. If you enter a question number which falls after a must enter field which is blank, the cursor instead stops at the must enter field.

Switch paths allows you to control the order in which records are presented when you select Next Form and Previous Form. The default order is by ID. With this path, the next record for the current ID is shown when you press Next Form. In Form order, the next record for the current form type is shown when you press Next Form. Using form order, for example, you could view all VDL records which have been entered. Selecting Switch Paths toggles the path. Note that the path has no effect in Add mode since after saving or canceling a record, the ID screen is always displayed.

13.4.4 Edits (and Problem Menu)

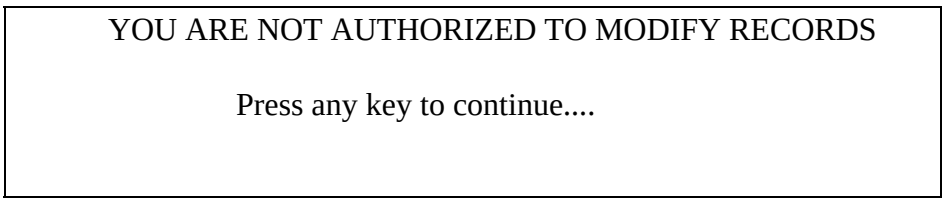
Each data field that you enter has an associated trio of status bytes which stores additional information about the field, such as whether the field is empty, missing, or contains an out-of-range value. The Problem option on the Add and Change menus gives you a way to provide this additional information.

The status bytes for a field are displayed in the bottom right of the grey bar above the record (they are called the “Field Status” there—see the previous picture). FAVORIT does not use the middle status byte and so it is always 'E' in FAVORIT. Also, the first status byte of a field is displayed as a light-grey letter after the given field. For example, in the following,



the light-grey 'A' after the field is its first status-byte and it indicates that the entered value was accepted by the DMS. Another example is that a light-grey 'E' appears after the field if the field is empty.

As you enter data values into a record, they are edited. If you do not have modify privileges, you will be alerted:

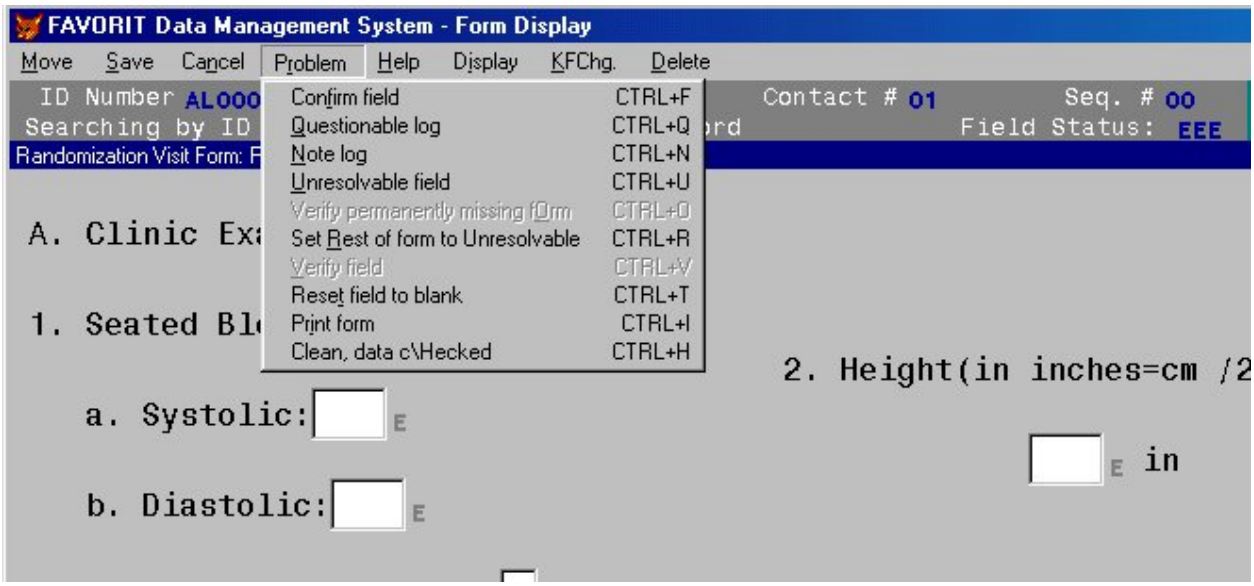


and the field’s prior value (blank or otherwise) is restored.

If a value fails an edit, for example if it is out of range or inconsistent with other values, an error window alerts you and gives the valid range:

> INVALID: Valid values are: xxxx
>
> Press any key to continue....

Press any key to clear the error and return to the field (you will notice that a 'D' for Dirty is made the first status byte of the field). If you made a keying mistake, retype the value. However, if the value is correct you must confirm it. Use the Problem menu to do this:



Choose Confirm field to confirm that an out of range value is accurate by setting the first status byte to 'C'.

Use Unresolvable field when a value cannot be collected or when the value you did collect is suspicious and should not be used in analysis. Unresolvable sets the first status byte to 'U' and, if the field is blank, fills the field with equal signs (==). Note that you can set a field to Unresolvable by keying the equal signs into the field rather than using the Problem menu.

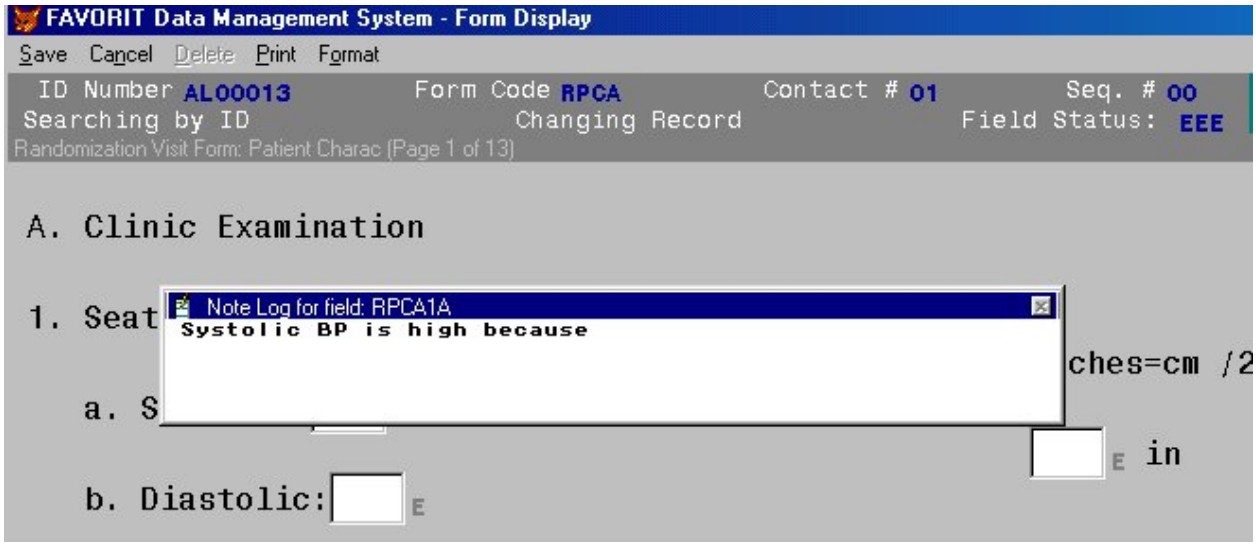
Set rest of form to Unresolvable fills all remaining fields on a form with equal signs and sets their first status byte to 'U'.

Reset field to blank removes the value in the field and sets the first status byte back to 'E' (empty).

Print form should be used only when a printer is attached to the DMS computer or laptop. It prints a copy of the form as it looks in the DMS. NOTE: Print form is not available in Version 1 of the DMS.

Clean, data cHecked sets a field's first status byte to 'H'. Do this when the Coordinating Center has run a cross-field data check and sent you a query about a possible inconsistency. This tells the CC that you have checked the value, it is correct and tells them not to query it again.

Note Log and Questionable Log allow you to attach a comment to a field. Choosing either option opens a window in which you can type comments.



If a note log or questionable log has already been entered for the field, it is shown in the window and you may edit the existing text. The window is empty if no log exists for the field.

There are four options on the menu bar. Choose Delete to delete the log displayed. To leave the log window without saving changes, select Cancel. Choose Save to save changes and return to the record. To print the log, choose Print.

Note logs and questionable logs function similarly but are used in different contexts.

Use a note log to comment on a value, for example, to explain an 'other' response.

Use a questionable log to mark a field which may be incorrectly recorded on the form and needs review. Questionable logs will not be often used in FAVORIT .

Adding a note or questionable log sets the third status byte to N for Note logs, Q for questionable logs or B for both.

13.4.5 Skips

Some fields are answered conditionally. That is, a certain response to one field can cause subsequent fields to be unnecessary or irrelevant. In the DMS these fields are skipped. After a response is entered into the trigger field, the cursor skips ahead to the next relevant field. This field might be on the same screen or several screens ahead.

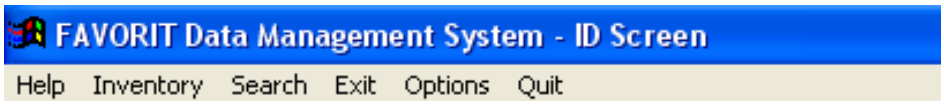
You cannot move to a skipped field using the Next Field or Prev Field keys. The only way to move to a skipped field is by using the Jump to Field option on the Move submenu. Once the cursor is positioned on a skipped field, you cannot enter a value into the field.

The first status byte of a skipped field is changed to indicate the field is skipped. The 1st status byte's value remain the same but is changed from upper to lower case.

13.4.6 Inventory Display

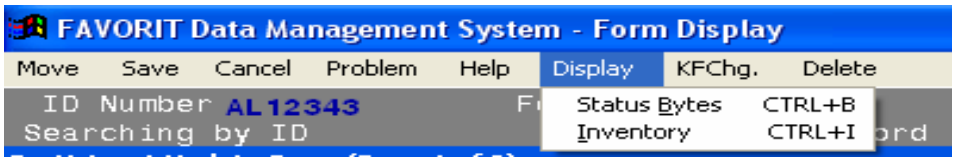
The DMS maintains an inventory of records entered for each participant. (In the past this inventory form has been called the CXI.) It can be displayed from the ID screen or when a data entry record is on the screen.

From the ID screen, to show the inventory choose Inventory from the menu (ALT+I).



If you have entered an ID, the inventory for that participant is shown. If you have not entered an ID, an inventory of the entire database is shown.

To show the Inventory for the current participant from a data screen, choose Display from the menu and, from the submenu select Inventory. (Alternatively you can use the shortcut key CTRL+I.)

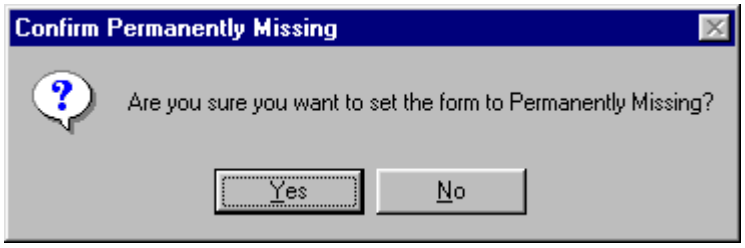


The Inventory Display lists the number of records entered for each form type in the database. Where applicable, a count of unverified records for the form type is also displayed (this count is not applicable to any forms in FAVORIT).

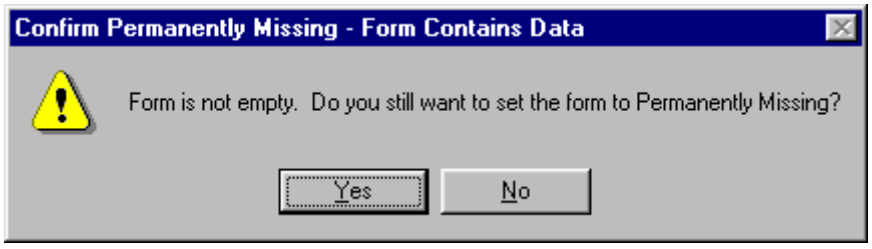
13.4.7 Permanently Missing Forms

If you are unable to collect an entire record of required data for a participant, enter the record into the DMS and set it to permanently missing. This tells the Coordinating Center staff that you will never be able to get the information so they will not query you.

A record can be set to permanently missing only in Add mode. To set a record to permanently missing, choose Perm. Miss. from the Add menu. You are prompted to confirm that the record is permanently missing:



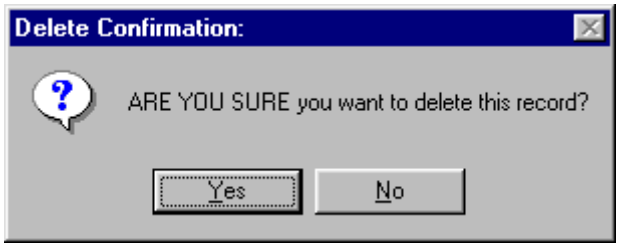
If you have already entered data into some fields and then decide to set the record to permanently missing, the fields will be blanked. You are prompted to confirm again:



When a record is set to permanently missing, the first status bytes for all fields are set to 'P'. When browsing the database and a permanently missing record is shown, a message informs you that the record is permanently missing. You cannot add data to any field.

13.4.8 Delete

To delete a record, select Delete from the Change Menu. You will be prompted to confirm the delete:



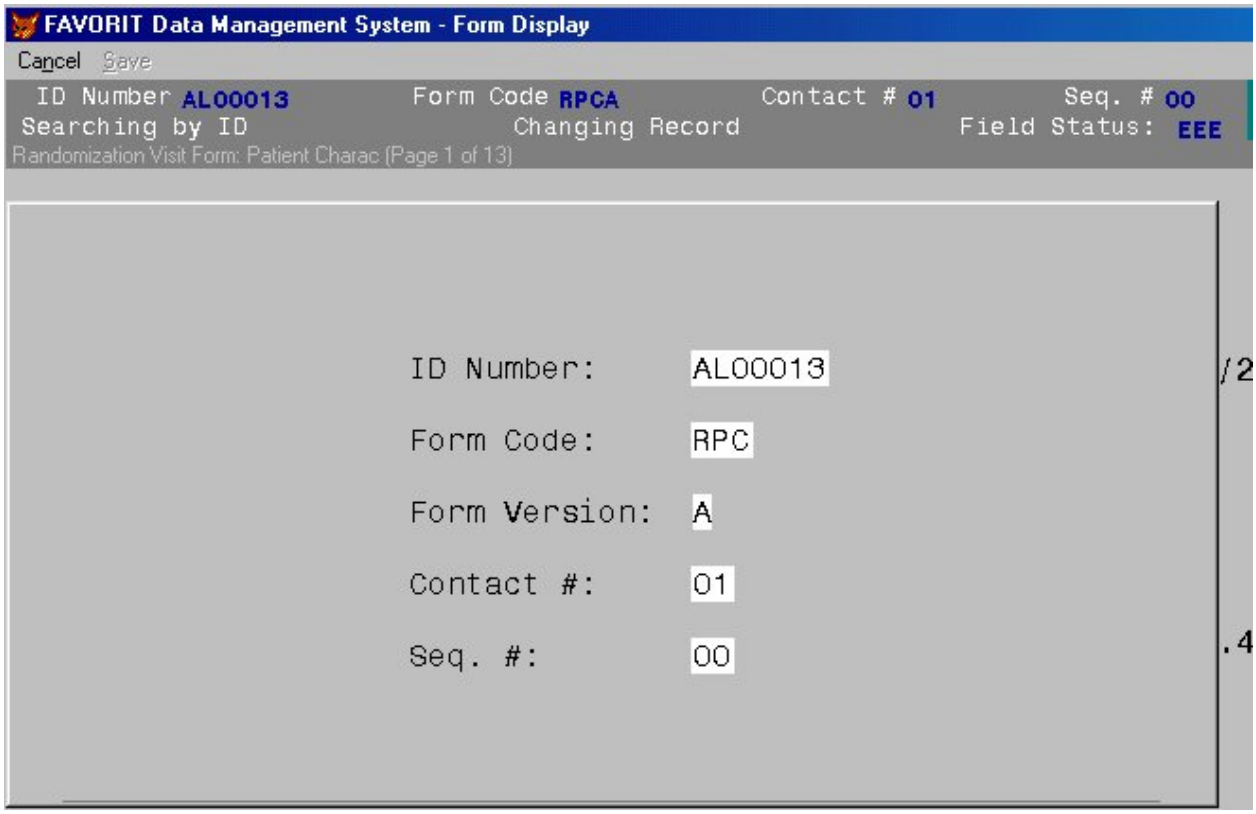
Press 'Yes' to delete the record or 'No' to return to the screen. After you delete a record, the ID screen is redisplayed.

If you do not have delete privileges, or if you are in Browse mode instead of Change, the Delete option on the menu will not be highlighted. Delete privileges are granted via the System Administrator utility.

13.4.9 Key Field Change

When a record is displayed for modification, most fields can be changed by simply entering a new value. However to change the key fields, the fields which identify the record, you must use KF Chg from the menu. With this option you can change the ID, the Contact and sequence number.

A screen similar to the ID screen will be shown:



Type in a new value for the field(s) you want to change.

If there is a record with the new keys in the database, the key field change is not accepted. When you are satisfied with the new values, go to the menu and select Save. To cancel the change, go to the menu and select Cancel.

The window will close and the record, with the new key fields, will be displayed. The Delete option is no longer highlighted. Deletes are not permitted after a key field change.

If you choose Cancel from the Change menu, the key field changes will be lost.

If you do not have Change privileges KFChg on the Change menu will not be highlighted.

13.4.10 Help

Help is an option on most of the primary menus in the DMS. It can be selected from the menu or by pressing F1. Help from the Add or Change menu lists a submenu with three options: field, screen or general. Select the type you want. If you choose General a list of topics is presented. If you choose Field or Screen information specific to the current field or screen is presented. Once a screen is shown you can view related topics or select from a list of all help topics.

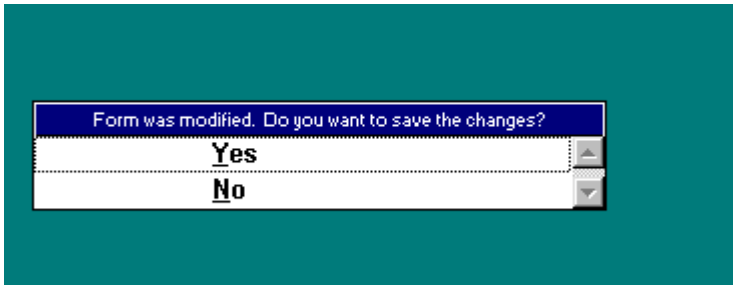
To return from help, press ESC or select Exit from the menu.

13.4.11 Save and Cancel

A record is automatically saved when you:

are in Add or Change mode and enter the last field on a record; or
use CTRL+PAGE UP or CTRL+PAGE DOWN to go to another record.

In the second case you are prompted:



You can also save a record manually by selecting Save from the Add or Change menu. There are some situations in which you must manually save a record:

a) When the response to a trigger field causes all remaining fields on a record to be skipped, you get the message:

Can't move forward from current field

because there is no field for the cursor to move to.

b) If you are in Add mode and must use an option from the problem menu (such as Confirm or Unresolvable) on the last field of a record, you get the message:

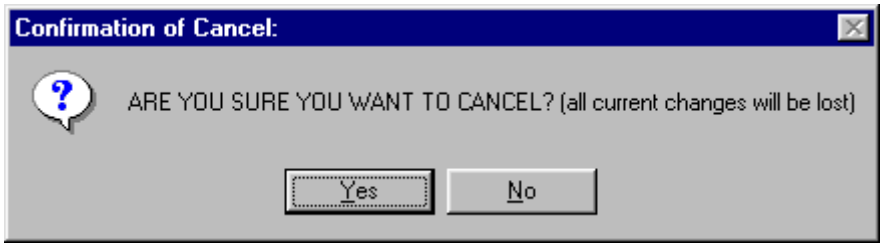
This is the last screen of the form

c) If you are in change mode and not on the last field of the record and want to return to the ID screen. Note that if you change the last field of the record in change mode, the record is automatically saved.

In any of these cases, select Save to save the record and return to the ID screen.

Save is not available when you are in Browse Only mode. See section 2.1 for a description of Browse Only mode.

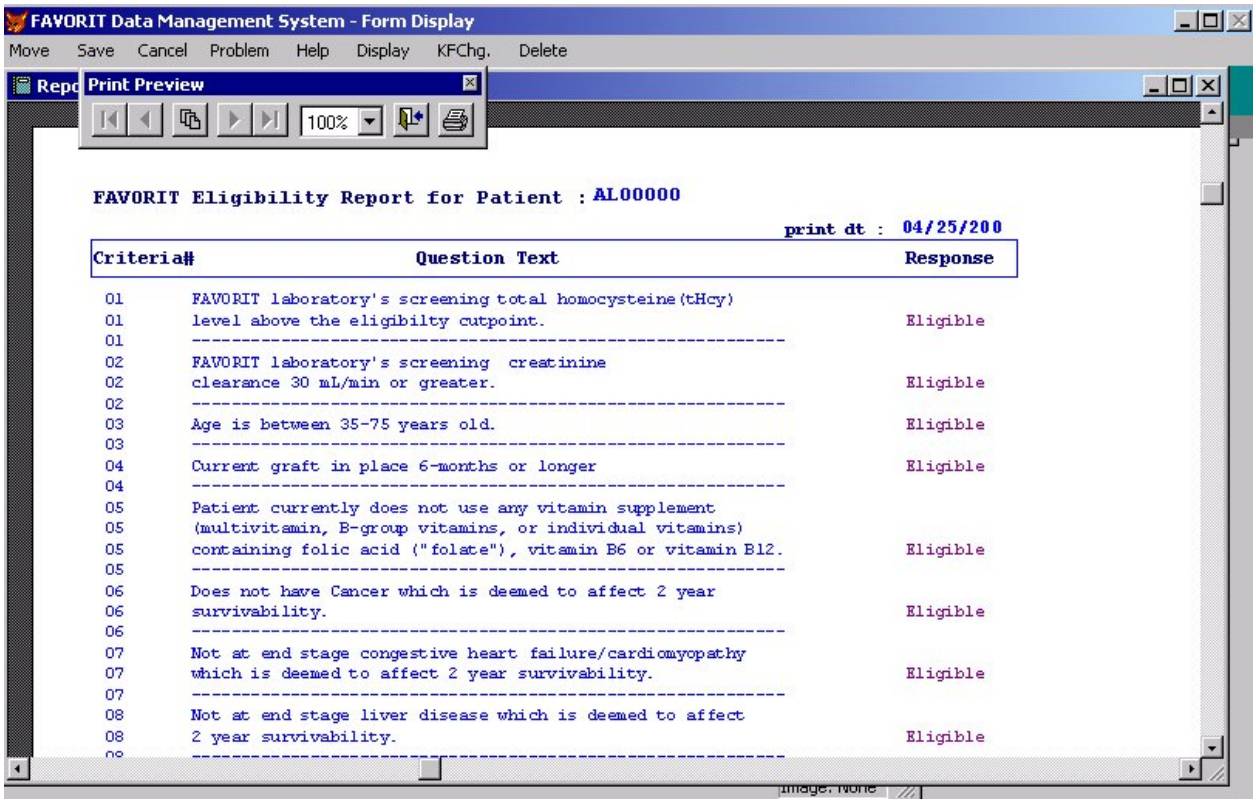
If you have entered incorrect information and want to cancel all changes, choose Cancel from the menu. You will prompted to confirm:



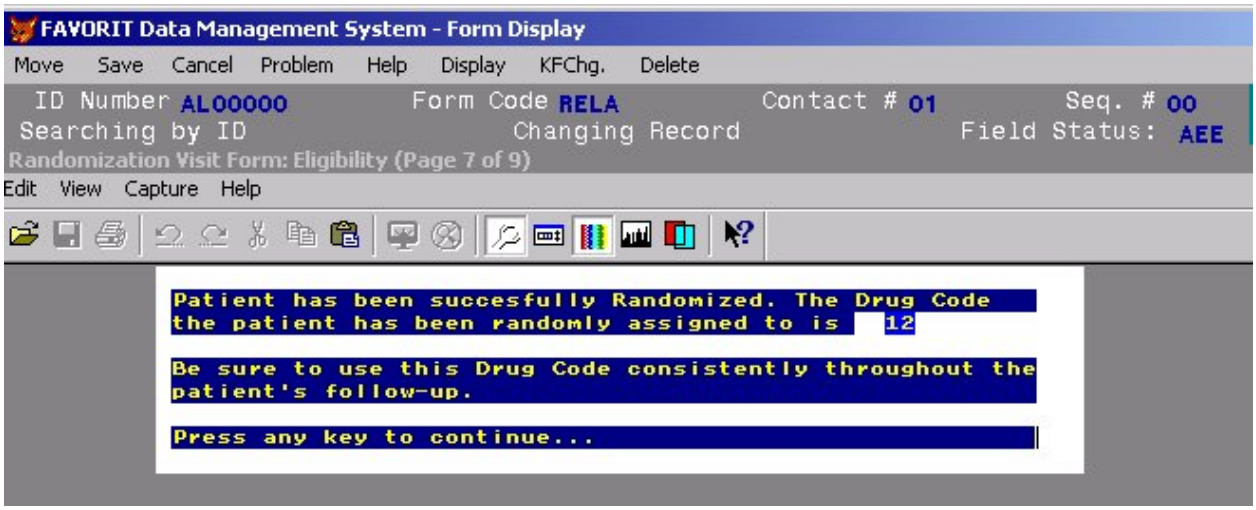
If you select 'Yes' the ID screen will be displayed.

13.4.12 Randomization

To randomize a patient, enter the patient ID, form REL (contact occasion 01 is the only allowable contact) on the ID screen. The REL form asks questions pertaining to patient eligibility. When you respond 'Y' to 'randomize a patient?', a report is displayed listing the eligibility criteria and whether the patient is eligible on each. (Note that the report lists criterion number not REL question number.)



If the patient is eligible on all criteria, the system assigns the patient a bottle code and display the following screen:



Once a patient is randomized, you can neither delete the REL form nor change its values. If you access the form, you are automatically forced into Browse only mode.

Two fields of the REL form are not entered by the user. Eligibility criteria on homocysteine and creatinine are determined by the coordinating center. Values for these questions are sent to the clinical centers. See the section below on Importing Eligibility Values.

13.4.13 Form Set Mode

Formsets are groups of forms which are displayed one after another automatically. When the user completes one form in the form set the system automatically saves that form and displays the next form in the set without returning to the ID screen.

In FAVORIT, form sets are not used in Version 1.0 of the DMS. The instructions below are for information in case we decide to implement them later.

To choose a form set from the ID screen, enter the form set mnemonic into the FORM field. Form sets are displayed in the drop down form list (displayed by pressing F3 when the cursor Is on the FORM field). The first form of the formset will be displayed.

If you must interrupt an interview in-progress, it is best to finish the current form. Then press ALT-F12 to display the menu and chose Cancel. When you resume the interview, the first form in the formset which has not been entered (the one you canceled) is displayed. If you have only completed a few questions on a form, you might choose to Cancel the current form which will cause those question to be repeated when you resume the interview.

To resume an interview, go to the ID screen and enter the same information you used to start the interview (i.e. ID#, Formset, Contact #) and the computer will automatically display the first form that you canceled.

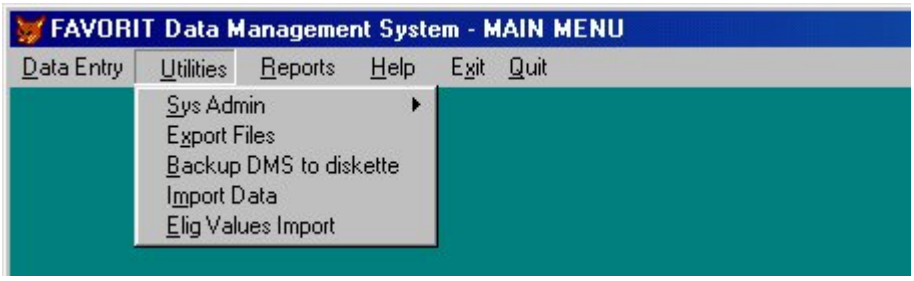
13.5 Utilities

The utilities are programs which are separate from the data entry functions of the DMS but which affect how it runs. The utilities are run by selecting Utilities from the main menu. A submenu lists the available options (see example below).

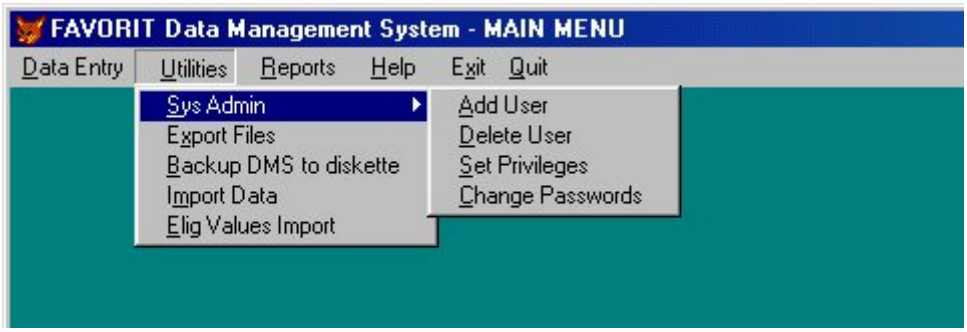
13.5.1 System Administration

To use the DMS you must log in using an ID and password. You use the password utility to assign IDs, passwords and privileges to users. Generally this system administration task is assigned to a single person called the data coordinator, or to the overall project coordinator for a site. Each user should be assigned an individual user ID and password and should always use this ID in the DMS. Users should be given access to only those tasks appropriate for their training and level of responsibility within the organizational structure of a clinical site. For instance, it may be appropriate to give the P.I. privileges to "browse" records, but not to "add", "change", or "delete" records. Whereas, a data entry specialist would need to have at least "add" and "change" privileges within the system.

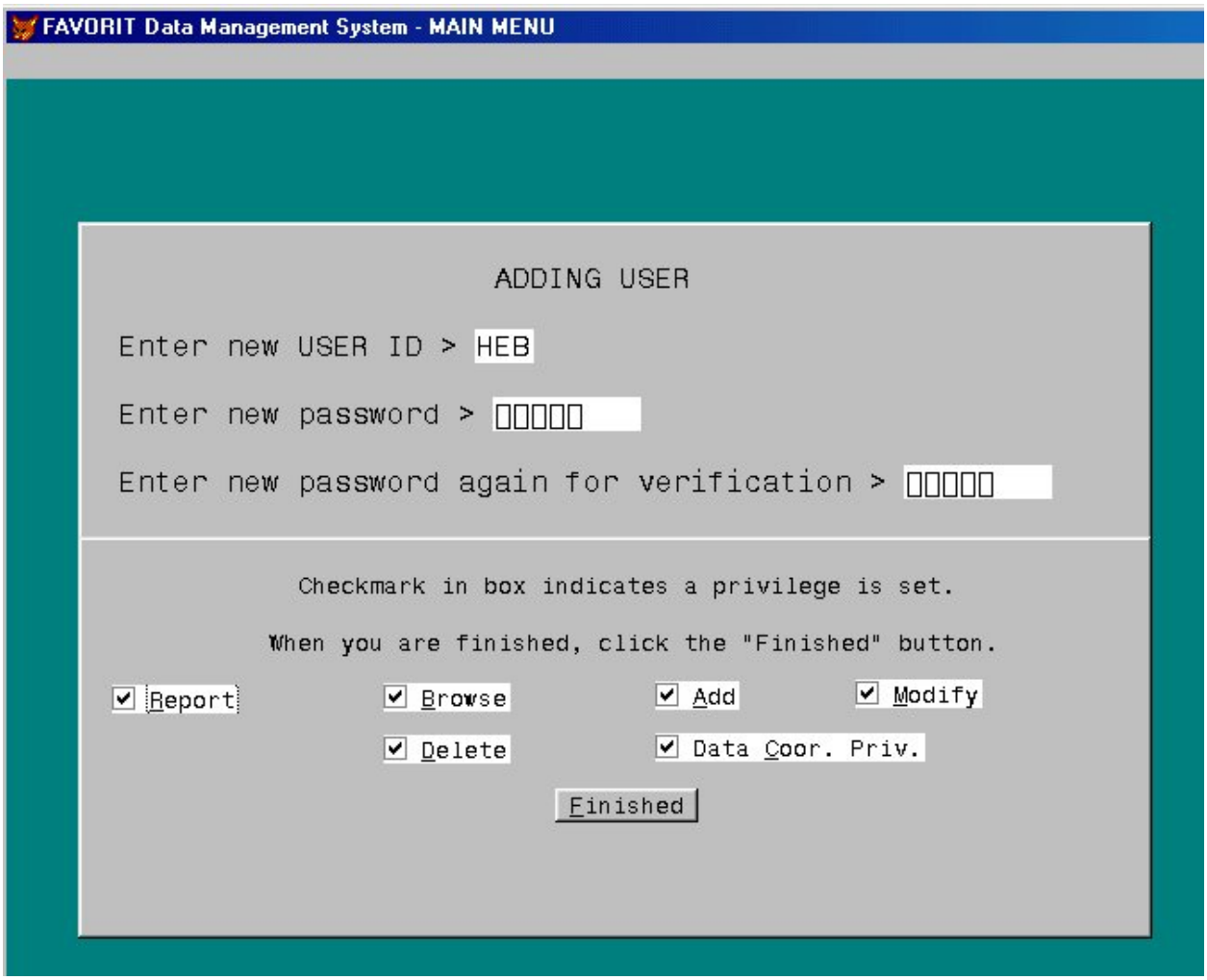
To run the password utility, choose Sys Admin from the Utility submenu.



Another submenu is then shown giving four choices:



To add new user IDs and passwords, choose Add User:



Enter the user's login ID and password. Enter the password again for verification. Choose which privileges the user is allowed.

Click on an option to select it. An 'X' in a box grants the associated privilege.

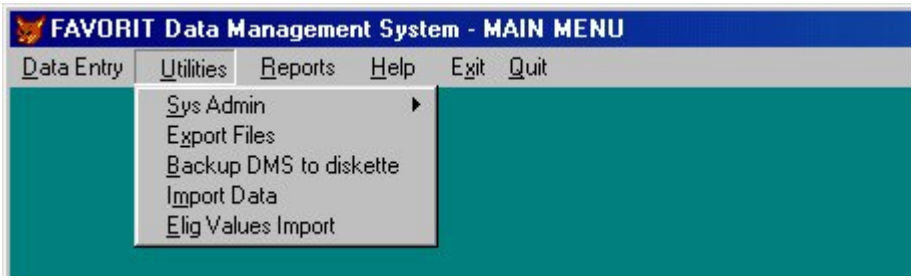
From the Sys Admin Utility you can also ‘Set Privileges' to change what a user is allowed to do in the DMS; remove users from the system with ‘Delete User’; and change a user's password with ‘Change Passwords’.

Only a user with data coordinator privileges can add users or modify privileges. Individual users can change their passwords.

The DMS is shipped with a default Data Coordinator ID and password. You can add your own data coordinator ID by creating a new ID and assigning that ID data coordinator privileges.

13.5.2 Eligibility Values Import

Two eligibility criteria on the REL form are determined by the coordinating center and imported into the DMS at the clinic. Files containing these values are sent to the clinic during data transfer. To add the values in the files to the DMS, go to the Utilities menu and choose 'Elig Values Import'.

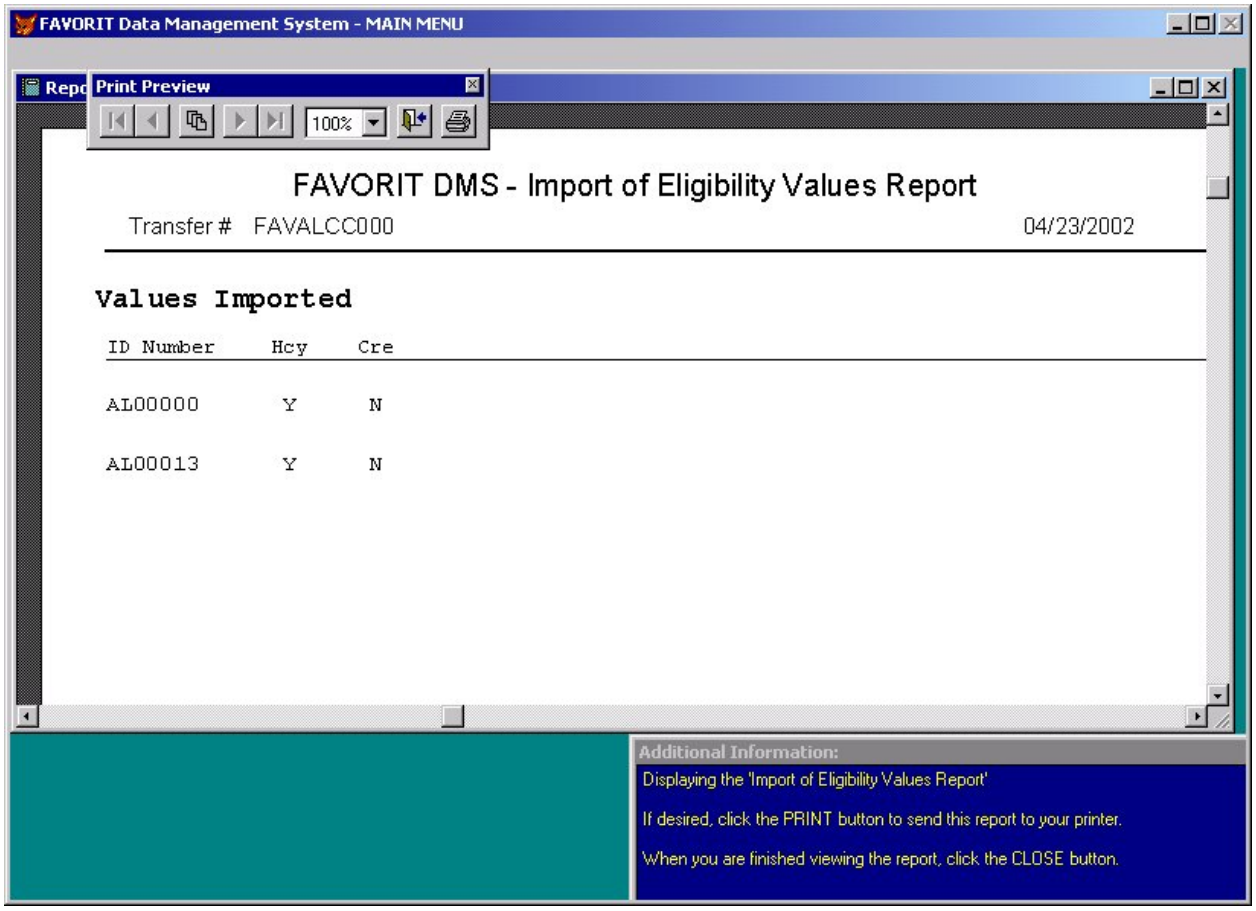


If there are files to import you will get the prompt:

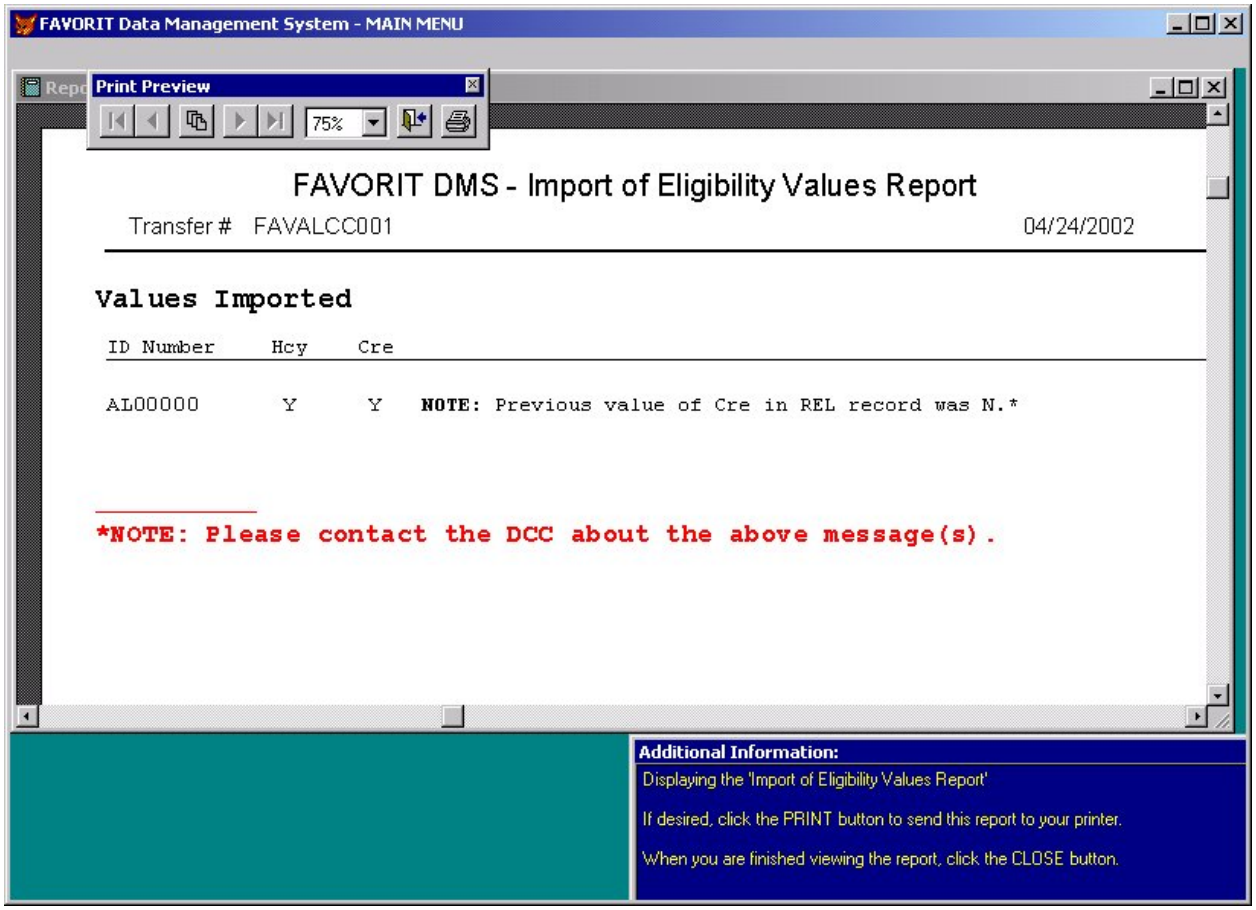
Choose 'Yes' to import the values.

The eligibility values import process adds the homocysteine and creatinine values in the import file to the RELA data table. For each Subject ID in the import file, the program checks whether there is an RELA form in the database. If there is, the values are inserted in the record. If there is not, the program inserts a new RELA record and adds the values. If a record exists with values present and the subject has NOT been randomized, the new values overwrite the old values. If the subject has been randomized, the new values are ignored. When values change you are asked to contact the coordinating center to make sure the correction is appropriate.

A report shows the status of each imported record:



The report above shows two imported records and the eligibility for the two criteria. These subjects have not been randomized.



The report above shows a single imported record. The eligibility value for Cre was N. The lab has, for some reason, rerun their analysis and a new value has been sent. As shown above, you may occasionally get a message to contact the coordinating center if a rare or unexpected event occurs.

If there are more files to import you will be asked whether you want to import the next file.

13.5.3 Secondary PC to Primary PC Backup and Export

At present, we do not have secondary computers at any of the FAVORIT sites.

Periodically data entered on the Clinical Center secondary computers (usually laptops) will be taken from the secondary pcs (Exported) and moved to the primary computer (Imported.) The export process involves several steps:

1. A complete backup of the DMS is made to diskette.
2. A data transfer diskette is created.
3. The laptop databases are cleared and all data is removed from the databases on the laptops.

The DMS Backup diskette is kept **with the secondary laptop (do not use the same set of backup diskettes on more than one pc).** The data transfer diskette is mailed or hand delivered to the data coordinator at the Clinical Center who is in charge of the primary computer.

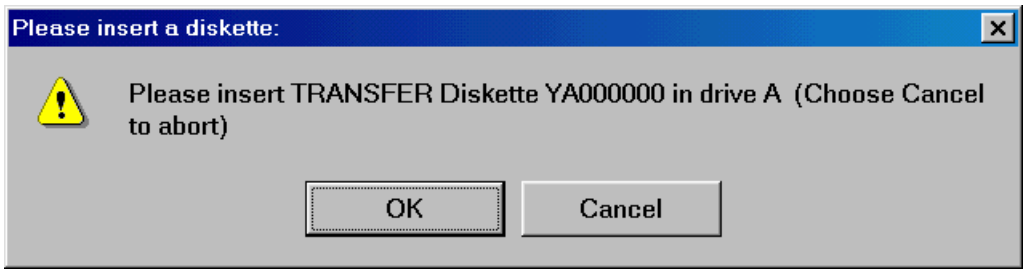
The backup of the secondary computer requires a 'backup diskette' which should be reused each time a backup is performed. The data transfer requires a blank, formatted high density 3½ inch diskette. After the Export is performed, **any changes** to records previously entered must be done **at the Clinical Center** on the primary computer because the record no longer exists on the secondary computer.

To start the export process, choose Export Files from the Utilities menu.

You will be asked to insert Backup Diskette number 1 into drive A. A status window is displayed for you to monitor the progress of the backup. If the backup requires more than one diskette, you will be asked to insert Backup Diskette 2, Backup Diskette 3, etc. (If you have never needed more than one diskette and are prompted for a second diskette, you may insert a formatted 3½ inch high density diskette. The diskette will be erased, then used to store backup data. Be sure to label the new diskette with the appropriate backup diskette number.)

A status window is displayed for you to monitor the progress of the backup. If the backup requires more than one diskette, you will be asked to insert Backup Diskette 2, Backup Diskette 3, etc. (If you have never needed more than one diskette and are prompted for a second diskette, you may insert a formatted 3.5 inch high density diskette. The diskette will be erased, then used to store backup data. Be sure to label the new diskette with the appropriate backup sequence number.)

After the backup has finished, the export begins. You will be asked to insert a blank diskette in drive A for the transfer files. Use a formatted 3.5" high density diskette. Press a key when the diskette is in the drive.



During export, two reports are produced. One lists the key fields of each record exported and is produced separately for each form type. This report is stored on the diskette but is not printed or displayed. The second is a summary which gives the number of adds, changes and deletes for each form type. Press **ESC** to clear the report from the screen. You are asked whether you want to print the summary report. Answer 'Yes' only if a printer is attached to the computer.

The export files are compressed into a single file which is copied onto the diskette. The export file is named FAVxxmmnnn.ZIP, where xx is the Clinical Center mnemonic, mm is the machine number for the secondary pc and nnn is the export file number. A message tells you the name of the file produced.

Please label the transfer diskette with this file name using the following format:

FAVORIT Transfer Diskette
Computer #: _____
Transfer # & Date:

Note: If you get a fatal error during Export, restart the export process using the same backup diskette and a new blank transfer diskette.

After successfully importing the data onto the primary pc, you can reuse the diskette for another export from this pc after first deleting the old files on the diskette. Use the labels supplied by the Coordinating Center.

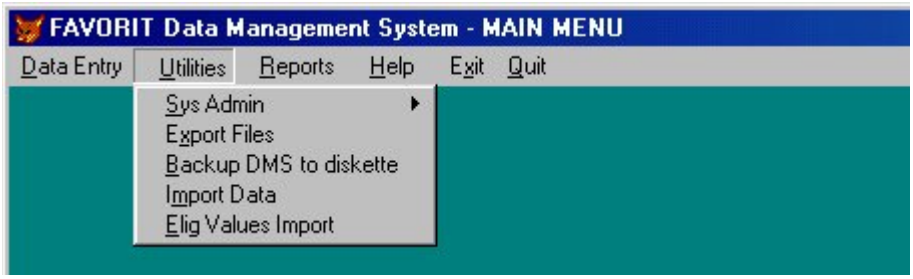
13.5.4 Clinical Center Import of Secondary PC Export Diskettes

At present, we do not have secondary computers at any of the FAVORIT sites.

FAVORIT study data keyed on secondary PCs are transferred to the primary computer on 3½" diskettes. At the Clinical Center, these diskettes must be *Imported* into the primary computer's database using the Import facility of the DMS.

Import Procedures

After logging into the DMS, select the UTILITIES menu option. The following menu is displayed:



To import a diskette, choose "Import Data" by pressing "M", or by using the arrows to highlight the desired option and pressing Enter.

Insert the laptop data transfer diskette in the A: drive and press Enter. If you have inserted a valid transfer diskette, the import program will update the desktop database with its contents and create an Import Summary Report. This report is first sent to the default printer, then to the screen for viewing. Press **Esc** to clear the report from the screen and continue the import process.

As data records are imported from the diskette to the desktop database, records are checked for consistency. If a record already exists in the database on the primary pc, it should not be coming in on a laptop transfer diskette. (A record with the same key field values should not be added more than once.) If this happens, the Import program notifies the user and prints the key fields of the duplicate record.

Once the diskette has been successfully imported, you will be asked whether you want to import another diskette. Select 'Yes' if you have another diskette to process. Select 'No' if you have no other diskettes to import.

Process another set of disks?
Yes
No

If you fail to insert a diskette when prompted, or if you insert a diskette which is not a data transfer diskette (or has become damaged), you will be prompted:

(Please insert a disk. ESC to abort.)

Note that DMS data transfer diskettes from each secondary pc must be imported in sequential order. If a diskette is skipped or processed out-of-order, you will be prompted:

You have inserted a diskette which is out of sequence
Current disk is #0002, received from AL01
(Prior disk was #0003, imported on 03/03/2002)
Yes – (Unexpected sequence is not OK)
No - (Unexpected sequence not OK)

If the unusual sequence of diskettes is expected, choose "Yes" to accept the diskette. *Otherwise, choose "No" and consult the DMS staff before processing more diskettes from this laptop.*

The DMS keeps backup copies of the content of data transfer diskettes on both the laptop and desktop systems. Thus, the laptop-desktop data transfer diskettes do not need to be stored for archival purposes. They can be erased (formatted) and returned to the laptop system for reuse.

Import Reports

The import program generates several types of reports which allow the data processor to determine the status of the desktop database. The *Import Summary Report* is generated once for each diskette. It is stored in a file named with the transfer file name in the import .ZIP archive. It is printed to the default printer as each diskette is processed.

FORM	Add		Change		Delete		KF Add		KF Del.		New to DB		Sub-total		Total	
	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
RELA	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0
RPCA	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0
SPPA	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0
VDLA	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Totals	1	0	3	0	0	0	0	0	0	0	2	0	3	0	1	0

A=Accepted R=Rejected
See error file for rejected records list

The columns of this report specify the types of records which existed on the diskette and how they were processed. Each column (Add, Change, etc) contains two sub-columns: **A**ccepted and **R**ejected. KF Add and KF Del stand for Key Field Add and Key Field Delete. The New to DB column is a count of all records added to the desktop database by this diskette. This number will be greater than the value in the Add column if any records were added and subsequently modified since the last export.

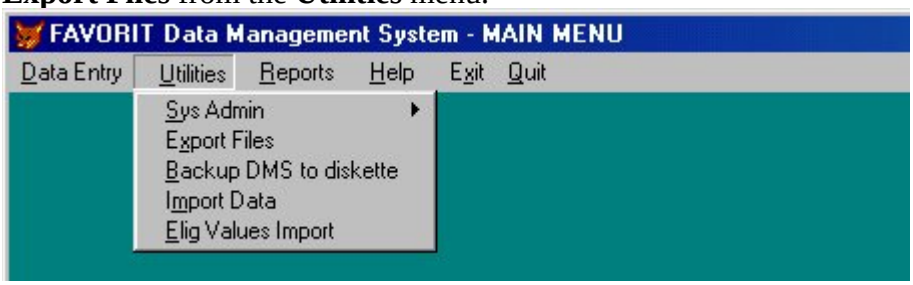
13.5.5 Clinical Center to Coordinating Center Export and DMS Backup

Every week data collected in the Clinical Centers will be sent to the Coordinating Center via modem or Internet. In addition, eligibility values computed by the Coordinating Center will be sent to the clinics. The export process includes several steps:

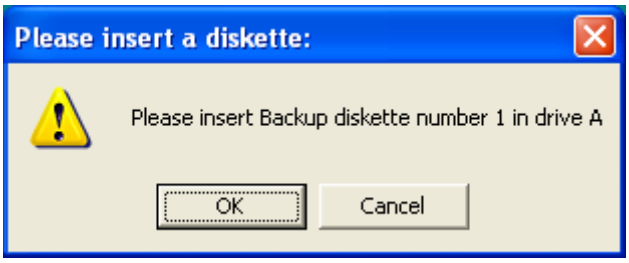
1. A complete backup of the DMS is made to a floppy diskette and the SECURE_DMS disk (memory key in the case of FAVORIT).
2. A data transfer file is created and put in a folder on the C drive..
3. The user exits the DMS and double-clicks on the FAVORIT Internet Data Transfer or FAVORIT Modem Data Transfer icon on the Windows 2000/Windows XP desktop.

The backup diskette is kept at the Clinical Center. The Backup procedure may require a series of 'backup diskettes' which should be reused each time a backup is performed.

To start the **Export to Coordinating Center** process, at the primary computer DMS choose **Export Files** from the **Utilities** menu.



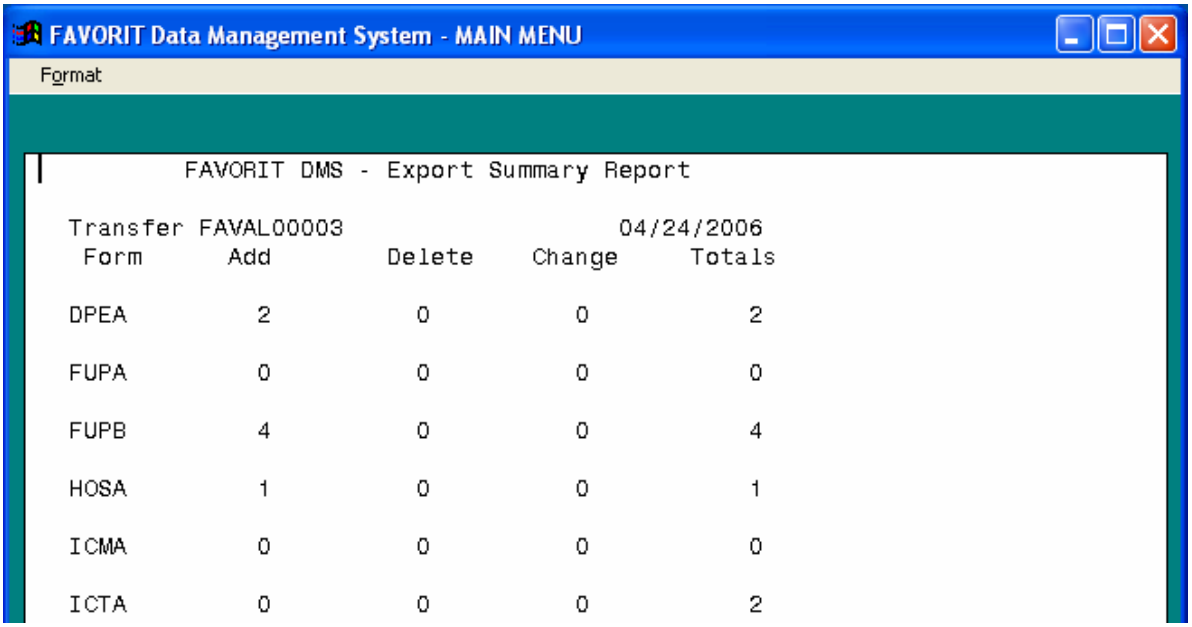
The data in the DMS is first backed to the FAVORIT memory key and then you are asked to insert the FAVORIT Backup Diskette.



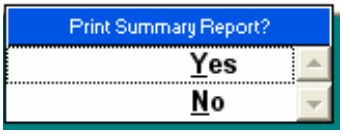
If you have not inserted the backup diskette, insert it and hit 'OK'. Then the data is backed up to the 3½" backup diskette(s) for the primary computer. A status window is displayed for you to monitor the progress of the backup.

After the backup has finished, the export begins. Records that—since the **last** export—have been added to the database, changed (either data change or key field change), or deleted are exported.

The "FAVORIT DMS – Export Summary Report" is then displayed. This report gives the number of adds, changes and deletes for each form type.

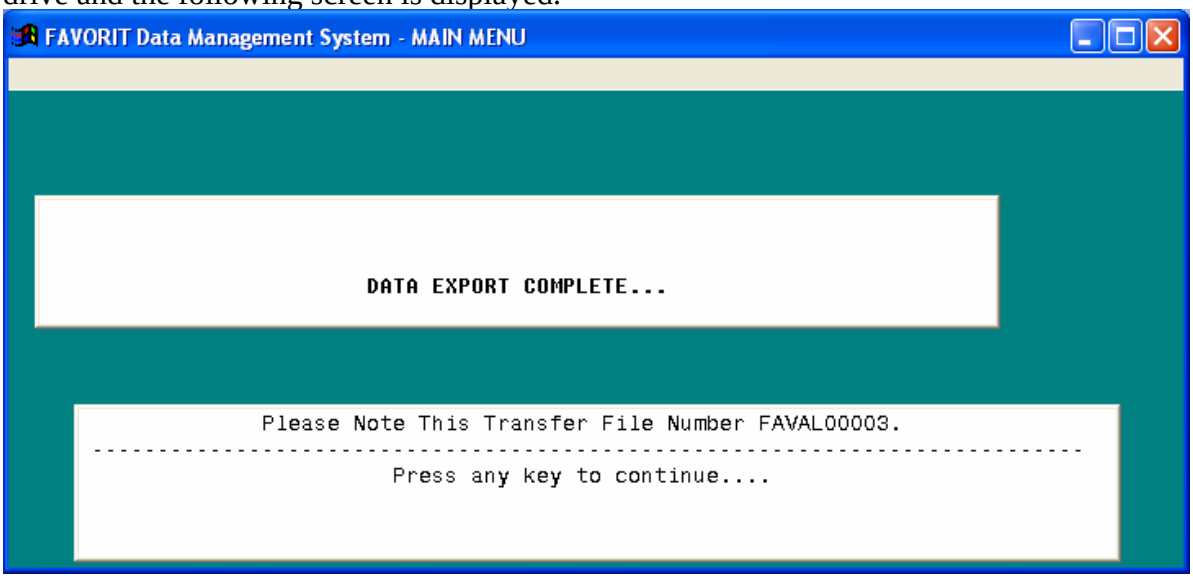


Hit 'Esc' to close report. You are asked whether you want to print the summary report.



Select 'Yes' to print the Summary Report. (Do **not** try to print the report if the computer is not connected to a local or network printer.) Select 'No' to continue the export process *without* printing the report.

Then, a compressed "transfer file" containing the exported records is created in a folder on the C drive and the following screen is displayed.



Using the name in the above screen-shot 'FAVAL00003', 'FAV' stands for 'FAVORIT', 'AL00' indicates that the export is from computer AL00 where 'AL' is the site mnemonic and '00' tells you that it is the primary computer (at present in FAVORIT, we do not have any secondary computers) , and '003' is the export sequence number.

The file is stored in the directory C:\FAVDMS\EXPORT\SEND. During the export process, one more report is produced which lists the key fields of each record exported and is produced separately for each form type. This report is not printed or displayed but is included in the data transfer file along with the Export Summary Report.

NOTE: If you get a fatal error during export, restart the export process using blank diskettes. You can later reformat any diskettes written to during the failed export process.

13.5.6 Data File Transfer between Coordinating Center and Clinical Centers

After you have completed the export, send the transfer file, via modem or Internet, to the Coordinating Center. During the same connection, the Coordinating Center will send you any available eligibility files and notification of any data needed to compute other eligibility values.

Exit the DMS by selecting QUIT from the Main Menu. From the Win2000/WinXP desktop double-click the icon **'FAVORIT Modem Data Transfer'** or the **'FAVORIT Internet Data Transfer'** icon.

A commercial communications program called pcAnywhere is used to connect your pc to a pc at the Coordinating Center. The program runs a script file which looks for files to be sent from the Coordinating Center to the clinics and files to send to the Coordinating Center from the clinics.

Do not reboot the computer, stop pcAnywhere or run the DMS after you start the script until the transfer is complete.

Please note that sometimes, during the first or second try, pcAnywhere will not be able to make a connection. Here, you will get a message that says "Timeout Looking for Connection." Click "OK" to clear that message. Then double-click again on the "FAVORIT Internet Data Transfer" or "FAVORIT Modem Data Transfer" icon to send the file.

The results of the data transfer are appended to a transfer log file (C:\FAVDMS\EXPORT\FVXFER.LOG) which is displayed in the Windows application called NOTEPAD. Go to the bottom of the file and examine the message written during the last transfer. If the transfer was successful and there are eligibility files to receive and data files to send, you should see messages like the following:

********FAVORIT Internet Data Transfer 04/15/02 03:00:13 ********
Eligibility values received successfully from DCC.

Data files sent successfully to DCC.
****

If there are no data files to send to the CC you will get messages like the following

********FAVORIT Modem Data Transfer 04/15/02 03:00:13 ********
Eligibility values received successfully from DCC.
No data files to send to DCC.
****

If there are no eligibility files to send to your site you will get messages like the following

********FAVORIT Internet Data Transfer 04/15/02 03:00:13 ********
No eligibility values to receive from DCC.
Data files sent successfully to DCC.
****

Finally if the Coordinating Center needs data from your site to compute some eligibility values, you will get messages like the following:

********FAVORIT Internet Data Transfer 04/15/02 03:00:13 ********
Eligibility values received successfully from DCC.
Report received from DCC that lists data the DCC needs. Enter DMS to view.
Data files sent successfully to DCC.
****

The log file is a complete record of the most recent transfers. Go to the bottom of the file and examine the date and time to be sure you are looking at the most current log message.

If the transfer was unsuccessful you will see an error message such as:

********FAVORIT Internet Data Transfer 04/15/02 03:00:13 ********
Error dialing Host ERROR Number: -15 Host: FVICSCC
****
In the case of Windows XP laptops, you get :

********* FAVORIT Internet Data Transfer 03/15/2006 17:18:16 *********
pcAnywhere connection failed. Try again.
****

In the above 2 cases, try again the next night, try again immediately, or call the CC for help.

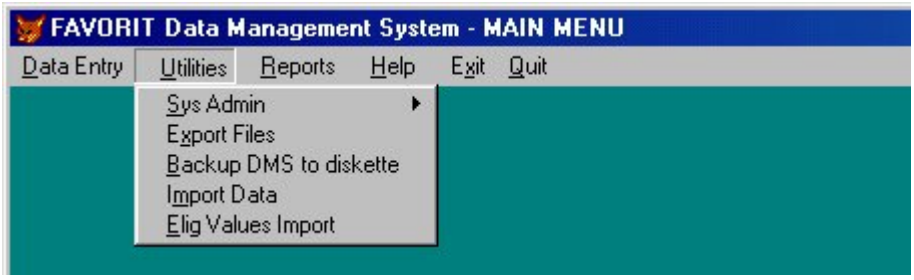
If you receive eligibility files, you must import them into the DMS. See section on Eligibility Values Import for instruction.

If you receive notice that the Coordinating Center needs data from your clinic, a report will be displayed the next time you log into the DMS.

To exit Notepad, choose File/Exit from the Notepad menu.

NOTE: If you have errors during file transfer, try again or call the CC.

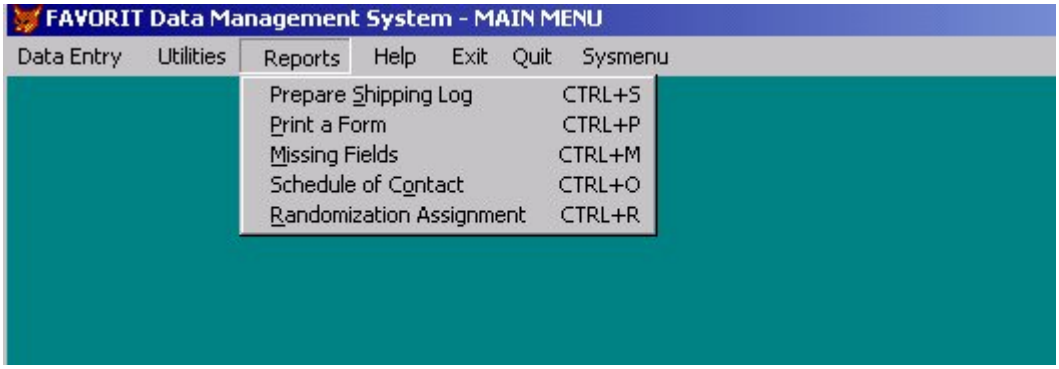
13.5.7 Backup DMS to Diskette



This backup is the same as the backup which occurs at the beginning of the Export process.
Floppies should be number sequentially to keep in proper order.
Please label the diskettes 'FAVORIT Backup Diskette Computer #.'

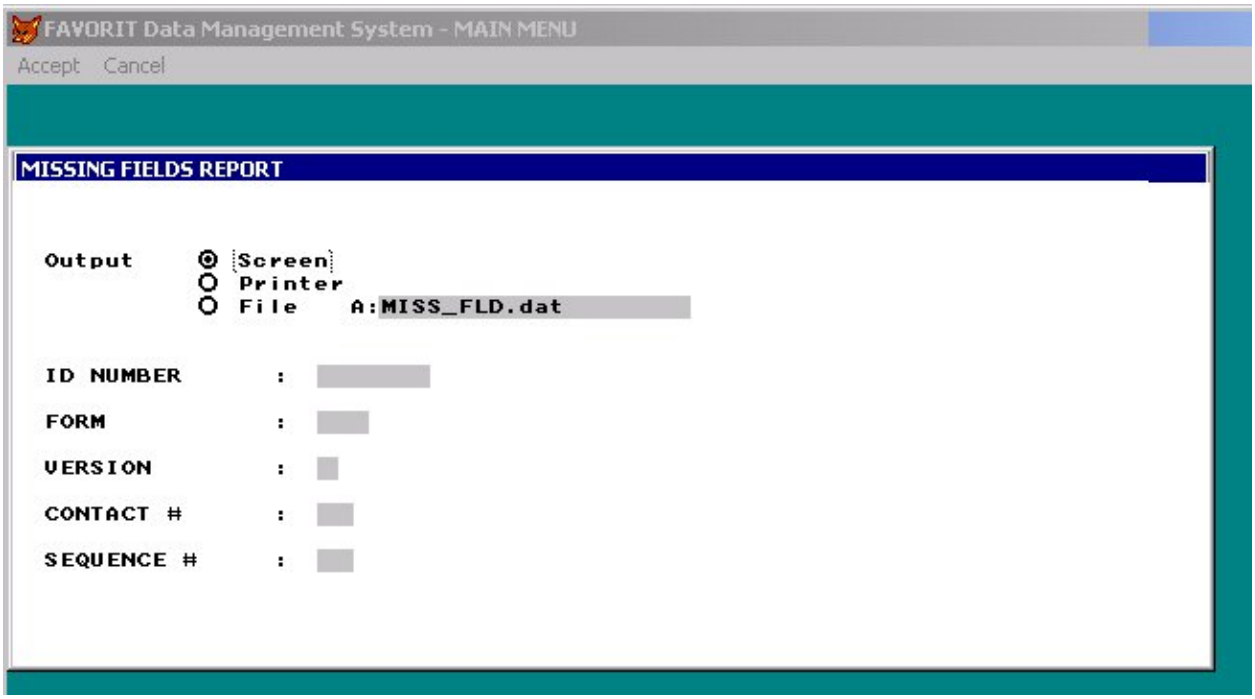
13.6 Reports

The FAVORIT DMS has several reports including a missing fields report, a print forms report, a shipping inventory report and participant scheduling report. To run reports, choose Reports from the main menu.



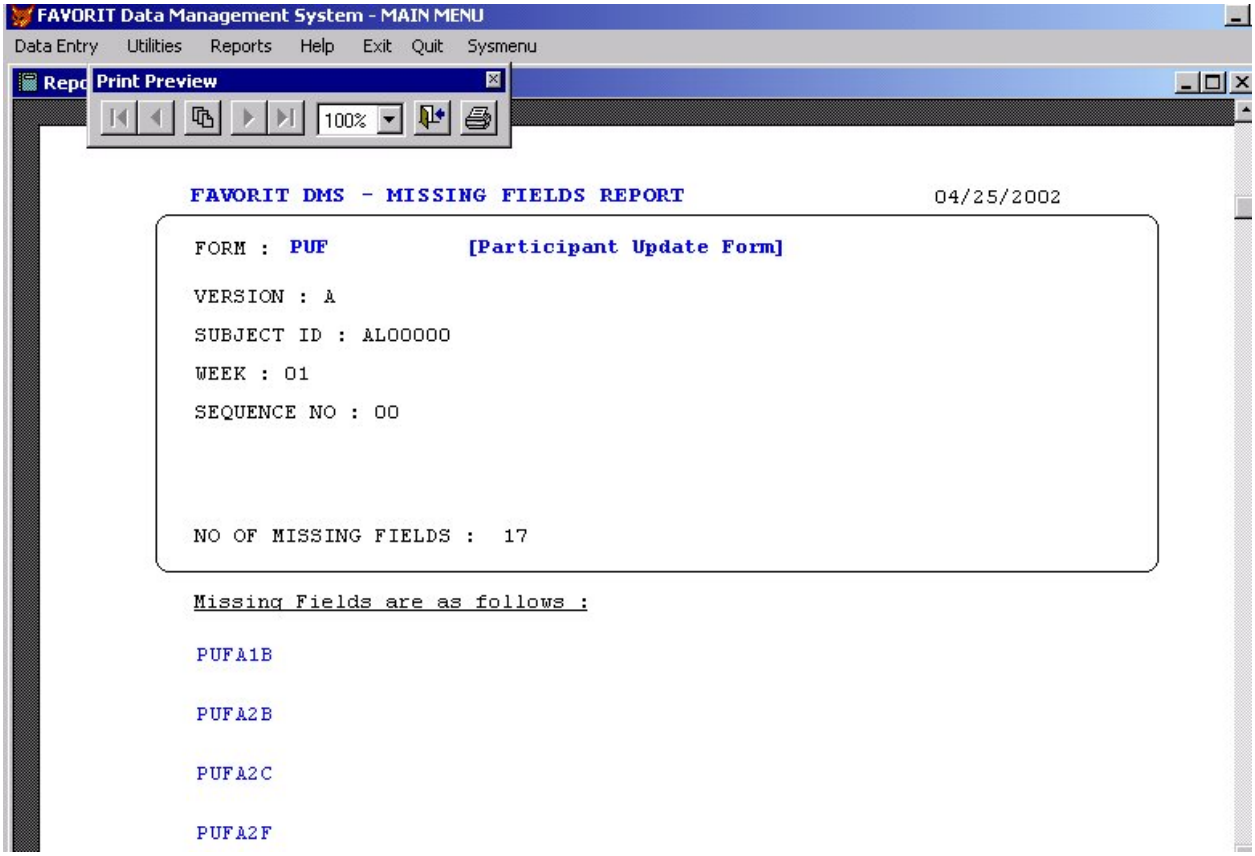
13.6.1 Missing Fields Report

The Missing Fields Report allows the user to list fields which are empty for a form. When you select this report option, the following screen is displayed.



Enter a FAVORIT ID and other key field information for the form of interest (Form, Version, Contact, Sequence #).

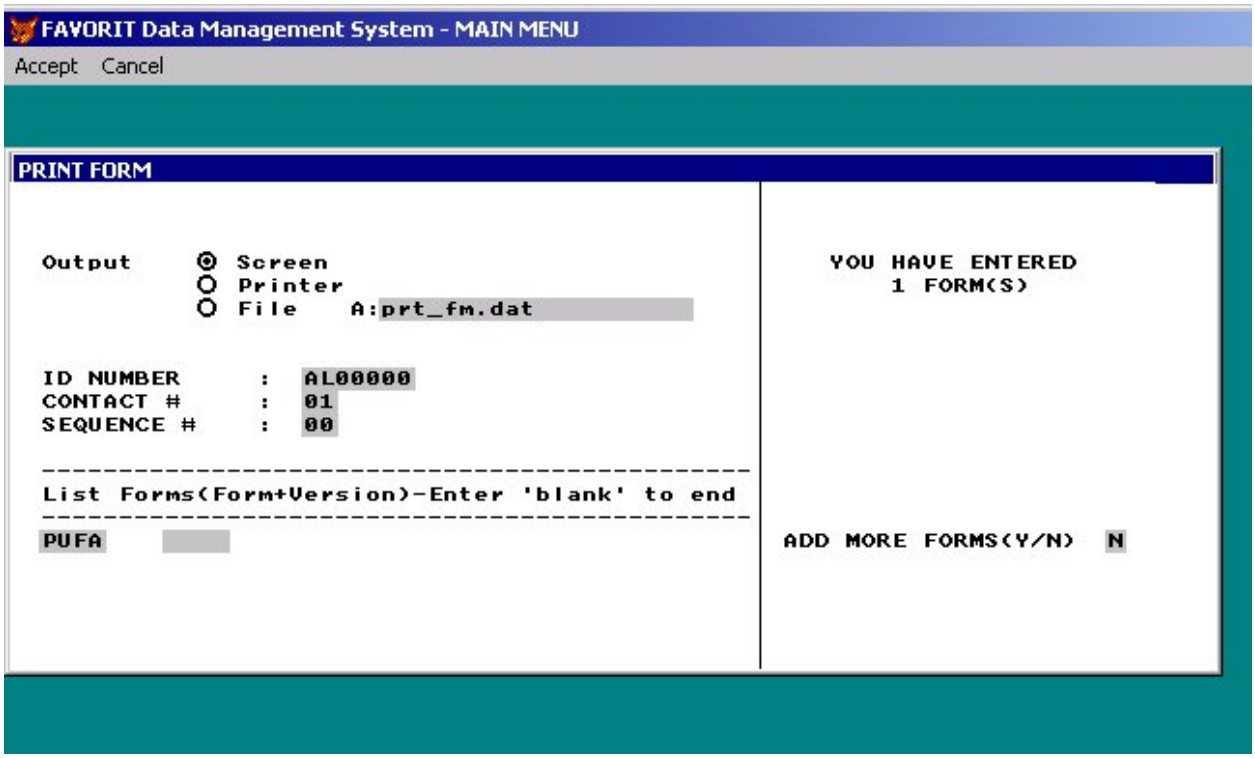
If the form exists in the database and has missing fields you get the following report:



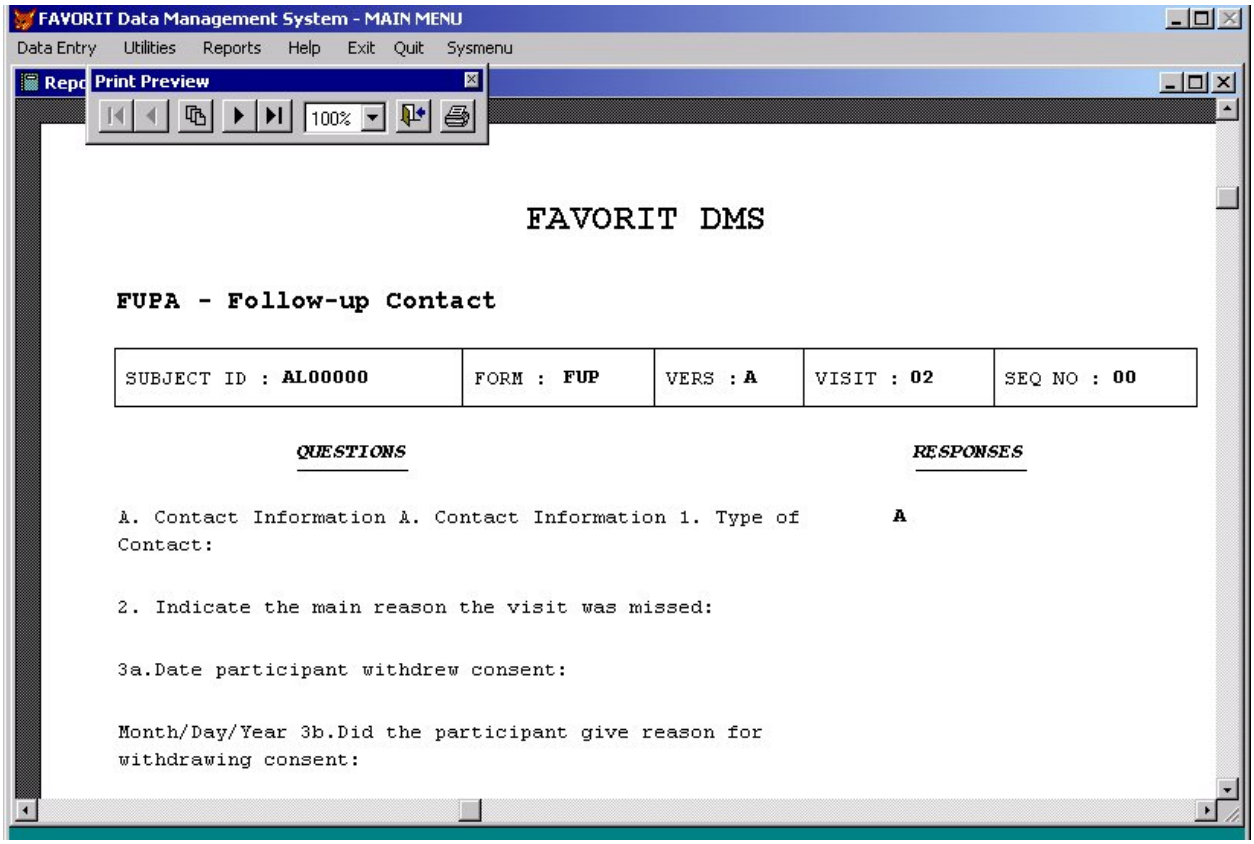
If the form does not exist in the database or if it has no missing fields, you are notified.

13.6.2 Print Form Report

The Print form report prints data from one or more forms in the database. The question (or prompt) plus the value (or response) is printed but the output is not formatted to look like the paper form or the DMS screen. To print a form choose that option from the report menu. On the displayed screen, shown below, enter the identifying information for the forms you want to print. Enter a list of forms. Enter a blank form to stop form list and respond 'N' to the prompt 'Add more forms'.



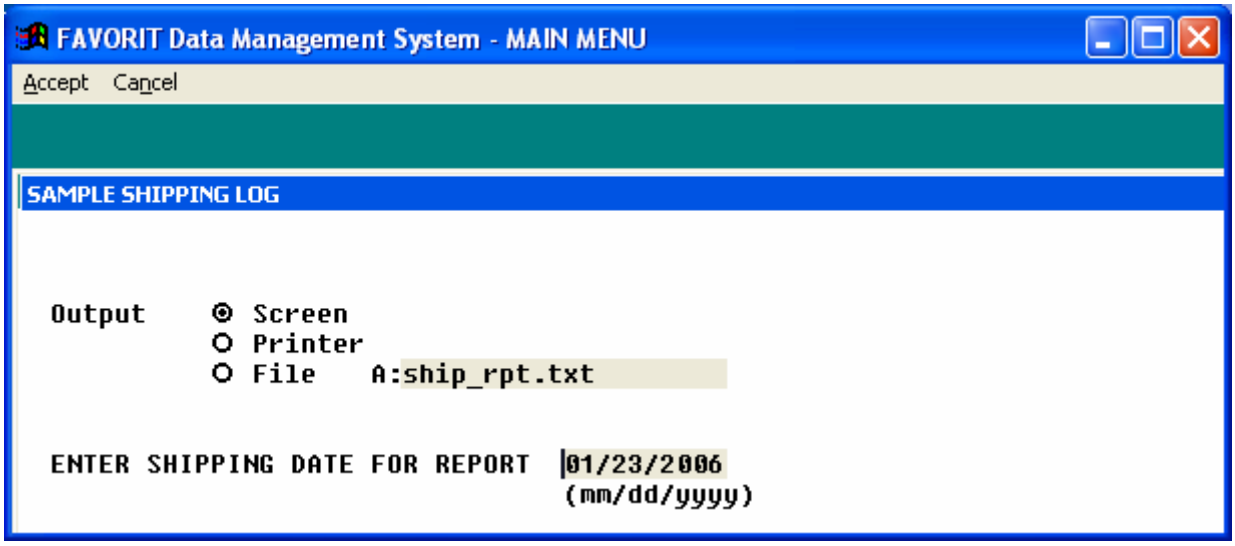
If the form exists a report is displayed:



13.6.3 Shipping Log

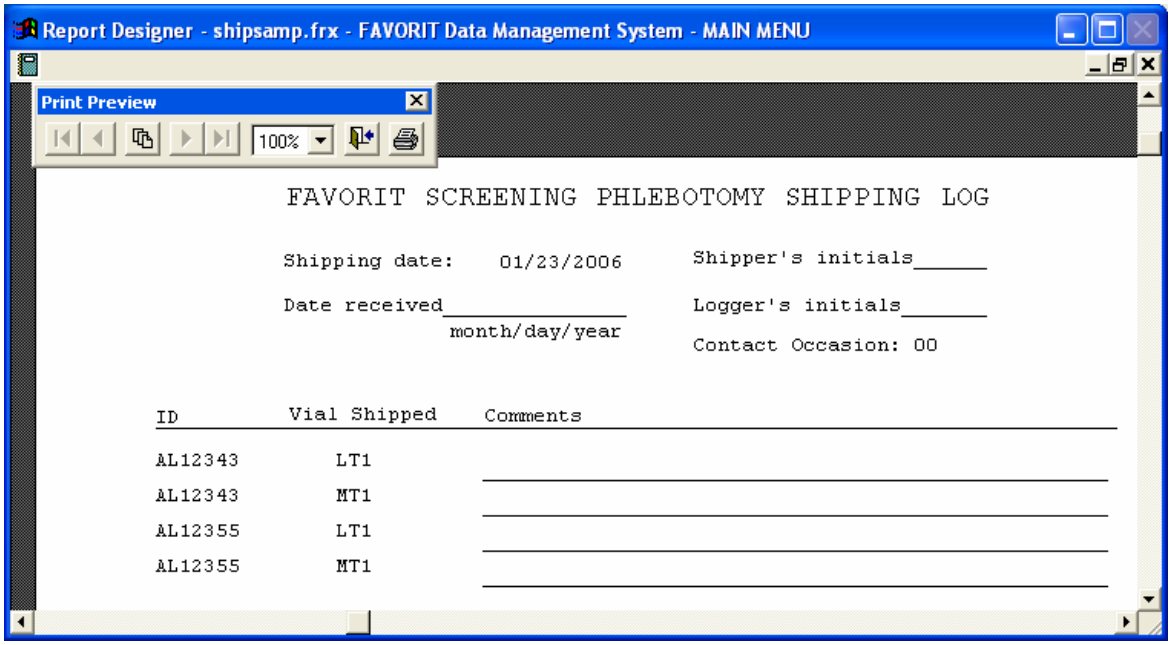
The shipping log provides the ability to generate a shipping log by shipping date. The report looks at SPP item 5c, SPP item 9c, SPP item 14c and SPP item 18c for shipping dates.

To run the report select 'Prepare Shipping Log' from the Reports Menu. The following screen will be displayed.



Enter the shipping date for the report, select an output option and then choose 'Accept' from the top menu.

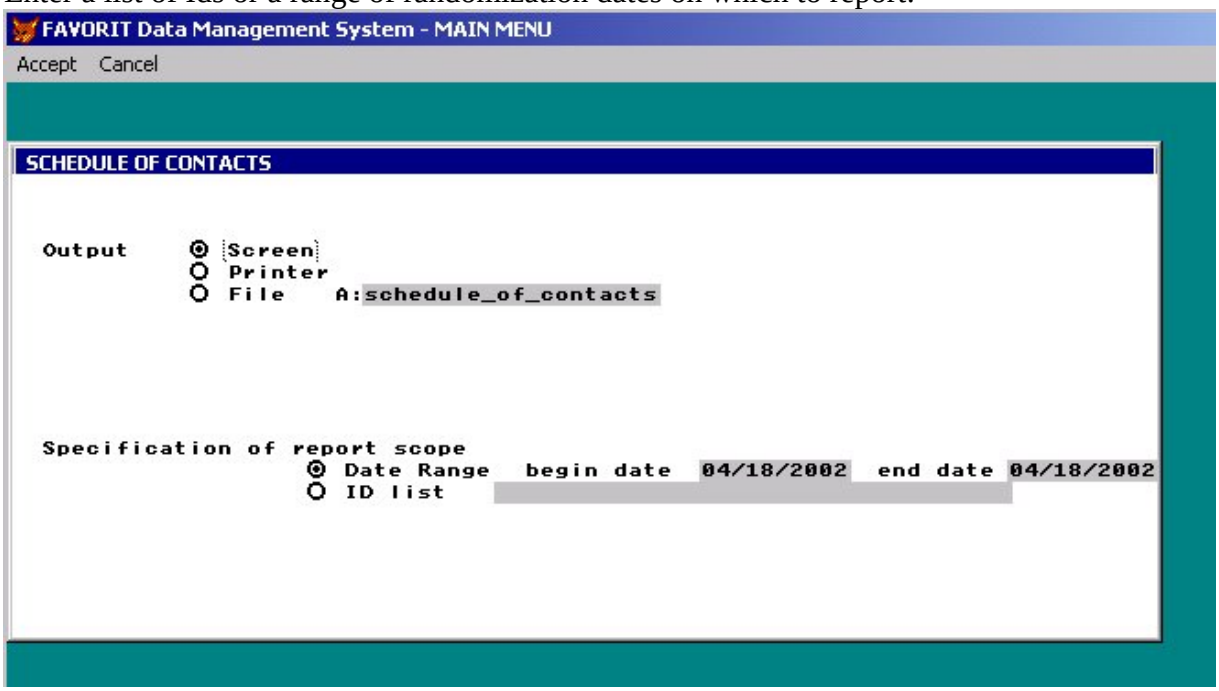
If any SPP records have values that match the date entered, the shipping report will be generated.


Report Designer - shipsamp.frx - FAVORIT Data Management System - MAIN MENU
Print Preview
100%
FAVORIT SCREENING PHLEBOTOMY SHIPPING LOG
Shipping date: 01/23/2006
Shipper's initials_____
Date received_____
month/day/year
Logger's initials_____
Contact Occasion: 00
ID
Vial Shipped
Comments
AL12343 LT1
AL12343 MT1
AL12355 LT1
AL12355 MT1

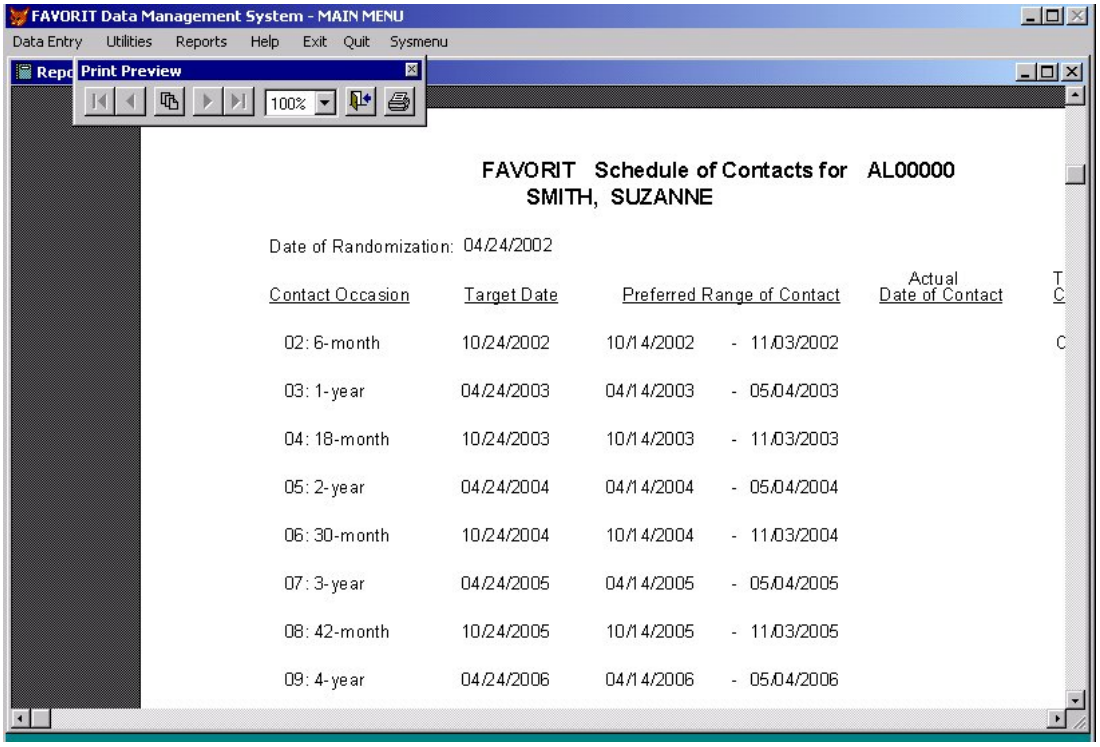
13.6.4 Schedule of Contacts Report

The patient scheduling report prints a list of contact occasions and the target date and range of target dates for each contact. If the contact has occurred, the date of actual contact is listed, along with the type of contact.

Enter a list of Ids or a range of randomization dates on which to report:

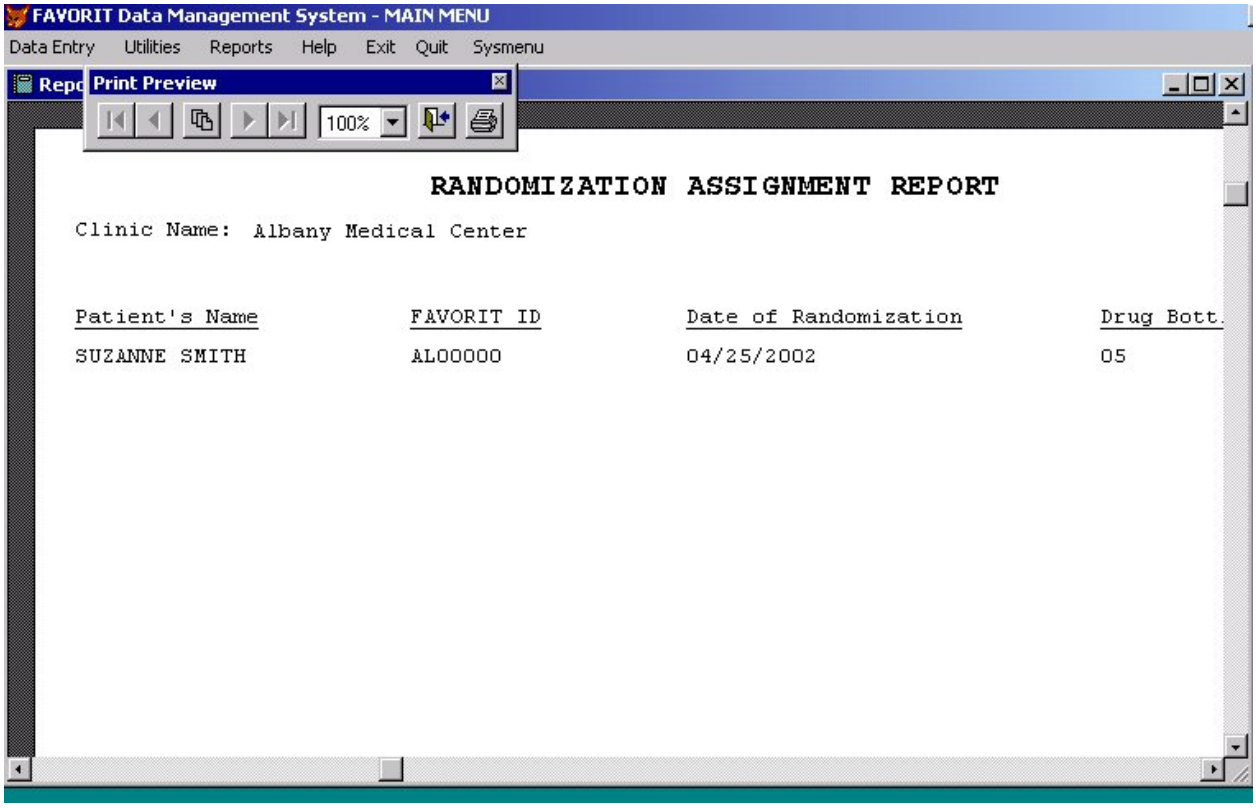


The report is generated for Ids which have been randomized and which have a PUF and a FUP form in the database. The report is shown below:


FAVORIT Data Management System - MAIN MENU
Data Entry Utilities Reports Help Exit Quit Sysmenu
Print Preview
100%
FAVORIT Schedule of Contacts for AL00000
SMITH, SUZANNE
Date of Randomization: 04/24/2002
Contact Occasion | Target Date | Preferred Range of Contact | Actual Date of Contact
02: 6-month 10/24/2002 10/14/2002 - 11/03/2002
03: 1-year 04/24/2003 04/14/2003 - 05/04/2003
04: 18-month 10/24/2003 10/14/2003 - 11/03/2003
05: 2-year 04/24/2004 04/14/2004 - 05/04/2004
06: 30-month 10/24/2004 10/14/2004 - 11/03/2004
07: 3-year 04/24/2005 04/14/2005 - 05/04/2005
08: 42-month 10/24/2005 10/14/2005 - 11/03/2005
09: 4-year 04/24/2006 04/14/2006 - 05/04/2006

13.6.5 Randomization Assignment Report

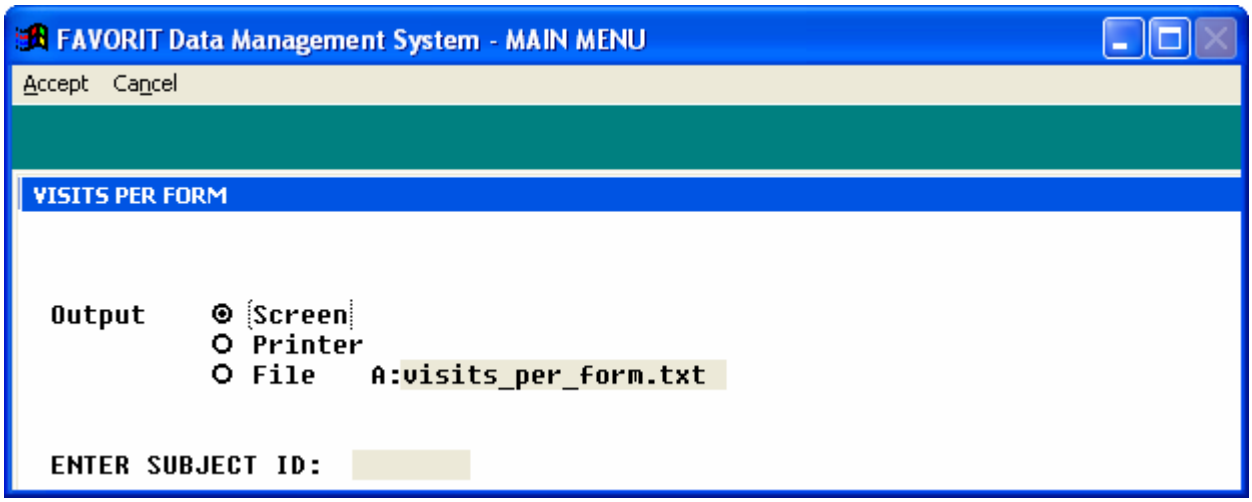
To produce a list of all subject Ids which have been randomized and the bottle code assignment for each ID, run the Randomization Assignment Report.



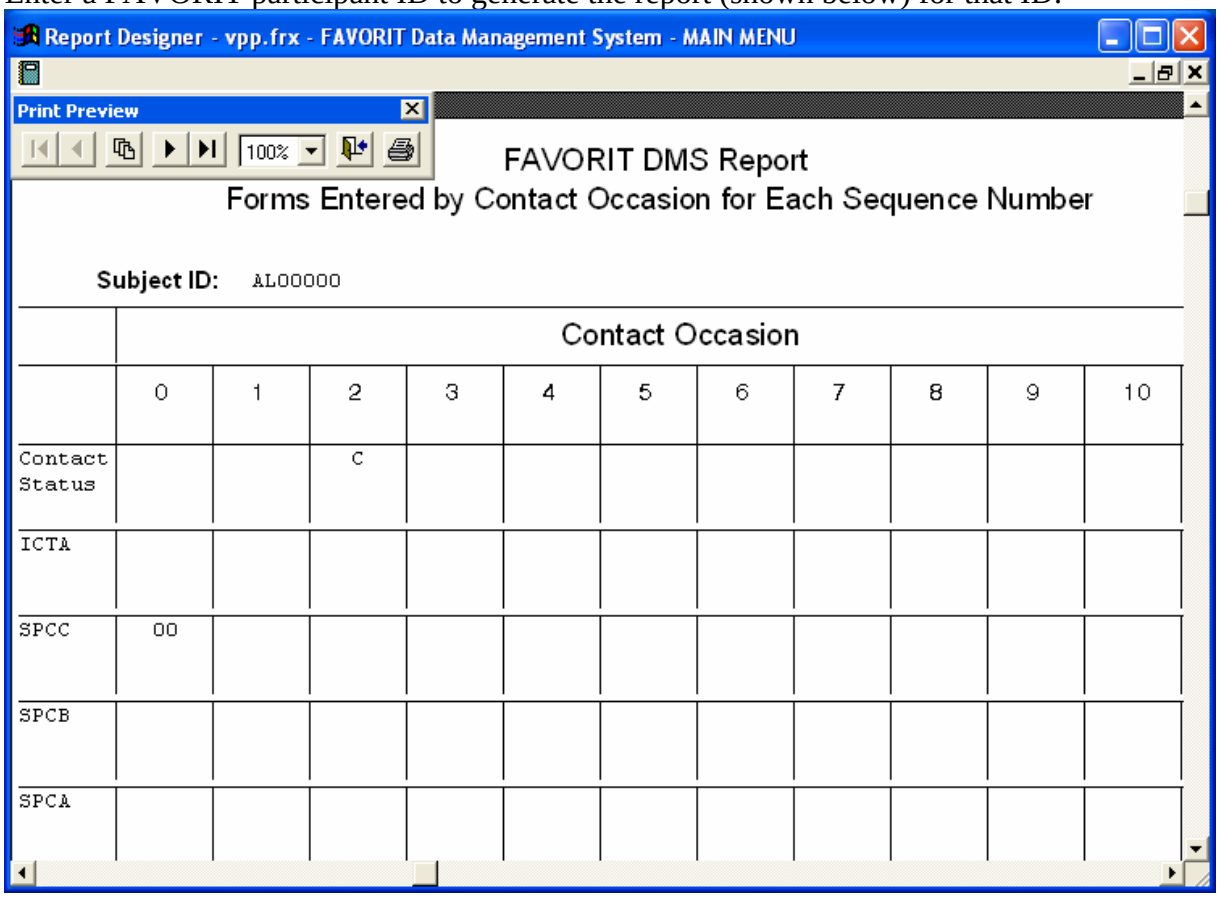
13.6.6 Forms per Visit Report

The Forms per Visit Report lists all the forms entered into the DMS for a particular ID. It also tells you the Contact Occasion and Sequence Number at which the forms were entered.

The following screen will be displayed when you select 'Forms per Visit' from the Reports Menu.



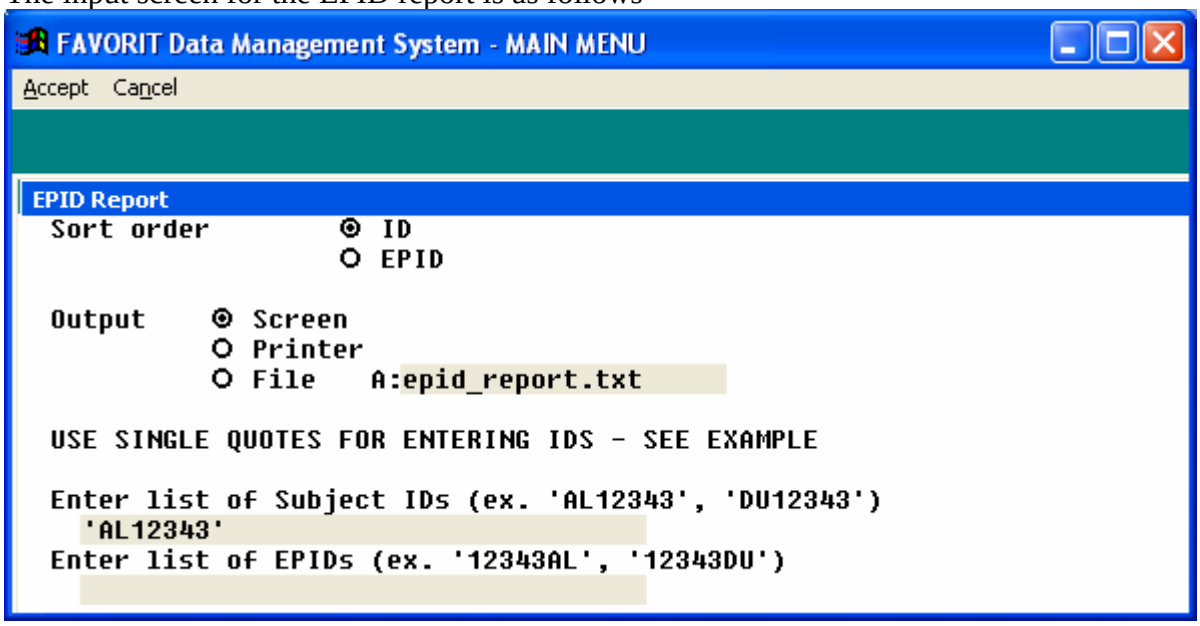
Enter a FAVORIT participant ID to generate the report (shown below) for that ID.



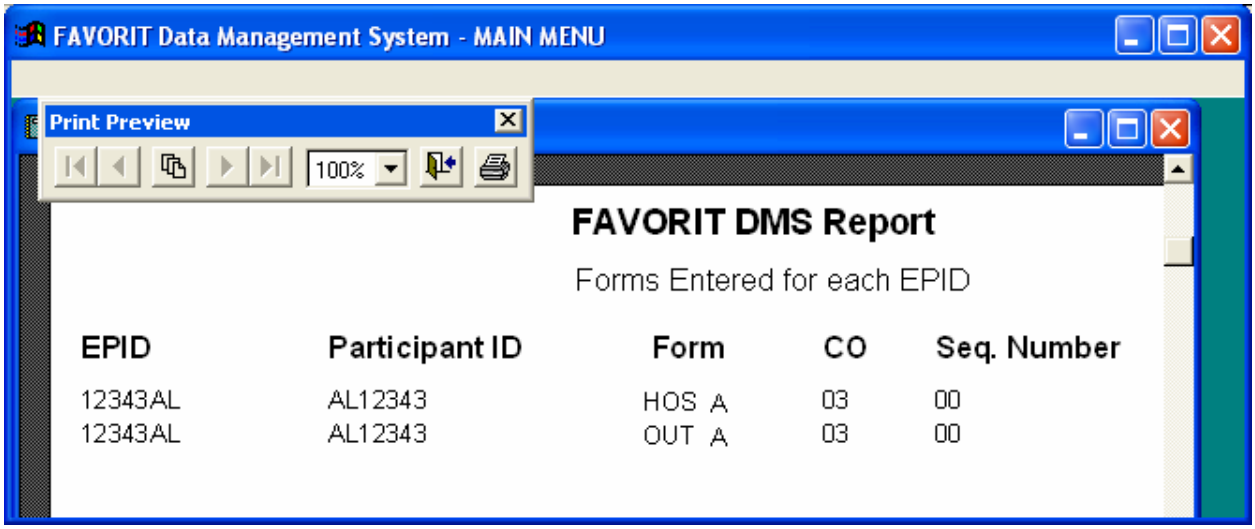
13.6.7 Epid Report

The EPID report is used to track all EPID's that have been assigned to a FAVORIT participant ID.

The input screen for the EPID report is as follows-



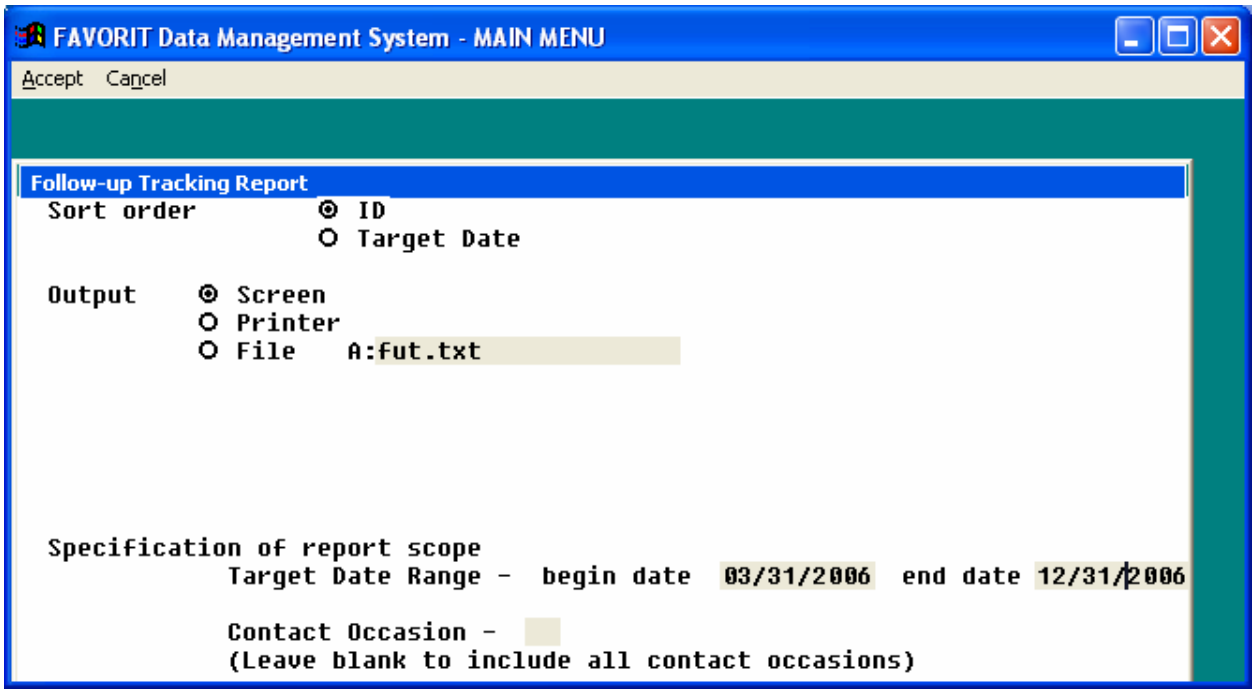
Enter a list of IDs or a list of EPIDs to get the report as shown below.



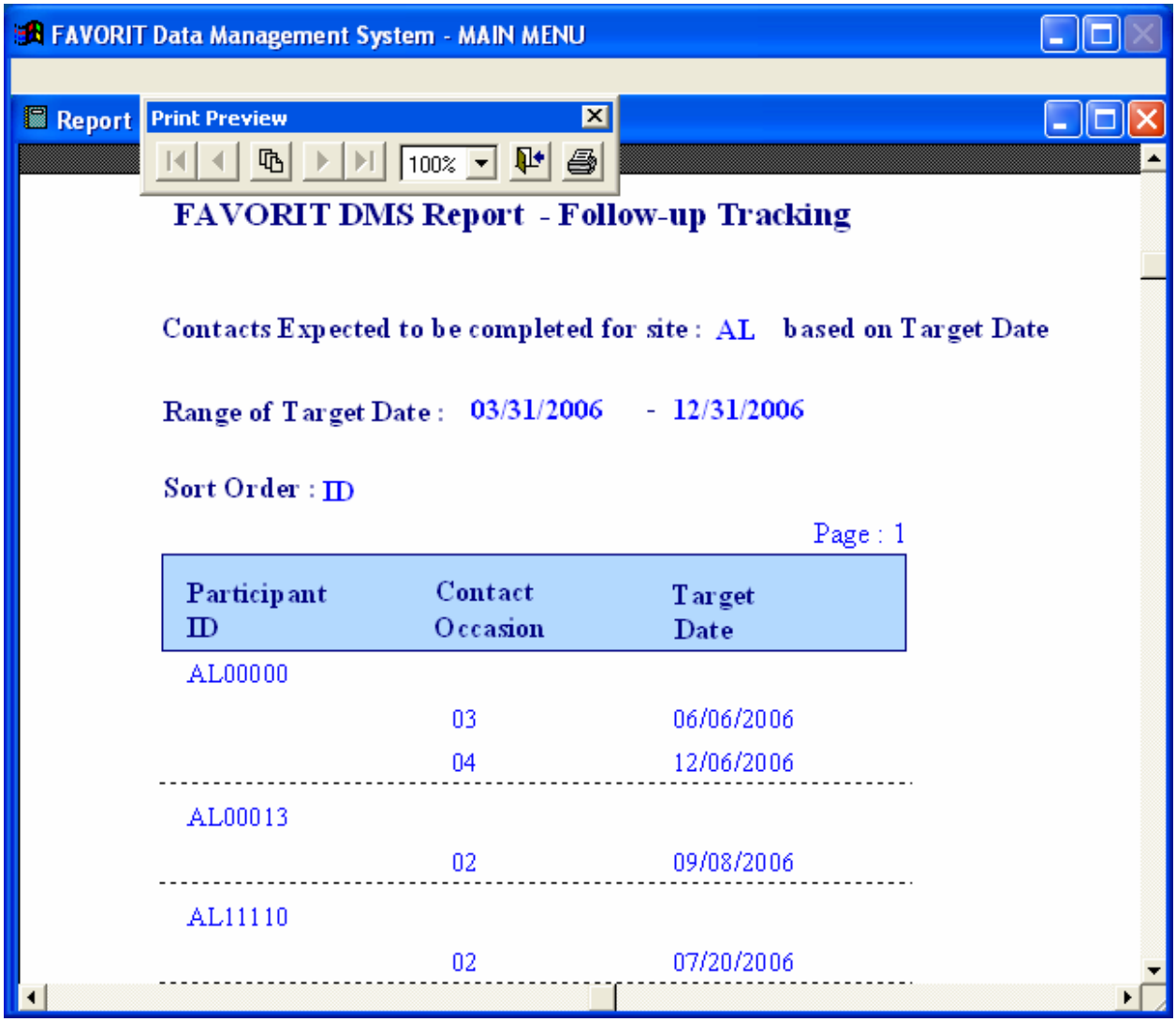
13.6.8 Follow-up Tracking Report

The Follow-up Tracking Report lists the Participant IDs, Contact Occasion and Target Dates for IDs which are due for a follow-up visit within the requested target-date range for a particular Contact Occasion or for all Contact Occasions. The report can be sorted by Participant ID or by Target Date. Visits that have been completed are skipped.

When you select 'Follow-up Tracking Report' from the Reports Menu, you will see the screen below.



Enter the required sort order (can be sorted by ID or by Target Date), Target Date range and required Contact Occasion to get the report shown below. You may leave the Contact Occasion blank to get all visits due for follow-up within the requested data range.


FAVORIT Data Management System - MAIN MENU
Report
Print Preview
100%
FAVORIT DMS Report - Follow-up Tracking
Contacts Expected to be completed for site : AL based on Target Date
Range of Target Date : 03/31/2006 - 12/31/2006
Sort Order : ID
Page : 1
Participant ID
Contact Occasion
Target Date
AL00000
03 06/06/2006
04 12/06/2006
AL00013
02 09/08/2006
AL11110
02 07/20/2006

13.7 Remote User Service (via pcAnywhere)

Sometimes the CC must call a Clinical Center computer to diagnose or repair a problem. To allow the CC computer to control the Clinical Center computer we use a product called pcAnywhere. Of course, if you have a problem you must call us first to discuss the problem and determine how to proceed.

To start pcAnywhere, double-click on the "Symantec pcAnywhere" icon on the desktop. There are two pcAnywhere objects which allow us to connect: FAVINT and FAVMOD in the older DELL laptops. The new sites with IBM laptops with pcAnywhere 10.5 have just one object, namely, FAV_H_CSCC. The person that you called at the CC will instruct you on which to select and what to do next.

The CC can then connect to your computer to diagnose and repair problems with the DMS.

In some situations it is not possible for your computer to receive a call on the available phone line or Internet connection, but it is possible for you to place a call. In this case, we will ask you to have your computer call us. If we determine that this connection method is required, we will give you a phone number for pcAnywhere to call or an Internet address for it to connect to.

While we are working on your computer using pcAnywhere, do not do anything on your computer unless we tell you to do so.

13.8 DMS Updates

During the course of the study, the CC will distribute updates to the Data Management System software. These updates may include new or updated forms for data collection, new features or reports, and corrections for errors which are detected in the system. **Each update should be installed as soon as possible after it is received at the Clinical Center**. Please run the Export process prior to installing an update.

Each update will include a memorandum detailing the changes to the DMS which are included in the update. Please insert this memorandum at the end of the Users Guide so that it includes the most up-to-date information about the DMS.

13.9 Appendix A - FAVORIT DMS Quick Start Instructions

These instructions are intended to serve as a quick reference for using the FAVORIT Data Management System (DMS), and are not a substitute for mastering the contents of the Users Guide.

Who do I contact if I have a problem / question / concern?

Contact Barbara Brown at the FAVORIT Coordinating Center.

Barbara Brown– Voice: (919) 962-3092 Email: barbw_brown@unc.edu

Starting the System

Log in to Windows with your Windows ID and password.
To start the Data Management System [DMS], use the mouse to double-click the "FAVORIT DMS" icon.
Log in to the DMS by keying your initials and your password. If you key the wrong initials, go ahead and key your password. You'll see the message "Login Failed." Key both in again.

Shutting Down the System

To shut down, you must first exit the DMS. If you are in the middle of a form, choose Save, then choose Quit. Now you will see the Windows desktop. Click the Start button (lower-left corner of the screen), choose Shut Down, then click OK. Wait for the computer to turn itself off.

What options are available during data entry?

Special Keys	
CTRL+U (or fill the field with equal signs)	When respondent refuses to answer a question, or if a question is not applicable (sets the field to missing).
CTRL+N	Open a Notelog. When finished, choose Save or Cancel to either keep or drop the note.
ALT+S	Saves the current form.
ALT+N	Cancel the form (you'll be asked "Are you sure?")
CTRL+PageDown	Save the current form and move to the next.

If you forget how to do something, browse through the menus. You'll find the Move and Problem menus particularly useful: the Move menu contains navigational items (move forward / back, etc.), and the Problem menu includes items for handling problems with a field (Confirm, Unresolvable, Notelog).

I have some strange error message on my screen. What do I do?

In rare cases, you might see an error message other than our Fatal Error display. Such messages typically have Close or Ignore as options -- choose Close rather than Ignore. Please make a note of what you were doing prior to seeing the message and notify the Coordinating Center.

Help! My laptop is frozen. What do I do?

Usually, the best thing to do is to restart the computer. Begin by shutting down the laptop. First, try pressing CTRL+ESC to open the Start menu, then choose Shut Down. If the Start menu doesn't appear, try turning off the computer by its power switch. Once the laptop is turned off, make sure all cables are connected tightly, then turn the unit on as you normally would.

13.10 Appendix B - FAVORIT Data Transfer Checklist

Below is a checklist of steps to follow when transferring FAVORIT data to the FAVORIT Coordinating Center and receiving eligibility values from the Coordinating Center.

1. RUN EXPORT (from the FAVORIT DMS.)

2. Exit the FAVORIT DMS.

3. From the Windows 2000 or Windows XP desktop, double-click on the "FAVORIT Internet Data Transfer" icon or the "FAVORIT Modem Data Transfer" icon.

4. Do not turn the computer off until the transfer has occurred.

5. Sometimes, during the first or second try, pcAnywhere (the communications software used for transferring the data) will not be able to make a connection. Here, you will get a message that says "Timeout Looking for Connection." Click "OK" to that message and then click "Cancel" when it automatically tries to send the file again (this automatic resending will not work). Then just double-click again on the "FAVORIT Internet Data Transfer" or "FAVORIT Modem Data Transfer" icon to send the file.

6. Check the computer screen to see that the transfer completed successfully. Go to the bottom of the displayed file and read the messages.

7. If the transfer failed, try again or call the FAVORIT Coordinating Center for assistance.

Chapter 14. Administrative Procedures

14.1 Overview

This chapter pertains to general issues that will be used throughout the study. It covers topics such as the correct usage of contact occasion and sequence numbers, filling out the paper forms as well as making corrections, adding revisions to the manual and to the forms and general filing guidelines.

14.2 Contact Occasions and Sequence Numbers

14.2.1 Contact Occasion

There can be up to 21 scheduled participant contact occasions in FAVORIT, and to facilitate data management, these are numbered 00-20. These numbers are used in the DMS and on paper forms and study documentation to identify and differentiate the various contacts.

In addition, prior to any patient contact, there is a Prescreening Form that is completed and this is the first data collection for a particular patient. This is followed by the Screening Contact, which is the first one with data that will be entered into the Data Management System (DMS). This Screening Contact has contact occasion (CO) # 00, and this 00 will be used on every Screening form to help identify it as a screening form. Similarly, the Randomization contact has CO # 01, and this number will be used on every randomization form to help identify it as a randomization form.

Following randomization (see table 14.1) there are scheduled contacts every six months, alternating between a telephone contact and a clinic visit, and this continues throughout the study follow-up period.

14.2.2 Sequence Numbers

Another field that is used to differentiate forms collected is called “Sequence Number”. For all of the forms at regularly-scheduled contact occasions the sequence number is simply 00.

Sequence Number becomes important with the forms that are collected between the scheduled contacts. For example, if on the 6-month telephone call you discover that the participant was hospitalized 2 months prior, so the hospitalization occurred between the randomization and 6-month contacts, the corresponding Hospitalization Form would be entered with the CO number for the regular contact immediately preceding its occurrence and the Sequence Number should be incremented.

Table 14.1: Sequence Number Increment Usage

Contact Description	Contact Occasion #	Sequence #
Prescreening		
Screening	00	00
Randomization	01	00
→ Hospitalization	**01**	**01**
6-month telephone	02	00
12 month clinic visit	03	00
18 month telephone	04	00
→ HOS and Outcomes	**04**	**01**
→ HOS and Outcomes	**04**	**02**
24-month clinic visit	05	00
30-month telephone	06	00

The use of contact occasion and sequence number is best illustrated with an example. In Table 14.1 at CO 01 there was one hospitalization form completed for a hospitalization that occurred between COs 01 and 02, that is, between the randomization visit and the 6-month telephone call. Therefore, the CO for the hospitalization form is 01 and the sequence number is 01.

At CO 04 there were two different hospitalization forms completed for hospitalizations that occurred between COs 04 and 05, that is, between the 18-month telephone call and the 24-month clinic visit. The CO for both hospitalization forms is 04 however; the sequence numbers are 01 and 02 respectively. The physical hospitalization forms may not have been filled out until **after** the 24-month visit because the coordinator may not have become aware of the hospitalization until this visit, but the date of the hospitalization was between the date of CO 04 and CO 05.

14.3 Making Corrections on the Paper Form

14.3.1 Background

FAVORIT uses a combination of direct data entry, paper first followed by entry, and forms collected on paper only (no entry) for data collection. The purpose of this document is to provide instructions for completing paper forms. It should be read carefully prior to working with any forms. Specific question-by-question instructions for each form (QxQ's) should be read prior to working with a form.

14.3.2 Form Structure

Most of the paper forms in FAVORIT are designed to correspond exactly to the computer screens used for data entry. For this reason, forms are organized by "screen" instead of by "page". Thus, any item on a paper form may be located in the same position on the corresponding computer screen, and vice versa. Most forms are structured as follows:

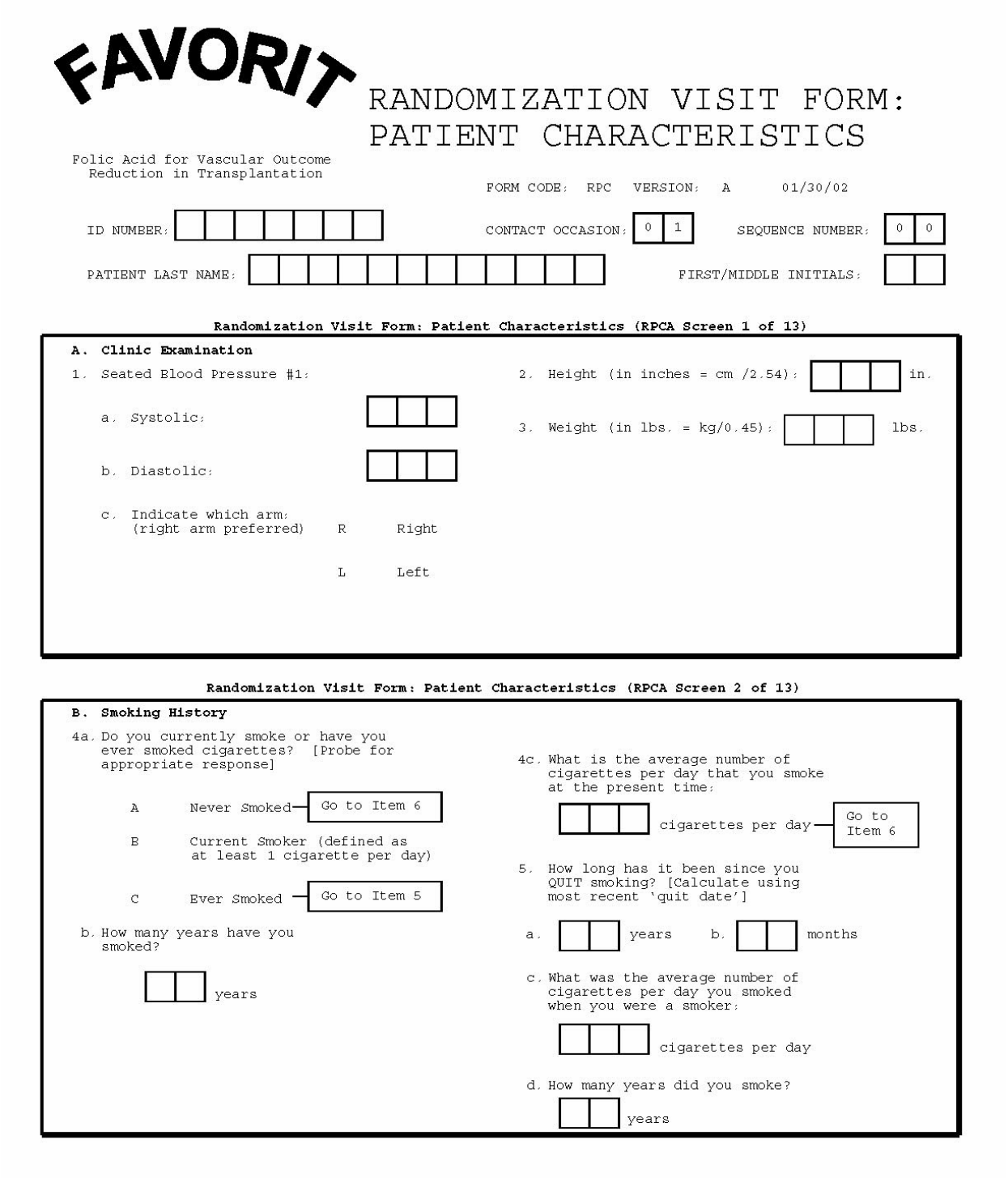
First page (see Figure 14.1 for an example first page):

1. Form Title
2. "Header" Information
    - Form Code
    - Version Number and Date
    - Participant's ID Number
    - Contact Occasion
    - Sequence Number
    - Participant's Last Name and Initials
3. First Screen of the Form

Following pages:

a. at the top of each page is a space to record the participant's ID, the contact occasion and the sequence number
b. at the top of each screen, Form Title, Code and Version and Screen number
c. Successive Screens

Figure 14.1: Example of FAVORIT Form - First Page



FAVORIT

RANDOMIZATION VISIT FORM:
PATIENT CHARACTERISTICS

Folic Acid for Vascular Outcome
Reduction in Transplantation

FORM CODE: RPC VERSION: A 01/30/02

ID NUMBER: | | | | | | | |

CONTACT OCCASION: 0 1

SEQUENCE NUMBER: 0 0

PATIENT LAST NAME: | | | | | | | | | | | | |

FIRST/MIDDLE INITIALS: | |

Randomization Visit Form: Patient Characteristics (RPCA Screen 1 of 13)

A. Clinic Examination

1. Seated Blood Pressure #1:

   a. Systolic: | | |

   b. Diastolic: | | |

   c. Indicate which arm:
   (right arm preferred) R Right

   L Left

2. Height (in inches = cm /2.54): | | | in.

3. Weight (in lbs. = kg/0.45): | | | lbs.

Randomization Visit Form: Patient Characteristics (RPCA Screen 2 of 13)

B. Smoking History

4a. Do you currently smoke or have you ever smoked cigarettes? [Probe for appropriate response]

   A Never Smoked — Go to Item 6

   B Current Smoker (defined as at least 1 cigarette per day)

   C Ever Smoked — Go to Item 5

b. How many years have you smoked?

   | | years

4c. What is the average number of cigarettes per day that you smoke at the present time:

   | | | cigarettes per day — Go to Item 6

5. How long has it been since you QUIT smoking? [Calculate using most recent 'quit date']

   a. | | years b. | | months

c. What was the average number of cigarettes per day you smoked when you were a smoker:

   | | | cigarettes per day

d. How many years did you smoke?

   | | years

14.3.3 General Instructions for Completing and Correcting Items on the Forms

All items fall into one of two main categories: (1) fill in the boxes, and (2) multiple choice. Techniques for completing each of these types of items, as well as making corrections, are described below. A general rule is to record information only in the spaces provided (except for some error corrections).

14.3.3.1 Fill In The Boxes: Recording Information

When alphabetic information is required, print the response beginning in the leftmost box using capital letters. Punctuation may be included.

Example: If the participant's last name were O'Reilly, it should be entered as follows:

LAST NAME:

O	'	R	E	I	L	L	Y				

If the response contains more characters than there are boxes, beginning with the first character enter as many characters as there are boxes. When this is entered into the DMS, add a "notelog" with the complete information (see chapter 13 for instructions on entering notelogs).

Example: If the participant's last name were Hobgoodnotting, it should be entered as follows:

LAST NAME:

H	O	B	G	O	O	D	N	O	T	T	I

Whenever numerical responses are required, enter the number so that the last digit appears in the rightmost box. Enter leading zeros where necessary to fill all boxes. (This does not apply to the address section or to any item which combines alphabetic and numeric information. Such items should be treated as alphabetic.)

Example: If the participant's diastolic blood pressure were 96, it should be coded as:

Diastolic:

0	9	6

It is possible that numeric fields could have a pre-printed number of decimal places. In this case, the QxQ instructions will specify the number of decimal places to be recorded. Instructions on how to round values to the expected number of decimal places are found in the QxQ instructions. When necessary, enter trailing zeros to fill the requested number of places to the right of the decimal point. Leading zeros may be needed so that all boxes to the left of the decimal are also filled.

In most cases when dates are recorded, slashes ("/") are used as the separator characters for month, day, and year. These are usually pre-printed in the response field. The format to be used to record dates is indicated below the boxes. If not, the QxQ instructions will indicate which format and separator to use. FAVORIT uses the U.S. order for recording dates (month/day/year). The QxQ instructions may also contain information on how to handle

partial dates. When necessary, use leading zeros within each date unit (month or day or year) so that each box is filled.

Example: Data collected on April 3, 2001 would be recorded as:

Date of data: | 0 | 4 | / | 0 | 3 | / | 2 | 0 | 0 | 1 |

FAVORIT usually records time using a 12-hour clock, with AM or PM indicated separately. In most cases, colons (":") are used as the separator character for hours and minutes, and are typically pre-printed in the response field. The format to be used is indicated below the boxes. If not, the QxQ instructions will indicate which format and separator to use. When necessary, use leading zeros within each time unit (hour or minute) so that each box is filled. Note that midnight is recorded as 12:00 AM, and noon is recorded as 12:00 PM.

Example: A time of fasting determination of 8:05 in the morning is recorded as:

a. Time of fasting determination: | 0 | 8 | : | 0 | 5 |
Hour Minute

b. A (AM)

P PM

14.3.3.2 Fill In The Boxes: Correcting Mistakes

If a number or letter is entered incorrectly, mark through the incorrect entry with an "X". Code the correct entry clearly above the original incorrect entry and the person making the corrections should initialize the correction, using his/her 3 initials, and record the date of the correction.

Example: If the participant's systolic blood pressure was actually 130, but was incorrectly entered:

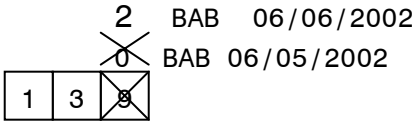
Systolic: | 1 | 3 | 9 |

The correction would look like:

0 BAB 06/05/2002

Systolic: | 1 | 3 | ~~9~~ |

If a mistake is made, corrected, and then it is discovered that the correction is incorrect, make a second correction as shown below:



14.3.3.3 Fill In The Boxes: Unknown Or Inapplicable Information

If an item of this type (either alphabetic or numeric) *does not apply* to the participant being interviewed, leave it **blank**. For example, if the participant does not have an "other" phone number, that item is left blank. Similarly, if the form provides spaces for three measurements, but only two are taken, the third space is left blank.

If the item *does apply*, but the response is unknown, mark through the box(es) with two horizontal lines (equal signs).

Example: The question "How long has it been since you QUIT smoking?" is asked, but the participant does not recall how long it was and is unable to provide an estimate. The question *does apply* because it has been established that the participant has previously smoked, but the *answer to this question is not known*. In this case, the response would look like:

How long has it been since you QUIT smoking? ☐☐ years

14.3.3.4 Multiple Choice: Recording Information

In this type of question several alternatives are given for the answer, each having a corresponding letter. When it is decided which alternative is most appropriate, circle the corresponding letter in the space provided. Always circle one letter only.

Example: If the participant indicates that they were told by their physician that their renal graft function has deteriorated, the response would look like:

Have you been told by your physician that your renal graft function has deteriorated?

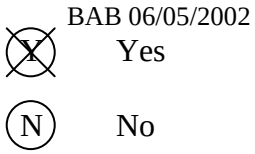
(Y) Yes

N No

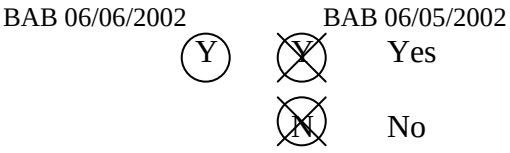
14.3.3.5 Multiple Choice: Correcting Mistakes

If a response is coded incorrectly, mark through the incorrectly coded response with an "X" and circle the correct response, initialize and date correction.

Example 1: The actual response is No, but Y was circled incorrectly. The correction looks like:



Example 2: If a mistake is made, corrected, and then it is discovered that the correction is incorrect, make a second correction as shown below:



14.3.4 Completing "Header" Information

The following guidelines should be observed in filling out the "header" information located at the top of the first page on all forms:

ID NUMBER: Write in the participant's 7-digit ID. The first two boxes contain the letter identifying the field center, followed by the 5-digit numeric portion of the ID number, **whenever possible use the preprinted paper labels** provided by the DCC.

Example: ID NUMBER:

A	L	1	1	1	1	2

CONTACT OCCASION: Fill in the appropriate contact occasion for the form. Use leading zeros. Note: This item may be pre-coded on some forms.

Example: For the Randomization visit the contact occasion should be recorded as:

CONTACT OCCASION:

0	1

SEQUENCE NUMBER: Fill in the appropriate sequence number. Use leading zeros. Note: This item may be pre-coded on some forms.

Example: For the first hospitalization that occurred after the third follow-up visit but before the fourth follow-up visit the sequence number should be recorded as:

SEQUENCE NUMBER:

0	1

PARTICIPANT LAST NAME: Code the response beginning in the leftmost box using capital letters. If the name contains more letters than there are boxes, beginning with the first letter enter as many letters as there are boxes. Punctuation (e.g., apostrophes and hyphens) and blanks may be entered as part of the last name. Follow the guidelines and examples given above for alphabetic "fill in the boxes" items.

FIRST/MIDDLE INITIALS: Record the participant's first initial in the first box and middle initial in the second box. If a female participant is married and uses a "maiden" name (father's surname) as a middle name, use that initial as the second initial. Otherwise, if the participant has more than one middle name, record only the first initial and the second initial. If there is no middle name, record the first initial in the first box and leave the second box blank.

Example 1: A participant's first initial is K, but he has no middle name. The entry would be as follows:

INITIALS: | K | |

Example 2: If the participant's full name is John Oscar Van Camp, Jr., and the participant specifies that his last name is "Van Camp, Jr", it should be entered as:

PARTICIPANT LAST NAME: | V | A | N | | C | A | M | P | , | | J | R | INITIALS: | J | O |

14.3.5 Skip Patterns ("Go to" Boxes)

Skip patterns occur in many multiple choice type items. Here, if a certain response is selected, it is necessary to skip over one or more items to the next applicable item. This is indicated by an arrow from the response which necessitates a skip to a box containing a "go to" statement. If that response is selected, the next item to be asked is the one indicated in the box. If the other response is selected, always proceed to the next item unless otherwise directed. The box will also indicate the screen containing the "go to" item if that item is not on the current screen.

Example: 18. Is this a Telephone Contact? Y Yes — Go to Item 20

N No

In this case, if the response is "Yes", skip to item 20. If the response is "No", proceed to the next question, item 19.

Occasionally, a skip pattern will occur in a fill-in type item. In those instances, specific instructions are provided on the form. Again, if the skip criteria are not satisfied, continue with the next item.

A few items will trigger a skip regardless of the response. For these, follow the instructions on the form.

14.4 Paper Forms versus DMS

14.4.1 Recording Responses

Most of the questions in the FAVORIT forms have precoded responses. There are a few questions, however, that you must write in a response to the question. Some questions have precoded responses as well as an "other" category. If the participant's answer does not fit into a precoded answer, you must specify the response. The recording practices below must be followed at all times to assure that the response recorded accurately reflects the participant's answers and to assure the questionnaire data can be converted to machine-readable form.

- You must listen to what the participant says and record the appropriate answer if the response satisfies the objective of the question.
- In recording answers to open-ended questions or "other" categories, print the response verbatim.
- Use a black ballpoint pen.
- Record in the white space below the questions any responses "that don't quite fit" in one of the response categories. Your notes will help the analysts in understanding points of confusion, difficulty, etc. Notes on paper forms can be entered as notelogs in the DMS.
- Print or write legibly.
- If a participant refuses to answer a question, write "refused" in the left margin beside the question and enter equal signs in the response field.
- A single answer choice code must be circled in each question to represent the participant's answer, unless the item states you can circle all that apply.

Some of the questions in the FAVORIT study ask about recall of events over time. You may assist the participant without violating probing rules by working with him/her on math or pinpointing dates or events. Another way to help pinpoint more accurate information is to ask the participant to think about time of year or season when an event occurred.

14.5 Numbered Memos:

The DCC will send various emails or memos. Updates to the forms, Manual of Procedures and QXQ's will be sent to the centers as numbered memos. Only memos that say DCC Memo # should be filed in a separate binder and placed next to the MOP. Memos should be filed from back to front with the most recent memo on top.

14.6 Adding Revisions to the Manual

All forms, MOP pages/chapters, MOP table of contents and QXQ's are saved in pdf format and are located on the FAVORIT website at (http://www.cscc.unc.edu/favorit/. The DCC

will send each Study Coordinator a numbered memo via email regarding revisions to the MOP.

14.6.1 1NSTRUCTIONS FOR NEW MATERIALS:

Forms:
Any new or corrected form will be available to print from the website. Forms should be replaced and copied for immediate use. Email confirmation to the DCC (Barbara Brown email address barbw_brown@unc.edu) when the revised forms are downloaded.

Manual:
The revised pages/chapters of the FAVORIT Manual of Procedures should be printed from the website and filed immediately in the MOP binder. Email confirmation to the DCC (Barbara Brown email address barbw_brown@unc.edu) when the revised pages/chapters are downloaded.

QXQ's:
Any new or corrected QXQ will be available to print from the website. They should be printed and filed immediately together with the appropriate form in the MOP binder. Email confirmation to the DCC (Barbara Brown email address barbw_brown@unc.edu) when the new QXQ's are downloaded.

14.6.2 INSTRUCTIONS FOR OUTDATED MATERIALS:

Manual & Forms Binder:
Take all outdated pages of the MOP, forms and QXQ's, attach to a copy of the appropriate numbered memo, and place in a permanent, chronological "Archive Manual" binder or file folder.

14.7 General Filing Instructions

All randomized participants should have either a binder or file folder filed in chronological order by participant ID. If the center prefers to file by last name there should be a cross-reference available with the corresponding ID number. It is important for centers to be able to communicate effectively with DCC by the participants' ID number. Data queries sent to the sites from the DCC will only identify participant ID numbers. **Remember, before sending any hospital records or forms to DCC, blind all personal information pertaining to the participant.**

Forms used for participants should be separated with index tab dividers or colored paper by the contact occasion/sequence #. This organization will expedite your response to data queries and facilitate site monitoring. For numerous hospitalizations, file by contact occasion and then by sequence number (example 1st hospitalization CO: 05, seq 01, 2nd hospitalization CO: 05, seq 02). Forms should be filed consistently with each contact occasion.

Laptops and participant data should be stored in a secured location.

File all non-randomized participant forms together in a file.

14.8 Techniques for Conducting the Questionnaire Interviews

14.8.1 Introduction

This section stresses the importance of interviewer-participant's perceptions and introduces the concept of the interview as a one-sided passing of information. The interviewer's most important technique is analytic listening. Listening affects the interviewer-participant relationship as well as the content of the interview. There are several hallmark barriers to listening that every skilled interviewer recognizes:

- interviewer expectations
- interviewer fatigue and/or boredom
- interviewer anxiety
- interviewer impulsiveness
- note taking
- tendency to evaluate
- distractions and interruptions

Although no one interviewer experiences every one of these during any one interview, four remedies to the above barriers to listening are often suggested:

- Be prepared; lack of organization is in and of itself a distraction.
- Involve yourself in the interaction.
- Concentrate on listening to what is being said and what you are recording.
- Integrate the messages; does a response require further clarification or does it present contradiction to a previous statement?

14.8.2 Response Styles

A structured interview, as is proposed here may sound like a conversation, but it is in fact not a conversation. It is rather one-sided passing of information. The interviewer can help to maintain control of the interview by controlling his/her response style. It should be recognized that a large portion of the impression that the participant has of the interviewer is based on the interviewer's voice and the manner with which the interviewer responds to the participant's comments. Along this line, the interviewer may **never** respond in an **evaluative or judgmental manner**. Such a response would indicate that the interviewer has made a judgment of the relative goodness, appropriateness, effectiveness, or rightness of the participant's statement. Thus if the participant says, "I think I must have had 3 or 4 strokes before my wife made me call the doctor," the interviewer should not say, "Well, maybe you should have called him sooner." That type of response suggests that the participant has made an error which may, in fact, have resulted in causing his current medical problems. It should be borne in mind that the interviewer, by announcing affiliation with a medical study and

conducting the interview in the hospital or clinic, has invested in him/herself the potential in the participant's mind for being part of the treatment staff, and this divestment of the role of caregiver while conducting the interview can be particularly difficult when the interviewer is at other times an active member of the institution's health care delivery system. Confronted with such a situation of answering an evaluative statement on the part of the interviewer, the participant may wish to terminate the interview.

A second type of response style is **interpretative** which might also be called teaching or preaching. An interpretative response is one which indicates that the interviewer's intent is to teach. This type of response is also not appropriate, as it would detract from the verbatim type of narrative that is required here. For instance, if the participant says that he/she experienced a sudden episode of right arm paralysis that went away after several hours, the interviewer should not say, "You probably had a TIA." We are interested in the participant's impression of what was happening, and not in the interviewer's impression. We are, further, interested in the facts that lead the participant to make an interpretative judgment, not in the interpretation itself.

A third response style that would be inappropriate would be **interrupting or sentence completion**. However slowly the participant is speaking, putting words in the participant's mouth, or not allowing the participant to finish thoughts will, in general, alter the information which the participant is attempting to give. However, long hesitations may be bridged by asking appropriate questions.

Appropriate response styles are discussed below. First, **supportive remarks** are ones that indicate that the intent of the interviewer is to reassure, to pacify, or to reduce the intensity of the participant's feelings. The general clucking, or understanding murmuring, are both supportive type remarks. Another, in response to the participant experiencing a sudden loss of sight might be, "That must have been difficult for you. Can you tell me what happened next?" Such remarks may help the participant to feel that the interviewer is still listening, is feeling empathy, and yet may not intrude on the flow of the conversation interview. Other supportive remarks, such as "Yes, my grandfather recently had a stroke, and it was a similar situation" probably will detract from the interview. Such remarks will certainly lengthen the interview in that the participant will probably want to go through the interviewer's grandfather's situation as well. While the interview with the participant may eventually be completed, discussion of the interviewer’s personal situation is non-productive and irrelevant.

The second appropriate response style is the **nondirective or understanding response** style. This is more frequently used when an interview includes a third person, acting as informant when the participant is experiencing a communication deficit. Should the participant's informant say, "My husband went into the bathroom, and then I heard a crash," the interviewer might respond by saying "I see." This is the general idea again of understanding murmuring or clucking. The interviewer also might repeat what the participant has just said, "Your husband went into the bathroom and you heard a noise." This may prompt the participant to elaborate.

A third appropriate response style which will be necessary in both types of interview is **probing**, although probing will be more restricted when the interviewer cannot speak directly with the participant who is reporting an event. A probe is a response that indicates that the interviewer's intent is to seek further information, to provoke further discussion along a certain line, to question the participant. Direct probes will be specific questions about details of what the participant has said. The interviewer is cautioned to limit the probes to those provided in this chapter. Another type of probe would be a request for clarification. Thus the interviewer might say, "I didn't understand that fully" or "Would you please elaborate on that?" Additional information on appropriate probing will be discussed in a later section.

14.8.3 Tempo of the Interview

Since the interview is focused on the participant, it must proceed at the pace which the participant, not the interviewer, finds comfortable. A deliberate, careful participant will be irritated and confused by having questions delivered too rapidly. It is well to remember that the participant is doing you a favor. If you go faster than s/he wants to, you give the impression that you are not really interested in what the answer is. If you go too slowly for a quick, decisive person, you will lose his/her interest, and s/he will be bored. Establishing the right tempo takes practice and observation.

14.8.4 Communication Traps

All interviewers, even those with a great deal of previous interviewing experience, should be aware of common communication problems in order to avoid them when conducting interviews. Some of these faults in communication are:

1. Anticipating and answering questions with the interviewer's own thoughts rather than the participant's. Thinking ahead and mentally finishing the participant's sentences will interfere with the interviewer's understanding of what the participant is really saying.
2. The interviewer hearing what he/she expects to hear rather than what is really being told to them. The interviewer must keep listening attentively.
3. Being drawn into the conversation personally by the participant. When dealing with an emotional participant there might be a tendency on the part of some participants to draw the interviewer into a discussion of his/her own similar experiences. The interviewer must guard against being made the "star" of the interview rather than the interview being centered on the participant. If this does occur, the interview will be longer than necessary or will fail to get the information needed.

14.8.5 Probing

Many participants will begin to pour out a good deal of information with little prompting. Others will have to be encouraged to give the information needed. With both types of participants, subsidiary questions may be needed to direct the conversation and elicit more complete answers.

A probe is a neutral, non-leading question designed to start an individual talking or to channel the conversation toward the information that is desired. Probes are used when an answer is unclear, incomplete, appears to be untrue or inconsistent, or when no response is given. There are precautions: do not interrupt the participant; do not give the impression that you are not listening; do not paraphrase the participant's words, and do not suggest an answer.

With the above caveats in mind, there are several types of probes which could be used effectively in the interview. Neutral probes include:

1. Silence: Silence is the most valuable probe. Many people react to silence. The interviewer who waits quietly and patiently will find a few seconds of silence is sufficient and the participant will often clarify a previously inadequate answer.

2. Repeating the question or a previous answer: If the answer given was irrelevant, be sure to repeat the question as stated in the questionnaire. In some cases, it will be necessary to remind the participant of the frame of reference, i.e., to remind him/her of a previous answer which led you to ask the current question.

3. Encouragement: "I see, un huh, hmm!" are effective. Without interrupting, the interviewer lets the participant know that s/he is still there and listening. Avoid comments like "okay" and "all right", which can be misinterpreted as being judgmental.

4. Definition: When asked by the participant for a definition of a term in the question, the interviewer can use the probes suggested in the instructions.

5. Clarification: Explanations to clear up an ambiguity: Could you explain that a little more?," "I'm sorry I didn't understand that." (This puts the onus of being unclear onto the interviewer rather than on the participant.)

6. Channeling is used with talkative individuals to focus on one aspect at a time "Tell me more about _____."

7. Continuation keeps the conversation moving with a non-verbal individual "And then what happened?", "What did you/he/she do then?"

8. Completion makes sure that all information on a participant is given before moving onto another area "Anything else?", "Can you tell me more about that?"

If the participant does not understand the question after one reading:

1. Repeat the question as worded, more carefully and slowly this time. (Often initial confusion is due to the interviewer's having read the questions too fast the first time. This extra time allows the participant to think and may be all that is needed for him/her to understand the question.)

2. Precede the (repeated) questions with a statement like, "Let me repeat the question....", so that the participant will understand that you are attempting to clarify the question for him/her.

If the participant still does not understand the question or asks you about its meaning:

1. Repeat the question exactly as worded.
2. Read the question slowly.
3. Precede the question with a phrase like, "The question I need to ask you..." so that again, the participant will not feel that you are simply impatiently reading the question as a command for his/her prompt answer.

Do not leave a probe dangling. Always record the response to a probe even if it's only "No" or "That's all I can think of."

Always cross-reference. When you probe to clarify a response, always indicate which response you are clarifying. There will be times when a participant will say something ambiguous and continue talking. When you probe to clarify the ambiguous response, indicate the question being clarified.

14.8.6 Neutral Probes That Do Not Suggest Answers

When needed to obtain more complete answers probes must be non-directive, i.e., the probe must not suggest any particular answer to the participant. Probes should be used whenever the participant is hesitant in answering a question; when he/she seems to have trouble expressing an answer clearly; when he/she seems too shy to speak to a potentially embarrassing question; whenever there is any reason for the interviewer to believe that the participant has not given a complete answer; and finally, reassuring probes are needed when a participant seems to lack confidence.

14.8.7 Examples of Neutral Probes

- How do you mean that?
- I would like your opinion....
- Can you tell me more about this?
- Can you give me an example? Or, for example?
- Can you explain that in a little more detail?
- How are you using the term....?
- How is that?
- If you had to choose, which would you say?
- What else can you tell me about that?

Table 14.2: Data Collection Schedule: Contact Occasion Numbers

Description	Contact Occasion #
Prescreening	
Screening	00
Randomization	01
6-month telephone	02
12 month clinic visit	03
18-month telephone	04
24-month clinic visit	05
30-month telephone	06
36 month clinic visit	07
42-month telephone	08
48 month clinic visit	09
54-month telephone	10
60 month clinic visit	11
66-month telephone	12
72 month clinic visit	13
78-month telephone	14
84-month clinic visit	15
90-month telephone	16
96-month clinic visit	17
102-month telephone	18
108-month clinic visit	19
Exit clinic visit	20

Chapter 2. Prescreening

2.1 Recruitment

2.1.1 Overview

Recruitment of participants has proven to be a major challenge to successful completion of large-scale trials. FAVORIT will incorporate several recruitment strategies to help maximize the number of eligible participants enrolled in the study. While recruitment is the primary responsibility of each clinical site, investigators must realize that strategies must be tailored to the population served. A strategy that may be ideal in one site may not work in another. Implementation may differ depending upon the characteristics of each transplant center. Each participating clinical center is experienced in recruiting participants for renal clinical trials and has developed its own preferred mechanism for recruiting participants.

The primary sources of participants for this trial are renal transplant recipients receiving follow-up care at participating sites. It is always important to have multiple strategies to maximize recruitment yield. A strategy for affluent participants may differ from one used to recruit underprivileged participants. A strategy for one ethnic group may not be successful for another. Whatever strategies are implemented, they should be adapted to not exclude minorities and underprivileged renal transplant patients.

Investigators should consider sending brief information letters to all physicians in the catchment area, describing the goals and methods of the study. This should be done before the study starts, and periodically throughout the recruitment phase. In addition, educational presentations to medical, nursing, and other health professional groups would help in recruitment. There should also be presentations to general audiences including, for example, transplant clubs and other health-conscious groups. Brochures describing the study should be placed in strategic patient locations. Past experience indicates that potential study subjects may be more likely to come to a screening exam if they have already heard of the study and physicians who know about the study are more likely to refer patients. Physicians allowing their patients to participate in FAVORIT should not lose their patients to the study investigator and should be kept informed of the participant's progress.

The most crucial and successful person for recruitment is an **energetic and dedicated Study Coordinator** who plays a central and multifaceted role. This individual is trained on all protocol details and is the local resource person for FAVORIT physicians, referring physicians, hospital administrators, participants, and the Data Coordinating Center and the Operations Center. The Study Coordinator should show dedication, honesty, and deal sympathetically with potential participants and their families. Concern for the overall health and well-being of study participants is imperative.

Participants and families should not feel as though the coordinator and physicians are solely interested in them as experimental subjects. Extension of clinic hours, familiarity with special needs and concerns, availability by telephone and pager are all important ways to demonstrate that study staff are sensitive and dedicated to the welfare of the participants.

The regular study follow-up telephone calls will help. Sending birthday and holiday cards should also be considered. Most importantly, FAVORIT personnel can serve as advocates for participants within the health care system.

2.1.2 Minority Recruitment

FAVORIT clinical sites should strive to recruit all available minority participants that meet basic eligibility criteria with respect to time since transplantation, age, and current graft function. Because minorities have generally been under represented in previous clinical trials, they are less likely to be familiar with medical terminology, may not know anyone who has been in a 'good' clinical trial, and may be suspicious of the medical system so more time and education may be necessary to recruit and retain minorities. In addition, because of the participant's family ties and living structure, additional time may be required to educate family members prior to the participant's enrollment.

Study Coordinators and other personnel with whom the participants will have contact should be sensitive to different cultural and ethnic attitudes and practices. For example, personnel should be aware of days of special significance when scheduling follow-up visits; this may help improve participant compliance. Whenever possible, individuals from different minorities should be represented in the study staff. Above all, the study staff should show dedication, honesty, and patience.

2.2 Prescreening Process

2.2.1 Overview

In brief, this process is designed to begin to identify renal transplant recipients who appear to meet basic eligibility criteria with respect to time since transplantation, age, and current renal graft function. Please also refer to the Prescreening Form, and note that all data collected are for paper form (not PC-based data management system) entry, only.

2.2.2 Initial Chart Review

All initial information relevant to this process is obtained by reviewing clinic charts approximately 6 to 12 -weeks in advance of a patient's next scheduled routine transplant clinic visit. In addition to establishing potential eligibility (i.e., with respect to age, transplant duration, and current renal graft function), the other main purpose of the chart review is to detect the presence of any conditions which might exclude a potential participant, especially serious conditions likely affecting 2-year survival.

2.2.3 Eligibility Requirements

The following information relevant to eligibility criteria is determined from the patient's medical record.

2.2.3.1 Age of Participant

Date of birth is obtained to calculate age. At the time of randomization (approximately 9-15 weeks from the time of prescreening), the patient must be 35-75 years old. If the prescreening indicates that a patient is younger than 35 years old, but will reach 35 years of age during the study recruitment period, the Study Coordinator should keep track of this

patient for future prescreening. If the prescreening indicates that the patient will reach 76 years of age or older at the estimated time of randomization, then the patient should not be screened any further. The age recorded on the Prescreening Form should be the patient's age at the time of prescreening.

2.2.3.2 Renal Graft Function

Date current graft performed is documented so that the elapsed time between the graft and anticipated date of screening can be determined. To be eligible for screening, the patient must have had a functioning renal graft for at least 6 months (as of the date of screening; it can be less than six months at prescreening).

2.2.3.3 Creatinine Clearance

Creatinine clearance (Ccr), estimated by the Cockcroft - Gault formula (Nephron 16: 31-41, 1976), must be 25 mL/min or greater to be eligible for the study. The patient's most recent (prior to prescreening) serum creatinine level and the date that the specimen for this determination was collected are recorded.

A Ccr calculator was provided along with the study's data management system. This calculator will accept values either in pounds or kilograms for weight, and mg/dL or µmol/L. If this calculator is not available, the estimated Ccr can be computed manually. In order to estimate creatinine clearance, the most recent serum creatinine value (mg/dL), weight (lbs), age (years), and sex of the patient must be known. Note that serum creatinine is recorded as mg/dL on the Prescreening form; if the laboratory value is recorded in units of µmol/L, these must be converted to mg/dL (creatinine in mg/dL = creatinine in µmol/L / 88.4). Also, weight is recorded in pounds on the Prescreening Form, but must be converted to kg for use in the Ccr calculation (weight in kg = weight in pounds / 2.2). Creatinine clearance is estimated as follows:

Males: (a) Ccr = [(140 – age) x (weight in kg)] / [72 x (serum creatinine in mg/dL)]

This is equivalent to:
(b) Ccr = [(140 – age) x (weight in pounds)] / [158.7 x (serum creatinine in mg/dL)]

Females: (c) Ccr = {[(140 – age) x (weight in kg)] / [72 x (serum creatinine in mg/dL)]} x 0.85

This is equivalent to:
(d) Ccr = {[(140 – age) x (weight in pounds)] / [158.7 x (serum creatinine in mg/dL)]} x 0.85

For example, for a male with a serum creatinine value of 1.9 mg/dL, a weight of 200 pounds, aged 50 years, using formula (a) first calculate weight in kg as 200 / 2.2046 = 90.7194.

Ccr = [(140 – 50) x (90.7194)] / (72 x 1.9)
Ccr = [(90) x (90.7194)] / 136.8
Ccr = 8164.746 / 136.8
Ccr = 59.68 that rounds to Ccr=60

Or, using formula (b):

Ccr = [(140 – 50) x (200)] / [158.7 x 1.9]
Ccr = (90 x 200) / 301.53
Ccr = 18,000 / 301.53
Ccr = 59.70 that rounds to Ccr = 60

For a female with a serum creatinine value of 186.0 µmol/L, a weight of 79.5 kg, aged 60 years, using formula (c) first calculate serum creatinine in mg/dL as 186.0 / 88.4 = 2.1041.

Ccr = {[(140 – 60) x 79.5] / (72 x 2.1041)} x (0.85)
Ccr = [(80 x 79.5) / (151.4952)] x (0.85)
Ccr= (6360 / 151.4952) x (0.85)
Ccr = 41.9815 x 0.85
Ccr = 35.68 that rounds to 36

Or, using formula (d): First, convert weight in kilograms to weight in pounds as 79.5 x 2.2046 = 175.2657.

Ccr = {[(140 – 60) x (175.2657)] / [158.7 x 2.1041]} x 0.85
Ccr= [(80 x 175.2657) / 333.9207] x 0.85
Ccr = (14,021.256 / 333.9207) x 0.85
Ccr = 41.99 x 0.85
Ccr = 35.69 that rounds to 36

The estimated creatinine clearance rate must be 25 mL/min or greater in order for the patient to be eligible for FAVORIT. The prescreening Ccr must be calculated using the most recent serum creatinine value and weight available in the patient's medical record. Age should be the patient's age at the time of prescreening.

2.2.4 Inclusion Criteria

In addition, inclusion into the trial requires the following:

1. Cognitive function adequate for patient to give accurate information
2. Geographically accessibility for follow-up
3. Informed consent
4. Adequate transportation facilities

2.2.5 Exclusion Criteria

2.2.5.1 Pregnancy, Lactation, or Child-bearing Potential

Gender is ascertained, and eligibility criteria specific to female patients are ascertained. Namely, if the patient is pregnant, lactating, or of child-bearing potential and not using birth control, then the patient will not be eligible for FAVORIT. The acceptable FAVORIT methods of birth control include: oral contraceptives, tubal ligation, intrauterine device, Depo-Provera and Norplant.

Note that these may be temporary exclusions, and if a female patient's status is expected to change during the study recruitment period, the Study Coordinator should keep track of this patient for future prescreening. Information on patient's status with respect to pregnant, lactating, or child-bearing potential and not using birth control should be verified on the prescreeeing telephone call (see section 2.2.6) unless the prescreening is stopped due to failure to meet other eligibility criteria.

If the patient has had a partial hysterectomy (removal of just the upper portion of the uterus, leaving the cervix intact, i.e., the ovaries and fallopian tubes are left intact) or a total hysterectomy (removal of the entire uterus and the cervix, i.e., removal of both ovaries and fallopian tubes) then they are considered not to be of child-bearing potential. Women who have reached menopause and have not had a menstrual period for 12 months or longer are also considered not to be of child-bearing potential.

2.2.5.2 End-Stage or Progressive Condition

The presence of any end-stage or progressive condition that is thought to limit the patient's life expectancy to less than two years is an exclusion criterion from the study. If deemed to affect 2-year survivability, conditions such as, but not limited to, cancer, end stage congestive heart failure, end stage liver disease, severe pulmonary disease, and progressive HIV make a patient ineligible for FAVORIT.

2.2.5.3 Other Conditions

The presences of other conditions that prevent reliable participation in the study make a patient ineligible for FAVORIT. Conditions such as refractory depression, severe cognitive impairment, and alcoholism or other substance abuse may prevent reliable participation in the study through non-adherence to the study medication, failure to participate in scheduled follow-up contacts, or other aspects of the FAVORIT protocol.

Patients who have experienced a myocardial infarction, stroke, percutaneous revascularization procedure (i.e., coronary, cerebrovascular, or lower extremity), or lower extremity amputation within the 2 months preceding prescreening or screening are ineligible for FAVORIT.

Patients who have had a coronary artery bypass graft, abdominal aortic aneurysm repair or carotid endarterectomy within the past five months of prescreening or screening are ineligible for FAVORIT.

If the patient has had a kidney and liver transplant they are ineligible for FAVORIT. Other multiple organ transplants such as heart and lung are ineligible for FAVORIT. However, kidney-pancreas transplant recipients with stable renal graft function (for at lease 6 months) as well as recipients of bone marrow transplants are eligible for study participation

2.2.5.4 Participating in Other Trials

Patients who are currently participating in any other trial that specifically involves cardiovascular disease risk factor management are ineligible for FAVORIT. If another study

is ongoing at a clinical site, and there is any question as to whether it specifically involves cardiovascular risk factor management, contact the Operations Center for clarification.

2.2.6 Prescreening Telephone Contact

Once potential eligibility of a given patient is established via chart review, the site Study Coordinator must contact that individual via telephone at least 5 to 6 weeks in advance of the patient's next scheduled routine clinic visit. During this telephone interview key additional information regarding pregnancy/reproductive status, vitamin supplement use, and willingness to provide random (non-fasting) blood samples is obtained to further clarify potential eligibility. Potential participants who report currently taking vitamin supplements that contain folic acid (folate), vitamin B_6, or vitamin B_{12} and are interested in participating in the trial must abstain from this vitamin supplement use to remain eligible. Verbal and follow-up written informed consent must be obtained from these individuals documenting their willingness to abstain from such intake. At the end of the prescreenin*g* process, potentially eligible participants should be scheduled for a screening appointment at their next regularly scheduled clinic visit.

Specific eligibility information to be queried in the prescreening telephone call includes:

Determine if the patient is willing to provide screening blood samples at their upcoming clinic visit to help determine their eligibility for the FAVORIT study. Participants who are not willing to provide the screening samples are ineligible for the study.

The patient's **current** use of any vitamin supplements that contain folic acid, vitamin B6 or vitamin B12 is queried. Current use of multivitamins, B-group vitamins, or individual folic acid, vitamin B6, or vitamin B12 are to be reflected on the form. Participants who are currently taking one or more of these supplements are asked if they would be willing to abstain from using any vitamin supplements containing folic acid, vitamin B6 and vitamin B12 for the 4-6 weeks prior to their next scheduled clinic visit (the visit that coincides with the FAVORIT screening visit). Only patients who do not take, or who agree to abstain (if currently using) from taking, multivitamins and any supplements containing folic acid, vitamin B6, and vitamin B12 will be eligible for screening.

For female patients whose reproductive status was not clear in the medical record, verify information on patient's status with respect to being pregnant, lactating, or of child-bearing potential and not using birth control during the prescreening telephone call.

Note if the patient does not qualify at this time to participate in the FAVORIT study then the Prescreening Form does not need to be filed along with those that do qualify. These forms can be filed as a group "Prescreened, Not Eligible".

Chapter 3. Screening

3.1 Overview

In brief, this visit, synchronized with a patient's regularly scheduled renal transplant clinic visit, is designed to: confirm basic eligibility criteria with respect to time since transplantation and age; further rule out potential exclusions; and obtain blood specimens for confirmation of FAVORIT-eligible creatinine-based GFR, and total homocysteine levels.

Screening visit can be done in two ways (depending on what is more convenient for the participant), a simple screening visit or a screening/baseline combination visit as described in section III.B.1.1 and III.B.1.2 in Chapter 1

The Screening Form (SCRC) is a paper only form, not PC-based data management system (DMS) entry. In contrast, if the patient undergoes screening phlebotomy, s/he will be assigned a study identification number, and all data included on the Screening Phlebotomy Forms (please refer to those forms) will be entered into the DMS.

3.2 Simple Screening Visit

This screening method is used for participants who, if eligible, will return to the transplant clinic within 120 days of screening for the clinic randomization. The simple screening visit includes reconfirmation of eligibility criteria and assessment of the development of any previously unidentified or interim (i.e., since the prescreening chart review and telephone interview) exclusion criteria, obtaining patient's written consent, weight, and blood collection. See table 3.2 for a full list of forms to be completed.

3.2.1 Changes in Eligibility/Exclusion Criteria

The eligibility and exclusion criteria are the same for the screening visit as those for the prescreening visit. In particular, at the screening visit: significant interim changes in renal graft function that may have resulted in dialysis-dependence, or a documented (i.e., interim serum creatinine values available) decline in creatinine-based GFR* below a FAVORIT-eligible level using the most recent creatinine values; confirmation that the patient is not excluded based on interim vitamin supplement use pattern; confirmation that the patient is not excluded based on participation in any other study involving cardiovascular disease risk factor management. See chapter 2: Prescreening, for more details of each of the specific criteria.

3.2.1.1 Ineligibility Before Phlebotomy Screening

If the patient does not meet the FAVORIT eligibility requirements or if s/he is excluded from the study by meeting an exclusion criterion, then the Study Coordinator will **not** need to: assign the patient an ID number, obtain an informed consent (unless required to document consent for abstaining from vitamin supplement use between prescreening and screening),

obtain the patient's weight or perform a phlebotomy. The individual should be thanked for his/her willingness to participate, and, when appropriate, considered for future recruitment.

3.2.2 Informed Consent

Informed consent must be obtained prior to screening weight determination and phlebotomy. If the patient agrees to participate in the study, informed consent is obtained and properly witnessed. Once the informed consent is obtained, complete the Informed Consent Tracking Form (ICT).

3.2.3 Obtaining Patient's Weight

Consented patients are then weighed with the patient in street clothes, shoes removed, and the weight is recorded in pounds on the Screening Form.

3.2.4 Venipuncture (Phlebotomy)

A phlebotomy is performed on each consented patient. In order to meet the FAVORIT eligibility criteria the patient must have a creatinine based estimate of GFR ≥ 25 mL/min and a random/non-fasting plasma tHcy level ≥12.0 μmol/L for men, or ≥11.0 μmol/L for women. The Central Lab screening data are forwarded to the Data Coordinating Center (DCC) where it will be merged with the clinical center screening data to determine if a patient has or has not met the tHcy & creatinine eligibility criteria. The notification of the estimated GFR and tHcy eligibility criteria will be returned automatically to each clinical center DMS at the time of the next data transfer initiated by the clinical center. Please also refer to both the Screening Phlebotomy Form and the detailed screening phlebotomy specimen collection, and MOP chapter 6: Specimen Collection and Processing.

3.2.4.1 Ineligibility Based on Central Laboratory Results

If the patient's lab values are measured and not in the FAVORIT ranges then the person is not eligible for the study. The individual should be contacted and informed of his/her ineligibility and cancellation of the scheduled randomization visit. If the patient continues to express interest in the study and if the clinical site believes s/he is a strong candidate for FAVORIT, the patient can be screened again at a future regularly scheduled clinical appointment. In this situation, the entire screening process must be repeated using a **new** participant ID number.

However, if the patient is ineligible because the Central Laboratory is unable to provide values for tHCY and/or creatinine based on the screening specimens, the clinical site will be notified of the problem. In some situations, for example if samples arrived thawed or there was a technical problem at the lab, clinical sites will be requested on the "Import of Eligibility Values Report" to send the archive sample(s) to the central laboratory. In other situations, for example if samples were hemolyzed, the clinical center will be offered the option of redrawing the patient's specimens using the **same** participant ID number as was originally used.

3.2.4.2 Authorized Redrawing of Screening Samples due to Technical Problems

Redrawing of screening specimens is only permitted when requested by the central laboratory. If the clinical site and patient agree to repeat the phlebotomy, the following procedure is to be followed.

1. Repeat the phlebotomy. If **both** tubes are collected (both lavender top and marble top), proceed with the following steps. Otherwise stop and notify the patient that he/she is not eligible.
2. Delete the original Screening Phlebotomy forms (SPC and SPP) from the data management system. Re-weigh the patient and add new Screening Phlebotomy Forms (using the original participant ID and contact occasion 00). **Add a notelog in the DMS to SPC item 4 (date of blood draw) indicating that this is a "Re-draw for specimens originally collected on <date>".**
3. Destroy all previously (originally) stored screening specimens for this patient (i.e., LT2, LT3, LT4, MT2, MT3).
4. Store all specimens from the re-drawn sample per screening protocol (see chapter 6).
5. Send appropriate vials to the Central Laboratory with the next scheduled shipment so tHcy and estimated GFR can be measured for evaluating the associated eligibility criteria. Write in the comments section of the shipping log that this is a redraw.

3.3 Screening/Baseline Combination Visit

For eligible participants who will be randomized over the telephone, much of the baseline data collection coincides with screening to become a screening/baseline combination visit. The clinical evaluation must be sufficient to establish eligibility and rule out any exclusion criteria. Six tubes of random blood are drawn, and a clean catch urine specimen is collected. A sample of participants will provide one additional tube of blood and/or a urine specimen for blind replicate quality control assessments. Blood pressure, height, weight, relevant medical history, regular medication use, and personal identifying information are obtained in addition to reconfirmation of eligibility criteria and assessment of the development of any previously unidentified or interim (i.e., since the prescreening chart review and telephone interview) exclusion criteria.

The first process in this visit should be to determine if the participant meets basic eligibility criteria, see section 3.2.1 and 3.2.1.1. If the participant meets the FAVORIT criteria then an informed consent must be obtained prior to obtaining the weight and phlebotomy, see sections 3.2.2 – 3.2.3. A phlebotomy is performed on each consented patient. This will consist of obtaining samples for both the screening and the baseline visit. See table 3.3 for a full list of forms to be completed.

3.3.1 Prior to the Screening/Baseline Combination Visit

Prior to the screening/baseline combination visit, the Study Coordinator reminds the participants to bring all vitamins and medicines that they have been using within the month prior to screening/baseline combination visit and the names of breakfast cereals/liquid or powered dietary supplements they are currently consuming.

3.3.2 Venipuncture, Blood Processing and Urine Collection

Phlebotomy is performed on each consented patient as well as a midstream clean catch urine specimen is also obtained. In order to meet the FAVORIT eligibility criteria the patient must have a creatinine based estimate of GFR ≥ 25 mL/min and a random/non-fasting plasma tHcy level ≥12.0 μmol/L for men, or GFR >=mL/min and a random/non-fasting plasma tHcy level ≥11.0 μmol/L for women, see section 3.2.4, 2.3.4.1 and 2.3.4.2 for more information regarding the screening venipuncture. Please also refer to the Screening Phlebotomy Form, Baseline/Randomization/Follow-up Phlebotomy Form, the detailed screening and baseline/randomization phlebotomy specimen collection forms, and MOP chapter 6: Specimen Collection and Processing.

3.3.3 Participant Information

Vital contact information regarding the participant as well as two informants (at least one of whom does not live with the participant) will be collected and recorded on the PUF. The participant information will be updated at every contact occasion by making changes to the baseline PUF.

3.3.4 Medication Interview

Next, the Study Coordinator conducts the medication use interview.

The medication survey includes regularly used aspirin and prescription drugs only. If the participant is prescribed medications but is currently **not** taking them, they should **not** be included on the Medication Survey Listing (MSL). **If the participant is taking aspirin daily, even if it is not prescribed, it should be recorded on the MSL**. The initial stage in this process involves filling out the paper only MSL form that lists the generic and brand names of each medication individually, as well as the source of this information (bottle label; participant list; verbal report). Study Coordinators must refer to the recording sheet when transferring this information to the Medication Survey Form (MSR) for data entry, while simultaneously consulting the complete FAVORIT medication thesaurus provided in the MOP (located behind the MSR form). The order of medication categories, subcategories, and individual medications on the medication thesaurus and data entry system screen(s) are identical.

The FAVORIT medication thesaurus will be routinely updated for both the US and Canada.

3.3.5 Blood Pressure, Height and Weight

In the next part of the screen/baseline combination visit, the participant's blood pressure is taken. A seated blood pressure is taken in the right arm (if possible; otherwise use of left arm is noted), and repeated again during the exam after at least a 5-minute interval. An average systolic and diastolic blood pressure is calculated by the DMS. If the average systolic value is between 180 and 199 mmHg (or higher) the participant's primary care physician should be notified. If the average systolic value is greater than or equal to 200 mmHg, the participant must immediately be examined by a physician. If the average diastolic value is between 100 and 109 mmHg (or higher) the participant's primary care physician must be notified. If the

average diastolic value is greater than or equal to 110 mmHg, the participant must be seen immediately by a physician.

Height and weight are obtained with the participant in street clothes with shoes removed, and recorded in inches and pounds on the Baseline/Randomization Visit Form: Patient Characteristics (RPC) form. A Body Mass Index (BMI) will be calculated by the DMS. If this value is greater than or equal to 40 the Study Coordinator should notify the participant's primary care physician.

3.3.6 Participant Characteristics Questionnaire

During this part of the clinic visit, the Study Coordinator questions the participants concerning their personal characteristics and records this information on the RPC.

Smoking history questions focus on current or former smoking, duration, intensity, and length of time since quitting. These questions pertain only to cigarettes; cigars and chewing tobacco are excluded.

Cardiovascular disease (CVD) history refers to the specific diagnoses listed on the RPC. Documentation from the participant's transplant clinic charts supersedes any information provided by verbal report. In either case, the source of this information is documented. Likewise, diabetes history is documented but does not distinguish between Type I and Type II diabetes. Previously diabetic participants who have received a pancreatic transplant are considered as diabetic for the RPC.

The questions related to cereal and liquid/powdered dietary supplements are designed to document regular intake of heavily fortified cereals, and/or liquid/powdered dietary supplements that contain large amounts of folic acid per serving.

The physical activity questions refer to the participant's activities during the past month. Activities are graded according to intensity and duration. To help aid the participant, response cards will be used along with these questions. These cards contain possible responses for each of the physical activity questions. The responses should also be read to the participants as the card is shown.

3.3.6.1 Physical Activity Scoring Technique

The DCC will compute a physical activity summary score based on the raw data using the Yale Physical Activity Survey scale. Scoring for the physical activity questions are divided into five categories; vigorous activity, leisurely walking, moving, standing and sitting.

See table 4.2.2 for a detailed description of the points assigned to each of the responses.

3.4 Schedule Randomization Visit or Call

Following completion of the screening procedures, **all** patients are scheduled for a randomization visit or randomization telephone contact (depending on what type of screen was completed), within 3 to 4-weeks of the screening visit. Prior to the scheduled randomization contact, **all** patients will be contacted to confirm their randomization clinic visit or telephone contact date, if eligible, based on the screening phlebotomy results.

3.4.1 120 Day Time Window

Patients must be randomized within 120 days after the screening visit. If the patient is not randomized within 120 days, then the patient is not eligible to be randomized. However, if the patient continues to be interested in the study, the patient can be screened again at a future regularly scheduled transplant follow-up clinic appointment. The entire screening process must be repeated using a new participant ID number.

[***** Renal function is determined based upon a calculation of creatinine clearance by the Cockcroft-Gault formula using the stand alone Excel program provided by the FAVORIT Data Center. Data required for this calculation include: sex; age; serum creatinine in mg/dL; and weight in/converted to kg]

Table 3.2 Summary of Data Collection Forms during Simple Screening Visit

Name of Form	Type of Processing
Screening Form	Paper Only
Screening Phlebotomy: Collection Form	DMS and/or Paper
Screening Phlebotomy: Inventory and Processing Form	DMS and/or Paper
Screening Phlebotomy: Shipping Log	Paper only or DMS Report Log
Informed Consent Tracking Form	DMS and/or Paper

Table 3.3 Summary of Data Collection Forms during the Screening/Baseline Combination Visit

Name of Form	Type of Processing
Screening Form	Paper Only
Screening Phlebotomy: Collection Form	DMS and/or Paper
Screening Phlebotomy: Inventory and Processing Form	DMS and/or Paper
Screening Phlebotomy: Shipping Log	Paper only or DMS Report Log
Informed Consent Tracking Form	DMS and/or Paper
Baseline/Randomization Visit Form: Patient Characteristics	DMS and/or Paper
Baseline/Randomization/Follow-up Phlebotomy Form: Collection	DMS and/or Paper
Baseline/Randomization/Follow-up Phlebotomy Form: Processing and Inventory	DMS and/or Paper
Medication Listing Form	
Medication Survey Form	DMS and/or Paper
Participant Update Form	DMS and/or Paper

Chapter 4. Randomization

4.1 Overview

Two types of randomization contacts can occur depending on the type of screening that took place for that participant. If the participant completed a Simple Screening Visit, then the participant returns for a **clinic randomization visit**. However, if the participant completed the Screening/Baseline Combination Visit, then the participant only needs to be contacted for a **telephone randomization contact**.

4.2 Summary of Exams & Procedures During Clinic Randomization Visit

The clinic randomization contact is designed to: confirm basic eligibility criteria, including FAVORIT-eligible creatinine-based GFR and total homocysteine levels, and rule out potential exclusions; record blood pressure and obtain height and weight; review regularly used medications with recording only of specific medications of interest to FAVORIT; review of smoking history; documentation of specific medical diagnoses, including hypertension, arteriosclerotic cardiovascular disease (CVD), and diabetes; review consumption of specific cereals, or liquid/powdered food supplements with high folic acid contents; review of physical activity patterns; and obtain blood specimens for FAVORIT analyses and the specimen bank.

Table 4.2.1 shows the forms and procedures used during the randomization visit.

4.2.1 Prior to the Clinic Randomization Visit

At the simple screening visit, the Study Coordinator will schedule the randomization visit. Additionally, the Study Coordinator reminds the participants to bring to the randomization visit all vitamins and medicines that they have been using within the month prior to randomization and the names of breakfast cereals/liquid or powered dietary supplements they are currently consuming.

4.2.2 Clinic Randomization Visit Components

The primary objectives of the randomization visit are to confirm eligibility, to obtain baseline information and to assign participants to a randomized vitamin (either high or low dose). The Randomization visit is composed of seven parts: (1) assessment of all inclusion and exclusion criteria, (2) randomization, bottle code assignment and distribution of multivitamins, (3) collection of personal information (4) medication survey, (5) blood pressure determinations and height and weight assessment, (6) participant characteristics including smoking history, cardiovascular disease history, dietary supplement, and physical activity, and (7) venipuncture and blood processing.

The forms required for the randomization visit are the:

- Randomization Visit Eligibility Form (REL)
- Baseline/Randomization Visit Patient Characteristic Form (RPC)
- Vitamin Distribution Form (VDL)
- Medication Listing Form (MSL)
- Medication Survey Form (MSR)
- Baseline/Randomization/Follow-up Phlebotomy Form: Collection (PHC)
- Baseline/Randomization/Follow-up Phlebotomy Form: Processing and Inventory (PHP)
- Participant Update Form (PUF).

When participants are unable to make the clinic visit, they cannot be randomized into the study. The Study Coordinator will contact the participant by phone or mail to reschedule the appointment and to document the reasons. The Study Coordinator should document every study-related attempt to contact the participant on the Record of Contacts Form.

4.2.3 Eligibility Criteria

In order to randomize the participant they MUST be present in the clinic. You cannot run the eligibility algorithm in the data management system prior to the clinic visit.

The randomization visit begins with the verification of all eligibility criteria. Some criteria may have changed since prescreening and screening. Diligence is necessary to identify the development of any significant interim changes in renal graft function that may have resulted in dialysis-dependence, or a documented (i.e., when interim serum creatinine values are available) decline in creatinine-based GFR below a FAVORIT-eligible level, and confirmation that the participant is not excluded based on interim vitamin supplement use. If the participant is ineligible, complete applicable sections of the REL. Do not complete other forms; the participant can be thanked for their time and allowed to leave.

4.2.4 Vitamin Distribution

If randomized, the participant will be assigned a bottle code by the DMS. The date the bottle code is assigned is the official date of randomization. After the participant receives a bottle code number, the participant is provided with a 12-month supply of study tablets. Participants are instructed to take one tablet a day. Refer to the Chapter 5, Vitamin Distribution, for details on the study vitamins.

4.2.5 Participant Information

Vital contact information regarding the participant as well as two informants (at least one of whom does not live with the participant) will be collected and recorded on the PUF. The participant information will be updated at every contact occasion by making changes to the baseline PUF.

4.2.6 Medication Interview

For the fourth part of the clinic visit, the Study Coordinator conducts the medication use interview.

The medication survey includes regularly used aspirin and prescription drugs only. If the participant is prescribed medications but is currently **not** taking them, they should **not** be included on the MSL. **If the participant is taking aspirin daily, even if it is not prescribed, it should be recorded on the MSL**. The initial stage in this process involves filling out the paper only MSL form that lists the generic and brand names of each medication individually, as well as the source of this information (bottle label; participant list; verbal report). Study Coordinators must refer to the recording sheet when transferring this information to the MSR form for data entry, while simultaneously consulting the complete FAVORIT medication thesaurus provided in the MOP (located behind the MSR). The order of medication categories, subcategories, and individual medications on the medication thesaurus and data entry system screen(s) are identical.

The FAVORIT medication thesaurus will be routinely updated for both the US and Canada.

4.2.7 Blood Pressure, Height and Weight

In the fifth part of the clinic visit, the participant's blood pressure is taken. A seated blood pressure is taken in the right arm (if possible; otherwise use of left arm is noted), and repeated again during the exam after at least a 5-minute interval. An average systolic and diastolic blood pressure is calculated by the DMS. If the average systolic value is between 180 and 199 mmHg (or higher) the participant's primary care physician should be notified. If the average systolic value is greater than or equal to 200 mmHg, the participant must immediately be examined by a physician. If the average diastolic value is between 100 and 109 mmHg (or higher) the participant's primary care physician must be notified. If the average diastolic value is greater than or equal to 110 mmHg, the participant must be seen immediately by a physician.

Height and weight are obtained with the participant in street clothes with shoes removed, and recorded in inches and pounds on the RPC form. A Body Mass Index (BMI) will be calculated by the DMS. If this value is greater than or equal to 40 the Study Coordinator should notify the participant's primary care physician.

4.2.8 Participant Characteristics Questionnaire

During this part of the clinic visit, the Study Coordinator questions the participants concerning their personal characteristics and records this information on the RPC.

Smoking history questions focus on current or former smoking, duration, intensity, and length of time since quitting. These questions pertain only to cigarettes; cigars and chewing tobacco are excluded.

Cardiovascular disease (CVD) history refers to the specific diagnoses listed on the RPC. Documentation from the participant's transplant clinic charts supersedes any information provided by verbal report. In either case, the source of this information is documented.

Likewise, diabetes history is documented but does not distinguish between Type I and Type II diabetes. Previously diabetic participants who have received a pancreatic transplant are considered as diabetic for the RPC.

The questions related to cereal and liquid/powdered dietary supplements are designed to document regular intake of heavily fortified cereals, and/or liquid/powdered dietary supplements that contain large amounts of folic acid per serving.

The physical activity questions refer to the participant's activities during the past month. Activities are graded according to intensity and duration. To help aid the participant, response cards will be used along with these questions. These cards contain possible responses for each of the physical activity questions. The responses should also be read to the participants as the card is shown.

4.2.8.1 Physical Activity Scoring Technique

The DCC will compute a physical activity summary score based on the raw data using the Yale Physical Activity Survey scale. Scoring for the physical activity questions are divided into five categories; vigorous activity, leisurely walking, moving, standing and sitting.

The frequency score is multiplied by the duration score for the vigorous activity dimension (Item 13 x Item 14 on the RPC) and for the leisurely walking dimensions (Item 15 x Item 16 on the RPC) to create a total daily duration score to correspond with similar information obtained from items 17, 18 and 19 on the RPC. Thereafter, each total daily duration index is multiplied by a weighting factor (based on the relative intensity of the activity dimension) and is summed to create an activity dimensions summary index, which is expressed as total units for each subject. See table 4.2.2 for a detailed description of the points assigned to each of the responses.

4.2.8.2 Venipuncture, Blood Processing and Urine Collection

Phlebotomy is performed on each participant. A midstream clean catch urine specimen is also obtained. Please refer to both the PHC and PHP forms, and chapter 6: Specimen Collection and Processing.

Following completion of the phlebotomy, Study Coordinators must schedule all participants for their 6-month telephone follow-up, and 12-month clinic follow-up visits. Prior to these scheduled visits Study Coordinators must contact all participants confirm their visit dates.

4.3 Summary of Procedures During Telephone Randomization Contact

The telephone randomization contact is designed to: confirm basic eligibility criteria, including FAVORIT-eligible creatinine-based GFR and total homocysteine levels, and rule out potential exclusions.

Table 4.3 shows the forms and procedures used during the telephone randomization contact.

4.3.1 Prior to the Telephone Randomization Contact

At the screening/baseline combination visit, the Study Coordinator will schedule the randomization telephone contact with the participant.

4.3.2 Telephone Randomization Components

The primary objectives of the telephone randomization contact are to confirm eligibility and to assign participants to a randomized vitamin (either high or low dose). The telephone randomization contact is composed of two parts: (1) assessment of all inclusion and exclusion criteria, and (2) randomization, bottle code assignment and distribution of multivitamins.

The forms required for the telephone randomization contact are the:

- Randomization Visit Eligibility Form (REL)
- Vitamin Distribution Form (VDL)

When participants are unable to be contacted by telephone, they cannot be randomized into the study. The Study Coordinator will contact the participant by mail to reschedule the call. The Study Coordinator should document every study-related attempt to contact the participant on the Record of Contacts Form.

4.3.3 Eligibility Criteria

In order to randomize the participant they MUST be on the telephone. You cannot run the eligibility algorithm in the data management system prior to the telephone randomization contact.

The telephone randomization contact begins with the verification of all eligibility criteria. Some criteria may have changed since prescreening and screening. Diligence is necessary to identify the development of any significant interim changes in renal graft function that may have resulted in dialysis-dependence, or a documented (i.e., when interim serum creatinine values are available) decline in creatinine-based GFR below a FAVORIT-eligible level, and confirmation that the participant is not excluded based on interim vitamin supplement use. If the participant is ineligible, complete applicable sections of the REL. Do not complete the VDL; the participant can be thanked for his/her time and allowed to end the call.

4.3.4 Vitamin Distribution

If randomized, the participant will be assigned a bottle code by the DMS. The date the bottle code is assigned is the official date of randomization. After the participant receives a bottle code number, the participant is mailed with a 12-month supply of study tablets. Participants are instructed to take one tablet a day. Refer to the Chapter 5, Vitamin Distribution, for details on the study vitamins.

4.4 Randomization Procedure

In order to satisfy assumptions necessary for the validity of the statistical analyses that will be used to evaluate the FAVORIT trial, eligible participants must be randomly assigned to treatments. To guard against randomizing participants who do not meet the eligibility criteria, it is necessary that the data used in defining eligibility be collected and entered immediately before randomization takes place. Eligibility will be confirmed after all necessary data are entered into the data management system by running the randomization program. The following procedure is to be followed to enroll and randomize participants.

4.4.1 Randomization Using the Data Management System

The Study Coordinator enters the data from the REL, sections A and B into the DMS. A FAVORIT nephrologist/transplant surgeon will be called upon to assess the patient's two year survivability prior to randomization, if the Study Coordinator needs further assessment. When the REL is being entered, the Study Coordinator may run the eligibility/randomization program on the data entry system. This program is triggered by responding "Yes" to the REL item "Do you want to randomize this patient?". The eligibility/randomization program verifies the patient's eligibility status, and prints a criterion-specific report of the patient's eligibility status. If the patient is found to be ineligible, the criteria indicating ineligibility is indicated in the report. A summary of the patient's status is printed at the bottom of the report. If it is found that the patient is ineligible for any reason, the patient is notified of such and thanked for his or her willingness to participate. If the participant meets all the qualifications for eligibility then the DMS will randomize the patient and the drug assignment bottle code is displayed on the screen and in the REL.

All eligibility items on the REL must be entered into the DMS.

4.4.2 Randomization Procedure When the DMS is Not Functional

Since the randomization procedure requires that eligibility data be entered on the clinical center DMS before obtaining a treatment assignment, a backup procedure is necessary if the clinical center computer is unusable.

As soon as it is realized that a randomization is anticipated and the DMS is nonfunctional, contact the DCC to arrange a remote randomization. First the DCC will attempt to solve the computer problem. If this fails, the Study Coordinator and the DCC will arrange a time to complete the randomization through the DCC.

After completing the REL eligibility items and determining that the patient appears eligible for randomization, the Study Coordinator will call the DCC. The DCC is normally staffed from 8 AM to 5 PM Eastern time, Monday through Friday.

In order for the DCC to perform the randomization, the following will need to occur.

1. The Study Coordinator will pre-arrange a time with the DCC to perform the randomization.
2. S/he will first send an updated copy (i.e., current) Randomization Assignment Table (blinding participant names) to the DCC. This table must show **all** randomizations completed to date.
3. The DCC will verify the Randomization Assignment Table against their database, and query discrepancies with the Study Coordinator.
4. The Study Coordinator will complete the REL form sections A. and B., and fax this form to the DCC.
5. The DCC staff will enter the REL into their DMS to establish eligibility.
6. If eligible, the DCC staff will manually determine the appropriate treatment assignment (bottle code number), and provide this by email or fax to the Study Coordinator along with an updated copy of the Randomization Assignment Table.
7. As soon as the clinical center microcomputer becomes operational, the REL must be entered on that system and randomization completed. This should result in the same bottle code as that provided by the DCC. If not, contact the DCC immediately. It is essential to enter any remote randomizations into the local data entry system **in the order** in which they were randomized, before using it for any further randomizations.

4.4.3 Randomization Assignment Table

The Study Coordinator must maintain a paper randomization assignment table for all randomized participants (see table 4.4). This table, which includes the participant's name, ID, date of randomization and the assigned drug bottle code, will be used when the data management system is inoperable. If desired, this form can also be generated by the DMS (Random Assignment Report), (see Chapter 13 Data Management, for more information on computer-generated reports), but must be generated following each randomization in order to be current. The Randomization Assignment Table should be filed in a convenient location for frequent updating.

4.5 Changes in a Participant's Eligibility

In some cases the condition leading to a participant's ineligibility may later change. In this case the Study Coordinator and/or the nephrologist/transplant surgeon should assess how to proceed and whether to schedule the participant for a future appointment.

If the change in eligibility is unrelated to the screening blood sample or the 120 day time window (for example, a patient of childbearing potential has begun taking an oral contraceptive, or a patient has reached age-eligibility), then the randomization visit may be re-scheduled. At that re-scheduled visit, all elements of the eligibility check must be updated in the DMS to reflect the current status of the participant. Then, the eligibility/randomization program can be run again.

If the participant is ineligible based on the final results of the screening blood sample or if they fail to be randomized within 120 days after the screening visit, then the participant cannot be randomized at this time. However, if the participant continues to be interested in the study and if the clinical site staff believe s/he is a strong candidate for FAVORIT, the patient can be screened again at a future regularly-scheduled transplant follow-up clinic appointment. The entire screening process must be repeated using a **new** participant ID number. However, note that many participants who are blood ineligible at the initial screening will remain ineligible at subsequent screenings.

Table 4.2.1 Forms Used During Clinic Randomization Visit

Form	Procedure
Randomization Visit Eligibility Form (REL)	Review of Eligibility Randomization Assignment and Bottle Code Assignment
Vitamin Distribution Log (VDL)	Vitamin distribution and date study medication started by participant
Participant Update Form (PUF)	Record participant and informant contact information
Medication Listing Form (MSL), Medication Survey Form (MSR)	Record medication inventory and related information
Baseline/Randomization Patient Characteristic Form (RPC)	Clinic Exam: Blood pressure, height, and weight Participant Characteristic Surveys: • Smoking history • CVD and diabetes history • Intake of dietary folic acid, vit. B6, & vit. B12 • Physical Activity
Baseline/Randomization/Follow-up Phlebotomy Collection Form (PHC), • Baseline/Randomization/Follow-up Processing and Inventory Form (PHP)	Random/non-fasting bloods for tHcy, folate, B6, B12, PLP, Lipid profile, creatinine, glucose, & fructosamine Midstream clean catch urine collection Processing and inventory information

Table 4.2.2 Scoring Technique for Physical Activity

VIGOROUS ACTIVITY	
About how many times during the past month did the patient participate in VIGOROUS activities that lasted at least 10 MINUTES and caused large increases in breathing and heart rate, or leg fatigue, or caused you to perspire?	**Frequency Score**
Not at all	Score =0
1-3 Times per month	Score=1
1-2 Times per week	Score=2
3-4 Times per week	Score=3
5 or more times per week	Score=4
About how long on the average did you do this (these) vigorous activities each time?	**Duration Score**
10-30 minutes	Score=1
31-60 minutes	Score=2
More than 60 minutes	Score=3
Vigorous Activity Index: Multiply Frequency Score x Duration Score from above x Weight (5) = _____	
LEISURELY WALKING	
Thinking about the walks you have taken during the past month, about how many times did you walk for least 10 MINUTES or more without stopping and which was NOT strenuous enough to cause large increases in breathing and heart rate, or leg fatigue, or cause you to perspire?	**Frequency Score**
Not at all	Score=0
1-3 Times per month	Score=1
1-2 Times per week	Score=2
3-4 Times per week	Score=3
5 or more times per week	Score=4
When you did this walking, for how many minutes on the average did you do it each time?	**Duration Score**
10-30 minutes	Score=1
31-60 minutes	Score=2
More than 60 minutes	Score=3
Leisurely Walking Index: Multiply Frequency Score x Duration Score from above x Weight (4) = _____	

Table 4.2.2 (continued)

MOVING	
About how many hours per day do you spend moving about on your feet doing things on a typical day during the past month? Please report on the time that you were ACTUALLY MOVING.	**Frequency Score**
Not at all	Score=0
Less than 1 hour per day	Score=1
1 to less than 3 hours per day	Score=2
3 to less than 5 hours per day	Score=3
5 to less than 7 hours per day	Score=4
7 or more hours per day	Score=5
Moving Index: Multiply Frequency Score x Weight (3) from above = _____	
STANDING	
Think about how much time you spend standing or moving around on your feet on an average day during the past month? About how many hours per day do you STAND?	**Frequency Score**
Not at all	Score=0
Less than 1 hour per day	Score=1
1 to less than 3 hours per day	Score=2
3 to less than 5 hours per day	Score=3
5 to less than 7 hours per day	Score=4
7 or more hours per day	Score=5
Standing Index: Multiply Frequency Score x Weight (2) from above = _____	
SITTING	
About how many hours did you spend sitting on an average day during the past month?	**Frequency Score**
Not at all	Score=0
Less than 3 hours	Score=1
3 to less than 6 hours	Score=2
6 to less than 8 hours	Score=3
8 or more hours	Score=4
Sitting Index: Multiply Frequency Score x Weight 1) from above =____	

Table 4.3 Forms Used During Telephone Randomization Contact

Form	Procedure
Randomization Visit Eligibility Form (REL)	Review of Eligibility Randomization Assignment and Bottle Code Assignment
Vitamin Distribution Log (VDL)	Vitamin distribution and date study medication started by participant

Table 4.4 Randomization Assignment Table

FAVORIT

Randomization Assignment Table

Clinic:_____

Patient's Name	FAVORIT ID	Date of Randomization	Drug Bottle Code
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12.			
13.			
14.			
15.			
16.			
17.			
18.			
19.			
20.			
21.			
22.			
23.			
24.			
25.			

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Chapter 5. Vitamin Distribution

5.1 General Description

Drugs will be prepared and distributed to each clinical center for 4000 participants (2000 in each of the two study arms). Participants will receive either a high or low dose multivitamin. Each participant will receive a kit, which will include a one-year supply of vitamins, in four bottles with 100 tablets per bottle. Each participant will receive a kit at the randomization visit and at annual follow-up visits until exiting from the study.

Pamlab,L.L.C. is the provider of the FAVORIT multivitamin. Pamlab, L.L.C. contracts with Anabolic Labs to manufacture the multivitamin and serve as the Vitamin Distribution Center (VDC). The responsibilities of the VDC will include bottling, labeling, storage and distribution of the study drug to the clinical sites according to a schedule provided by the FAVORIT Data Coordinating Center (DCC).

5.2 Procurement

5.2.1 Description of the Vitamin

There will be two different formulations of drugs manufactured and distributed during the study. These are classified as the low dose vitamins and the high dose vitamins. The two formulations will be identical with respect to color, size, shape, odor, markings, weight and taste both in whole and in broken conditions. All vitamins contain the standard components of a multivitamin tablet except for variations in the folic acid, B_6 and B_{12} content. Table 5.1 lists the specific components of each type of tablet.

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5.2.2 Shelf Life

The shelf life for the vitamins is still being established, but expected to be approximately 18 to 24 months. Once the shelf life is verified, it means that the ingredients within the tablet have been assayed and determined to be active and stable for at least the specified period following the manufacture of the tablet. This factor has a major impact on the time line for manufacturing, packaging and distribution of the vitamins. A bottle of study vitamins will need to have an expiration date of at least thirteen months beyond the date it is distributed to the participant in order to insure that it will not expire before the next scheduled resupply visit.

5.2.3 Initial Shipment of Vitamins

The clinics will receive an initial shipment of vitamins that are expected to last for four months of recruitment. The subsequent schedule for manufacture, packaging and shipment to the clinics will depend on the pattern of recruitment.

It is expected that Batch #1 of the study vitamins will be manufactured in June 2002. The number of low and high dose vitamins in the initial batch will be determined jointly by the DCC based on projections for recruitment and by the VDC based on manufacturing

parameters. In an attempt to balance the uncertainties of recruitment, the volume of the vitamins for the first batch will be based on equal recruiting across the 20 clinics. Each clinic will recruit approximately eight participants per month, to meet the goal of at least 2000 study participants over a 24-month period.

5.2.4 Subsequent Shipments

The VDC will manufacture, bottle, label, and distribute subsequent batches of multivitamins as needed. The volume of vitamins in the subsequent batches will be determined jointly by the DCC based on recruitment patterns and by the VDC. Each site can expect to have sufficient, appropriately-coded drug available for all randomized participants.

5.3 Vitamin Distribution Center

5.3.1 Study Drugs

The study will require the packaging of vitamins for 2000 participants in each of the two treatment arms (total of 4000 participants). The high dose pills and the low dose pills will be bottled in separate runs in identical bottles such that without knowledge of the coding scheme, the bottles are indistinguishable as to dose without a laboratory analysis. The coding information developed by the DCC will be provided only to the VDC. Each bottle will contain 100 vitamins. Each bottle will be labeled with a code number provided by the DCC. The VDC will maintain a back-up supply of the bottles from each run. These will be used for QC and for emergency replacements at clinical centers.

5.3.2 Labeling of Bottles

The study vitamin bottle labels will be similar to that in Figure 5.1. Labels will indicate the name of the study, code number, dosage instructions, lot number, storage instructions, expiration date, number of vitamins in the bottle, and any other information required by law.

The DCC provided the VDC with a list indicating the code number assignments for the low dose and for the high dose labels. The VDC will prepare and affix coded labels to the appropriate bottles. Labels for the bottles of treatment vitamins will be identical in every way except for the assigned code numbers. Each of the low and high doses will be assigned a fixed number of codes by the DCC, e.g., 8 different code numbers for low dose and 8 different code numbers for high dose vitamins for a total of 16 codes.

5.3.3 Packaging of Bottles

Labeled bottles will be prepackaged into kits of four bottles each for dispensing to participants. Therefore, generally kits instead of individual bottles will be shipped to the clinical centers. Kits will be packed in boxes for shipping, with a label affixed to the outside of the box indicating the box number and description of the contents, the bottle codes that are contained in the case and the number of bottles for each code. Vitamins will be packaged in a childproof container.

5.3.4 Scheduling

Given the 24-month shelf life of the multivitamin preparations, the bottling and labeling will need to be done in numerous batches over the course of the study. The schedule of

manufacture, packaging, and shipment to clinics will also depend on the pattern of recruitment. The actual packaging and distribution schedule may require adjustment during the course of the study to reflect changes in the pattern of recruitment and follow-up visits.

The first shipment, VDC Order #1 (Batch #1), to each of the 20 clinics, will be a total of 72 kits , four each of the 1-16 codes and one kit of each back-up coded A-H.

5.4 Clinic Responsibilities

Careful management of study vitamin supplies has a major impact on the quality of the study. Each clinical center has specific responsibilities with respect to storage, distribution, record-keeping, disposal, replacement, and unmasking information related to study vitamins.

Each clinic will identify a "Clinic Vitamin Distributor". This should be a pharmacist, Study Coordinator, or a responsible person with knowledge of receiving, dispensing and inventory of drugs. The name, the clinic address and the clinic telephone number of this person will be maintained by the DCC. The Study Coordinator will notify the DCC of any changes to the Clinic Vitamin Distributor (personnel, address, phone, etc.), or to vitamin storage, distribution, record-keeping, or disposal procedures.

5.4.1 Storage

Each clinic is required to provide space for up to 200 kits of study vitamins. The study vitamins should be stored in a secure, cool, dry place at room temperature and away from direct sunlight. The storage location will vary depending on the policies of the institution. In some clinics the study vitamins will be maintained in the hospital pharmacy and requisitioned by study personnel as needed. In other clinics the study vitamins may be stored in a secure locker or cabinet within the research facility.

5.4.2 Distribution to Participants

The vitamins will be prepared and distributed to participants at the randomization visit and annually throughout follow-up (e.g., contact occasions 03, 05, 07, 09, 11, 13, 15, 17, and 19) in kits containing 4 bottles, with 100 vitamins per bottle. Each year, the participants will receive one kit consisting of four bottles. Careful identification of the proper bottle codes and checking of the bottle expiration dates before distribution will be essential in avoiding errors related to this procedure.

Clinic Vitamin Distributors will need to be particularly careful in checking the expiration date before distribution to a participant. The expiration date must be at least 13 months after the date of distribution to a participant. In addition, the DCC will notify the clinics when remaining vitamins in a batch must be disposed due to impending expiration. (See section 5.4.5 on disposal).

5.4.2.1 Vitamins at Randomization and Follow-up

At the randomization of a participant, the study vitamin assignment code will be generated by the clinic computer on the Randomization Eligibility Form (REL). The coordinator will retrieve a kit with the indicated code, carefully check the expiration date on the bottles, and enter the required information on the Vitamin Distribution Log (VDL) and on the paper Randomization Assignment Table. If the vitamins are dispensed from a pharmacy, the Study Coordinator will need to call the pharmacy confirm that the participant received the vitamins and to obtain the lot number and expiration date.

Study Coordinators should call the participant approximately seven days after the randomization visit to follow-up on possible vitamin side effects. If the participant reports any problems with the vitamins on this call (or any time between the scheduled contact) the Study Coordinator should fill out a Follow-up Contact Form (FUP), completing only the sections on side effects and the administrative information.

The follow-up vitamins will be distributed at the clinic follow-up visits. At each of these visits, the study drug assignment code should be verified on the FAVORIT data management system (in the REL form) before retrieving a resupply of study vitamins. The Study Coordinator will then retrieve a kit with the indicated code, check the expiration date on the bottles, and enter the required information on both the paper and DMS versions of the VDL.

If a follow-up clinic visit is missed or changed to a later date, vitamins may need to be delivered to the participant. Whenever vitamins are delivered to the participant, the participant must confirm receipt with a signature. This is available through insured mail, express mail, Federal Express, and other couriers. Instruct the participant to call the clinic on the day the vitamins are received. At that time the participant will be told to begin taking the study vitamins. Record the date drug started on the paper and DMS versions of the VDL. This will be needed to calculate compliance at the return visit. IMPORTANT: If the participant does not call within three days of sending the vitamins, the Study Coordinator should contact the participant to confirm delivery and ascertain when the participant began taking the vitamins.

5.4.2.2 Back-up Vitamin Kits

Each clinic has been supplied with back-up vitamin kits. These kits are distinguished from regular kits by letter bottle codes (e.g., J, K, M, N, P, Q, R, S) as opposed to numeric bottle codes. On rare occasions, clinics may be out of stock for a numeric vitamin code when it is time to distribute vitamins to a participant. If this occurs, a clinic must distribute a back-up code. This is done by contacting the DCC with the participant ID, assigned bottle code number, reason for need of a back-up (e.g., participant came in early for a visit) and a list of back-up bottle codes in stock (letter codes, number of each) . The DCC will assign a back-up code for that one distribution.

5.4.3 Instruction to Participants

The Study Coordinator will instruct the participant to keep all vitamin bottles, and to bring them to the next clinic follow-up visit. These will be used for bottle and pill counts.

The participant should be instructed to **not** take a study vitamin on the day of the clinic follow-up visit. Study Coordinator should send a note home with the participant reminding them of these instructions.

The participant is given the kit of study vitamins and instructed as follows.

a. Vitamins are for the participant's use only;
b. Take one tablet daily, preferably at a regular time; say in the morning with breakfast. However, if the regular time is missed, it should be taken later in the same day.
c. Vitamins should be stored at room temperature away from direct sunlight or heat;
d. Take any other medications on their regular schedule;
e. If participant forgets to take study vitamin and realizes it the next day, do not take two pills in the same day;
f. If planning a trip or staying away overnight, remember to take the FAVORIT vitamins along;
g. Keep the bottle of vitamins out of the reach of children

In general, emphasize the importance of taking **one pill every day and give the participant a reminder to return all bottles, including empty ones, to the next clinic visit and to refrain from taking a study vitamin on the day of the next clinic visit.**

5.4.4 Record Keeping

At the beginning of the study and at subsequent intervals during the study, a shipment of drugs will be sent to the Clinic Vitamin Distributor. This person will be responsible for receiving the vitamin shipment, verifying the contents of the shipment against the packing list, storing the vitamins, and monitoring the distribution of the study vitamin by reviewing the VDL. This person will periodically, at the request of the DCC, provide a current inventory of the vitamins available for distribution.

5.4.4.1 Verifying Shipment of Vitamins

Each case of study vitamins that is received by the clinic will be accompanied by a packing list that indicates the number of bottles in the case for each code group. The Clinic Vitamin Distributor will verify that the case contains the items listed on the packing list by comparing the codes on the list with those in the case. Check off each bottle/kit on the packing list as you locate it within the case. If all is satisfactory, sign and date the packing list. Make a copy of the verified packing list and send it to the DCC. If there is a discrepancy between the contents of the box and the packing list, notify the DCC immediately so that a correction can be made.

5.4.4.2 Maintaining the Vitamin Distribution Log (VDL)

The VDL form will be maintained for all vitamins that are distributed. This form will document the details of distribution for each bottle to facilitate the DCC tracking of vitamins available at the clinical sites and for calculating participant vitamin adherence rates. As study vitamin is assigned to a participant, enter the required information for each bottle as

indicated on the log as well as any comments. As will be seen in 5.4.6 the log is also used when a lost vitamin is replaced. All information must be entered on both the paper and DMS versions of the VDL.

5.4.5 Disposal of Excess or Expired Vitamins

Study vitamins should not be discarded or destroyed without approval from the DCC. In general, vitamins will be disposed of on a regular schedule around the time that a batch is expiring. Following authorization from the DCC, the coordinator will arrange for disposal according to the policy of the local institution. Disposal will include unused vitamins returned by a participant, vitamins unusable because of the expiration date, and drugs remaining at the conclusion of the study. Each Study Coordinator is requested to send a statement to the DCC briefly describing the disposal method to be used (incinerate, bury, flush down drain, etc.) at that site.

5.4.6 Replacing Lost Study Medication

If a participant reports that her/his vitamins have been lost or stolen, it will be important to replace the vitamins as soon as possible. Verify the bottle code assigned to that participant in the clinic database (from the REL form). Note the time that the participant is expected to return for his/her next resupply of vitamins. Retrieve a bottle(s) with the appropriate code and expiration date following the usual routine for documenting distribution information in the VDL. If the code number is not available in the clinic's inventory, the Study Coordinator will call the DCC to have them assign a backup bottle code (a code letter A through H) to the participant. At that time the Study Coordinator can request from the DCC new kits for the missing codes. Note in the comment field on the log how many bottles were sent, why (replacement of lost vitamins) and when vitamins were lost. Also note on the VDL the number of tablets that were lost.

If the participant is picking up the replacement vitamins in person, instruct them to begin taking one pill a day on the day they receive the replacement bottle(s). If the replacement is being sent to the participant see section 5.4.2.1 for shipping instructions.

5.4.7 Unmasking Policy

Given the expectation that there will be a relative lack of serious side effects associated with the use of multivitamins, unmasking should rarely be required. In most cases, adverse events will not be affected by a multivitamin preparation and the event may be managed without knowledge of treatment assignment and without discontinuing study medication if the treating physician thinks this is appropriate. Investigators should later attempt to reinstitute the study medication in participants who discontinue it. It is expected that study investigators will be aware of and adhere to this principle. However, private physicians and emergency room physicians will not be aware of the nature of the study and may require some explanation if an adverse event occurs. The principal investigator of the clinical center should always be contacted when requests for unmasking are made. Frequently, a conversation with the physician managing the adverse event will help avoid the perceived need for unmasking.

5.5 Data Coordinating Center

The primary tasks of the DCC with respect to drug distribution are to provide the list of codes for each dose group to the VDC, to monitor the storage, shipping, ordering, and disposal of vitamins, and to manage the quality control system.

5.5.1 Coding List

Before the beginning of the study, the DCC provided the VDC a paper list of the codes to be assigned to high dose and to low dose. The DCC will also monitor that the vitamins are bottled according to this list, as described in the quality control section below.

5.5.2 Vitamin Reorders to Manufacturer & VDC Shipments to Clinics

Approximate quantities for initial manufacture and shipment of vitamins, both from manufacture to VDC and from VDC to clinic, are specified in section 5.3. The DCC will monitor use of the vitamins and will notify the VDC of needs for additional shipments to the clinics. The VDC will not ship until receiving a list specifying the clinic-specific number of bottles/kits per code group to be shipped. The DCC will also monitor the overall supply of vitamins, and will notify the manufacturer when the second and subsequent batches need to be prepared and shipped.

5.6 Quality Control

The primary objectives of quality control with respect to vitamin distribution are to insure:

a. participants are always given the correct doses,

b. vitamins are not given out to be used beyond their expiration date,

c. potency of the vitamins remains above the prescribed level for B_6, B_{12}, and folic acid until the expiration date, and

d. high and low dose preparations remain indistinguishable to participants and FAVORIT investigators.

Prior to the first distribution of the vitamins, samples of each dose will be received by the DCC and tested by an independent laboratory. Similar testing will be conducted with each manufactured batch, and with vitamins returned by participants for pill count.

5.6.1 Indistiguishability of Doses

The vitamins are to be manufactured to be indistinguishable, in whole or broken form, with respect to smell, taste, appearance, size, weight, color, and any other characteristic that would allow participants or FAVORIT investigators to distinguish between the doses without complex laboratory investigation.

5.6.2 Potency Testing

At each testing, a masked sample of vitamins will be sent to an independent laboratory for analysis of quantity of B_6, B_{12}, and folic acid per tablet. Vitamins are not acceptable if the amounts of B_6, B_{12}, or folic acid fall more than 50% below or above the target amounts.

5.6.2.1 Pre-Study

A sample of both the high and low dose will be tested to confirm potency and labeling. Masked samples will be sent to the laboratory. The laboratory will return results of the analyses to the DCC within two weeks, in a format worked out between the laboratory and the DCC beforehand. The DCC will inspect samples of the two doses for indistinguishability.

5.6.2.2 At Time of Participants' Return of Bottles for Pill Count

To assure that the vitamins remain of required potency under conditions of actual usage, a random sample of returned vitamins will be requested from the clinical sites by the DCC for testing. The entire bottle with remaining vitamins will be sent to the DCC, with the participant's ID. Each bottle's contents will be sent to the testing laboratory.

Table 5.1 "High dose and Low dose" Vitamin Formulation Contents

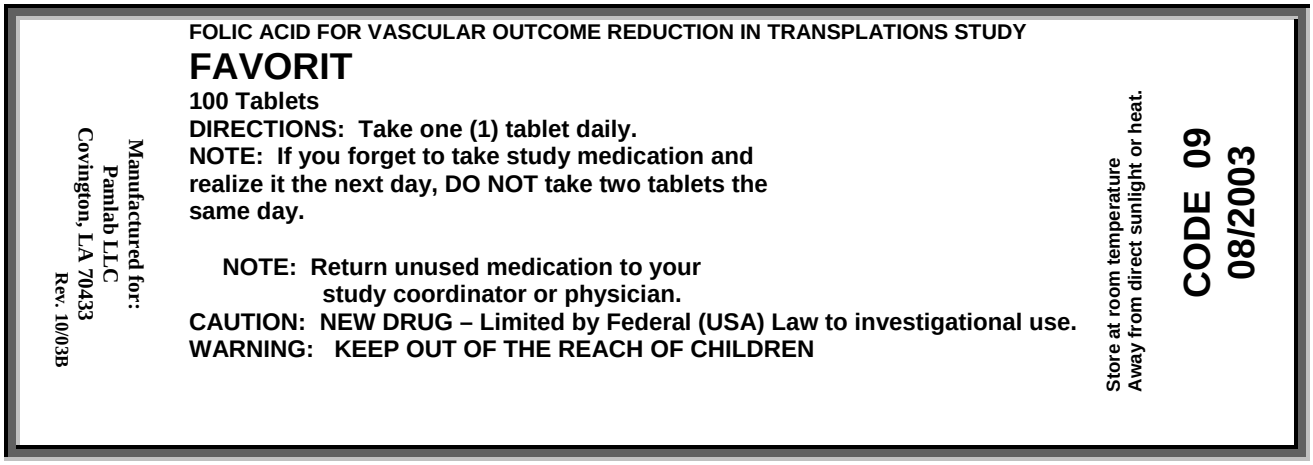
COMPONENT	"High" Formulation	"Low" Formulation
Vitamin B6 (Pyridoxine HCl)*	***50 mg***	**1.4 mg**
Folic acid**	***5.0 mg***	**0.0 mg****
Vitamin B12*	***1.0 mg***	**2.0 mcg**
Vitamin B1 (Thiamine HNO3)*	***1.5 mg***	**1.5 mg**
Vitamin B2 (Riboflavin)*	***1.5 mg***	**1.5 mg**
Vitamin C (Ascorbic Acid)*	***60 mg***	**60 mg**
d-Biotin***	***300 mcg***	**300 mcg**
Niacinamide*	***20 mg***	**20 mg**
Pantothenic Acid (Calcium Pantothenate)***	***10 mg***	**10 mg**

* All values, (i.e., low or "high" formulations), at or above the Estimated Average Requirement (EAR)

** Fortification of all enriched cereal grain flour provides an average of 340 mcg/d folate to non-supplement users

*** All values, (i.e., placebo or "active" formulations), at or above the Average Intake (AI), since EAR is unknown

Figure 5.1 Sample of Study Drug Label Provided by VDC



Chapter 6. Specimen Collection and Processing

6.1 Overview

Participants will be seen at the FAVORIT clinics Monday through Friday. Scheduling should allow for adequate time to meet the critical requirements associated with the handling and processing of samples. It is recommended that no more than two to three participants be screened per day; this could vary depending upon each clinic's capabilities (i.e., equipment, personnel, etc).

6.2 Policy of Ultra-Low Freezer

6.2.1 Installation

The Revco Ultima II freezer will need to be installed according to the manufacturer's specifications by a qualified electrician from your institution and must comply with local codes. Site preparation requires availability of the proper receptacle, a dedicated circuit and a phone line to install the electronic remote alarm system. The freezer's operating temperature will be set at **–80°C**. The high alarm temperature set point should be at **–60°C** and the low alarm temperature set point should be at **–90°C.**

The installation instructions list the required voltage, rated amperage, circuit breaker size and NEMA electrical plug information. These instructions are found in the manual that is shipped inside the freezer.

6.2.2 IntrLogic™ Microprocessor Control

The IntrLogic™ Microprocessor Control system on the Revco Ultima II freezer is equipped with a comprehensive management system for temperature control, electrical power, alarm and monitoring functions. Each Study Coordinator is responsible for knowing how to operate, maintain and interpret each feature of the system.

6.2.3 Daily Monitors

The displays listed below are part of the management control system and must be monitored daily. These displays are located on the front panel of the freezer. Major adverse events should be documented.

- Lifeguard Alert
- Automatic Incident Monitor
- Extreme Ambient Alert
- Clean filter light
- Low Battery Alarm
- Voltage Boost Indicator
- Temperature changes

6.2.4 Remote Alarm System

Each Ultima II Revco freeze comes with an electronic remote alarm system to be installed on the unit and connected to a dedicated outgoing phone line. The remote alarm must be programmed to sound in the event of a power or temperature failure and must be programmed to dial 4 emergency phone numbers. When the alarm is activated the system will automatically dial the numbers (in order of priority) until the alarm call is answered. Suggested numbers would be the Study Coordinator's daytime phone number, nighttime number and additional number, and the facility's Physical Plant number. Do not use a pager number.

6.2.5 Responding to the Alarm

Assess the situation and try to identify the type of failure. The facility's Physical Plant department may need to get involved. If electrical failure is the problem and the situation cannot be corrected within a short period of time, (i.e., one hour) proceed with backup plan, unless freezer is connected to a back-up generator. If temperature failure is problem, try to determine the reason for the failure and correct it. Check the temperature of the freezer 30 minutes after the initial alarm to verify that the temperature is back in range. If the temperature continues to rise above –60^{o}C and/or cannot stabilize back to -80^{o}C after the 30 minutes, proceed with the backup plan to move the samples to an alternative ultra low freezer set at -80^{o}C. During an electrical or temperature failure, opening and closing the freezer should be avoided unless absolutely necessary. Also, always exercise the necessary safety procedures when handling and transporting specimens and use protective gloves. A thermal type of protective glove could be useful when moving and/or handling a large amount of specimens in the freezer.

When the freezer door is open, the temperature being monitored can rise very quickly. The longer the door is open and the less full the unit is, the quicker the temperature will rise. Therefore it is important to work quickly when placing specimens into or removing specimens from the freezer. However, there will be occasions when the normal daily operation of placing samples into and/or removing samples from the freezer will cause the alarm to go off. Further, the rate at which the temperature returns back to the set point will vary and could take longer than 30 minutes, especially if the freezer is not full to capacity. Under these conditions it is not necessary to unload specimens to the backup freezer **as long as the temperature continues to decline back to –80^{o}C and stabilizes within 60 to 90 minutes.**

6.2.6 Back-Up Plan

Each Study Coordinator must develop and document a clearly defined backup plan in the event of prolonged power and/or temperature failure to ensure the stability of the stored samples. A specific alternative ultra low freezer must be identified, (location and area documented) in the event that specimens must be removed from the FAVORIT Revco freezer. The remote dialup procedure, contact persons and phone numbers should also be documented in this plan. The conditions in which specimens will be moved and procedure for transport should also be included. After developing this plan with the guidelines outlined in this policy, the plan must be submitted to Gayle Perrone for review. After approval, the plan will be ready for implementation.

6.2.7 Inventory Systems

Each freezer has the Revco Inventory System that is designed for flexibility, easy storage and retrieval and accurate inventory management. The shelving racks and boxes with dividers enhance the value of the freezer by saving space and improving inventory management. Study Coordinators are required to use this system and develop a management inventory tracking system which maintains the position of each box placed within the freezer, (i.e., identification of shelf number, rack number and position number within the rack).

Questions or concerns should be directed to Joyce McKenney at the Operations Center, between 9am to 5pm (Eastern time), Monday through Friday.

6.3 Screening

6.3.1 Time Table

- Sample Collection: Monday through Friday, Week 1
- Shipment: Monday, Week 2
- Receipt/Inventory of Samples: Tuesday, Week 2
- Analysis: Wednesday and Thursday, Week 2
- Entry of Data: Friday, Week 2
- Release of Data: Monday, Week 3

**Holiday Schedule*: Timetable will be adjusted accordingly. Each site is responsible for notifying appropriate personnel of the holiday schedule.

6.3.2 Forms

The following three phlebotomy forms will be used during the Screening Visit:

- Screening Phlebotomy Form: Collection (SPC) –PC data entry.
- Screening Phlebotomy Form: Processing & Inventory (SPP) – Paper form filled out during processing and entered into the FAVORIT data management system (DMS) at the completion of day's activities.
- Screening Phlebotomy Shipping Log (SPS) – Either paper form or computer generated report based on shipping date. Site will keep the original and a copy will accompany specimens shipped to the Central Lab.
-

6.3.3 Specimen Collection

The following steps occur prior to specimen processing:

1. Prior to blood collection, place the provided specimen collection labels onto appropriate vacutainer tubes. Labels will contain the following information:

    Barcode
    ID#
    Contact Occasion#
    Vacutainer Type

Place specimen collection labels on tubes atop manufacturer's labels to allow for a glass window (do not wrap). This will ensure you will be able to see the sample and make aliquots correctly (this is critical for the removal of the buffy coat). If a label is missing or unusable prepare a hand-printed label containing all pertinent information with water resistant ink (i.e., cryomarker) that can withstand temperature below –80^{o}C, only printing on the white part of the label. Additional labels will be provided, as needed.

Start the on-line Screening Phlebotomy Form: Collection. If desired, this form can be completed on paper and entered into the DMS before the end of the day.

2. The following specimens will be obtained from the participants (this will also be the priority order of draw):
    - Lavender Top tube, EDTA Additive (LT), 5 mL
    - Marble Top tube (MT), 5 mL

3. Completion of blood collection:
    a. Immediately after sample collection, invert the 5 mL Lavender Top tube (LT) 5-10 times, cover in foil and place directly on wet ice until sample is centrifuged.
    b. Allow the 5 mL Marble Top tube (MT) to clot (20 minutes) at room temperature prior to centrifuging.
    c. All vacutainers are spun in a refrigerated centrifuge at 2800 RPM at 4^{o}C for 15 minutes. **This should be done within 30 minutes of collection**.

       If the above is not possible, the Lavender Top tube will sit on wet ice and the Marble Top tube (after the 20 minute clotting period) will go into the refrigerator. If this occurs, then **centrifugation must be done within 3 hours** post-draw. Also, if specimen processing is to take place at a different site from collection, it is extremely critical to transport the samples under the conditions described above. The Lavender Top tube will remain on wet ice and the Marble Top tube should be kept in a cold environment, (i.e.~2 - 4^{o} C) but do not place directly on ice. This may be accomplished by the use of a cooler and cold packs. **Do not place MT tube directly on top of cold pack.**

    d. Complete the on-line Screening Phlebotomy Form: Collection (SPC).

6.3.4 Specimen Processing

1. Label tubes with the provided aliquot labels, which will have the following information:

    Barcode
    ID#
    Contact Occasion #
    Aliquot Code

If a label is missing or unusable prepare a hand-printed label using water resistant ink (i.e. cryomarker), only print on the white part of the label. Additional blank labels will be provided, as needed.

2. The working (paper form) Screening Phlebotomy Form: Processing& Inventory will be completed during specimen processing.

   Immediately following centrifugation, aliquot samples (into labeled tubes) according to the schedule below (see figures 1 and 2):

Sample Processing Schedule: Screening Visit

Specimen/ Container Type	#	Test Request	Aliquot Volume	Aliquot Code	Storage Instructions	Special Instructions
EDTA Plasma/ 5 mL Lavender Top Vacutainer	1	Total Homocysteine (tHcy) Plasma Archive Buffy Coat (WBC's) RBC Archive	1000 µL 1000 µL ~500 µL 1000 µL	LT1 LT2 LT3 LT4	Wkly Ship Hold Hold Hold	Foil/Wet Ice **See ****
Serum/5 mL Marble Top Vacutainer	1	Creatinine Glucose Serum Archive	750 µL 750 µL 1000 µL	MT1 MT2 MT3	Wkly Ship Hold Hold	

**** Special Handling for Total Homocysteine/Buffy Coat**
In order to avoid elevated plasma homocysteine values and ensure optimal retrieval of the buffy coat, sample should be centrifuged and processed within 45 minutes from time of collection (See Note in Section 3c of Specimen Collection).

Plasma should be removed immediately after centrifugation using a transfer pipet (careful to not disturb buffy coat) and placed initially into a 5 mL borosilicated glass tube, from which LT aliquots will be made. Remember to hand label the glass tube to indicate the vacutainer it represents (i.e. LT).

The buffy coat is the hazy white film found between the plasma and the red blood cell (RBC) layers. The key to successful removal is to use one continuous skimming motion. Use a new transfer pipet to ensure maximum suction. Do not be concerned if your buffy coat contains RBC's or residual plasma, this is unavoidable (at least 500 µL is required for sample aliquot therefore it is imperative to retrieve the entire WBC layer). If the buffy coat is disturbed, recentrifuge and proceed with processing.

3. To ensure there are no remaining white blood cells or plasma, suction off the top layer of the remaining red blood cells. Gently invert the Lavender Top tube before making the RBC aliquots.

4. Upon completion of aliquotting, all tubes should be tightly capped and frozen; remaining cells should be discarded.

5. **All processing should be COMPLETED within 4 hours of specimen collection. Samples should be placed IMMEDIATELY into the FAVORIT freezer.**

   Note: If for some reason the tubes must be re-centrifuged, keep the original centrifugation time on the SPP.

6.3.5 Archived Samples

The following aliquots, some of which are labeled 'archive', should not be collected at the screening visit if the patient refuses to sign a specimen banking consent form:

LT3 - Buffy Coat
LT4 - RBC Archive

Other aliquots labeled 'archive' are primarily backups for serum and plasma samples drawn at the Screening visit. In the event of breakage or loss during shipment or equipment malfunction at the Central Lab, the samples labeled 'archive' (except for Buffy Coat, RBC archive mentioned above) would be used as backups so that assays could successfully be performed. If designated backup samples were not utilized, these samples would also be available for the purpose of approved ancillary studies for participants with a signed specimen banking consent form. The designated backup samples for the screening visit are:

LT2 - Plasma Archive
MT3 - Serum Archive

6.3.6 Storage

For temporary storage (specimens to be shipped), MT1 (Creatinine) and LT1 (tHcy) should be stored in the FAVORIT freezer at –80^{o}C, shielded from light, and in an upright position until time of shipping. The boxes should be labeled as follows:

FAVORIT Study
Eligibility Specimens
"SITE" Name
Shipping Date

For long-term storage (specimens stored on-site), there should be a storage box for each aliquot type (LT2, LT3, LT4, MT2, and MT3). Specimens must be stored in the FAVORIT freezer at –80^{o}C, in the yellow storage boxes, with aliquots in an up-right position. It is extremely important to inventory the boxes' contents. Boxes should be labeled as follows:

Aliquot Type
Contact Occasion
Box Number
Beginning and End Dates

For example, all LT2 aliquots from the Screening visit, CO 00, will be stored together. You may need more than one box for the complete set of LT2 aliquots from CO 00, therefore it is

important to include the box number in the labeling. Similarly, all LT3 aliquots from CO 00 will be stored together, and the same will hold true for the remaining aliquots. It is important not to get confused in the respect that similar aliquot types from other COs do not get stored together. Each aliquot type from a specific CO gets stored in a separate box. For example, LT2 aliquots from CO 00 will be in a different box from LT2 aliquots from CO 01. A typical box label may read as follows:

Aliquot Type **LT2**
Contact Occasion **00**
Box 2
11/17/02 – 04/24/03

From this example the label informs one that this box of specimens is the second box of aliquot type LT2, collected at the Screening visit, (CO 00) and from subjects who Screening visits were between the dates of November 17, 2002 and April 24, 2003. One also knows that another box (i.e., Box #1) exists for LT2 aliquots from the Screening visit and that the dates would be from the beginning of the study (June 2002) through about November 17, 2002.

Once specimens have been properly stored then all information from the Screening Processing/Inventory Form (SPP) should be entered into the DMS. It is important to do this at the completion of each day because data entry sheets will accumulate quickly.

6.3.7 Blind Replicates

Periodically, each site will be notified to collect blind replicate specimens at the screening visit. When notified, the site should collect, process, and ship/store a complete set of tubes and vials as normally done at the screening visit. These will be used to perform quality control measurements on Central Lab analyses by matching analysis results on blind replicate samples with results on original participant samples. The entire process is called blind replicate matching (BRM). Information regarding the BRM will be collected on the SPP.

BRM aliquot vials are made from additional tubes drawn specifically for BRM, and are labeled with the BRM ID rather than the patient's FAVORIT ID (see BRM form and QxQ instructions for details). To minimize the burden on patients, no more than one tube for BRM purposes is drawn per patient. Therefore, for screening visit BRM, one extra tube is drawn from two patients: an LT from one patient, and a MT from another.

6.3.8 Specimen Transport/Shipping

Specimens (LT1 and MT1) will be stored temporarily at the collection site and will be transported weekly to the Central Lab located at the USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University (Boston, MA). On Monday, the previous week's specimens should be shipped using an overnight mail service (i.e., UPS) to ensure arrival at the HNRCA by Tuesday (see figure 4). Each site, including US and Canada, will have its own account number which will be billed to the Operations Center.

The boxes being shipped should contain a shipping log, identifying the content and number of specimens per box. The shipping log should be placed in a plastic bag and attached to the outside lid of the Styrofoam container in order to maintain list integrity. **It is extremely important that specimens remain completely frozen during this process.**

Each storage box being sent should be secured by placing a rubber band around its middle and placed into a polyfoam shipping container. Approximately 3-5 lbs of dry ice are necessary to keep the specimens frozen for 48 hours. A layer of dry ice should be placed below and above each box with most of the dry ice concentrated on the top of the boxes. Any air space should be filled with a packing material (i.e. newspaper, packing peanuts). Once the polyfoam container is filled, the lid should be secure with packing tape and placed into its companion cardboard carton. Place the following labels on the outside of the cardboard carton:

- Return Address
- "Keep Frozen" Label
- Completed Dry Ice Label
- Air Bill
- Address Label:

  ATTN: Stephanie Valliere, Rm 805
  USDA Human Nutrition Research Center On Aging
  Tufts University
  711 Washington Street
  Boston, MA 02111
  Tel. (617) 556-3168

The tracking number for each shipment should be sent via email or fax to the Central Lab.

6.4 Receipt at Central Lab

Upon arrival of specimens, the contents of each box will be checked against the shipping log. Specimens will be distributed to the technicians who will perform sample analysis for Total Homocysteine and Creatinine. Samples will be stored at –80^{o}C prior to and for one week following analysis.

The Central Lab will notify the site if there is a problem with the shipment.

6.5 Central Lab Data Entry/Result Reporting

Technicians will enter results in the database. The Lab Coordinator will review the final data set and release results to the Data Coordinating Center. The Central Lab will notify that clinic if any creatinine value is greater than 3.5 mg/dL for their participants, and will email a copy of this notification to Barbara Brown at the DCC.

6.6 Randomization and Follow-up Visits

6.6.1 Forms

The following forms will be used during the Randomization and Follow-up visits:

- Baseline/Randomization/Follow-up Phlebotomy Form: Collection (PHC) – PC data entry.
- Baseline/Randomization/Follow-up Phlebotomy Form: Processing & Inventory (PHP) – Paper form filled out during processing and entered into DMS at the end of day.

6.6.2 Specimen Collection

The following steps occur prior to specimen processing:

1. Prior to blood collection, place the provided specimen collection labels onto appropriate vacutainer tubes. Labels will contain the following information:

   Barcode
   ID#
   Contact Occasion #
   Vacutainer Type

   Place specimen collection labels on tubes atop manufacturer's labels to allow for a glass window (do not wrap). This will ensure you will be able to see the sample and make aliquots correctly (this is critical for the removal of the buffy coat). If a label is missing or unusable prepare a hand-written label containing all pertinent information using water-resistant ink (i.e. cryomarker) only print on the white part of the label. Additional labels will be provided, as needed.

   Start the on-line Randomization/Follow-up Phlebotomy Form: Collection. If desired, this form can be completed on paper and entered into the DMS before the end of the day.

2. The following specimens will be obtained from the volunteers (this will also be the priority order of draw):
   - 2 Lavender Top tubes, EDTA Additive (LT), 10 mL
   - 1 Marble Top tube (MT), 10 mL
   - 1 Light Blue Top tube, Sodium Citrate Additive (BT), 4 mL
   - 1 Midstream Clean Catch Urine in Sterile Container (UR)

3. For urine collection, a midsteam clean catch urine is required. The urine sample should be aliquotted immediately upon receipt. If not possible, refrigerate until processing occurs.

4. Completion of blood collection

    a. Immediately after sample collection, invert the two 10 mL Lavender Top tubes (LT) 5-10 times, cover with foil and place directly on wet ice until samples are spun.

    b. The 10 mL Marble Top tube (MT) should be inverted 2-3 times and allowed to clot (20 minutes) at room temperature prior to spinning.

    c. Invert the 4 mL Light Blue Top tube (BT) 2-3 times after collection to ensure proper distribution of the sodium citrate and place on wet ice.

    d. All vacutainer tubes are spun in a refrigerated centrifuge at 2800 RPM at 4^{o}C for 15 minutes. **This should be done within 30 minutes of collection**.

    If the above is not possible, the Lavender Top tubes and the Light Blue Top tube will sit on wet ice, the Marble Top tube (after the 20 minute clotting period at room temperature) is refrigerated. If this occurs, then centrifugation must be done within 3 hours post-draw. Also, if specimen processing is to take place at a different site from collection, it is extremely critical to transport the samples under the conditions described above. The Lavender Top tubes and the Light Blue Top tube will remain on wet ice. The Urine Sample and the Marble Top tube should be kept in a cold environment, (i.e.~2 - 4^{o} C) but do not place the Marble Top tube directly on ice. This may be accomplished by the use of a cooler and cold packs. Do not place MT tube directly on top of cold pack.

    e. Complete the Randomization/Follow-up Phlebotomy Form: Collection (PHC).

    If for some reason the tubes must be re-centrifuged, keep the original centrifugation date on the PHP.

6.6.3 Specimen Processing

1. Label tubes with the provided aliquot labels, which will contain the following information:

    Barcode
    ID
    Contact Occasion #
    Aliquot Code

    If label is missing or unusable prepare a hand-printed label using water-resistant ink (i.e. cryomarker), only print on the white part of the label. Additional blank labels will be provided, as needed.

2. The working (paper form) Randomization/Follow-up Form: Processing & Inventory will be completed during specimen processing.

    Immediately following centrifugation, aliquot samples (into labeled tubes) according to the schedule below (see figure 3):

Sample Processing Schedule: Randomization & Follow-up Visits

Specimen/ Container Type	#	Test Request	Aliquot Volume	Aliquot Code	Storage Instructions	Special Instructions
EDTA Plasma/ 10 mL Lavender Top Vacutainer	1	Total Homocysteine (tHcy) B12/folate PLP Plasma Archive-1 Buffy Coat (WBC's) RBC Archive-1 RBC Archive-2	500 µL 1000µL 500 µL 1000 µL ~500 µL 1000µL 1000µL	LT1 LT2 LT3 LT4 LT5 LT6 LT7	–80°C	Foil/Wet Ice **See ****
EDTA Plasma/ 10 mL Lavender Top Vacutainer	1	Lipid Profile: Chol/Trig HDL LDL Plasma Archive-2	 500µL 500µL 500µL 1000µL	 LT8 LT9 LT10 LT11	–80°C	Foil/Wet Ice **See ****
Serum/10 mL Marble Top Vacutainer	1	Creatinine Glucose Fructosamine Serum Archive –1 Serum Archive - 2	500 µL 500 µL 500 µL 1000 µL 1000 µL	MT1 MT2 MT3 MT4 MT5	–80°C	
Sodium Citrate Plasma/4 mL Light Blue Top Vacutainer	1	Plasma Archive –1 Plasma Archive - 2	1000 µL Remainder	BT1 BT2	–80°C	Foil/Wet Ice **See ****
Urine/5 oz. Sterile Midstream Urine Collection Container	1	Creatinine Micro Albumin Urine Archive	1500 µL 1500 µL 1500 µL	UR1 UR2 UR3	–80°C	

**** Special Handling Instructions**

In order to avoid elevated plasma homocysteine values and ensure optimal retrieval of the buffy coat, sample should be centrifuged and processed within 45 minutes from time of collection.

All plasma (from LT and BT) should be removed immediately after centrifugation, using a new transfer pipette for each tube (careful to not disturb buffy coat), and placed initially into 5 mL borosilicated glass tubes, from which LT and BT aliquots will be made. Hand label each glass tube as to indicate which vacutainer it represents (i.e. LT or BT).

The buffy coat is the hazy white film found between the plasma and the red blood cell (RBC) layers. The key to successful removal is to use one continuous skimming motion. Use a new transfer pipette to ensure maximum suction. Do not be concerned if your buffy coat contains RBC's or residual plasma, this is unavoidable (at least 500 µL are required for the sample aliquot therefore it is imperative to retrieve the entire WBC layer). If the buffy coat is disturbed recentrifuge and proceed with processing.

The Lavender Top tubes (LT) are treated identically and considered interchangeable in the event one sample is compromised

3. To ensure there are no remaining white blood cells or plasma, suction off the top layer of the remaining red blood cells. Gently invert the LT tube before making the RBC aliquots.

4. When left standing, a urine sample may settle out of solution. It is necessary to re-suspend the sample prior to aliquotting. This is done by gently swirling the specimen sufficiently to provide thorough mixing.

5. Upon completion of aliquotting, all tubes should be tightly capped and frozen; remaining cells are discarded.

6. All processing should be COMPLETED within 4 hours of specimen collection. Samples should be placed IMMEDIATELY in the FAVORIT freezer.

6.6.4 Archived Samples

The following aliquots, some of which are labeled 'archive', are not collected at randomization and follow-up visits if the patient refuses to sign a specimen banking consent form:

LT5 - Buffy Coat
LT6 - RBC Archive 1
LT7 - RBC Archive 2
BT1 - (Do not draw 4 ml Blue Top tube)
BT2 - (Do not draw 4 ml Blue Top tube)

Other aliquots labeled 'archive' are primarily backups for serum and plasma samples drawn at Screening and Randomization and Follow-up visits. In the event of breakage or loss during shipment or equipment malfunction at the Central Lab, the samples labeled 'archive' (except for Buffy Coat, RBC archive, and BT1 and BT2 mentioned above) would be used as backups so that assays could successfully be performed. If designated backup samples were not utilized, these samples would also be available for the purpose of approved ancillary studies for participants with a signed specimen banking consent form. The designated backup samples for randomization and follow-up visits are:

LT4 - Plasma Archive 1
LT11 - Plasma Archive 2
MT4 - Serum Archive 1
MT5 - Serum Archive 2
UR3 - Urine Archive

6.6.5 Storage

For long-term storage, there should be a storage box for each aliquot type (i.e., LT2–LT11, MT2–MT5, BT1, BT2 and UR1–UR3). Specimens must be stored in the FAVORIT freezer at –80^{o}C, in the yellow storage boxes, with aliquots in an up-right position. It is extremely important to inventory the boxes' contents. Boxes should be labeled as follows:

Aliquot Code
Contact Occasion #
Box Number
Beginning and End Dates

For example, all LT2 aliquots from the randomization visit, CO 01, will be stored together. You may need more than one box for the complete set of LT2 aliquots from CO 01, therefore it is important to include the box number in the labeling. Similarly, all LT3 aliquots from CO 01 will be stored together, and the same will hold true for the remaining aliquots. It is important not to get confused in the respect that similar aliquot types from other CO s do not get stored together. Each aliquot type from a specific CO gets stored in a separate box. For example, LT2 aliquots from CO 01 will be in a different box from LT2 aliquots from CO 00, 02, 03, etc. A typical box label may read as follows:

Aliquot Type **LT2**
Contact Occasion **02**
Box 2
12/03/03 – 05/10/04

From this example the label informs one that this box of specimens is the second box of aliquot type LT2, collected at the 12 Month visit, (CO 02) and from subjects who's 12 Month visits were between the dates of December 3, 2003 and May 10, 2004. One also knows that another box (i.e., Box #1) exists for LT2 aliquots from the 12 Month visit and that the dates would be from the June 2003 through about December 3, 2003.

Once specimens have been properly stored then all information from the Randomization/Follow-up Form: Processing & Inventory should be entered into the DMS. It is important to do this at the completion of each day because data entry sheets will accumulate quickly.

6.6.6 Blind Replicates

As for the screening visit, each site will periodically be notified to collect blind replicate specimens from participants at the randomization and follow-up visits. When notified, the site will collect, process, and ship/store a complete set of tubes and vials as normally done at that visit. These specimens will be used to perform quality control measurements on Central Lab analyses by matching analysis results on blind replicate samples with results on original participant samples. The entire process is called blind replicate matching (BRM). Information regarding the BRM will be collected on the PHP.

BRM aliquot vials are made from additional tubes drawn specifically for BRM, and are labeled with the BRM ID rather than the participant's FAVORIT ID (see BRM form and QxQ instructions for details). To minimize the burden on participants, no more than one blood tube for BRM purposes is drawn per participant. Therefore, for a randomization or follow-up visit BRM, one extra tube is drawn from four participants: 1st LT, 2nd LT, MT and BT all from different participants. The BRM urine sample is taken from one of these participants.

Note that since several participants at one type of visit are required for completing the BRM, it may take several days or weeks to compile the complete BRM vial set.

6.7 Combination Visit: Screening/Baseline

Sites will be given the option of combining the Screening and Randomization visit. In this event, it will be called a Screening/Baseline Combination visit.

6.7.1 Forms

The following forms will be used during the Screening/Baseline Combination visit:

- Screening Phlebotomy Form: Collection (SPC) –PC data entry.
- Screening Phlebotomy Form: Processing & Inventory (SPP) – Paper form filled out during processing and entered into the FAVORIT data management system (DMS) at the completion of day's activities.
- Screening Phlebotomy Shipping Log (SPS) – Either paper form or computer generated report based on shipping date. Site will keep the original and a copy will accompany specimens shipped to the Central Lab.
- Baseline/Randomization/Follow-up Phlebotomy Form: Collection (PHC) – PC data entry.
- Baseline/Randomization/Follow-up Phlebotomy Form: Processing & Inventory (PHP) – Paper form filled out during processing and entered into DMS at the end of day.

6.7.2 Specimen Collection

Specimen collection and order will remain the same. Screening tubes to be drawn first followed by Baseline tubes (as shown below):

Screening: 1) Lavender Top tube, EDTA Additive (LT), 5 mL
2) Marble Top tube (MT), 5 mL

Baseline: 3) 2 Lavender Top tubes, EDTA Additive (LT), 10 mL
4) 1 Marble Top tube (MT), 10 mL
5) 1 Light Blue Top tube, Sodium Citrate Additive (BT), 4 mL
6) 1 Midstream, Clean Catch Urine in Sterile Container (UR)

All tubes may be centrifuged together. Specimen handling and the processing schedule will remain unchanged; please refer to Section 6.3.4 for Screening visit and 6.6.3 for Baseline visit. Due to the large volume of blood collected during the combined visit, please be aware of the likelihood of a short draw. The serum from Screening and Baseline are interchangeable, as is the plasma from Screening and Baseline tubes.

6.7.3 Storage

LT1 and MT1 aliquots are stored temporarily in the –80°C freezer before they are sent to the Central Lab. Screening aliquots collected during the combined visit will be stored as usual in the appropriate long-term storage boxes. Please see section 6.3.6 for specific details.

Baseline aliquots, should remain in the rack and placed directly into the –80°C freezer until it is determined if the participant is randomized. If randomized, the Baseline samples will be placed in the corresponding Randomization long-term storage boxes. Please refer to section 6.6.5 and follow as directed.

Non-randomized samples will be stored separately in a BASELINE box. All aliquots from a given participant should be kept together in one box, which will be sent to the repository. If you cannot put a subject's complete sample-set in the same box, it is recommended to start a new one.
A typical box label may read as follows:

BASELINE
Box 1
12/03/03 – 05/10/04

6.8 Sorting Procedure

6.8.1 Removal/Transport of Storage Boxes from -80º C Revco Freezer

1. The coordinating center will generate a clinic-specific list of retrieval IDs for each site. It will include the subject number, contact occasion number, aliquot type, box location, and date of blood draw.

2. After removing storage boxes from the -80º C Revco freezer, it is imperative that all samples remain frozen throughout the sorting process. Only remove boxes that contain IDs from your list.

a. If samples are to be sorted at a location that is different from that of the Revco freezer, place boxes in a cooler during transport. Dry ice should be used if the distance is substantial (i.e., driving is involved). Wet ice is sufficient for short- term travel or just the cooler if the transport time is negligible. Upon arrival at your destination, the boxes should be kept in the cooler or placed in a -20º C unit.
If you must use the cooler to hold your boxes during the sorting process, use dry ice during transport regardless of time or distance.

b. If the samples are being sorted in close proximity to the -80º C Revco, either, remove one box from the freezer at a time, or remove a portion of boxes and place in a -20º C unit or cooler with dry ice. This is a better option since frequent opening and closing of the Revco door can effect the temperature enough to trigger the alarm.

6.8.2 Sorting Samples

1. Fill the insulated tray with wet ice. Place your polypropylene rack in the ice at a deep enough level to provide adequate cooling to prevent thawing. When you are ready to sort, only remove one box at a time and place in the ice tray with your rack (See Figure 5). The first sample pulled should be the first ID from your list. Check mark the ID number after the sample is removed from the storage box or note if the aliquot is missing. Place the sample in the first position of the rack, the second sample pulled in the second position, and so forth.

2. After all pertinent samples have been removed from a box, place back in the designated cool area (cooler or refrigerator) before taking the next storage box. When the rack is full, transfer the samples to a yellow storage box for shipping. The samples should be placed in the box in the exact order they are in the rack, which should correspond sequentially to your list (See figures 6 and 7). Mark the location of position one by placing an arrow on the outside of the box (See yellow box in Figure 1). When the box is full, label it as Box 1 and indicate the first and last ID contained in the box as follows:

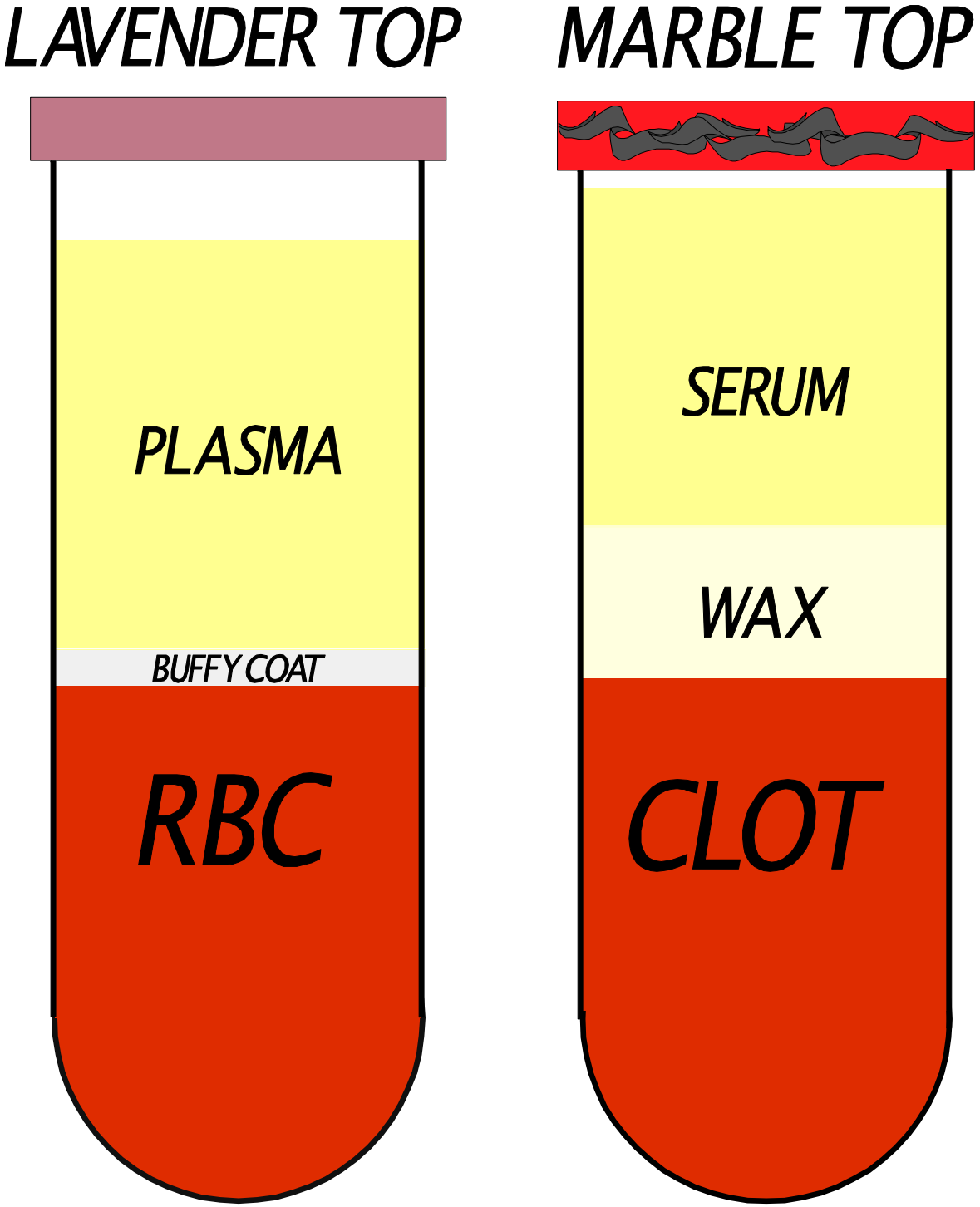
Box 1
AL00000 - AL00100

Store appropriately until time of shipment. Place in -80°C Revco or keep in cool space if you intend on shipping immediately after completion.

6.8.3 Shipment of Samples

1. The shipping procedure is similar to that done on a weekly basis. The polyfoam shipping containers used for the weekly ship will be sufficient to ship one large, yellow storage box (100 samples). Please use at least 4lbs (1.8 Kg) of dry ice per container.

* In order to provide an adequate amount of dry ice to keep the samples frozen during transit no more than one box per container is allowed, unless you have access to or we provide you with, the larger sized polyfoam shipping boxes.


LAVENDER TOP
PLASMA
BUFFY COAT
RBC
MARBLE TOP
SERUM
WAX
CLOT

FAVORIT

Specimen Collection Scheme for SCREENING VISIT

Centrifuge at 2800 rpm for 15 min. at 4°c (+/- 2°)

Gently Invert LT Vacutainer to mix EDTA into the Whole Blood.
Wrap LT in FOIL and place on wet ICE until Centrifuged.

5 mL LT

LT 1	LT 2
total Homocysteine	Plasma Archive
1000 µL	1000 µL

Use a clean transfer pipet to suction off the entire WBC layer

LT 3
Buffy Coat
Not Quantified (appx 500 µL)

Make sure no visible plasma or WBCs remain in LT.
Gently invert LT several times to mix RBCs.

LT 4
RBC Archive
1000 µL

Allow MT Vacutainer to
CLOT AT ROOM TEMP
for 20 min. before Centrifuging.

5 mL MT

MT 1	MT 2	MT 3
Creatinine	Glucose	Serum Archive
750 µL	750 µL	1000 µL

Aliquots LT1 and MT1 are
SHIPPED WEEKLY
to the Central Lab.

Figure 3

FAVORIT

Specimen Collection Scheme for:
RANDOMIZATION AND YEARLY FOLLOW-UP VISITS

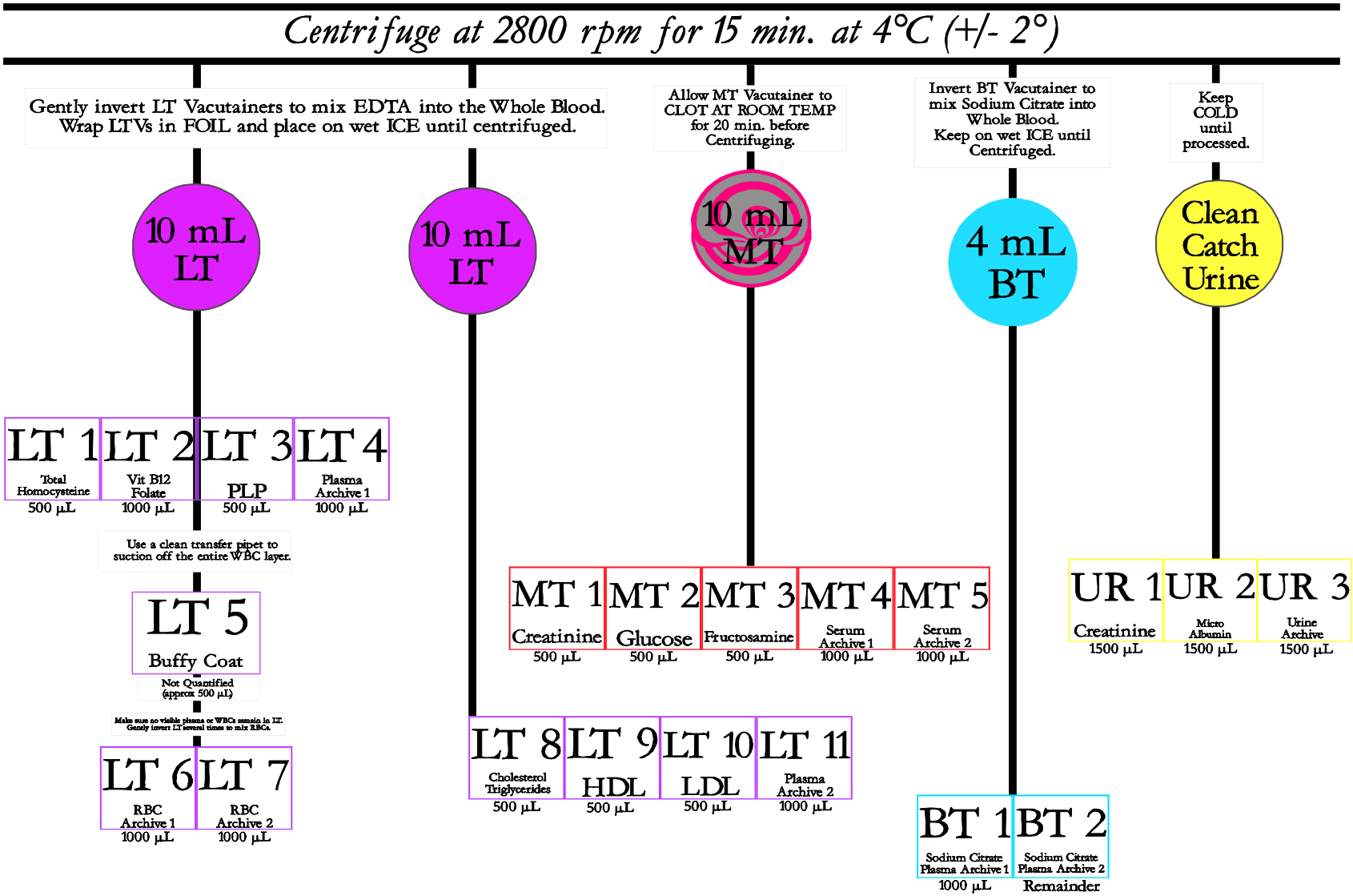


Figure 4: UPS shipping slip

Figure 5: Sorting of Samples from Storage Boxes

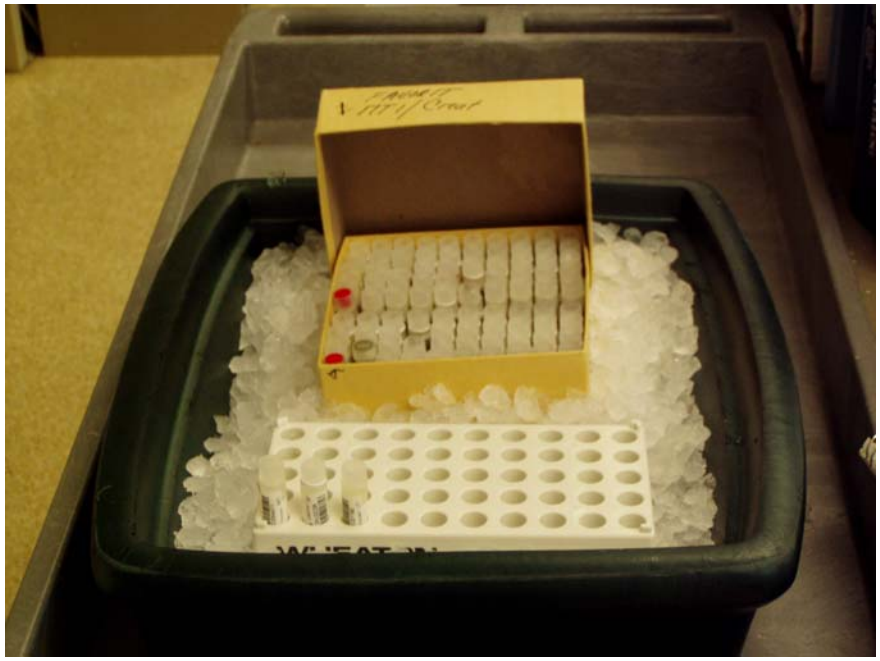


Figure 6: Transferring Sorted Samples to Shipping Box (First sample, in position 1, corresponds to first ID on list)

Figure 7: Transferring of Sorted Samples (Full rack is placed into shipping box; box number and ID's (first and last) are noted on cover; position 1 is indicated by arrow)

Chapter 7. Follow-Up

7.1 Overview

During the follow-up period (which will end on a common date for all participants – this date will be provided by the DCC), clinical and telephone follow-up contacts with participants will alternate every six months, or until the occurrence of death, with one exception. Participants who develop dialysis-dependent end stage renal disease will be followed until their first primary outcome occurs, after which mortality surveillance sufficient to distinguish CVD from non-CVD death continues until the end of the follow-up period.

In brief, the clinic follow-up visits are designed to: capture all hospitalizations, particularly those related to potential arteriosclerotic cardiovascular disease (CVD) outcomes, and/or major renal graft dysfunction; record blood pressure (twice) and obtain height and weight; review prescription medications with recording of specific medications of interest to FAVORIT; review of smoking history; document specific medical diagnoses, including hypertension, arteriosclerotic CVD diagnoses, and diabetes; document interim intake of both study-related and unrelated vitamin supplements; review interim intake of specific cereals, or liquid/powdered food supplements with high folic acid content; review interim physical activity patterns; obtain blood and urine specimens for the FAVORIT specimen bank; determine study vitamin compliance; and record any possible adverse reactions to the study vitamin.

The telephone follow-up visits are designed to: capture all interim hospitalizations, particularly those related to potential arteriosclerotic CVD outcomes, and/or major renal graft dysfunction; document interim intake of both study related and unrelated vitamin supplements; determine study vitamin compliance; and determine any possible adverse reactions to the study vitamin.

Whenever it is discovered that a participant has been hospitalized, a Hospitalization Form (HOS) is completed, preferably with information from the participant's hospital records.

Detailed information on deaths, including hospitalization data, is completed for all participants who die during the course of the study.

7.2 Summary of Data Collection During Follow-up

See table 7.1. After randomization, follow-up procedures continue until the exit visit. The one exception to this is that participants who develop dialysis-dependent end stage renal disease will be followed until their first primary outcome occurs, after which mortality surveillance sufficient to distinguish CVD from non-CVD death continues until the common end date for the study.

7.3 Detailed Follow-up Procedures

7.3.1 Overview

At the randomization visit, the Study Coordinator will schedule both the telephone contact and the clinic follow-up visit. The Coordinator will verify that the principal investigator or his/her designee is available to perform the examination. The coordinator will remind the participant that routine follow-up consists of both clinic visits and interim telephone interviews. Routine clinic visits are scheduled every 12 months, allowing ± ten days from the visit due date and **always scheduling from the date of randomization** (not from the date of the last visit). Two to three weeks before a participant's due date for a regularly scheduled clinic visit, the Study Coordinator reminds the participant of his/her clinic visit with a postcard, followed up by a phone call a few days before the scheduled visit. Additionally, the Study Coordinator reminds the participant to bring in all vitamins and medicines on both telephone and postcard reminders.

A telephone interview is conducted between each clinic visit, so alternating clinic visits and telephone contacts take place at 6 month intervals. Every scheduled contact, in clinic or by telephone, includes the Follow-up Contact Form (FUP) and verification of contact information on the Participant Update Form (PUF). Clinic follow-up visits will also need the Follow-up Visit Phlebotomy Collection and Processing and Inventory Forms (PHC and PHP), Medication Listing Form (MSL), Medication Survey Form (MSR) and Vitamin Distribution Log (VDL). The Hospitalization Form (HOS) should be filled out whenever the participant reports he/she is hospitalized for any condition during the follow-up. The Outcomes Documentation Form (OUT) should be completed for all participations who die during the course of the study. Additionally the Informant Interview Form (INF) should be completed whenever an informant is used.

7.3.2 Clinic Follow-up Visit

7.3.2.1 Overview

A summary of data collected during follow-up is provided in Table 7.1. The follow-up visits focus on assessment of vitamin compliance and side effects, and the development of any potential interim arteriosclerotic CVD outcomes, and/or major renal graft dysfunction. Information on participant contact information, consent, and selected risk factors is updated.

7.3.2.2 Modifications to Informed Consent

At any time during follow-up, a participant may modify their Informed Consent. If a participant indicates a change in their consent to participate in the study, complete an Informed Consent Modifications or Withdrawal Form (ICM).

7.3.2.3 Blood Pressure/Height and Weight

Seated blood pressure is taken in the right arm (if possible; otherwise use of left arm is noted), and repeated again during the exam, using the same arm, after at least a 5-minute interval. Height and weight are obtained with the participant in street clothes, and recorded in inches, and pounds.

7.3.2.4 Medication Survey

The interim medication survey includes regularly used prescription drugs only. The initial stage in this process involves filling out a paper-only MSL on which the data collector lists the generic or brand names of each medication individually, as well as the source of this information (bottle label, participant list, or verbal report). Study Coordinators must refer to the recording sheet when transferring this information to the PC-based data entry system, while simultaneously consulting the complete FAVORIT medication thesaurus provided. The order of medication categories, subcategories, and individual medications in the medication thesaurus and on the MSR are identical.

7.3.2.5 Follow-up Contact Form

The interim smoking history related questions focus on current or former smoking, duration/intensity, and length of time since quitting.

Interim diabetes history is documented but does not distinguish between Type I and Type II diabetes. Documentation from the participant's transplant clinic charts supersedes any information provided by verbal report, only. In either case, the source of this information is documented.

Participant compliance to the study vitamins is documented on the FUP. The Coordinator will perform the pill count only during clinic visits. During both clinic visits and telephone interviews the participant is asked to report any adverse reactions to the vitamins.

The questions related to cereal and liquid/powdered dietary supplements are designed to document regular, interim (past 3 months) intake of heavily fortified cereals, and/or liquid/powdered dietary supplements that contain large amounts of folic acid per serving.

The physical activity questions refer to the participant's interim activities during the past month. Activities are graded according to intensity and duration. The Data Coordinating Center (DCC) will tally a physical activity summary score based on these raw data.

7.3.2.6 Venipuncture, Blood Draw

Refer to both the PHC and PHP forms and chapter 6: Specimen Collection and Processing.

7.3.2.7 Vitamin Distribution

Upon completion of the follow-up clinic visit, the participant is provided with a 12-month supply of study tablets. All participants are then scheduled for their next follow-up telephone contact and clinic visit, keeping in mind the target dates are based on the date of randomization.

If a follow-up clinic visit is missed or changed to a later date, vitamins will need to be delivered to the participant through Federal Express or other couriers. Instruct the participant to call the clinic on the day the vitamins are received. At that time the participant will be told to begin taking the study vitamins. Record the “date bottle started” in the comment field of the VDL. This will be needed to calculate compliance at the return visit. Vitamin delivery

must be confirmed: if the participant fails to call, the coordinator will need to call the participant and confirm that s/he received the vitamins.

7.3.3 Telephone Follow-up Interview

Participants are scheduled for a telephone interview six months after their randomization and thereafter between clinic visits until the conclusion of the study. The Study Coordinator will send a reminder postcard to the participant two weeks before the scheduled telephone interview. If that scheduled date and time is not convenient, the Coordinator requests a phone call from the participant to reschedule the telephone interview.
At the prearranged time, the Study Coordinator calls the participant and conducts the interview. The interview consists of administering the Follow-up Contact Form and updating the participant information on the PUF. Upon completion of the telephone follow-up contact, the date of the next clinic follow-up visit is confirmed.

7.3.4 Use of Informant

During the course of the study, it may be necessary to use an informant instead of the participant in order to collect data. For example, if a participant becomes extremely ill or incompetent to respond to interview questions, an informant should be used. Informants are also used in the event of the participant's death or loss to follow-up. An "informant" would be someone who responds on behalf of the participant. The informant must be someone very close to the participant, such as a relative, caretaker, or friend, who is in frequent (e.g., daily) direct contact with the participant. During follow-up, if a participant is contacted but unable to respond, an informant should be used.

It is important to distinguish between data collection from the participant versus from an informant. When the informant is responding for the participant, the Informant Interview Form is completed.

7.4 Changes in Follow-up Status

7.4.1 Telephone Contact Replaces Clinic Visit

If a participant is unable to attend clinic visits due to severe health problems, geographical relocation, or another reason, but agrees to respond to the FAVORIT telephone interview, this information must be indicated on the FUP. This will inform the DCC and the Operations Center that follow-up is continuing, but data forms relating to a clinic visit will not be forthcoming for this contact occasion. The participant should continue to receive his/her study vitamins every 12-months via Federal Express or another courier. If at a later point in time the participant is able to resume clinic visits, record this appropriately on the FUP completed at the clinic visit either by indicating a regularly scheduled clinic visit or a clinic visit replacing a telephone contact.

7.4.2 Informant Response During Telephone Contact

If a participant is unable to communicate clearly due to severe health problems that develop or worsen during follow-up, an informant may be used if they can provide accurate data and are willing to assist the participant (see section 7.3.4).

7.4.3 Clinic Visit Replaces Telephone Contact

Occasionally, it may be appropriate to replace a telephone contact with a clinic visit, for example if a participant has missed previous clinic visits but is now available for a visit. In this situation, the usual follow-up clinic visit forms should be administered, but vitamins should NOT be distributed. **Vitamins should only be dispensed at the annual anniversary of the randomization visit.** Indicate that a clinic visit is replacing the telephone contact on the FUP so the DCC will know what forms are expected for this contact occasion.

7.4.4 Participant Stops Taking Study Vitamins

If a participant decides to stop taking the study vitamins they can and should remain in the study. Always try to convert the participant to resume taking the study vitamins. Continue collecting clinic and telephone visits for participants not taking the study vitamin. If a participant is not willing to come to the clinic then only conduct telephone follow-up contacts. If participants are not willing to participant in telephone contacts then contact the participant, or if unable to directly contact the participant, contact a reliable source every six months using the INF and ascertain at a minimum the participant's mortality status.

7.4.5 Participant transfers from one FAVORIT Site to Another

When a participant moves out of the clinic area, the clinic should notify the DCC. Together the DCC and the clinic should determine if the participant can be transferred to another FAVORIT clinic. If yes, the clinic should complete sections A and B of the Transfer Form (TRN) and mail a package to the new clinic containing a copy of the TRN, copies of all case report forms, medical records and labels to the coordinator at the new clinic. The original site coordinator should also fax a copy of the TRN to the Data Coordinating Center.

The clinic the participant is transferring to should then complete section C of the TRN form once the participant makes his or her first clinic visit or telephone contact. The coordinator at the new clinic should fax a copy of the completed TRN form to the DCC.

Warning: The transition of a participant from one FAVORIT clinic to another is potentially a time when the participant can be lost to follow-up or experience a possible undetected endpoint. Communication lines between the original clinic, new clinic and the participant should remain open and strong. The original clinic is responsible for the participant until he or she makes the first official visit at the new clinic.

7.4.6 Participant is Deceased

When the clinical center staff becomes aware that a participant is deceased, a FUP for the next contact occasion can be completed, even if the target window for that contact is not yet open. The type of contact is "missed" with the reason being that the participant is deceased. Refer to the INF and OUT forms, and to chapter 10: Endpoints for more information.

7.4.7 Dropout and Follow-up to Dropout

7.4.7.1 Definition

A participant is a dropout if the participant is living and is not completing follow-up contacts. Examples of a dropout include a participant who refuses telephone contacts as well as clinic

visits, a participant who has moved and cannot be located, or a participant with a long-term illness preventing his/her participation in a clinic visit or telephone interview who has no informant available.

7.4.7.2 Following the Dropout for Minimum Morbidity/Mortality Information

Contact the participant, or if unable to directly contact the participant, contact a reliable source every six months using the INF and ascertain at a minimum the participant's mortality status. If possible, determine if the participant has been hospitalized. To the fullest degree possible, document possible events and hospitalizations per protocol instructions. Because each participant has already signed a consent form and a medical release form hospital records should be obtainable.

7.5 Attempted Recovery of Dropout

7.5.1 Overview

When applied systematically, dropout recovery methods have been demonstrated in clinical trials to re-engage participants who have become inactive. While not originally conceptualized in this manner, this approach incorporates the use of good reflective-listening and directive skills to elicit barriers to participation from subjects. This information is then used to problem-solve with participants about methods to overcome the identified participation barriers. Finally, an essential component of dropout recovery is the application of motivational interviewing methods in an attempt to further elicit and clarify participants' personal reasons for continued participation.

The approach to dropout recovery will involve the following steps: (1) contact the participant; (2) identify reasons for withdrawal; (3) negotiate solutions to overcome barriers; and (4) apply motivational interview methods. If the coordinator is not successful in re-engaging the participant, then the principal investigator should initiate contact. Contact by a new member of the staff results in new perspectives and is encouraged in dropout recovery.

7.5.2 Contact the Participant

Attempt to contact the participant. When a participant has missed one follow-up clinic visit or phone contact without advance warning, the Study Coordinator should then contact the participant. The Study Coordinator will first attempt to contact the participant directly by phone. If the participant has an unlisted telephone number, then an attempt should be made to contact the informant of the participant. The informant may be asked to have the participant contact the study staff or to find out whether the participant is still in the area and his/her status. The Study Coordinator may also send a letter by certified mail, asking the participant to contact the study staff. Record the results of all attempts to contact the participant either by phone or by mail on a contact log.

Attempt to contact the participant's physician. Record the result of the attempt to contact the participant's personal physician, by letter or by phone, for the participant's current address and/or vital status. Other staff in the physician's office, such as the nurse, may also be asked to provide this information.

Other sources to investigate the participant's whereabouts include participant's employer, internet directories, Social Service agencies, the Department of Motor Vehicles, the Police Department, etc. In each instance record the results of the inquiry.

7.5.3 Identify Reasons for Missed Contacts

7.5.3.1 Participant Is Deceased

Record date of death on the OUT and proceed with obtaining the death certificate and copies of any hospital records related to the death.

7.5.3.2 Participant is Lost to Follow-up

All efforts to locate participant have failed. Record whether participant's vital status is known or unknown and last date of known contact with FAVORIT on the contact log.

7.5.3.3 Participant has Moved Away

The participant has moved away and cannot or will not return for follow-up appointments and any scheduled follow-up visits. If the participant has moved to another FAVORIT center area, it may be possible to keep the participant in the trial for follow-up visits. The DCC should be contacted for instructions on how to transfer participant from one center to another.

7.5.3.4 Participant Refuses to Continue in Study

Identify reasons for withdrawal and identify whether barriers to participation are solvable. It is important to use good reflective-listening and directive skills to elicit barriers to participation from participant. To the extent possible, determine the willingness of participant to work on solutions to overcome barriers to participation. If participant has dropped out of active treatment and is absolutely unwilling to return, negotiate continued participation in telephone follow-up assessment. If the participant cannot be converted to continue telephone contact at a minimum, record participant reasons for withdrawal and date of withdrawal on the ICM form.

7.5.4 Negotiate Solutions to Overcome Barriers

7.5.4.1 Logistical Obstacles

When major barriers to participation involve logistical obstacles, first try to identify alternative strategies that the participant may use to overcome these obstacles. If these alternative strategies fall short or are not available, then offer reasonable logistical assistance provided by study resources.

7.5.4.2 Lack of Motivation

Use motivational interviewing techniques to encourage participants to reconsider their decision to withdraw. Suggest an in-home visit to discuss re-entry into study with the participant. If family members play in role in decision to dropout, then try to schedule a phone call or home visit with family members present.

7.5.4.3 Recurrent Event

If participant is temporarily hospitalized, or otherwise unable to continue follow-up visits because of a recurrent event or other health condition, discuss importance of returning to follow-up after recovery. Conduct weekly follow-up phone calls to determine status of participant.

7.6 Locating Difficult-to-Follow Participants

If the initial call to the number provided by the participant at last contact is unsuccessful, the first telephone tracing step is to contact Directory Assistance in an attempt to verify the address provided and to obtain a new telephone listing. If the address is verified and the phone number is unlisted, send a letter to the participant requesting that the participant telephone the Study Coordinator. If attempts to obtain a listing or verify the address are unsuccessful, secondary sources will be used. Identifying the order of tracing sources for all cases is difficult, though the typical tracing will follow the procedures described below.

The first step is to obtain from the participant's record the contact person (informant) listed by the participant at enrollment and during follow-up contacts. The informants are contacted to determine if they can provide the current address or telephone number of the participant. If successful, the Study Coordinator enters the updated location information on the PUF and attempts to contact the participant.

If these attempts to contact are not successful within two weeks, the Study Coordinator should consider a home visit if possible. If this is not successful, depending on the participant's last residence, a decision is made whether to consult the city directory information or to contact the local post office. If the address is rural, the postmaster is called, since, in rural areas, the postal carrier or postmaster typically knows individuals in their service area. Since address corrections should have been obtained from the mailing of the advance letter, calls are not made to urban post offices.

7.7 How Close to the Next Contact Should a Contact be Scheduled?

The goal for scheduled contacts is the target date ±ten days (target date is determined based on the number of months since randomization). For example, the goal for scheduling a six-month contact is within the period six months ±ten days from the date of randomization. FAVORIT would rather have data outside the time period than no data at all, but it would be a waste of resources to schedule two contacts too close together. Scheduled contacts for randomized participants should be at least 30 days apart. When a participant contact (clinic visit or phone) cannot be scheduled prior to 30 days before the next required contact, then it must be considered skipped (i.e., there will be no data collected for this contact occasion). It is important that we send the participant the study vitamins regardless of whether or not they participate in the scheduled follow-up visit. If the participant misses a scheduled clinic visit the Study Coordinator will mail the study vitamins to the participant either by Federal Express or some other courier. The next contact should then be scheduled, making it a clinic visit if at all possible.

Since the clinic visits gather more information than the telephone contact, it is especially important that these visits not be skipped.

7.8 Follow-up for Dialysis Dependent Participants

7.8.1 Overview

Participants who develop dialysis-dependent end stage renal disease, i.e., expected to be on dialysis for three months or longer or until a transplant is received, prior to experiencing a primary endpoint, will be followed indefinitely until their first primary outcome occurs.

If the participant states he/she is currently on dialysis (during a follow-up contact or any other time) the study coordinator will complete an Initiation of Dialysis Fax Notification Form (DIA). This form includes information regarding the expected duration of dialysis as well as a signature from the site investigator.

7.8.2 Follow-up for dialysis dependent participants

Once it has been established that the participant has developed dialysis-dependent end stage renal disease the clinical sites will be notified as early as possible when a primary outcome has been classified.

Renal transplant recipients who become dialysis-dependent after experiencing a primary outcome will undergo only mortality surveillance sufficient to determine CVD vs. non-CVD death until the end of the study. The study coordinator should contact the participant every six months and complete the Dialysis post-event surveillance form (DPE). The participant will no longer take the study medication once mortality surveillance begins.

7.9 Participant Exit from the Study

7.9.1 Overview

Due to the early close out of the FAVORIT study, exit visits will occur from July 25 to December 31, 2009, capturing hospitalizations and procedures occurring on or before June 24, 2009. The final (exit) visit should take place as a brief/phone contact either over the phone or in the clinic. It is similar to a regularly scheduled brief/telephone follow-up visit (see 7.3.3), with the exception that vitamins are not dispensed and the Exit (EXT) Form is also completed. The Exit Form collects information on the participant's and the coordinator's guess of the participant's treatment assignment. If the participant is a dropout but his/her location is known, a final effort should be made to contact the participant to complete a phone follow-up to capture events that occurred through July 24, 2009.

7.9.2 Completeness of Exit Visit

Once IRB approval is obtained for the close-out/extended contact consent form, the participant notification letters should be sent immediately to each participants. This letter formally notifies participants of the closure of the study and requests that all unused medications be returned (see figure 7.1). You will need to obtain notification that the letters have been received by the participants (i.e., certified mail or UPS).

7.9.2.1. Follow-up visits that occurred between June 25, 2009 and receiving IRB closeout approval

a) Follow-up forms (FUP) completed between June 25, 2009 and when IRB approval is obtained can be used as the participant's final (exit) FUP.
b) update the Participant Update Form (PUF) with current contact information
c) collect vitamins and complete items 6 through 8 on the Vitamin Distribution Form (VDL) for the previous contact
d) complete the Exit Visit Form (EXT), please use the same contact occasion for the EXT form that you used on the FUP
e) complete a Hospitalization Form (HOS) if the participant was hospitalized or had an outpatient procedure, prior to June 25, 2009. Complete an OUT and INF if appropriate
f) mail the participant the new "Closeout" Informed Consent Form
g) enter the signed "Closeout" informed consent information into the new ICM version C
h) approximately April 2010 clinical site coordinators, Investigators or other staff will need to distribute the unblinding letters, results and the participant certification of appreciation

7.9.2.2. Follow-up visits that occur after getting IRB approval

a) complete an FUP

- **If the visit is over the phone** enter either B 'regularly scheduled brief/phone contact' or C 'Brief/phone contact replacing clinic visit' for item 1. Complete the rest of the form as you would for a brief/phone contact.

- **If the visit is being completed in the clinic (in order to track participant travel reimbursement and to track how many exit visits were obtained in the clinic)** you will need to enter FUP item 1 as either A 'regularly scheduled clinic visit' or D 'clinic visit replacing brief/phone contact'. You can set items 5-8 as permanently missing (equal signs ===) as blood pressure, height and weight are not needed for the exit visit. Complete items 9-21, for item 21 'Is this a brief phone contact' enter "Yes". This will skip you to the administrative section of the form

Complete items b-h above (section 7.9.2.1).

7.9.2.3. Surveillance Only Participants

For participants who are being followed for surveillance only, you will need to complete a Dialysis Post-Event Surveillance form (DPE) and items b and d-h above (section 7.9.2.1).

7.9.2.4. No Extended Follow-up

For all participants who did not agree for extended follow-up, please be sure to that they have completed an Exit Visit Form (EXT) and are offered the opportunity to sign the new closure consent.

7.9.3 Recognition of Participation in the Study and Study Results

In spring 2010 the participant will be mailed a certificate of appreciation (see figure 7.2) for his or her commitment to the FAVORIT study. If consent is provided, participants will be informed by the clinical site, DCC or Operations center of the results of the study in writing after the final results are available but before they have been published. Participants will also be unblinded to their treatment at this time. Each clinical site is responsible for identifying the staff person who will be responsible for distribution these materials and responding to participant queries.

Table 7.1 Summary of Data Collection During Scheduled Follow-up

DATA COLLECTIONFORMS†	Telephone/Brief Contact†	Clinic Contact†	Exit Contact††
Contact Occasion	**02**	**03**	**last contact**
Follow-Up Contact Form (FUP)	X	X	X
Participant Update Form (PUF)	X	X	X
Medication Listing Form (MSL)		X	
Medication Survey Form (MSR)		X	
Vitamin Distribution Log (VDL)		X	X*
Randomization/Follow-Up Visit Phlebotomy Collection Form (PHC)		X	
Randomization/Follow-Up Visit Phlebotomy Processing and Inventory Form (PHP)		X	
Exit Form (EXT)			X
Informed Consent Modifications or Withdrawal Form (ICM)			X
Schedule Follow-up Telephone Interview and Follow-up Clinic Visit	X	X	

† Informed Consent Modifications or Withdrawal Form (ICM), Hospitalization Form (HOS), Outcomes Documentation Form (OUT) and the Informant Interview Form (INF) use when needed.

†† Hospitalization Form (HOS), Outcomes Documentation Form (OUT) and the Informant Interview Form (INF) use when needed.

* Compliance only (no vitamins are distributed at this visit).

Figure 7.1

August 20, 2009

[PARTICIPANT NAME]
[ADDRESS LINE 1]
[ADDRESS LINE 2]
[CITY, STATE, ZIP]

Dear [MR/MS & PARTICIPANT'S LAST NAME HERE]:

Greetings from your FAVORIT clinic! You have been a valuable part of the Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) study over the past years, and we are writing to let you know that the study has reached an early conclusion.

Some time during the next [X] months, we will be contacting you for a final study interview. We also want your permission to contact you in the future so we can share the results of this important study, once they become available next Spring. At that time, we will tell you whether you were given the high dose or the low dose multivitamin. We need to update your address and all contact information to make sure you receive this information. Also, if we are funded for additional follow-up or a related new study, we would like your permission to contact you about this.

Since the study is closing, it is important that you stop taking the study vitamins and return all FAVORIT bottles and unused vitamins to us. *There are no safety issues with the FAVORIT multivitamin.* Enclosed is a prepaid addressed mailer that you can drop in the mail with your returned bottles and vitamins. You can also return the signed form indicating your agreement with future contact after the Exit interview, or you can indicate that you prefer no further contact.

We extend our sincere thanks to you for your dedicated participation in the study, and we will be glad to answer any questions you might have. It is important to remember there are no safety concerns with either dose of FAVORIT multivitamin.

We thank you again for your participation in FAVORIT and hope you will continue to support clinical research studies. The information learned in FAVORIT is very important for understanding cardiovascular disease risk in people who receive kidney transplants and for future clinical research.

Sincerely,

[LOCAL PI'S NAME] [Coordinator's name]
(TYPED Name of Local PI)

Enclosure(s)

Figure 7.2


FAVORIT
FOLIC ACID FOR VASCULAR OUTCOME REDUCTION IN TRANSPLANTATION
NATIONAL INSTITUTES OF HEALTH
Certificate of Appreciation
On behalf of the National Institutes of Health and the FAVORIT Clinical Trial, we are pleased to present this certificate in recognition of your dedicated and valued participation toward the successful completion of the FAVORIT study
<insert name>
John Kusek, PhD
Senior Scientific Advisor
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
, MD
Co-Principal Investigator
Local Hospital
Andrew Bostom, MD
Principal Investigator
The FAVORIT Project

Chapter 8. Compliance

8.1 Overview

The power of the study to show an effect of treatment (if one exists) will be reduced if participants do not take their prescribed vitamins according to protocol. To ensure that the resources spent and the efforts of Study Coordinators are maximized, it is desirable to minimize non-compliance. Compliance will be monitored throughout the trial. Adherence to the study protocol and follow-up procedures will be examined through data reports and monitoring visits. Participant compliance with taking the study vitamin will be assessed both by pill count and by folate levels analyzed centrally. Through dietary assessment, the study will monitor participants' food intake to determine additional nutritional intakes of folate.

8.2 Follow-up Visits and Telephone Contacts

Reports describing adherence to the study visit schedule as well as to the study vitamin, clinic-specific and overall, will be distributed to the Principal Investigators (PIs), Study Coordinators, Operations Center, the Executive Committee, and the Data and Safety Monitoring Board. Steps will be taken within the trial structure to assist a clinic with lagging performance. Reasons for missed visits and phone calls should be noted on the Record of Contacts Form at each site. Each contact is to be scheduled within ten days of the target date for that contact, and all attempts should be made to complete a contact within the window. However, to avoid missing data altogether, a contact can be conducted outside of this window provided that it does not fall too close to the next scheduled contact. Refer to the Follow-up chapter for a detailed discussion of visit scheduling.

8.3 Pill Count

Tablet counts will be recorded on the Follow-Up Contact (FUP) form. Non-compliance with study vitamins, operationally defined as < 75% compliant by pill count, will be tracked site by site, and routinely reported to the Executive Committee. Periodic reports will also be distributed to the PIs, Study Coordinators, and Data and Safety Monitoring Board. Study Coordinators will encourage non-compliant participants to improve their compliance by reminding them of the instructions to take one tablet daily, and focusing, for example, on synchronizing study vitamin intake with their usual immunosuppressive drug intake (drugs which renal transplant recipients typically take with a high consistency). The clinic physician should also be involved in measures to increase adherence. However, even at higher levels of adherence (say 80% or even 90%), every effort should be made to improve them until they reach 100%. When participants do not take several consecutive days of vitamins, efforts must be made to get participants back to the protocol schedule of taking one a day. If a consistent pattern of non-adherence develops, the coordinator should alert the study physician who should use this information at a future visit to explore concealed adherence problems.

Because statistical analysis will be based upon intention to treat, non-compliant participants will be encouraged to adhere to protocol schedule but will not be removed from the study. We assume that non-compliance will occur equally in both treatment groups. Participants will continue to be seen in clinic every year and telephoned between clinic visits.

8.3.1 Clinic Visit Procedures

At each clinic visit, the participant will bring the vitamin bottles dispensed at the previous visit. The Study Coordinator will record the number of bottles the participant brought to the clinic as well as the total number of bottles dispensed to the participant (this information can be found on the Vitamin Distribution Log). The Study Coordinator will record the number of remaining pills, including pills in any unopened bottles. Returned bottles containing pills will be retained by the Study Coordinator, and will be handled according to directions provided by the Data Coordinating Center (DCC) and Vitamin Distribution Center (VDC).

8.3.2 Phone Contact Procedures

At telephone contacts, the Study Coordinator will ask the participant to count the tablets remaining in any open vitamin bottles which were dispensed at the previous visit. The Study Coordinator will also ask the participant for the number of unopened vitamin bottles the participant has. At which point, the Study Coordinator will record the total number of pills, which includes both the number of tablets in opened bottles, and the tablets in unopened bottles.

Pill counts at the 6-month telephone contacts **are necessary**. Remind participants at the reminder call/postcard that they will be asked to count their remaining pills during the 6-month telephone contact.

8.4 Analysis of Folate

In addition to using pill count to determine a measure of compliance, plasma will be drawn at the yearly clinic visits and sent to the Central Lab for blind analysis of folate, vitamin B_{12} and PLP levels. Results will be available only to the DCC.

Because the plasma homocysteine levels and folate results could unblind the investigators, individual data will be restricted to the DCC and Data and Safety Monitoring Board. Group data on the efficacy of the treatment to lower homocysteine will be made available to the Executive Committee, if approved by the Data and Safety Monitoring Board. Discrepancies between pill count and folate laboratory results will be monitored by the DCC.

8.5 Dietary Assessment

It is very important to ascertain the baseline level of consumption of the nutrients included in the intervention. This is particularly important for FAVORIT since variations in dietary folate and vitamin B_6 and B_{12} can have a marked effect on homocysteine levels and thereby may influence cardiovascular risk. The Study Coordinators will question the participants on

their intake of folate-containing cereals and grains and supplements at randomization and at all follow-up clinic visits.

Chapter 9. Adverse Events

9.1 Overview

Because vitamins occur naturally in foods, and we have found no placebo-controlled evidence of adverse reaction of the doses comparable to or greater than those contained in the study medications when given to either maintenance dialysis or renal transplant patients, it is very unlikely that true adverse effects from the study medication will occur.

During follow-up telephone and clinic visits, study participants will be asked to report possible adverse reactions to the vitamins. All reported side effects are recorded. Symptoms suggesting severe allergy are grounds for discontinuation of treatment. Mild gastrointestinal distress, presumably representing placebo effect, will be discussed with the participant. At each contact the participant will be asked about these possible adverse study vitamin reactions, and responses will be recorded on the appropriate forms. Participants required to discontinue study vitamin treatment or refusing to take it will not be removed from the study. Investigators will attempt to re-institute study vitamins in participants who choose to discontinue them.

9.2 Side Effects and Adverse Event Monitoring and Review

9.2.1 Side Effects and Adverse Event Monitoring

The study will routinely collect information on all reported side effects of the study medication, all hospitalizations, and all deaths. All possible side effects reported by the participant are recorded on the Follow-up Contact Form. All hospitalization discharge diagnoses and codes are reported on the Hospitalization Form. Information on all deaths is collected through the Outcomes Documentation Form, with supplemental information coming from the Hospitalization and Informant Interview Forms, when appropriate.

9.2.2 Review of Reported Side Effects and Adverse Events

This information will be reported to the FAVORIT Data and Safety Monitoring Board (DSMB) on a regular basis (as requested by the DSMB, and at least annually). The DSMB will review this information both in aggregate and by blinded treatment assignment.

9.3 FDA Reporting Requirements

Based on the FAVORIT IND approval, the study is required to report the following to the Food and Drug Administration:

1. Any unexpected fatal or life-threatening adverse experience **associated with the use of the drug**,
2. Any adverse experience **associated with the use of the drug** that is both serious and unexpected.

Dr. John Kusek of the NIDDK, as sponsor of the IND, will handle this reporting. Refer to section 9.3.2 for reporting procedures for the clinical sites and DCC.

9.3.1 Definitions Related to Serious Adverse Experiences

FDA 21CFR312.32 defines **associated with the use of the drug** as "There is a reasonable possibility that the experience may have been caused by the drug."

An **unexpected adverse drug experience** is defined as (FDA 21CFR312.32) "Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure only referred to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure only listed cerebral vascular accidents. 'Unexpected', as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product."

A **life-threatening adverse drug experience** is defined as (FDA 21CFR312.32) "Any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death."

A **serious adverse drug experience** is defined as (FDA 21CFR312.32) "Any adverse drug experience occurring at any dose that results in any of the following outcomes: Death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse."

9.3.2 Reporting Process for Serious Adverse Experiences (SAE)

Any adverse experience **associated with the use of the multivitamin** that is both serious **and** unexpected must be reported immediately to the Data Coordinating Center (DCC) by submitting a Death or Serious Adverse Event Fax Notification (DSA) and an Adverse Drug Reaction Report (FDA Form 3500A). These forms and instructions for completing them can be found in the forms section of the FAVORIT Manual of Procedures. The two forms should be completed and faxed to the DCC at (919)962-3265 (attention to Myra A. Carpenter and Lisa Gravens-Mueller). The DSA should be faxed immediately; FDA Form 3500A must be

faxed within one working day of identifying an SAE. Also, send an e-mail alerting the DCC to the forthcoming fax. An acknowledgement from the DCC will be provided within one working day of receipt. If acknowledgement is not received, contact the DCC by phone.

Within two working days of receipt at the DCC of FDA Form 3500A, a letter revealing the participant's study vitamin dose will be attached, and the packet (FDA Form 3500A plus unmasking letter) will be submitted to Dr. John Kusek at NIDDK. As the IND sponsor, Dr. Kusek will decide whether to forward the information to the FDA (through submitting an IND safety report), the FAVORIT DSMB, and all study investigators (masked safety report). Although the FDA and DSMB will be notified of the study dose, study investigators will **not** be unblinded to the dose (high or low). NIDDK and/or the Data and Safety Monitoring Board will have three working days to contact the DCC staff with any recommendations. These recommendations will be acted upon by the DCC the same or next working day.

The timetable for reporting experiences that will be submitted to the FDA is as follows:

- As soon as possible and within one working day of identifying a possible serious adverse event (SAE), the Principal Investigator will submit a DSA and FDA Form 3500A to the DCC by fax (with e-mail notification).
- Upon receipt of faxed forms, DCC will send acknowledgement to the form originator.
- Within two working days of receipt of the faxed forms, the DCC will submit SAE packet to the NIDDK IND Sponsor.
- As soon as possible and within 7 calendar days after the IND Sponsor's initial receipt of information regarding unexpected fatal or life-threatening adverse experience associated with the use of the vitamin, the Sponsor will submit a telephone or fax notification to the FDA, FAVORIT DSMB, and study investigators.
- As soon as possible and within 15 calendar days after the IND Sponsor's initial receipt of information regarding any adverse experience associated with the vitamin that is both serious and unexpected, the Sponsor will submit a written IND safety report to the FDA, FAVORIT DSMB, and study investigators.
- NIDDK and the FAVORIT DSMB will have 3 working days to notify the DCC with any recommendations. These recommendations will be acted upon at the DCC by the next working day following receipt.

The clinical site reporting a serious adverse experience is responsible for reporting to their local IRB in accordance with the requirements set forth by that IRB. Notification of SAEs reported by other sites is discussed above.