

## APPENDIX F

### LABORATORY PROCEDURES

#### **SPECIMEN COLLECTION PROCEDURES**

The following five procedures, with the exception of the Four-Glass Test, will be performed only during the screening phase of the study. The Four-Glass Test will be repeated for microscopy study only at the brief clinic visit, and for culture and microscopy at the extensive clinic visit. *It is mandatory that the following procedures be performed using sterile technique.*

#### ***FOUR GLASS TEST PROCEDURE***

The four glass urine test will be performed by the physician or designated health care professional.

#### **Equipment:**

Non-sterile gloves, sterile urine cup, microscope, Colombia agar with 5% sheep blood, 10 µl loop.

#### **Procedure:**

*Important:* The patient should be instructed to be abstinent (no ejaculation) for the two (2) days before the four-glass test procedure.

1. Uncircumcised men should retract their foreskin.
2. The head of the penis should be cleansed with an alcohol pad.
3. The patient should void approximately 10ml (VB1) directly into a sterile container.
  - A) A portion of this specimen (5ml) should be transferred into a test tube, centrifuged and examined as described below (see microscopy methods).
  - B) The remaining portion should be sent to the laboratory for culture (see culture methods).
4. The mid-stream sample (VB2) should be collected. This is done by having the patient place the sterile container into his urine stream while he is voiding and at least 5ml (Range 5-200ml) should be collected.
  - A) A portion should be sent to the laboratory for culture (see culture methods).

- B) A portion of this specimen should be tested with a dipstick for pH, glucose, and protein. Record results on the case report form.
- C) A portion of the specimen should be centrifuged (5 mls) and examined as described below (see microscopy methods).
5. EPS (Expressed Prostatic Secretion) – during/after prostatic massage collect the EPS on a sterile surface (example: lid of a sterile urine cup).
- A) The volume should be estimated in drops. About 5 $\mu$ L should be examined at 400X power and WBCs, and RBCs recorded per HPF. Note yeast as present or absent.
- B) Using a 10 $\mu$ L sterile, disposable loop spread the EPS onto Columbia agar plus 5% sheep blood and incubate at 37°C for 5 days. Record results at 48 hours and 5 days. Isolate colonies and send to laboratory for identification.
- Interpretation:            1 colony = 100 CFU/ml  
                                  10 colonies = 1000 CFU/ml  
                                  100 colonies = 10,000 CFU/ml
- C) Any remaining volume of EPS should be restored in cryovials and snap frozen in liquid nitrogen for storage later at -70°C.
6. Collect the first 10ml of post massage urine (VB3) within 30 minutes (Range 5-30ml).
- A) A portion of the specimen should be sent to the laboratory for culture (see culture methods).
- B) A portion of the specimen should be centrifuged (5 mls) and examined as described below (see Microscopy Method I).

## **ALTERNATIVE MICROSCOPY AND CULTURE METHODS**

### **Method I. VB1, VB2, and VB3 Microscopy**

#### **Procedure:**

1. Centrifuge 5 mls. of urine for 5 minutes at maximum table top speed (1500 rpm).
2. Pour off supernatant, break up pellet using a disposable pipette and place a drop onto a clean microscope slide, and cover with a cover slip.
3. Examine using a microscope at 400X power.

4. Count WBC, RBC, and yeast (average of three fields rounded to nearest whole number). Record results on the case report form.

## **Method II. VB1, VB2, and VB3 Culture**

A minimum of 1.0 ml of urine is generally required for hospital laboratories for microbiological culture. Each hospital laboratory should have a Standard Operating Procedure (SOP) and these should be kept on file in each Clinical Center (and at the DCC) for examination. For those Clinical Centers that do their own culture, the following is a standard microbiological procedure for urine culture.

### **Procedure:**

1. Pipette 100  $\mu$ l of urine into a sterile test tube containing 0.9 ml of sterile phosphate buffered (pH 7.2) saline (PBS) and another 100  $\mu$ l directly onto a Colombia agar with 5% sheep blood.
2. Spread the plate using a sterile loop (or a sterile plate spreader).
3. Dip a sterile 10  $\mu$ l loop into the diluted urine and plate onto Colombia agar with 5% sheep blood.  
Interpretation:  

|                      |                                 |
|----------------------|---------------------------------|
| ➤ 100 $\mu$ l plate: | 1 colony = 10 CFU/ml            |
|                      | 10 colonies = 100 CFU/ml        |
|                      | 100 colonies = 1000 CFU/ml      |
| ➤ 10 $\mu$ l plate:  | 1 colony = 1000 CFU/ml          |
|                      | 10 colonies = 10,000 CFU/ml     |
|                      | 100 colonies = 100,000 CFU/ml   |
|                      | >100 colonies = >100,000 CFU/ml |
4. Isolated colonies are sent to a laboratory for identification using standard urine culture identification techniques.

## APPENDIX G

### LABORATORY PROCEDURES

#### ***SEMEN SAMPLE PROCEDURE***

The physician or designated health care professional will instruct the patient.

#### **Equipment:**

Sterile specimen cup, sterile pipette, sterile cryovials.

#### **Procedure:**

1. The patient should be instructed to be abstinent (no ejaculation) for the two (2) days before the semen sample collection.
2. Patient should wipe the glans penis with an alcohol prep. The patient should be instructed to avoid touching the inside of the container with the penis.
3. The sample is produced by masturbation into a sterile specimen container.
4. After the sample is obtained the lid is replaced on the container.
5. The specimen is kept at ambient temperature for 30 minutes.
6. At the end of the 30 minute interval an aliquot of the whole semen is submitted for culture (see VB1, VB2, VB3 Culture above).
7. After the aliquot is submitted for culture the remaining semen is pipetted with a sterile, disposable pipette into a sterile 15 ml conical centrifuge tube.
8. The semen is centrifuged in a standard table top laboratory centrifuge for 10 min at 1500 RPM. This translates into 450-500g with a typical rotor diameter.
9. The supernatant (seminal plasma) is aspirated with a sterile pipette and placed in 0.5 ml aliquots into sterile cryovials labeled with the patient ID number, date and labeled "Seminal plasma." The 1.8 ml Nunc vials are ideal.
10. The samples are placed in a freezer and stored at  $-70^{\circ}\text{C}$ . Record the number of vials.
11. Specimens collected at home must be transported on ice to the clinical center immediately.

#### **Peroxidase Staining Procedure:**

Prepare Benzidine-Cyanosine stock solution:

1. Dissolve 62 mg Benzidine (Sigma catalog# B3383) in 24 ml Methanol.
2. Add 75mg Cyanosine. ( Phloxine B, Sigma catalog # P2759)
3. Add 26 ml boiling deionized or triple distilled  $\text{H}_2\text{O}$ . (Solution will precipitate if room temperature water is used).

4. Store in brown bottle (solution is light sensitive) at room temperature for less than 1 year.

Immediately prior to counting sample, make Benzidine-Cyanosine working solution:

1. Combine 1 ml Benzidine-Cyanosine stock solution and 4 uL 3% H<sub>2</sub>O<sub>2</sub>.
2. Mix semen and Benzidine-Cyanosine working solution in 1:1 ratio: 20uL semen with 20uL working solution.
3. Place 20uL of combination on microscope slide and coverslip.
4. Scan slide under microscope at low power to assure even distribution of cells.
5. Count number of WBC per 5 high power fields (400X) after 5-10 minutes. WBC will be stained brown. Round cells which are not WBC (immature spermatids) will not be stained brown. The background will be pink. There may also be bubbles as a result of the H<sub>2</sub>O<sub>2</sub>.

### ***SERUM SAMPLE PROCEDURE***

To be performed by medical technologist or appropriate health care professional.

#### **Equipment:**

Tourniquet, Vacutainer apparatus, centrifuge, cryovials.

#### **Procedure:**

1. Place patient in a comfortable seated position providing a surface on which to rest patient's arm at approximately the level of the heart.
2. Place a tourniquet around the upper arm and ask patient to open and close fist several times to accentuate venous filling.
3. After identifying a suitable vein for venipuncture cleanse the skin surface several times with an alcohol prep swab.
4. Using the Vacutainer (Beckton-Dickinson) needle apparatus, insert the needle bevel edge up in to the vein at approximately a 45 degree angle. Use caution in inserting the needle so as to avoid going through the back wall of the vessel.
5. Once needle is in the lumen of the vein, engage Vacutainer collection tube into the needle apparatus and once blood starts to flow into the tube remove the tourniquet with your free hand. Use Becton Dickinson SST brand serum separation tubes –

“tiger top” - red/gray silicone lubricated rubber stoppers, 9.5 ml draw containing inert barrier material and clot activator on interior walls (Becton Dickinson reorder number 6510).

6. After blood has filled the tube, completely remove the needle from the vein and apply a 2" X 2" gauze pad over the puncture site and ask the patient to apply pressure with the opposite hand. Discard the needle into an appropriately labeled biohazard sharps container. After homeostasis has been achieved, place a Band-Aid over the venipuncture site.
7. After collection gently invert tube 5 times and allow to clot for 30 minutes at room temperature.
8. Centrifuge at 1,000 - 1,300 X G for 10 minutes to separate serum (top layer) from clotted material.
9. Remove rubber stopper and pipette 0.5 ml aliquots of serum into 4-6 cryovials.
10. Label vials and store in ultra cold freezer (-70°C).

### ***URETHRAL SWAB PROCEDURE***

This test will be performed by the urologist or designated health care professional.

#### **Equipment:**

Alcohol prep, cotton tipped or calcium alginate swab, Colombia agar with 5% Sheep Blood plate.

#### **Procedure:**

1. Preparation of the penis:
  - have the patient lying supine and retract the foreskin, if present.
  - wipe glans with an alcohol swab.

2. Collection of specimen:

- remove sterile swab from culture collection kit, taking care not to touch it to any surface.
- holding penis at the base of the glans with the left hand, use right hand to insert cotton swab into urethra approximately 1-2 inches and slowly remove with a swirling motion.
- immediately plate swab onto Colombia media with 5% sheep blood.

3. Culture report:

- report presence and type of bacteria at 5 days.

***UROFLOW STUDY PROCEDURE***

This test will be performed by the urologist or designated health care professional.

**Equipment:**

Urodynamic monitor, portable ultrasound machine.

**Procedure:**

1. On arrival, the patients should be asked to drink water or other fluids: the men should be instructed to drink enough fluids to give the patient a feeling of bladder fullness (up to a point that the men would normally void). The patient's bladder should not be uncomfortably full. At this point, it is important to perform the uroflow study without delay.
2. Any of the standard uroflow machines may be used. The results must include:
  - 1) total voided volume (in cc's),
  - 2) maximum void velocity (cc's / sec),
  - 3) average void velocity (cc's / s)
  - 4) flow strip.

The uroflow machine's accuracy and internal variability should be provided by the company and documented for study purposes.

3. The uroflow procedure itself should be performed in the standard fashion. Either sitting or standing (whichever technique the man normally uses) the man should void to completion into the uroflow container. This should mimic the man's normal voiding pattern as closely as possible. If there is less than 150 cc's of voided urine

or the patient states that the stream was not typical for his voiding pattern, the study should be repeated.

4. Post-uflow, an abdominal ultrasound will be performed to measure post-void residual, which will be reported in cc's. Regular ultrasound machines with curvilinear or linear arrays or ultrasound units specifically designed to measure bladder volume may be used. Because of the variety of types of ultrasounds which will be available to the researchers, it is critical that each center determine and document the accuracy and variability of their particular ultrasound's determination of post-void residual.

#### **SPECIMEN LABELING AND STORAGE**

Specimens will be stored at each clinical center as described in the specific procedures above. Every specimen should be labeled with the following information: CPC Study, date of procedure, patient initials, and patient ID. Specimens are not to be used for any purpose other than CPC Study research purposes. Access to study specimens will be controlled by a sub-group of the PP&AS Committee, entitled the Committee on Access to CPC Study Data and Patient Specimens.