DAISY

Islet Autoantibody Testing

Measurement of islet autoantibodies

Assays 1994-2010: Blood samples obtained by venipuncture or a finger stick will be used. We are currently using radio-binding assays for insulin, GAD_{65}, and ICA512 (IA-2) autoantibodies, IAA, GAA, and ICA512AA respectively. IAA are measured in a standard radioimmunoassay[145] incorporating competition with unlabeled insulin and precipitation with polyethylene glycol and in a new microassay. The former requires 600 μl of serum (150 μl duplicates with and without unlabeled insulin). The inter-assay coefficient of variation is 10.3% at low positive levels[145]. In the most recent proficiency testing this assay gave 100% sensitivity and specificity. The microassay, based on protein A/G precipitation, requires 5 μl of serum and appears to be more specific than the large volume assay. GAA are measured in triplicate using a modification of the radio-binding assay with in vitro transcribed and translated human GAD65 and precipitation with protein A-sepharose[146]. The results are expressed as an index: GAA index = (sample cpm - negative control cpm) / (positive control cpm - negative control cpm). The inter-assay coefficient of variation is 10.7% (n=24). The Immunology of Diabetes Society Workshop (IDS1995) the assay gave 82% sensitivity and 99% specificity using sera from new onset diabetic patients aged less than 30 years. The ICA512 (IA-2) autoantibodies are measured in a system similar to the GAA assay with the recombinant human ICA512 protein transcribed and translated in vitro with ^{35}S labelling[147]. The most recent modification of the assay utilizes clone consisting of amino acids 256 to 979 of human ICA512. The inter-assay coefficient of variation is 9.6% (n=12). In IDS1995 the assay gave 73% sensitivity and 100% specificity. Over 90% newly diagnosed T1D patients and ICA positive relatives followed to diabetes[33-36] are positive for either GAD_{65} or ICA512 using the combined assay. The GAA and ICA512AA are measured in a combined assay using ^{3}H-labelled GAD_{65} and ^{35}S-labelled ICA512 autoantigens and Packard Top beta-counter with protein A sepharose and 96 well filtration.

Quality control All samples with GAA or ICA512AA levels exceeding the 99th percentile of the distributions in 198 unrelated non-diabetic persons ages 0.4 - 67.5 years (i.e., GAA >0.032 and ICA512AA>0.16, respectively) and a random 10% of the remaining samples are blindly retested for quality assurance. The IAA testing were repeated if the levels are above 79 nU/ml, rather than above the 99th percentile of non-diabetic distribution (42 nU/ml) because of the large serum required relative to that obtained in these young children. With development of IAA microassay, all positive samples (index >0.01) are re-tested using blinded duplicates.

2010-Harmonized Assays for GAD65 and IA-2 (ICA512): Labs from the Type 1 Diabetes Genetics Consortium compared results from samples included in the Diabetes autoantibody Standardization Program and developed standard methods for reporting in WHO units/ml(1).

The cutoff of 20 DK units/ml of GADA65 antibody was established as the 98th percentile of 500 healthy control subjects. A receiver operating characteristic (ROC) curve among the newly diagnosed (within 2 weeks) patients with diabetes (n=50). The cutoff of 20 DK units corresponded to 86% sensitivity and 98% specificity.

The cutoff of 5 DK units/ml of IA-2 antibody was established as the 99.4th percentile of 500 healthy control subjects. A receiver operating characteristic (ROC) curve among the newly diagnosed (within 2 weeks) patients with diabetes (n=50). The cutoff of 5 DK units corresponded to 64% sensitivity and 99.4% specificity.

QA: All samples are run in triplicate and assays are run in duplicate, along with three standard samples (1x high positive, 1x low positive, 1x negative control serum samples). Positive samples, above the 99th percentile index, are repeated, in duplicate in a separate assay. A third assay will be implemented if the 1st and 2nd results do not agree. The result reported is the mean value of the two assays in agreement.

ZnT8 antibody 2011: This assay was developed in the lab of John Hutton(2) and implemented in 2011. The assay is not a standard procedure for all participants at every visit. If a participant is currently, or newly positive for GADA, IAA, and/or IA-2A, then the ZnT8 assay is performed on the repeated assay and for subsequent visits for those who confirm positive for any islet autoantibodies. To identify any participants who may be positive for ZnT8 but negative for other islet autoantibodies, all enrolled participants who completed a clinic visit and blood draw during the year of 2012 were tested for ZnT8A. In addition, participants who had previously tested positive for any islet autoantibodies, retrospective analysis of ZnT8A was completed for multiple previous time points, if serum was available, to determine earlier presence of ZnT8A.

The ZnT8A assay is a radio-binding assay, similar to GAA and IA-2A. The cutoff index is 0.02, which corresponds to 60% sensitivity and 99% specificity.

1) Measurement of islet cell antibodies in the Type 1 Diabetes Genetics Consortium: efforts to harmonize procedures among the laboratories. Clin Trials. 2010 Aut;7(1_supplement): S56-64. doi: 10.1177/1740774510373496.

2) Diabetes antibody standardization program: first proficiency evaluation of assays for autoantibodies to zinc transporter 8. Lampasona V, Schlosser M, Mueller PW, Williams AJ, Wenzlau JM, Hutton JC, Achenbach P. Clin Chem. 2011 Dec;57(12):1693-702. doi: 10.13733/clinchem.2011.170662. PMID: 21980171.