

### **GAD65 Autoantibody Radioimmunoassay for HABC Ancillary Study AS04-51**

For this ancillary study, available stored sera from all Health ABC participants with self-reported physician diagnosed and undiagnosed diabetes, were tested for autoantibodies against GAD<sub>65</sub>. For diabetic participants identified at baseline and follow-up, year 1 and year 6 (or last available follow-up serum sample) sera were used for GAD65 autoantibody testing.

Blood specimens stored as part of Health ABC were used in the following assay. All specimens were required to have been stored at -70° C and have been through no more than two-freeze-thaw cycles. The autoantibody assays were performed in the Laboratory of Immunogenetics at the Brehm Center for Type 1 Diabetes and Analysis, Internal Medicine and Immunology, University of Michigan Medical School, Ann Arbor, MI. All samples assayed for autoantibodies against GAD65 were detected in triplicate using *in vitro* transcribed/translated <sup>35</sup>S-[Met]-labeled recombinant human GAD65, as originally described (1,2). The GAD65 construct used for this study was kindly donated by Dr. Åke Lernmark. The results are expressed as an index (index = sample cpm - negative control cpm/positive control cpm - negative control cpm) as previously reported (3). The cut-off point for the assay was established as the 99th percentile of autoantibody values calculated using 280 control subjects for the radioimmunoassays and corresponded to 0.069 for GAD65 AA. The inter-assay coefficient of variation (CV) was 13.2% (n=7), and the intra-assay CV was 12.2% (n=11) as previously reported (3). In house laboratory thresholds for positivity gave excellent performance in multiple proficiency workshops. Proficiency workshop results organized by the University of Florida, Gainesville (1995, 1996 and 1997), and the Diabetes Autoantibody Standardization Program (DASP, 2000, 2003, 2005, 2007), organized by WHO were as follows: 76-100% sensitivity, 90-100% specificity (100% specificity 3 times), and 100% validity for GAD65 autoantibodies; 48-84% sensitivity, 98-100% specificity, 87.5% validity and 91.6% consistency in the 1996, 2000 and 2003, 2005 and 2007 (4).

### **References**

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## Health ABC Ancillary Study 04-51 Data Documentation

**AS Title:** Islet Cell Autoimmunity and B-cell Deterioration in a Biracial Elderly Cohort with Diabetes

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**Data File Name:** 1. AS04-51\_HABC\_AS\_GAD\_data\_2009\_12.sas7bdat

**Laboratory Assessment:** Autoantibody to GAD65

**Component Description** Health ABC stored sera were used to identify individuals at risk for developing autoimmune diabetes mellitus in adulthood. Measurements were made of autoantibodies (AA) to glutamic acid decarboxylase (GAD65) in baseline and follow-up sera from Health ABC participants

**Eligible Sample** In summary, participants classified as having diabetes at baseline and follow-up and a sample of non-diabetic controls were tested. Specifically the following group specimens were selected for GAD65AA testing.

- 1) all prevalent diabetics based on reported history of diabetes (treated and untreated)
- 2) all new diabetics at baseline based on fasting and two-hour glucose measurements
- 3) all new diabetics at follow-up based on history, diabetes medication and fasting and two-hour glucose
- 4) a sample of non-diabetic normal glucose tolerant (at baseline and follow-up) controls frequency matched to the diabetics on age, gender, race, clinic site and duration in the study

**Description of Laboratory Methodology** Autoantibodies to GAD 65 were detected in triplicate by immunobinding of serum with in vitro transcribed/translated recombinant <sup>35</sup>S-[Met]-labeled recombinant human GAD65. The results are reported as an index based on the counts per minute of the positive and negative control serum used in each plate (index=sample cpm-negative control cpm/positive control cpm-negative control cpm) (1).

**Laboratory Quality Control** The inter-assay coefficient of variation (CV) was 13.2% (n=7) and the intra-assay CV was 12.2% (n=11) (2). In house laboratory thresholds for

**and Monitoring** positivity gave excellent performance in multiple proficiency workshops. Proficiency workshop results organized by the University of Florida, Gainesville (1995, 1996 and 1997), and the Diabetes Autoantibody Standardization Program (DASP, 2000, 2003, 2005, 2007), organized by WHO were as follows: 76-100% sensitivity, 90-100% specificity (100% specificity 3 times), and 100% validity for GAD65 autoantibodies; 48-84% sensitivity, 98-100% specificity, 87.5% validity and 91.6% consistency in the 1996, 2000 and 2003, 2005 and 2007 (3).

**Analytic Notes** The cut-off point for positivity for the GAD65AA assay was an index of 0.069 (4) and was established as the 99th percentile of autoantibody values calculated using 280 healthy control subjects, which have been described previously (2). Samples with values below this index are coded as being GAD65 AA negative.

- References**
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  2. Pietropaolo, M., Peakman, M., Pietropaolo, S.L., Zanone, M.M., Foley, T.P., Becker, D.J., and Trucco, M. Combined analysis of GAD65 and ICA512(IA-2) autoantibodies in organ and non-organ specific autoimmune diseases confers high specificity for insulin-dependent diabetes mellitus. *J Autoimmun* 11:1-10, 1998.
  3. Bingley PJ, Bonifacio E, Mueller PW, and Participating Laboratories 2003 Diabetes Antibody Standardization Program: First Assay Proficiency Evaluation. *Diabetes* 52:1128-1136, 2003.
  4. Barinas-Mitchell E, Kuller LH, Pietropaolo S, et al. The prevalence of the 65-kilodalton isoform of glutamic acid decarboxylase autoantibodies by glucose tolerance status in elderly patients from the cardiovascular health study. *The Journal of clinical endocrinology and metabolism* 2006;91:2871-7.