A Comparative Analysis of the Sensitivity, Specificity, Concordance, and the 5-year Predictive Power of Diabetes-related Autoantibody Assays

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Running title: Islet Autoantibodies Comparison

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Abstract Word Count:	194
Highlights Word Count	98
Total Word Count:	3947
Total Number of Tables:	4
Total Number of Figures:	1

Abstract

This study compares novel type 1 diabetes-related autoantibody assays developed to improve upon the standard radiobinding assay (RBA). Samples from 1505 individuals, followed for 5 years or to clinical type 1 diabetes, originally tested by RBA were aliquoted and sent blindly to 5 laboratories (BDC, IDR, DRI, MSD, Enable) to be tested by electrochemiluminescence (ECL) assays, Luciferase Immuno Precipitation System (LIPS) assays, multiplex antibody detection by agglutination-PCR (ADAP) assays, and N-terminally truncated GAD65 or IA2β autoantibody RBAs (tGADA/IA2βA).

Findings: The fraction of samples that were concordant for negative/positive interpretations across all assays were 79.7% (GADA), 65.2% (IA-2A), 36.2% (IAA), and 67.5% (ZnT8A). The assays with the highest Youden index for predicting the previous RBA results differed by autoantibody: 0.65 LIPS(IDR) for IAA, 0.91 ECL(BDC) for ZnT8A, 0.82 tGADA RBA(IDR) for GADA, 0.91 ECL(MSD and BDC) for IA-2A. The Youden index for predicting 5-year type 1 diabetes varied significantly across assays and was highest for LIPS(DRI) for all autoantibody combinations, with little variation in the respective maximum Youden index. The discordance between assays makes it problematic to interpret positivity when comparing results from different assays. Longitudinal autoantibody assessments should be tested with the same assay.

Article Highlights

Inter-assay concordance and 5-year diabetes prediction of islet cell autoantibody detection using the radiobinding assay (TrialNet RBA), two independently developed multiplex electrochemiluminescence detection methods (ECL), the luciferase immune precipitation system (LIPS), detection by agglutination-PCR (ADAP), and truncated GADA (tGADA), and IA2βA RBAs are reported.

- There was considerable discordance that varied by type of autoantibody across the assays.
- T1D prediction was relatively high and uniform, implying confirmation of increased diabetes risk among those who are multiple autoantibody positive, although substantial false positive rates need to be considered when autoantibodies alone are used for screening to identify high diabetes risk.

Type 1 diabetes-related autoantibodies (islet autoantibodies) have long been recognized as harbingers of type 1 diabetes (T1D). Yet not all individuals in whom these autoantibodies have been detected will progress to clinical disease and not all progress at the same rate. Longitudinal studies have made it clear that the presence of multiple autoantibodies with or without metabolic dysregulation is associated with increased diabetes risk and that sequential stages leading to diabetes are identifiable long before clinical disease (1-3). Progression from diabetes-related autoantibodies detection to clinical T1D varies widely among those at risk according to phenotypic characteristics or autoantibody profile and is inversely related to the age at which the autoantibodies appear.

The identification of individuals who have islet autoantibodies and are progressing to clinical diabetes (4), necessitating exogenous insulin, is important since research studies have shown that close monitoring can lead to interventions that reduce the incidence of diabetic ketoacidosis and allow for treatments that can delay progression (5,6). Prospective studies for the prevention or delay in progression from autoimmunity to T1D depend upon identifying individuals at elevated risk.

Early recognition of individuals at elevated risk for T1D and the effect it may have on reducing rates of diabetic ketoacidosis or treatment with approved prevention therapy has led to increased screening outside of a research setting. The laboratories offering autoantibody testing currently use different types of assays. Differences among these assays make interpretation difficult and there is limited information available on their comparability or their prognostic value for T1D onset.

Autoantibodies against four major proteins expressed by beta cells are currently in clinical use to characterize T1D risk (7,8). These are autoantibodies against insulin (IAA), GAD65 (GADA), tyrosine phosphatase-related insulinoma antigen 2 (IA-2A), and zinc transporter 8 (ZnT8A). The RBA has been the ‘gold’ standard assay for measuring autoantibodies because of the superior sensitivity and specificity

compared to other assay platforms and the low serum volume required. (9) However, the time-limited nature of the radioactive labels, the associated regulatory requirements, and the expense of radioimmunoassays have driven the search for alternative assay technologies. Furthermore, though the detection of two or more autoantibodies has been a reliable marker for early disease, the disease relevance of single autoantibodies detected by RBA is far more heterogeneous, with only a ~ 15% 10-year cumulative risk of single autoantibody-positive, genetically-susceptible individuals progressing to clinical disease (1).

Since the introduction of RBAs over 20 years ago, methods have been developed to detect autoantibodies that may improve their predictive abilities. Competition binding assays to identify high-affinity autoantibodies, particularly for IAA and GADA, have demonstrated improved prediction of single autoantibody individuals at highest risk of progression to multiple autoantibodies and clinical disease (10-12). Variations of the conventional antibody targets, such as truncation of the n-terminus of GAD65 and IA-2-related member of the protein tyrosine phosphatase family (IA-2β), have both been reported to successfully enhance disease-relevant signals and predict progression (13-16).

Bridge-ELISA assays and three novel non-radioactive islet autoantibody assay formats have shown comparable, if not superior, sensitivity and specificity compared to the RBA in islet autoantibody standardization workshops (9, 17). The first of the latter three methods uses electrochemiluminescence (ECL) detection. Exploiting the bivalent binding of IgG, the antigen anchored to the plate captures the autoantibody, which in turn captures the fluid-phase antigen carrying a luminescent label (18). The signal is formed when the autoantibody bridges both the bound and the labeled antigen. The ECL detection method has demonstrated effective filtering out of low-affinity single autoantibodies detected by RBA whose positivity did not correlate with disease progression. Furthermore, ECL reports an ability to improve prediction of time to diabetes in autoantibody-positive relatives (18-21). The assay has been

recently translated into a multiplex platform which can screen for all four islet autoantibodies simultaneously (22). Another approach, the Luciferase Immuno Precipitation System (LIPS), replaces the radioactive label with a luciferase reporter on the antigen. (23) That is, this method substitutes light-emitting recombinant antigens to generate autoantibody profiles with quantifiable titers. For IAA detection, it has shown high concordance with the RBA and improved discrimination of progressors to T1D (24). LIPS uses 1μL of serum per replicate test. The multiplex Antibody Detection by Agglutination-PCR (ADAP) assay takes advantage of the agglutination capacity of antibodies to aggregate antigen-DNA conjugates, driving ligation of DNA strands, thereby producing a new and distinct PCR reporter which can be amplified and quantified (25). At the IASP 2018 workshop, ADAP demonstrated the highest sensitivity, and specificity on par with the highest performing assays for GADA detection (17). Advantages include being able to multiplex up to 6-8 antigen targets using a single 4μL sample and its use of a fluid-phase antigen, overcoming epitope masking and conformational distortion that can occur with solid-phase capture assays. Autoantibodies that react to variants of known antigens have also been developed. N-terminally truncated GAD65 (96-585) appears to improve the prediction of diabetes compared to the conventional assay using full length GAD65 as antigen. (26) Detection of high affinity autoantibodies to conventional antigens has also been shown to improve prediction. High affinity anti-insulin antibodies of a of > 10^9 l/mol threshold distinguished groups that progressed to multiple autoantibody positivity or overt diabetes. (10)

This paper reports on the results of a comparison of different islet autoantibody assays with regard to inter-assay concordance and type 1 diabetes prediction over a 5 year period. Assays are compared with respect to single and multiple autoantibody detection at initial screening and by each autoantibody type. T1D developed in 36% (542/1505) in the 5 year follow up period.

Research Design and Methods

Study Population: The TrialNet Pathway to Prevention study (TNPTP) is one of the largest ongoing prospective studies, with objectives to study the pathogenesis and natural history of T1D, and to facilitate the risk assessment and recruitment of individuals who might qualify for T1D prevention trials. In the TNPTP study, relatives of individuals with T1D are screened for the presence of islet autoantibodies (GAD65A, IA-2A, and IAA) using the radiobinding assay. ZnT8A is measured on those who are positive for at least one other islet autoantibody. Those positive for at least one autoantibody were then followed longitudinally for the development of T1D. Additionally, a random sample of autoantibody negative individuals are also followed in the same way for the development of T1D. The details on the screening and follow-up schemes have been previously described (27). This study consists of all participants from the TNPTP study that have been followed for 5 years or until a diagnosis of T1D, which ever came first, N=1505 (Table 1). All subjects (and/or their parents) signed a written consent form approved by the participating study site's human subjects committee. All have had islet autoantibodies tested at the BDC, using the RBA assay. The initial screening test samples were used in this study and aliquots from these samples were distributed to the other participating labs in a blinded fashion.

Autoantibody assays:

Radiobinding Assay at the Barbara Davis Diabetes Center (BDC), Aurora, Colorado (RBA BDC).

GADA, IA-2A, IAA and ZnT8A were measured by radioimmunoassay in the TrialNet Core laboratory. Prior to June 2010, autoantibodies of GAD65 and IA-2 were tested in a combined assay using 3H-leucine-labeled GAD65 (glutamic acid decarboxylase 65) and 35S-methionine-labeled ICA512 (islet cell autoantigen 512). (27) Since June 2010, the laboratory has performed the harmonized GADA and IA-2A assays for the National Institute of Diabetes and Digestive and Kidney Diseases Consortia (28). Autoantibody positivity was defined using threshold indexes/units of GADA >0.032, harmonized

GADA >20 NIDDK units/ml, ICA512A >0.049, harmonized IA-2A>5 NIDDK units/ml, mIAA >0.01 and ZnT8A>0.02.

Multiplex Electrochemiluminescence Detection Method (ECL) done by the Barbara Davis Diabetes Center, Aurora, Colorado (ECL BDC). Using the Meso Scale Discovery (MSD) U-PLEX® platform biotinylated capture entities are coupled to U-PLEX Linkers, which self-assemble onto unique spots on the MSD U-PLEX 96-Well SECTOR™ plates.

Multiplex Electrochemiluminescence Detection Method (ECL) developed by Meso Scale Diagnostics, LLC., Rockville, Maryland (ECL MSD). Similar to the protocol above, linkers were bound to biotinylated antigens (human GAD65, insulin, ZnT8, or IA2), and combined to create a U-PLEX linker-coupled biotin antigen mix. Sample concentrations were determined based on the respective calibration curves and presented as arbitrary Units/mL for insulin and ZnT8 autoantibodies and WHO-anchored Units/mL for GAD65 and IA2 autoantibodies.

Multiplex Antibody Detection by Agglutination-PCR (Multiplex ADAP) done by Enable Biosciences, South San Francisco, California. The ADAP protocols began by mixing 4µL of serum with 8µL of ADAP conjugates mixture. Next, 25µL of the mixtures were mixed with 25µL of pre-amplification product and subjected to thermocycling. The amplified products were then diluted for subsequent real-time qPCR quantification. The assay readout ΔCt is defined as the difference of Ct value between blank and the samples as previously reported. The value of ΔCt is proportional to the initial amplicon concentrations in the PCR plate well. This amplicon concentration is then also proportional to the amount of target antibodies present in the samples.

Luciferase Immuno Precipitation System (LIPS) done by the Diabetes Research Institute (DRI), IRCCS Ospedale San Raffaele, Milan, Italy. The LIPS assay is based on the immunoprecipitation in liquid phase of luciferase tagged recombinant proteins with test sera. For use in LIPS, the San Raffaele DRI lab developed and validated several T1D recombinant autoantigens that are tagged with a nanoluciferase reporter (ZZ) and expressed by transfection in Expi293F cells. In this study the following recombinant autoantibodies were used: N-terminally truncated GAD65 (aa 96–585) (26, 29), insulin (24), IA-2 intracellular domains (aa 609-979) (29), IA-2β intracellular domains (aa 644-1015), dimeric ZnT8 cytoplasmic domain (aa 258- 369) (29) encoding for the arginine and tryptophan aa 325 polymorphism, respectively. Autoantibody binding competition in the IAA LIPS was performed with two different concentrations of unlabeled recombinant insulin to discriminate low- and high-affinity IAA (31).

Truncated GADA and IA2βA radiobinding assays, and IAA LIPS assay done by the Institute of Diabetes Research (IDR), Helmholtz Munich. Autoantibodies against N-terminally truncated GAD65 (tGADA) and IA-2β (IA2βA) were measured according to the NIDDK harmonized radiobinding assay protocol (28). In vitro transcribed/translated and [^{35}S]-labeled recombinant human N-terminally truncated GAD65 (aa 96–585) and the intracellular domain of IA-2β (aa 644–1,015) (both plasmids from Vito Lampasona, San Raffaele Institute, Milan, Italy) were used as antigens to detect tGADA (31) and IA2βA (16), respectively. IAA were measured using the pIAA LIPS assay with nanoluciferase-tagged human proinsulin as antigen (25).

Results are compared in terms of their concordance and ability to detect individuals who will progress to T1D within 5 years. The assays were chosen because they performed well in the IASP or there have been prior publications indicating improved islet cell autoantibody detection.

Diagnosis of Diabetes: Diabetes was diagnosed according to ADA criteria: 1). Presence of unequivocal hyperglycemia including acute metabolic decompensation (diabetic ketoacidosis); 2). Fasting plasma glucose ≥ 126 mg/dL (7 mmol/L); or 2-hr plasma glucose during an OGTT ≥ 200 mg/dL (11.1 mmol/L); or random plasma glucose ≥ 200mg/dL (11.1 mmol/L) accompanied by symptoms of polyuria, polydipsia, and /or weight loss. The criteria in 2) must be met on two consecutive tests. If a diabetes diagnosis was not made by year 5, the follow up time was truncated to 5 years (censored).

Statistical Methods: All P-values are two-sided. Sensitivity and specificity, negative and positive predictive values (NPV and PPV) were computed along with the Youden's J index and the predictive summary index (PSI). The Youden J index is the sum of the sensitivity and specificity minus one. The PSI is the sum of the negative and positive predictive value minus one. The maximum Youden J Index corresponds to the specific measurement threshold that maximizes the difference between the true positive rate and the false positive rate over all possible cut-point values. The 1505 participants included in the analysis provide ≥80% statistical power for detecting differences of 5% (2-sided test of significance) or more. Spearman rank correlation analysis was used throughout to describe the association between pairs of variables. As an alternative to adjustments for multiple testing, 95% confidence intervals (CI) are provided. SAS 9.4 (SAS Institute Inc, Cary, NC) was used to assess the baseline characteristics.

Data and Resource Availability

The datasets generated during and/or analyzed during the current study will be made available for request at the NIDDK Central Repository (NIDDK-CR) website, Resources for Research (R4R), https://repository.niddk.nih.gov/studies/trialnet/.

Results

Between March 2004 and August 2019, a total of 1505 screened relatives had either been followed to T1D onset or for a minimum of 5 years since screened (Table 1). 220 (14.6%) were randomly selected autoantibody negative controls, 555 (36.9%) single autoantibody positive and 730 (48.5%) multiple-autoantibody positive at initial screening according to the TrialNet RBA assay used. The high variability of autoantibody positivity across the laboratories that participated in this study, based on laboratory-defined thresholds, is shown in Table 2. Among those with a single autoantibody, GADA was the most frequently detected across all assays (range 57.2%-97.1%). The second most frequently detected single-positive autoantibody was IAA (range 1.0%-34.5%), except for the ECL (BDC), ADAP and ECL (MSD) which was IA2A (23.9%) and ZnT8A (14.4%), respectively. The most common autoantibody pairs detected, among those with two autoantibodies, were GADA/IAA for the RBA and all 3 LIPS assays (range 51.8%-67.7%). GADA/ZnT8A was the most common pair detected by the ECL (BDC) (42.7%), and ECL (MSD) (46.1%) assays. The GADA/IA2A pair was detected most frequently by the ADAP (43.0%) assay, but this was only slightly higher than the 40.2% GADA/IAA pair frequency in this same assay. Autoantibody pairs that did not include GADA were relatively low in frequency across all assays (<20%). Autoantibody triplets almost always included GADA, IA2A and ZnT8A, except in the ADAP assay where the triplet GADA/IA2A/IAA was most common (47.5%). Since there were no ZnT8A results from the IDR, the relative percentages, by number and type of autoantibody, are not comparable with the other assay data presented. Across all assays, the percentage of children developing T1D within 5 years increased with increasing numbers of autoantibodies, with the largest increase coming with two or more autoantibodies detected at screening.

The correlations between assay titers is strongest for GADA (ranging from 0.82 (RBA with ECL (BDC) to 0.95 (tGADA, LIPS with RBA)) and weaker for IA2A (ranging from 0.49 (IA2βA LIPS with ADAP) to 0.78 (IA2A LIPS with IA2A ECL (MSD)), IAA (ranging from 0.21 (RBA with ECL(MSD)) to 1.0

(HiStd LIPS with HiIdx LIPS), and Znt8A (ranging from 0.58 (LIPS(DRI) with ADAP) to 0.81 (LIPS with ECL(MSD)) (Supplemental Figures S1-S4). The variability among assays can also be viewed in the interpretation (positive/negative) using the assay specific thresholds (indicated in bottom sections of the supplemental figures). Using those thresholds, the percentage of samples that are concordant in their positive/negative status interpretation, by autoantibody and across all assays, ranges from a high of 79.7% for GADA to a low 36.2% for IAA. Agreement across all assays for IA2A and ZnT8A interpretation (combining positive and negative) was also high at 65.2% and 67.5%, respectively (Supplemental Figure S5). The corresponding discordance rates were 20.3%, 34.8%, 64.8% and 32.5% for GADA, IA2A, IAA and ZnT8A, respectively.

Using RBA as the "gold standard", Table 3 shows the assay performance using the laboratory and assay specific thresholds for positivity in detecting RBA autoantibodies by assay and autoantibody type. The assays with the highest Youden J index for predicting the RBA autoantibody result differed by autoantibody: 0.65 LIPS(IDR) for IAA, 0.91 ECL(BDC) for ZnT8A, 0.82 tGADA RBA(IDR) for GADA, 0.91 ECL (MSD and BDC) for IA2A.

Table 4 describes assay performance for the progression to T1D within 5 years. Since the incidence of T1D increased markedly if two or more autoantibodies were detected, the analysis is based upon different combinations of two to four autoantibodies. Assay performance varied according to the specific types of autoantibodies detected. The LIPS (DRI) assays had the highest Youden J index (28) values for all autoantibody pairs. Whenever the pair included IAA, the LIPS Hildx outperformed the other LIPS assays, albeit the differences were small. The LIPS (DRI) assay also had the highest sensitivity for T1D, although when the autoantibody pair included IAA, it was the LIPS Loldx that outperformed the rest. As expected, when there were three positive autoantibodies, sensitivity decreased, and specificity increased. Specificity generally tracks inversely with sensitivity and the emphasis on sensitivity versus specificity

depends somewhat on how the results are to be used. As in the case with the autoantibody positive pairs, the LIPS (DRI) outperformed the other assays on sensitivity (and PPV) but trailed the others in specificity (and NPV). ROC curves (Supplemental Figure S6-S9) showed little improvement in Youden J index result by changing the assay-specific positivity thresholds near the existing thresholds. The single positive autoantibody results highlight the difference in performance between the assays across the different autoantibodies.

Prior studies (33) have shown that detection of a single autoantibody does not statistically significantly increase T1D risk. Increased risk is generally accepted when two or more autoantibodies are detected. Since T1D is defined to include the detection of at least one autoantibody, the sensitivity of a single autoantibody by any of the assays analyzed has a sensitivity approaching 1.0 and a corresponding lower specificity. When two or more autoantibodies are detected, the LIPS Loldx had the highest sensitivity (range 0.76-0.92) and the RBA (tGADA) & RBA (IA2βA) and LIPS (pIAA) (IDR) combination had the highest specificity. The highest Youden index of 0.52 was achieved by the ECL (BDC). (Figure 1)

When examined by age group (Supplemental Table 1), there is a clear drop in sensitivity for progression to clinical T1D within 5 years among individuals 20 years of age and older compared to the younger age groups. Specificity is only slightly improved, but the sensitivity drops by 20% or more across all the assays.

Discussion

The population studied included all individuals who have a first or second degree relatives diagnosed with T1D, have been screened for autoantibodies in the TrialNet Pathway to Prevention Study and followed to diabetes or for a minimum of 5 years. A potential limitation is the possible under reporting of individuals who had ZnT8A by RBA as their only autoantibody, since TrialNet tested for ZnT8A only if another autoantibody was detected. Prior TrialNet analyses have shown that presence of ZnT8A

alone was a very rare occurrence. As per that study, autoantibody negative individuals, using the RBA assay, are not systematically followed except for a random subset. From a population perspective, the prevalence of autoantibody positivity is close to 5% (32). The positive and negative predictive values of detected autoantibodies are affected by their prevalence. Hence, while we think the sensitivity and specificity estimates would likely be the same in samples drawn from a population without a diabetes affected family member, the positive and negative predictive values may not because the incidence of islet autoantibodies is lower in that population as compared to relatives. The larger numbers in this study, which is the largest study of its kind to date, make the sensitivity, specificity and Youden index estimates more precise. Other strengths of the study include the systematic, uniform follow up duration and criteria for diabetes diagnosis.

While assay development has been stimulated by the desire to improve autoantibody detection, enable multiplex testing to reduce costs and sample volumes, or to move away from dependence on radioactive isotopes, there have been only limited attempts to directly compare detection rates or to ascertain whether the likelihood of progression to clinical disease is comparable across assays in those who are screened autoantibody positive prior to diagnosis. The IASP workshop is much more limited in the number of samples, generally includes samples from individuals diagnosed with type 1 diabetes and, therefore, cannot answer these questions. The analysis presented here allows for an examination of assay performance that can be interpreted according to how autoantibody screening is to be used. It shows that the differences in autoantibody detection rates are very much under appreciated.

Consider the use of autoantibody screening to determine whether an individual was at elevated diabetes risk. Should a test detect only a single autoantibody, it would naturally be repeated at a later time to determine persistence or epitope spreading leading to the detection of multiple autoantibodies. The variability shown in this study makes it important to know which assay is used and to use the same assay

at both time points. The interpretation of the lack of detection of any autoantibodies also depends upon which assay is used, at least when considered relative to the radiobinding assay. Also, assay performance varied depending upon the autoantibody detected and no assay, apart from the LIPS assay performed uniformly well across them all. The variable degree of concordance between assays for the same autoantibody and the differences seen according to the type of autoantibody make it problematic to extend the interpretation of positivity when comparing results from different assays. Of note, the discordance was not limited to values near the assay threshold, suggesting that a simple re-definition of the assay thresholds will not necessarily suffice. Rather, the results suggest a need for assay harmonization as has been attempted by the NIDDK harmonized assay for GADA and IA2A. But it is doubtful whether the different methods lend themselves to harmonization. As long as islet autoantibody screening programs continue to use assays without an attempt to standardize results, the full potential of screening will not be realized.

The need to annotate autoantibody results by the assay used for detection is also important to distinguishing between type 1 and type 2 diabetes in individuals who meet the glycemic thresholds. Insulin deficient, autoantibody negative cases, may have to be reinterpreted in light of the potential for false negative results.

The T1D predictive ability varies relatively little across assays, however. The clinical significance of the difference depends upon how the assay results are to be used (e.g., screening or ruling out T1D). All of the assays had relatively low specificity for the 5 year period following testing which should be included in the messaging to individuals tested and the need for subsequent monitoring (33).

Specificity declined with increasing participant age. The age of the individual screened for autoantibodies suggests an increasing role of factors, other than autoantibodies, in predicting the 5-year

T1D incidence. For example, those over the age of 20 found to be autoantibody positive may have a greater than 5-year window used in this study before experiencing elevated T1D rates. The interaction of age and autoantibody status maybe accounted for in how the study population was constructed. TrialNet autoantibody screening is cross-sectional. So, unlike a prospective longitudinal study, there is no way to know how long an individual might have had detectable autoantibodies before screening and those who have had autoantibodies for a longer period of time prior to screening (i.e., an older population at study entry) might have a slower rate of progression as compared to others. The incidence of autoantibodies is greatest in younger ages and those who seroconvert to autoantibody positive during that time have a faster rate of progression. This corresponds to a higher 5-year false positive rate as seen in the older age group. So, a limitation in this study is how the study population was constructed and the drop in assay performance might not have anything to do with age per se. An important message is that no matter how islet autoantibodies are detected, when multiple are present they correspond to elevated T1D risk but it might not be apparent over a 5-year window of time.

Finally, there are other factors, such as genetics and glycemic status that should also be considered in identifying individuals at diabetes risk and, while the detection of islet autoantibodies is an early marker, it is only a milepost along the path to diabetes.

Acknowledgements Jeffrey Krischer and Lu You are the guarantors of this work, had full access to all the data, and take full responsibility for the integrity of data and the accuracy of data analysis.

Author Contributions

Jeffrey Krischer, Sarah Muller, and Lu You researched data, contributed to the discussion, wrote the manuscript, and reviewed/edited manuscript. All other authors also reviewed the manuscript and contributed to its content. The funding sources had no role in the manuscript's content.

Conflict of Interest

The authors declare that they have no conflicts of interest.

External Funding Acknowledgement

The Type 1 Diabetes TrialNet Study Group is a clinical trials network currently funded by the National Institutes of Health (NIH) through the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Allergy and Infectious Diseases, and The Eunice Kennedy Shriver National Institute of Child Health and Human Development, through the cooperative agreements U01 DK061010, U01 DK061034, U01 DK061042, U01 DK061058, U01 DK085461, U01 DK085465, U01 DK085466, U01 DK085476, U01 DK085499, U01 DK085509, U01 DK103180, U01 DK103153, U01 DK103266, U01 DK103282, U01 DK106984, U01 DK106994, U01 DK107013, U01 DK107014, U01 DK106993, UC4 DK117009, and Breakthrough T1D (formerly JDRF). The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of NIH or Breakthrough T1D.

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Table 1. Characteristics of study population by RBA assay result at baseline.

	IA Negative		Single IA+		Multiple IA+	
	T1D-Free	Developed T1D	T1D-Free	Developed T1D	T1D-Free	Developed T1D
N	212	8	456	99	295	435
Age at Baseline (Yrs., Mean, SD))	7.9 (7.9)	8.6 (8.2)	20.6 (14.7)	18.2 (14.5)	14.5 (12.1)	11.0 (8.7)
Age Group at Baseline (yrs.)						
Age 0-9	163 (76.9%)	6 (75.0%)	162 (35.5%)	44 (44.4%)	143 (48.5%)	256 (58.9%)
Age 10-19	40 (18.9%)	1 (12.5%)	112 (24.6%)	20 (20.2%)	91 (30.8%)	138 (31.7%)
Age 20+	9 (4.2%)	1 (12.5%)	182 (39.9%)	35 (35.4%)	61 (20.7%)	41 (9.4%)
Gender						
Female	93 (43.9%)	4 (50.0%)	269 (59.0%)	47 (47.5%)	155 (52.5%)	229 (52.8%)
Male	119 (56.1%)	4 (50.0%)	187 (41.0%)	52 (52.5%)	140 (47.5%)	205 (47.2%)
(Missing)	0	0	0	0	0	1
Ethnicity						
Hispanic or Latino	17 (8.0%)	0 (0.0%)	40 (8.8%)	7 (7.1%)	21 (7.1%)	38 (8.7%)
Not Hispanic or Latino	195 (92.0%)	8 (100.0%)	416 (91.2%)	92 (92.9%)	274 (92.9%)	397 (91.3%)
Race						
White	188 (94.9%)	7 (87.5%)	394 (92.5%)	89 (96.7%)	259 (94.9%)	374 (93.5%)
African American	2 (1.0%)	1 (12.5%)	10 (2.3%)	1 (1.1%)	5 (1.8%)	11 (2.8%)
Asian	3 (1.5%)	0 (0.0%)	9 (2.1%)	1 (1.1%)	5 (1.8%)	5 (1.2%)
Other	5 (2.5%)	0 (0.0%)	13 (3.1%)	1 (1.1%)	4 (1.5%)	10 (2.5%)
(Missing)	14	0	30	7	22	35

Table 2. Frequency of autoantibody detection by assay based on laboratory-defined thresholds for positivity. The numbers in parentheses are the percent of cases in category defined by the umber of autoantibodies detected. The IDR did not report ZnT8A results, so the results in this table only reflect the other autoantibodies. Thus, the distribution of the number of samples positive for one or more autoantibodies is not comparable to the other assay results.

	Types of IA	RBA	ECL (BDC)	LIPS LoIdx (DRI)	LIPS HiIdx (DRI)	LIPS HiStd (DRI)	ADAP (Enable)	ECL (MSD)	tGADA/IA2betaA (IDR)
0	None	214	477	301	339	350	352	396	393
1	GADA	393 (70.9%)	305 (97.1%)	159 (57.2%)	195 (69.6%)	218 (74.4%)	262 (69.5%)	285 (78.9%)	477 (80.7%)
	IA2A	36 (6.5%)	5 (1.6%)	6 (2.2%)	8 (2.9%)	9 (3.1%)	90 (23.9%)	15 (4.2%)	41 (6.9%)
	IAA	121 (21.8%)	3 (1.0%)	96 (34.5%)	58 (20.7%)	47 (16.0%)	25 (6.6%)	9 (2.5%)	73 (12.4%)
	ZnT8A	4 (0.7%)	1 (0.3%)	17 (6.1%)	19 (6.8%)	19 (6.5%)	0 (0.0%)	52 (14.4%)	NA
2	GADA IA2A	65 (20.8%)	56 (25.5%)	23 (8.8%)	24 (10.6%)	32 (14.2%)	125 (43.0%)	83 (25.0%)	206 (54.9%)
	GADA IAA	100 (32.1%)	42 (19.1%)	176 (67.7%)	140 (61.9%)	117 (51.8%)	117 (40.2%)	26 (7.8%)	148 (39.5%)
	IA2A IAA	6 (1.9%)	1 (0.5%)	5 (1.9%)	3 (1.3%)	2 (0.9%)	13 (4.5%)	4 (1.2%)	21 (5.6%)
	GADA ZnT8A	80 (25.6%)	94 (42.7%)	37 (14.2%)	44 (19.5%)	56 (24.8%)	20 (6.9%)	153 (46.1%)	NA
	IA2A ZnT8A	41 (13.1%)	21 (9.5%)	9 (3.5%)	7 (3.1%)	11 (4.9%)	15 (5.2%)	61 (18.4%)	NA
	IAA ZnT8A	20 (6.4%)	6 (2.7%)	10 (3.8%)	8 (3.5%)	8 (3.5%)	1 (0.3%)	5 (1.5%)	NA
3	GADA IA2A IAA	37 (13.9%)	35 (11.6%)	57 (20.4%)	56 (19.6%)	48 (16.3%)	145 (47.5%)	7 (2.3%)	144 (100.0%)
	GADA IA2A ZnT8A	149 (55.8%)	197 (65.0%)	95 (34.1%)	108 (37.8%)	141 (47.8%)	114 (37.4%)	258 (84.0%)	NA
	GADA IAA ZnT8A	54 (20.2%)	55 (18.2%)	100 (35.8%)	93 (32.5%)	81 (27.5%)	32 (10.5%)	37 (12.1%)	NA
	IA2A IAA ZnT8A	27 (10.1%)	16 (5.3%)	27 (9.7%)	29 (10.1%)	25 (8.5%)	14 (4.6%)	5 (1.6%)	NA
4	GADA IA2A IAA ZnT8A	151	191	386	373	340	180	107	NA
	(Missing)	7	0	1	1	1	0	2	2

Table 3. Summary of assay performance relative to the RBA. NPV and PPV are negative and positive predictive values, respectively. PSI is the predictive summary index. This table uses the local laboratory thresholds for positivity.

IA	Assay	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
GADA	GADA ECL (BDC)	0.87 (0.85,0.89)	0.84 (0.81,0.88)	0.72 (0.68,0.76)	0.76 (0.72,0.79)	0.92 (0.91,0.94)	0.68 (0.64,0.72)
	tGADA LIPS (DRI)	0.90 (0.89,0.92)	0.79 (0.75,0.82)	0.69 (0.65,0.73)	0.79 (0.76,0.83)	0.90 (0.88,0.92)	0.69 (0.65,0.73)
	GADA ADAP (Enable)	0.88 (0.86,0.90)	0.81 (0.77,0.84)	0.68 (0.64,0.72)	0.75 (0.71,0.79)	0.91 (0.89,0.93)	0.66 (0.62,0.70)
	GADA ECL (MSD)	0.87 (0.85,0.89)	0.88 (0.85,0.91)	0.76 (0.72,0.79)	0.76 (0.73,0.80)	0.94 (0.93,0.96)	0.71 (0.67,0.74)
	tGADA (IDR)	0.91 (0.89,0.92)	0.91 (0.89,0.94)	0.82 (0.79,0.85)	0.82 (0.79,0.85)	0.96 (0.94,0.97)	0.78 (0.74,0.81)
IA2A	IA2A ECL (BDC)	0.96 (0.93,0.98)	0.95 (0.92,0.97)	0.91 (0.87,0.94)	0.97 (0.95,0.99)	0.93 (0.89,0.96)	0.90 (0.86,0.94)
	IA2A LIPS (DRI)	0.99 (0.98,1.00)	0.86 (0.82,0.90)	0.85 (0.81,0.89)	0.99 (0.98,1.00)	0.83 (0.78,0.87)	0.82 (0.77,0.87)
	IA2A ADAP (Enable)	0.89 (0.84,0.93)	0.72 (0.67,0.77)	0.61 (0.55,0.68)	0.90 (0.87,0.94)	0.68 (0.63,0.74)	0.59 (0.52,0.66)
	IA2A ECL (MSD)	0.96 (0.94,0.99)	0.95 (0.92,0.97)	0.91 (0.87,0.94)	0.97 (0.96,0.99)	0.92 (0.89,0.96)	0.90 (0.86,0.94)
	IA2βA RBA (IDR)	0.80 (0.74,0.85)	0.99 (0.97,1.00)	0.79 (0.73,0.84)	0.88 (0.84,0.91)	0.98 (0.95,1.00)	0.86 (0.81,0.90)
IAA	IAA ECL (BDC)	0.52 (0.48,0.56)	0.92 (0.90,0.94)	0.44 (0.39,0.49)	0.79 (0.76,0.81)	0.77 (0.73,0.81)	0.56 (0.51,0.61)
	IAA (LoIdx) LIPS (DRI)	0.85 (0.82,0.88)	0.58 (0.54,0.61)	0.43 (0.38,0.47)	0.88 (0.85,0.91)	0.51 (0.48,0.55)	0.39 (0.35,0.43)
	IAA (HiIdx) LIPS (DRI)	0.87 (0.84,0.90)	0.68 (0.65,0.71)	0.55 (0.51,0.59)	0.91 (0.89,0.93)	0.59 (0.55,0.62)	0.49 (0.45,0.54)
	IAA (HiStd) LIPS (DRI)	0.85 (0.82,0.88)	0.77 (0.74,0.79)	0.62 (0.58,0.66)	0.91 (0.89,0.93)	0.66 (0.62,0.69)	0.56 (0.52,0.61)
	IAA ADAP (Enable)	0.76 (0.73,0.80)	0.86 (0.84,0.89)	0.63 (0.59,0.67)	0.87 (0.85,0.90)	0.75 (0.71,0.78)	0.62 (0.58,0.66)
	IAA ECL (MSD)	0.20 (0.16,0.23)	0.90 (0.88,0.92)	0.10 (0.06,0.14)	0.68 (0.66,0.71)	0.51 (0.44,0.58)	0.19 (0.12,0.26)
	pIAA LIPS (IDR)	0.68 (0.64,0.72)	0.97 (0.95,0.98)	0.65 (0.61,0.69)	0.85 (0.83,0.87)	0.91 (0.88,0.94)	0.77 (0.73,0.80)
ZnT8A	ZnT8A ECL (BDC)	0.98 (0.96,0.99)	0.93 (0.92,0.95)	0.91 (0.89,0.93)	0.99 (0.98,0.99)	0.88 (0.86,0.91)	0.87 (0.84,0.90)
	ZnT8A LIPS (DRI)	0.98 (0.96,0.99)	0.83 (0.81,0.85)	0.81 (0.78,0.83)	0.99 (0.98,0.99)	0.75 (0.72,0.79)	0.74 (0.71,0.77)
	ZnT8A ADAP (Enable)	0.67 (0.63,0.71)	0.97 (0.96,0.98)	0.64 (0.60,0.68)	0.84 (0.82,0.87)	0.93 (0.91,0.96)	0.77 (0.74,0.81)

IA	Assay	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
	ZnT8A ECL (MSD)	0.92 (0.90,0.94)	0.80 (0.78,0.83)	0.72 (0.69,0.76)	0.95 (0.94,0.97)	0.71 (0.68,0.75)	0.66 (0.63,0.70)

Table 4 Summary of assay performance by multiple positive islet autoantibody (IA) combinations relative to the prediction of T1D within 5 years. This table uses the local laboratory thresholds for positivity.

IA Combination	Assay	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
GADA & IA2A	RBA	0.49 (0.45,0.53)	0.86 (0.84,0.88)	0.35 (0.30,0.39)	0.75 (0.72,0.77)	0.66 (0.61,0.71)	0.41 (0.36,0.46)

IA Combination	Assay	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
	ECL (BDC)	0.59 (0.55,0.63)	0.83 (0.81,0.86)	0.43 (0.38,0.47)	0.78 (0.76,0.81)	0.67 (0.63,0.71)	0.45 (0.40,0.50)
	LIPS (DRI)	0.68 (0.64,0.72)	0.80 (0.78,0.83)	0.48 (0.44,0.53)	0.82 (0.79,0.84)	0.66 (0.62,0.70)	0.48 (0.43,0.52)
	ADAP (Enable)	0.65 (0.61,0.69)	0.78 (0.75,0.81)	0.43 (0.38,0.47)	0.80 (0.77,0.82)	0.62 (0.58,0.66)	0.42 (0.37,0.47)
	ECL (MSD)	0.55 (0.51,0.59)	0.83 (0.81,0.86)	0.38 (0.33,0.43)	0.77 (0.74,0.79)	0.65 (0.61,0.69)	0.42 (0.37,0.47)
	RBA (tGADA & IA2βA) (IDR)	0.45 (0.41,0.50)	0.89 (0.87,0.91)	0.35 (0.30,0.39)	0.74 (0.72,0.77)	0.70 (0.65,0.75)	0.45 (0.39,0.50)
GADA & IAA	RBA	0.40 (0.36,0.44)	0.87 (0.85,0.89)	0.27 (0.22,0.31)	0.72 (0.69,0.75)	0.63 (0.58,0.68)	0.35 (0.29,0.41)
	ECL (BDC)	0.39 (0.35,0.43)	0.89 (0.87,0.91)	0.28 (0.23,0.32)	0.72 (0.70,0.75)	0.66 (0.61,0.71)	0.38 (0.32,0.44)
	LIPS LoIdx (DRI)	0.74 (0.70,0.78)	0.67 (0.64,0.70)	0.41 (0.36,0.46)	0.82 (0.79,0.85)	0.56 (0.52,0.59)	0.38 (0.33,0.42)
	LIPS HiIdx (DRI)	0.71 (0.68,0.75)	0.71 (0.68,0.74)	0.43 (0.38,0.47)	0.82 (0.79,0.84)	0.58 (0.55,0.62)	0.40 (0.35,0.44)
	LIPS HiStd (DRI)	0.65 (0.61,0.69)	0.76 (0.73,0.79)	0.41 (0.37,0.46)	0.80 (0.77,0.82)	0.60 (0.56,0.64)	0.40 (0.35,0.45)
	ADAP (Enable)	0.55 (0.51,0.59)	0.82 (0.79,0.84)	0.37 (0.32,0.42)	0.76 (0.74,0.79)	0.63 (0.59,0.67)	0.40 (0.34,0.45)
	ECL (MSD)	0.17 (0.14,0.21)	0.91 (0.90,0.93)	0.09 (0.05,0.12)	0.66 (0.64,0.69)	0.53 (0.46,0.60)	0.19 (0.12,0.27)
	RBA (tGADA) & LIPS (pIAA) (IDR)	0.35 (0.31,0.39)	0.90 (0.88,0.92)	0.25 (0.21,0.29)	0.71 (0.69,0.74)	0.66 (0.60,0.71)	0.37 (0.31,0.43)
GADA & ZnT8A	RBA	0.49 (0.45,0.53)	0.82 (0.80,0.85)	0.31 (0.26,0.36)	0.74 (0.71,0.77)	0.61 (0.56,0.65)	0.35 (0.30,0.40)
	ECL (BDC)	0.61 (0.57,0.65)	0.78 (0.76,0.81)	0.39 (0.34,0.44)	0.78 (0.75,0.81)	0.61 (0.57,0.65)	0.39 (0.34,0.44)
	LIPS (DRI)	0.68 (0.64,0.72)	0.74 (0.71,0.77)	0.42 (0.38,0.47)	0.81 (0.78,0.83)	0.60 (0.56,0.64)	0.40 (0.36,0.45)
	ADAP (Enable)	0.42 (0.38,0.46)	0.88 (0.86,0.90)	0.30 (0.25,0.34)	0.73 (0.70,0.75)	0.66 (0.61,0.71)	0.39 (0.33,0.44)
	ECL (MSD)	0.57 (0.52,0.61)	0.74 (0.71,0.77)	0.31 (0.26,0.36)	0.75 (0.72,0.78)	0.55 (0.51,0.59)	0.30 (0.25,0.35)
IA2A & IAA	RBA	0.32 (0.28,0.36)	0.95 (0.94,0.96)	0.27 (0.23,0.31)	0.71 (0.69,0.74)	0.78 (0.73,0.84)	0.50 (0.44,0.56)
	ECL (BDC)	0.33 (0.29,0.37)	0.93 (0.92,0.95)	0.26 (0.22,0.30)	0.71 (0.69,0.74)	0.73 (0.67,0.78)	0.44 (0.38,0.50)
	LIPS LoIdx (DRI)	0.61 (0.57,0.65)	0.85 (0.83,0.87)	0.47 (0.42,0.51)	0.80 (0.77,0.82)	0.70 (0.66,0.74)	0.50 (0.45,0.54)
	LIPS HiIdx (DRI)	0.61 (0.57,0.65)	0.86 (0.84,0.88)	0.47 (0.42,0.52)	0.80 (0.77,0.82)	0.71 (0.67,0.75)	0.51 (0.46,0.56)

IA Combination	Assay	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
	LIPS HiStd (DRI)	0.56 (0.52,0.60)	0.88 (0.86,0.90)	0.44 (0.40,0.49)	0.78 (0.76,0.81)	0.73 (0.69,0.77)	0.51 (0.46,0.56)
	ADAP (Enable)	0.46 (0.41,0.50)	0.89 (0.87,0.91)	0.35 (0.30,0.39)	0.74 (0.72,0.77)	0.70 (0.65,0.75)	0.45 (0.39,0.50)
	ECL (MSD)	0.15 (0.12,0.18)	0.95 (0.94,0.97)	0.10 (0.07,0.13)	0.67 (0.64,0.69)	0.64 (0.56,0.73)	0.31 (0.22,0.40)
	RBA (IA2βA) & LIPS (pIAA) (IDR)	0.25 (0.21,0.29)	0.97 (0.96,0.98)	0.22 (0.18,0.26)	0.70 (0.67,0.72)	0.82 (0.77,0.88)	0.52 (0.46,0.58)
IA2A & ZnT8A	RBA	0.48 (0.44,0.53)	0.89 (0.87,0.91)	0.37 (0.33,0.42)	0.75 (0.73,0.78)	0.71 (0.67,0.76)	0.47 (0.41,0.52)
	ECL (BDC)	0.53 (0.49,0.57)	0.86 (0.83,0.88)	0.39 (0.34,0.43)	0.76 (0.74,0.79)	0.68 (0.63,0.72)	0.44 (0.39,0.49)
	LIPS (DRI)	0.64 (0.60,0.68)	0.82 (0.80,0.85)	0.46 (0.41,0.51)	0.80 (0.78,0.83)	0.67 (0.63,0.71)	0.47 (0.42,0.52)
	ADAP (Enable)	0.39 (0.35,0.43)	0.88 (0.86,0.90)	0.27 (0.23,0.32)	0.72 (0.69,0.75)	0.65 (0.60,0.71)	0.37 (0.32,0.43)
	ECL (MSD)	0.53 (0.49,0.57)	0.85 (0.83,0.87)	0.38 (0.34,0.43)	0.76 (0.74,0.79)	0.67 (0.62,0.71)	0.43 (0.38,0.48)
IAA & ZnT8A	RBA	0.34 (0.30,0.38)	0.93 (0.91,0.94)	0.26 (0.22,0.31)	0.71 (0.69,0.74)	0.72 (0.67,0.78)	0.43 (0.37,0.50)
	ECL (BDC)	0.35 (0.31,0.39)	0.92 (0.90,0.93)	0.26 (0.22,0.31)	0.71 (0.69,0.74)	0.70 (0.65,0.76)	0.42 (0.36,0.48)
	LIPS LoIdx (DRI)	0.62 (0.58,0.66)	0.80 (0.78,0.83)	0.42 (0.37,0.47)	0.79 (0.76,0.81)	0.64 (0.60,0.68)	0.43 (0.38,0.48)
	LIPS HiIdx (DRI)	0.61 (0.57,0.65)	0.82 (0.80,0.84)	0.43 (0.38,0.48)	0.79 (0.76,0.81)	0.66 (0.61,0.70)	0.45 (0.40,0.49)
	LIPS HiStd (DRI)	0.57 (0.53,0.61)	0.85 (0.83,0.87)	0.42 (0.37,0.47)	0.78 (0.75,0.80)	0.68 (0.64,0.72)	0.46 (0.41,0.51)
	ADAP (Enable)	0.30 (0.26,0.34)	0.93 (0.92,0.95)	0.23 (0.19,0.27)	0.70 (0.68,0.73)	0.71 (0.65,0.77)	0.42 (0.35,0.48)
	ECL (MSD)	0.16 (0.13,0.19)	0.93 (0.91,0.95)	0.09 (0.05,0.12)	0.66 (0.64,0.69)	0.56 (0.48,0.64)	0.22 (0.14,0.30)
GADA & IA2A & IAA	RBA	0.27 (0.23,0.31)	0.96 (0.94,0.97)	0.23 (0.19,0.27)	0.70 (0.67,0.72)	0.78 (0.72,0.84)	0.48 (0.41,0.54)
	ECL (BDC)	0.30 (0.26,0.34)	0.94 (0.92,0.95)	0.24 (0.20,0.28)	0.70 (0.68,0.73)	0.73 (0.67,0.78)	0.43 (0.37,0.49)
	LIPS LoIdx (DRI)	0.57 (0.53,0.61)	0.86 (0.84,0.88)	0.43 (0.38,0.48)	0.78 (0.76,0.81)	0.70 (0.65,0.74)	0.48 (0.43,0.53)
	LIPS HiIdx (DRI)	0.56 (0.52,0.60)	0.87 (0.85,0.89)	0.43 (0.39,0.48)	0.78 (0.75,0.80)	0.71 (0.67,0.75)	0.49 (0.44,0.54)
	LIPS HiStd (DRI)	0.52 (0.48,0.57)	0.89 (0.87,0.91)	0.41 (0.37,0.46)	0.77 (0.74,0.79)	0.73 (0.69,0.77)	0.50 (0.45,0.55)
	ADAP (Enable)	0.42 (0.38,0.46)	0.90 (0.88,0.92)	0.32 (0.28,0.37)	0.73 (0.71,0.76)	0.70 (0.66,0.75)	0.44 (0.38,0.49)

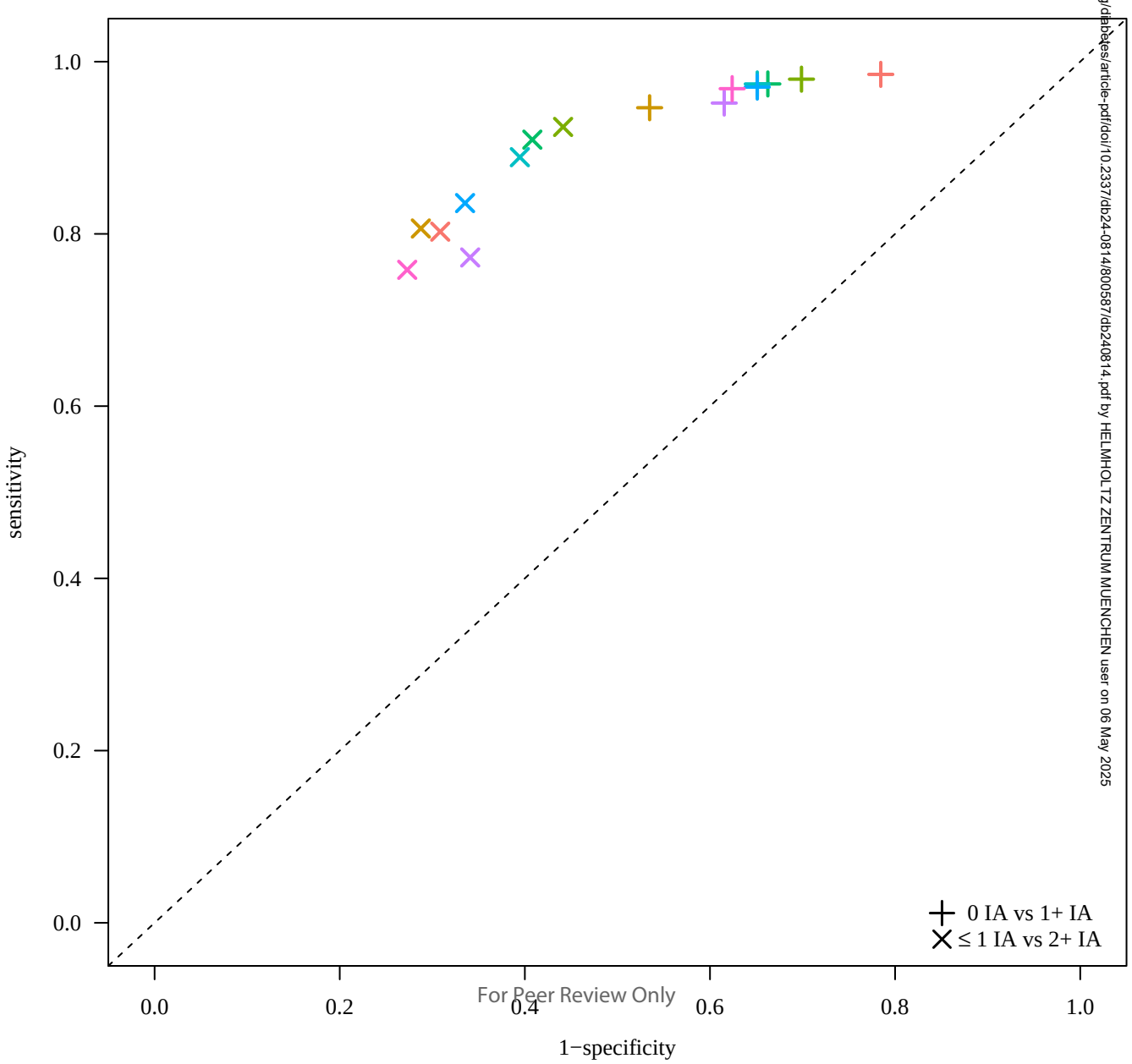
IA Combination	Assay	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
	ECL (MSD)	0.14 (0.11,0.17)	0.96 (0.95,0.97)	0.10 (0.06,0.13)	0.66 (0.64,0.69)	0.65 (0.56,0.74)	0.31 (0.22,0.40)
	RBA (tGADA) & RBA (IA2βA) & LIPS (pIAA) (IDR)	0.22 (0.18,0.25)	0.97 (0.96,0.98)	0.19 (0.15,0.23)	0.69 (0.66,0.71)	0.82 (0.76,0.88)	0.51 (0.44,0.58)
GADA & IA2A & ZnT8A	RBA	0.39 (0.34,0.43)	0.91 (0.89,0.92)	0.29 (0.25,0.34)	0.72 (0.70,0.75)	0.70 (0.64,0.75)	0.42 (0.36,0.48)
	ECL (BDC)	0.48 (0.44,0.52)	0.87 (0.85,0.89)	0.35 (0.30,0.40)	0.75 (0.72,0.77)	0.67 (0.63,0.72)	0.42 (0.37,0.47)
	LIPS (DRI)	0.60 (0.55,0.64)	0.83 (0.81,0.86)	0.43 (0.38,0.48)	0.79 (0.76,0.81)	0.67 (0.63,0.71)	0.46 (0.41,0.50)
	ADAP (Enable)	0.37 (0.32,0.41)	0.90 (0.88,0.92)	0.27 (0.22,0.31)	0.72 (0.69,0.74)	0.67 (0.62,0.73)	0.39 (0.33,0.45)
	ECL (MSD)	0.45 (0.40,0.49)	0.87 (0.85,0.89)	0.32 (0.27,0.36)	0.74 (0.71,0.76)	0.66 (0.61,0.71)	0.40 (0.34,0.45)
GADA & IAA & ZnT8A	RBA	0.27 (0.23,0.30)	0.94 (0.92,0.95)	0.21 (0.16,0.25)	0.69 (0.67,0.72)	0.71 (0.65,0.77)	0.40 (0.33,0.47)
	ECL (BDC)	0.32 (0.28,0.35)	0.92 (0.91,0.94)	0.24 (0.19,0.28)	0.71 (0.68,0.73)	0.70 (0.64,0.75)	0.40 (0.34,0.46)
	LIPS LoIdx (DRI)	0.57 (0.53,0.61)	0.82 (0.79,0.84)	0.39 (0.34,0.44)	0.77 (0.75,0.80)	0.64 (0.60,0.68)	0.41 (0.36,0.46)
	LIPS HiIdx (DRI)	0.56 (0.52,0.60)	0.83 (0.81,0.85)	0.39 (0.34,0.44)	0.77 (0.75,0.80)	0.65 (0.61,0.69)	0.42 (0.37,0.47)
	LIPS HiStd (DRI)	0.53 (0.48,0.57)	0.86 (0.84,0.88)	0.39 (0.34,0.43)	0.76 (0.74,0.79)	0.68 (0.63,0.72)	0.44 (0.39,0.49)
	ADAP (Enable)	0.28 (0.24,0.32)	0.94 (0.92,0.95)	0.22 (0.17,0.26)	0.70 (0.67,0.72)	0.71 (0.65,0.77)	0.41 (0.34,0.48)
	ECL (MSD)	0.15 (0.12,0.18)	0.94 (0.92,0.95)	0.09 (0.05,0.12)	0.66 (0.64,0.69)	0.57 (0.49,0.65)	0.23 (0.15,0.32)
IA2A & IAA & ZnT8A	RBA	0.27 (0.23,0.30)	0.97 (0.95,0.98)	0.23 (0.19,0.27)	0.70 (0.68,0.73)	0.81 (0.76,0.87)	0.52 (0.45,0.58)
	ECL (BDC)	0.28 (0.24,0.32)	0.94 (0.93,0.96)	0.22 (0.18,0.26)	0.70 (0.67,0.72)	0.73 (0.67,0.79)	0.43 (0.36,0.49)
	LIPS LoIdx (DRI)	0.54 (0.50,0.58)	0.87 (0.85,0.89)	0.41 (0.36,0.46)	0.77 (0.75,0.80)	0.70 (0.66,0.75)	0.48 (0.42,0.53)
	LIPS HiIdx (DRI)	0.54 (0.49,0.58)	0.88 (0.86,0.90)	0.42 (0.37,0.47)	0.77 (0.75,0.80)	0.72 (0.68,0.77)	0.49 (0.44,0.54)
	LIPS HiStd (DRI)	0.50 (0.46,0.54)	0.90 (0.88,0.92)	0.40 (0.35,0.44)	0.76 (0.74,0.79)	0.74 (0.69,0.78)	0.50 (0.45,0.55)
	ADAP (Enable)	0.26 (0.22,0.30)	0.94 (0.93,0.96)	0.21 (0.17,0.24)	0.69 (0.67,0.72)	0.73 (0.66,0.79)	0.42 (0.35,0.49)
	ECL (MSD)	0.13 (0.10,0.16)	0.96 (0.95,0.97)	0.09 (0.06,0.12)	0.66 (0.64,0.69)	0.64 (0.55,0.73)	0.31 (0.21,0.40)

IA Combination	Assay	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
GADA & IA2A & IAA & ZnT8A	RBA	0.22 (0.19,0.26)	0.97 (0.96,0.98)	0.19 (0.16,0.23)	0.69 (0.66,0.71)	0.80 (0.74,0.86)	0.49 (0.42,0.56)
	ECL (BDC)	0.26 (0.22,0.29)	0.95 (0.93,0.96)	0.20 (0.16,0.24)	0.69 (0.67,0.72)	0.73 (0.66,0.79)	0.42 (0.35,0.49)
	LIPS LoIdx (DRI)	0.50 (0.46,0.54)	0.88 (0.86,0.90)	0.38 (0.33,0.43)	0.76 (0.73,0.78)	0.70 (0.66,0.75)	0.46 (0.41,0.51)
	LIPS HiIdx (DRI)	0.49 (0.45,0.54)	0.89 (0.87,0.91)	0.38 (0.34,0.43)	0.76 (0.73,0.78)	0.72 (0.67,0.76)	0.47 (0.42,0.53)
	LIPS HiStd (DRI)	0.46 (0.42,0.50)	0.91 (0.89,0.92)	0.37 (0.32,0.41)	0.75 (0.73,0.77)	0.74 (0.69,0.78)	0.49 (0.43,0.54)
	ADAP (Enable)	0.24 (0.21,0.28)	0.95 (0.94,0.96)	0.19 (0.15,0.23)	0.69 (0.66,0.71)	0.73 (0.66,0.79)	0.42 (0.35,0.49)
	ECL (MSD)	0.13 (0.10,0.16)	0.96 (0.95,0.97)	0.09 (0.06,0.12)	0.66 (0.64,0.69)	0.64 (0.55,0.74)	0.31 (0.21,0.40)

Figure 1. Receiver Operating Curve (ROC) for 5-year T1D by assay. Parameters listed are Sensitivity (SEN), Specificity (SPE), Youden Index (YOU). The thresholds used are assay specific.

Diabetes

Assay	0 IA vs 1+ IA SEN (95% CI)	0 IA vs 1+ IA SPE (95% CI)	0 IA vs 1+ IA YOU (95% CI)	≤ 1 IA vs 2+ IA SEN (95% CI)	≤ 1 IA vs 2+ IA SPE (95% CI)	≤ 1 IA vs 2+ IA YOU (95% CI)
RBA	0.99 (0.98,1.00)	0.22 (0.19,0.24)	0.20 (0.17,0.23)	0.80 (0.77,0.84)	0.69 (0.66,0.72)	0.49 (0.45,0.54)
ECL (BDC)	0.95 (0.93,0.97)	0.47 (0.43,0.50)	0.41 (0.37,0.45)	0.81 (0.77,0.84)	0.71 (0.68,0.74)	0.52 (0.47,0.56)
LIPS LoIdx (DRI)	0.98 (0.97,0.99)	0.30 (0.27,0.33)	0.28 (0.25,0.31)	0.92 (0.90,0.95)	0.56 (0.53,0.59)	0.48 (0.44,0.52)
LIPS HiIdx (DRI)	0.97 (0.96,0.99)	0.34 (0.31,0.37)	0.31 (0.28,0.34)	0.91 (0.89,0.93)	0.59 (0.56,0.62)	0.50 (0.46,0.54)
LIPS HiStd (DRI)	0.97 (0.96,0.99)	0.35 (0.32,0.38)	0.32 (0.29,0.36)	0.89 (0.86,0.92)	0.61 (0.57,0.64)	0.49 (0.45,0.54)
ADAP (Enable)	0.97 (0.96,0.98)	0.35 (0.32,0.38)	0.32 (0.29,0.35)	0.84 (0.80,0.87)	0.66 (0.63,0.69)	0.50 (0.46,0.54)
ECL (MSD)	0.95 (0.93,0.97)	0.38 (0.35,0.42)	0.34 (0.30,0.37)	0.77 (0.74,0.81)	0.66 (0.63,0.69)	0.43 (0.39,0.48)
RBA (tGADA) & RBA (IA2ßA) & LIPS (pIAA) (IDR)	0.97 (0.95,0.98)	0.38 (0.35,0.41)	0.34 (0.31,0.38)	0.76 (0.72,0.79)	0.73 (0.70,0.76)	0.49 (0.44,0.53)



Supplemental Table, Figures, and Appendix

Table S1. Summary of assay performance by multiple positive islet autoantibody (IA) combinations relative to the prediction of T1D within 5 years by age group. This table uses the local laboratory thresholds for positivity.

IA Combination	Assay	Age 0-9						Age 10-19						Age 20+					
		Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
GADA & IA2A	RBA	0.47 (0.42, 0.53)	0.88 (0.86, 0.91)	0.36 (0.30, 0.42)	0.72 (0.68, 0.76)	0.73 (0.67, 0.79)	0.45 (0.38, 0.52)	0.57 (0.49, 0.64)	0.80 (0.75, 0.85)	0.37 (0.28, 0.46)	0.74 (0.69, 0.79)	0.65 (0.57, 0.73)	0.39 (0.30, 0.49)	0.39 (0.28, 0.50)	0.86 (0.82, 0.90)	0.25 (0.13, 0.37)	0.82 (0.78, 0.87)	0.46 (0.34, 0.58)	0.28 (0.15, 0.41)
	ECL (BDC)	0.59 (0.53, 0.64)	0.87 (0.83, 0.90)	0.45 (0.39, 0.52)	0.76 (0.73, 0.80)	0.74 (0.69, 0.80)	0.50 (0.44, 0.57)	0.67 (0.60, 0.75)	0.77 (0.71, 0.82)	0.44 (0.35, 0.53)	0.78 (0.73, 0.83)	0.65 (0.58, 0.73)	0.43 (0.34, 0.52)	0.43 (0.32, 0.54)	0.85 (0.80, 0.89)	0.27 (0.15, 0.39)	0.83 (0.78, 0.87)	0.46 (0.34, 0.57)	0.29 (0.16, 0.41)
	LIPS (DRI)	0.67 (0.62, 0.72)	0.83 (0.79, 0.86)	0.50 (0.43, 0.56)	0.79 (0.76, 0.83)	0.72 (0.66, 0.77)	0.51 (0.45, 0.57)	0.79 (0.72, 0.85)	0.73 (0.67, 0.78)	0.51 (0.43, 0.60)	0.84 (0.79, 0.89)	0.65 (0.59, 0.72)	0.49 (0.41, 0.58)	0.51 (0.40, 0.63)	0.82 (0.77, 0.87)	0.33 (0.21, 0.46)	0.85 (0.80, 0.89)	0.46 (0.36, 0.57)	0.31 (0.20, 0.43)
	ADAP (Enable)	0.66 (0.61, 0.71)	0.79 (0.76, 0.83)	0.45 (0.39, 0.52)	0.78 (0.74, 0.82)	0.68 (0.62, 0.73)	0.46 (0.39, 0.52)	0.71 (0.64, 0.78)	0.71 (0.65, 0.77)	0.42 (0.33, 0.51)	0.79 (0.74, 0.84)	0.62 (0.55, 0.69)	0.41 (0.32, 0.50)	0.47 (0.36, 0.58)	0.82 (0.77, 0.87)	0.28 (0.16, 0.41)	0.83 (0.79, 0.88)	0.44 (0.33, 0.55)	0.27 (0.16, 0.39)
	ECL (MSD)	0.52 (0.46, 0.58)	0.86 (0.83, 0.89)	0.38 (0.32, 0.45)	0.73 (0.70, 0.77)	0.71 (0.65, 0.77)	0.45 (0.38, 0.52)	0.64 (0.57, 0.72)	0.78 (0.73, 0.83)	0.42 (0.33, 0.51)	0.77 (0.72, 0.82)	0.66 (0.58, 0.73)	0.43 (0.33, 0.52)	0.46 (0.35, 0.57)	0.83 (0.79, 0.88)	0.29 (0.17, 0.41)	0.84 (0.79, 0.88)	0.45 (0.34, 0.57)	0.29 (0.17, 0.41)
	RBA (tGADA & IA2βA) (IDR)	0.46 (0.40, 0.51)	0.91 (0.88, 0.94)	0.37 (0.31, 0.43)	0.72 (0.68, 0.76)	0.77 (0.71, 0.83)	0.49 (0.42, 0.56)	0.55 (0.47, 0.62)	0.83 (0.78, 0.88)	0.38 (0.29, 0.47)	0.74 (0.69, 0.79)	0.68 (0.60, 0.76)	0.42 (0.32, 0.51)	0.25 (0.15, 0.34)	0.92 (0.88, 0.95)	0.16 (0.06, 0.27)	0.80 (0.75, 0.85)	0.47 (0.32, 0.63)	0.27 (0.11, 0.44)
GADA & IAA	RBA	0.49 (0.43, 0.54)	0.83 (0.79, 0.86)	0.32 (0.25, 0.38)	0.71 (0.67, 0.75)	0.65 (0.59, 0.71)	0.36 (0.29, 0.43)	0.36 (0.29, 0.44)	0.85 (0.81, 0.90)	0.22 (0.13, 0.30)	0.67 (0.62, 0.72)	0.62 (0.52, 0.72)	0.29 (0.18, 0.40)	0.12 (0.05, 0.19)	0.96 (0.94, 0.98)	0.08 (0.00, 0.15)	0.78 (0.73, 0.83)	0.47 (0.25, 0.70)	0.25 (0.03, 0.48)
	ECL (BDC)	0.47 (0.41, 0.53)	0.87 (0.83, 0.90)	0.34 (0.27, 0.40)	0.71 (0.68, 0.75)	0.70 (0.63, 0.76)	0.41 (0.34, 0.48)	0.36 (0.28, 0.43)	0.84 (0.80, 0.89)	0.20 (0.11, 0.29)	0.67 (0.62, 0.72)	0.60 (0.50, 0.70)	0.27 (0.16, 0.38)	0.16 (0.07, 0.24)	0.96 (0.94, 0.99)	0.12 (0.04, 0.20)	0.79 (0.74, 0.83)	0.57 (0.36, 0.78)	0.36 (0.14, 0.58)
	LIPS LoIdx (DRI)	0.82 (0.77, 0.86)	0.64 (0.60, 0.68)	0.46 (0.40, 0.52)	0.84 (0.80, 0.88)	0.60 (0.55, 0.65)	0.44 (0.38, 0.50)	0.75 (0.69, 0.82)	0.61 (0.55, 0.67)	0.36 (0.27, 0.45)	0.79 (0.73, 0.85)	0.56 (0.49, 0.62)	0.35 (0.26, 0.44)	0.41 (0.30, 0.52)	0.78 (0.73, 0.83)	0.19 (0.07, 0.31)	0.81 (0.77, 0.86)	0.36 (0.26, 0.46)	0.17 (0.06, 0.29)

IA Combination	Assay	Age 0-9						Age 10-19						Age 20+					
		Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
	LIPS HiIdx (DRI)	0.82 (0.77, 0.86)	0.67 (0.62, 0.71)	0.48 (0.42, 0.54)	0.85 (0.81, 0.88)	0.62 (0.57, 0.66)	0.46 (0.40, 0.52)	0.70 (0.63, 0.77)	0.65 (0.59, 0.71)	0.34 (0.25, 0.44)	0.77 (0.71, 0.82)	0.56 (0.49, 0.63)	0.33 (0.24, 0.42)	0.33 (0.22, 0.43)	0.87 (0.82, 0.91)	0.19 (0.08, 0.31)	0.81 (0.76, 0.86)	0.42 (0.30, 0.55)	0.23 (0.10, 0.37)
	LIPS HiStd (DRI)	0.75 (0.71, 0.80)	0.70 (0.65, 0.74)	0.45 (0.39, 0.52)	0.81 (0.77, 0.85)	0.62 (0.57, 0.67)	0.43 (0.37, 0.49)	0.65 (0.57, 0.72)	0.72 (0.67, 0.78)	0.37 (0.28, 0.47)	0.76 (0.70, 0.81)	0.61 (0.53, 0.68)	0.36 (0.27, 0.46)	0.26 (0.16, 0.36)	0.91 (0.87, 0.94)	0.17 (0.07, 0.28)	0.80 (0.76, 0.85)	0.47 (0.32, 0.61)	0.27 (0.11, 0.42)
	ADAP (Enable)	0.67 (0.62, 0.72)	0.75 (0.71, 0.79)	0.42 (0.36, 0.49)	0.78 (0.74, 0.82)	0.64 (0.59, 0.69)	0.42 (0.35, 0.48)	0.52 (0.44, 0.59)	0.79 (0.74, 0.85)	0.31 (0.22, 0.40)	0.71 (0.66, 0.77)	0.62 (0.54, 0.70)	0.34 (0.24, 0.43)	0.16 (0.07, 0.24)	0.96 (0.94, 0.99)	0.12 (0.04, 0.20)	0.79 (0.74, 0.83)	0.57 (0.36, 0.78)	0.36 (0.14, 0.58)
	ECL (MSD)	0.18 (0.14, 0.23)	0.92 (0.90, 0.95)	0.11 (0.06, 0.16)	0.63 (0.60, 0.67)	0.61 (0.51, 0.71)	0.24 (0.14, 0.35)	0.13 (0.08, 0.18)	0.91 (0.87, 0.95)	0.04 (0.00, 0.11)	0.61 (0.56, 0.66)	0.49 (0.34, 0.64)	0.10 (0.00, 0.26)	0.22 (0.13, 0.32)	0.90 (0.86, 0.94)	0.12 (0.02, 0.23)	0.79 (0.75, 0.84)	0.40 (0.26, 0.55)	0.20 (0.04, 0.35)
	RBA (tGADA) & LIPS (pIAA) (IDR)	0.47 (0.41, 0.52)	0.86 (0.83, 0.89)	0.32 (0.26, 0.39)	0.71 (0.67, 0.75)	0.68 (0.62, 0.74)	0.39 (0.32, 0.47)	0.28 (0.21, 0.35)	0.90 (0.86, 0.94)	0.18 (0.10, 0.26)	0.66 (0.60, 0.71)	0.64 (0.53, 0.76)	0.30 (0.18, 0.42)	0.05 (0.00, 0.10)	0.97 (0.95, 0.99)	0.02 (0.00, 0.07)	0.77 (0.72, 0.82)	0.33 (0.07, 0.60)	0.10 (0.00, 0.37)
GADA & ZnT8A	RBA	0.47 (0.41, 0.52)	0.83 (0.79, 0.86)	0.29 (0.23, 0.36)	0.70 (0.67, 0.74)	0.64 (0.58, 0.70)	0.34 (0.27, 0.42)	0.60 (0.52, 0.67)	0.78 (0.73, 0.83)	0.38 (0.28, 0.47)	0.75 (0.69, 0.80)	0.64 (0.56, 0.71)	0.38 (0.29, 0.48)	0.34 (0.23, 0.44)	0.86 (0.82, 0.90)	0.20 (0.08, 0.31)	0.81 (0.76, 0.86)	0.43 (0.30, 0.55)	0.24 (0.10, 0.37)
	ECL (BDC)	0.59 (0.54, 0.65)	0.80 (0.76, 0.83)	0.39 (0.32, 0.45)	0.75 (0.71, 0.79)	0.66 (0.60, 0.71)	0.40 (0.34, 0.47)	0.72 (0.65, 0.79)	0.72 (0.67, 0.78)	0.45 (0.36, 0.54)	0.80 (0.75, 0.85)	0.63 (0.56, 0.70)	0.43 (0.34, 0.52)	0.43 (0.32, 0.54)	0.82 (0.77, 0.87)	0.25 (0.13, 0.37)	0.82 (0.78, 0.87)	0.42 (0.31, 0.53)	0.24 (0.12, 0.36)
	LIPS (DRI)	0.68 (0.62, 0.73)	0.76 (0.72, 0.80)	0.43 (0.37, 0.50)	0.78 (0.74, 0.82)	0.64 (0.59, 0.70)	0.43 (0.36, 0.49)	0.79 (0.72, 0.85)	0.69 (0.63, 0.75)	0.47 (0.39, 0.56)	0.83 (0.78, 0.88)	0.62 (0.55, 0.69)	0.45 (0.37, 0.54)	0.49 (0.37, 0.60)	0.77 (0.71, 0.82)	0.25 (0.13, 0.38)	0.83 (0.78, 0.88)	0.39 (0.29, 0.48)	0.22 (0.11, 0.33)
	ADAP (Enable)	0.44 (0.39, 0.50)	0.87 (0.83, 0.90)	0.31 (0.24, 0.37)	0.70 (0.67, 0.74)	0.68 (0.62, 0.75)	0.38 (0.31, 0.46)	0.47 (0.39, 0.54)	0.86 (0.81, 0.90)	0.32 (0.23, 0.41)	0.71 (0.66, 0.76)	0.68 (0.59, 0.77)	0.39 (0.29, 0.49)	0.25 (0.15, 0.34)	0.92 (0.89, 0.95)	0.17 (0.07, 0.27)	0.80 (0.75, 0.85)	0.49 (0.33, 0.64)	0.29 (0.12, 0.45)
	ECL (MSD)	0.56 (0.50, 0.61)	0.77 (0.73, 0.81)	0.33 (0.26, 0.39)	0.73 (0.69, 0.77)	0.61 (0.56, 0.67)	0.34 (0.27, 0.41)	0.64 (0.57, 0.72)	0.69 (0.63, 0.74)	0.33 (0.23, 0.42)	0.74 (0.69, 0.80)	0.57 (0.50, 0.65)	0.32 (0.22, 0.41)	0.45 (0.34, 0.56)	0.74 (0.68, 0.79)	0.19 (0.06, 0.31)	0.82 (0.77, 0.87)	0.34 (0.25, 0.43)	0.16 (0.05, 0.26)

IA Combination	Assay	Age 0-9 Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Age 10-19 Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Age 20+ Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
IA2A & IAA	RBA	0.40 (0.34, 0.45)	0.93 (0.91, 0.96)	0.33 (0.27, 0.39)	0.70 (0.67, 0.74)	0.80 (0.73, 0.86)	0.50 (0.43, 0.57)	0.31 (0.24, 0.38)	0.95 (0.92, 0.97)	0.25 (0.18, 0.33)	0.68 (0.63, 0.73)	0.79 (0.69, 0.89)	0.47 (0.35, 0.58)	0.04 (0.00, 0.08)	0.98 (0.97, 1.00)	0.02 (0.00, 0.07)	0.77 (0.72, 0.82)	0.43 (0.06, 0.80)	0.20 (0.00, 0.57)
	ECL (BDC)	0.38 (0.33, 0.44)	0.93 (0.91, 0.95)	0.31 (0.25, 0.37)	0.70 (0.66, 0.73)	0.78 (0.71, 0.85)	0.48 (0.40, 0.55)	0.31 (0.24, 0.39)	0.89 (0.85, 0.93)	0.21 (0.13, 0.29)	0.67 (0.61, 0.72)	0.66 (0.55, 0.76)	0.32 (0.21, 0.44)	0.13 (0.05, 0.20)	0.97 (0.95, 0.99)	0.10 (0.02, 0.18)	0.79 (0.74, 0.83)	0.59 (0.35, 0.82)	0.37 (0.14, 0.61)
	LIPS LoIdx (DRI)	0.68 (0.63, 0.73)	0.83 (0.80, 0.87)	0.51 (0.45, 0.57)	0.80 (0.76, 0.83)	0.72 (0.67, 0.78)	0.52 (0.46, 0.59)	0.65 (0.58, 0.73)	0.80 (0.75, 0.85)	0.46 (0.37, 0.55)	0.78 (0.73, 0.83)	0.68 (0.61, 0.76)	0.46 (0.37, 0.55)	0.26 (0.16, 0.36)	0.94 (0.91, 0.97)	0.20 (0.10, 0.30)	0.81 (0.76, 0.85)	0.56 (0.39, 0.72)	0.36 (0.20, 0.53)
	LIPS HiIdx (DRI)	0.68 (0.63, 0.74)	0.85 (0.81, 0.88)	0.53 (0.47, 0.59)	0.80 (0.77, 0.84)	0.74 (0.69, 0.79)	0.55 (0.49, 0.61)	0.64 (0.57, 0.72)	0.81 (0.77, 0.86)	0.46 (0.37, 0.55)	0.78 (0.73, 0.83)	0.69 (0.62, 0.77)	0.47 (0.38, 0.56)	0.24 (0.14, 0.33)	0.94 (0.91, 0.97)	0.18 (0.08, 0.28)	0.80 (0.76, 0.85)	0.55 (0.38, 0.72)	0.35 (0.17, 0.52)
	LIPS HiStd (DRI)	0.63 (0.58, 0.69)	0.85 (0.82, 0.88)	0.48 (0.42, 0.55)	0.78 (0.74, 0.82)	0.73 (0.68, 0.79)	0.52 (0.45, 0.58)	0.59 (0.51, 0.67)	0.87 (0.83, 0.91)	0.46 (0.37, 0.55)	0.76 (0.71, 0.81)	0.75 (0.67, 0.82)	0.51 (0.42, 0.60)	0.20 (0.11, 0.29)	0.96 (0.94, 0.98)	0.16 (0.07, 0.25)	0.80 (0.75, 0.84)	0.60 (0.41, 0.79)	0.40 (0.20, 0.60)
	ADAP (Enable)	0.57 (0.51, 0.62)	0.85 (0.82, 0.88)	0.42 (0.35, 0.48)	0.75 (0.71, 0.79)	0.71 (0.65, 0.77)	0.46 (0.39, 0.53)	0.41 (0.33, 0.49)	0.87 (0.83, 0.91)	0.28 (0.19, 0.36)	0.69 (0.64, 0.74)	0.67 (0.58, 0.76)	0.36 (0.25, 0.47)	0.12 (0.05, 0.19)	0.99 (0.97, 1.00)	0.10 (0.03, 0.18)	0.79 (0.74, 0.83)	0.75 (0.51, 0.99)	0.54 (0.29, 0.78)
	ECL (MSD)	0.16 (0.12, 0.20)	0.96 (0.95, 0.98)	0.12 (0.08, 0.17)	0.64 (0.60, 0.67)	0.74 (0.64, 0.85)	0.38 (0.27, 0.49)	0.13 (0.08, 0.18)	0.95 (0.92, 0.98)	0.08 (0.02, 0.14)	0.62 (0.58, 0.67)	0.64 (0.47, 0.80)	0.26 (0.09, 0.43)	0.12 (0.05, 0.19)	0.94 (0.91, 0.97)	0.06 (0.00, 0.14)	0.78 (0.73, 0.83)	0.38 (0.18, 0.57)	0.15 (0.00, 0.35)
	RBA (IA2βA) & LIPS (pIAA) (IDR)	0.32 (0.27, 0.37)	0.96 (0.94, 0.97)	0.28 (0.22, 0.33)	0.68 (0.65, 0.72)	0.82 (0.76, 0.89)	0.51 (0.43, 0.58)	0.23 (0.17, 0.30)	0.97 (0.94, 0.99)	0.20 (0.13, 0.27)	0.66 (0.61, 0.71)	0.82 (0.71, 0.93)	0.48 (0.36, 0.60)	0.01 (0.00, 0.04)	1.00 (1.00, 1.00)	0.01 (0.00, 0.04)	0.77 (0.72, 0.81)	1.00 (1.00, 1.00)	0.77 (0.72, 0.81)
IA2A & ZnT8A	RBA	0.49 (0.43, 0.55)	0.91 (0.88, 0.93)	0.40 (0.34, 0.46)	0.73 (0.70, 0.77)	0.78 (0.72, 0.84)	0.51 (0.44, 0.58)	0.57 (0.50, 0.65)	0.82 (0.77, 0.87)	0.39 (0.30, 0.48)	0.75 (0.69, 0.80)	0.67 (0.60, 0.75)	0.42 (0.32, 0.51)	0.27 (0.17, 0.37)	0.92 (0.89, 0.96)	0.20 (0.09, 0.30)	0.81 (0.76, 0.85)	0.52 (0.37, 0.68)	0.33 (0.17, 0.49)
	ECL (BDC)	0.53 (0.47, 0.58)	0.88 (0.85, 0.91)	0.41 (0.35, 0.47)	0.74 (0.70, 0.78)	0.75 (0.69, 0.80)	0.49 (0.42, 0.55)	0.64 (0.56, 0.71)	0.79 (0.74, 0.84)	0.43 (0.33, 0.52)	0.77 (0.72, 0.82)	0.66 (0.59, 0.74)	0.43 (0.34, 0.52)	0.32 (0.22, 0.43)	0.87 (0.83, 0.91)	0.20 (0.09, 0.31)	0.81 (0.76, 0.86)	0.44 (0.31, 0.57)	0.25 (0.11, 0.38)

IA Combination	Assay	Age 0-9						Age 10-19						Age 20+					
		Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
	LIPS (DRI)	0.64 (0.58, 0.69)	0.83 (0.80, 0.87)	0.47 (0.41, 0.53)	0.78 (0.74, 0.81)	0.71 (0.66, 0.77)	0.49 (0.43, 0.56)	0.77 (0.70, 0.83)	0.77 (0.71, 0.82)	0.53 (0.45, 0.62)	0.83 (0.79, 0.88)	0.68 (0.61, 0.75)	0.52 (0.43, 0.60)	0.38 (0.27, 0.49)	0.86 (0.81, 0.90)	0.24 (0.12, 0.36)	0.82 (0.77, 0.87)	0.45 (0.33, 0.57)	0.27 (0.14, 0.40)
	ADAP (Enable)	0.42 (0.37, 0.48)	0.87 (0.84, 0.90)	0.29 (0.23, 0.36)	0.70 (0.66, 0.74)	0.68 (0.61, 0.75)	0.38 (0.30, 0.45)	0.41 (0.33, 0.49)	0.86 (0.81, 0.90)	0.26 (0.18, 0.35)	0.69 (0.64, 0.74)	0.65 (0.56, 0.74)	0.34 (0.23, 0.45)	0.21 (0.12, 0.30)	0.94 (0.91, 0.97)	0.14 (0.05, 0.24)	0.79 (0.75, 0.84)	0.50 (0.33, 0.67)	0.29 (0.12, 0.47)
	ECL (MSD)	0.55 (0.49, 0.60)	0.88 (0.85, 0.91)	0.42 (0.36, 0.49)	0.75 (0.71, 0.78)	0.75 (0.69, 0.80)	0.49 (0.43, 0.56)	0.61 (0.53, 0.69)	0.79 (0.74, 0.84)	0.40 (0.31, 0.49)	0.75 (0.70, 0.81)	0.66 (0.58, 0.73)	0.41 (0.32, 0.50)	0.32 (0.21, 0.42)	0.86 (0.82, 0.90)	0.18 (0.06, 0.29)	0.81 (0.76, 0.85)	0.41 (0.28, 0.53)	0.21 (0.08, 0.35)
IAA & ZnT8A	RBA	0.42 (0.37, 0.48)	0.90 (0.87, 0.93)	0.32 (0.26, 0.38)	0.70 (0.67, 0.74)	0.73 (0.67, 0.80)	0.44 (0.36, 0.51)	0.31 (0.24, 0.38)	0.92 (0.89, 0.96)	0.23 (0.15, 0.31)	0.67 (0.62, 0.72)	0.72 (0.61, 0.83)	0.39 (0.27, 0.51)	0.05 (0.00, 0.10)	0.98 (0.97, 1.00)	0.04 (0.00, 0.09)	0.77 (0.73, 0.82)	0.50 (0.15, 0.85)	0.27 (0.00, 0.62)
	ECL (BDC)	0.41 (0.35, 0.46)	0.90 (0.87, 0.92)	0.30 (0.24, 0.37)	0.70 (0.66, 0.74)	0.72 (0.65, 0.79)	0.42 (0.34, 0.49)	0.33 (0.26, 0.41)	0.90 (0.86, 0.94)	0.23 (0.15, 0.31)	0.67 (0.62, 0.72)	0.68 (0.58, 0.78)	0.35 (0.24, 0.47)	0.13 (0.05, 0.20)	0.98 (0.96, 1.00)	0.11 (0.03, 0.18)	0.79 (0.74, 0.83)	0.62 (0.39, 0.86)	0.41 (0.17, 0.65)
	LIPS LoIdx (DRI)	0.69 (0.64, 0.74)	0.77 (0.73, 0.81)	0.46 (0.39, 0.52)	0.79 (0.75, 0.83)	0.66 (0.61, 0.71)	0.45 (0.39, 0.51)	0.66 (0.59, 0.73)	0.76 (0.70, 0.81)	0.42 (0.33, 0.51)	0.77 (0.72, 0.83)	0.64 (0.57, 0.71)	0.41 (0.32, 0.50)	0.24 (0.14, 0.33)	0.92 (0.88, 0.95)	0.15 (0.05, 0.25)	0.80 (0.75, 0.85)	0.46 (0.31, 0.62)	0.26 (0.10, 0.42)
	LIPS HiIdx (DRI)	0.70 (0.64, 0.75)	0.79 (0.75, 0.83)	0.49 (0.42, 0.55)	0.80 (0.76, 0.84)	0.68 (0.63, 0.74)	0.48 (0.42, 0.55)	0.63 (0.55, 0.70)	0.77 (0.72, 0.82)	0.40 (0.31, 0.49)	0.76 (0.71, 0.81)	0.64 (0.57, 0.72)	0.40 (0.31, 0.49)	0.22 (0.13, 0.32)	0.92 (0.89, 0.96)	0.15 (0.05, 0.25)	0.80 (0.75, 0.84)	0.47 (0.31, 0.64)	0.27 (0.10, 0.44)
	LIPS HiStd (DRI)	0.65 (0.60, 0.71)	0.80 (0.77, 0.84)	0.46 (0.39, 0.52)	0.78 (0.74, 0.82)	0.68 (0.63, 0.74)	0.47 (0.40, 0.53)	0.58 (0.51, 0.66)	0.83 (0.78, 0.87)	0.41 (0.32, 0.50)	0.75 (0.70, 0.80)	0.69 (0.61, 0.77)	0.44 (0.35, 0.54)	0.20 (0.11, 0.29)	0.95 (0.93, 0.98)	0.15 (0.06, 0.24)	0.80 (0.75, 0.84)	0.56 (0.37, 0.74)	0.35 (0.16, 0.55)
	ADAP (Enable)	0.37 (0.32, 0.42)	0.90 (0.87, 0.93)	0.27 (0.21, 0.33)	0.69 (0.65, 0.72)	0.71 (0.64, 0.78)	0.39 (0.31, 0.47)	0.28 (0.21, 0.35)	0.93 (0.90, 0.96)	0.21 (0.13, 0.28)	0.66 (0.61, 0.71)	0.72 (0.61, 0.83)	0.38 (0.26, 0.51)	0.06 (0.01, 0.12)	1.00 (0.99, 1.00)	0.06 (0.01, 0.12)	0.78 (0.73, 0.82)	0.83 (0.54, 1.00)	0.61 (0.31, 0.91)
	ECL (MSD)	0.18 (0.13, 0.22)	0.94 (0.92, 0.96)	0.12 (0.07, 0.16)	0.64 (0.60, 0.67)	0.66 (0.56, 0.76)	0.29 (0.19, 0.40)	0.13 (0.07, 0.18)	0.93 (0.89, 0.96)	0.05 (0.00, 0.11)	0.62 (0.57, 0.67)	0.53 (0.37, 0.69)	0.14 (0.00, 0.31)	0.16 (0.08, 0.24)	0.91 (0.88, 0.95)	0.07 (0.00, 0.16)	0.78 (0.74, 0.83)	0.35 (0.19, 0.51)	0.14 (0.00, 0.30)

IA Combination	Assay	Age 0-9 Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Age 10-19 Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Age 20+ Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
GADA & IA2A & IAA	RBA	0.33 (0.27, 0.38)	0.94 (0.92, 0.96)	0.27 (0.21, 0.33)	0.68 (0.65, 0.72)	0.79 (0.72, 0.86)	0.47 (0.39, 0.55)	0.27 (0.20, 0.34)	0.95 (0.92, 0.98)	0.22 (0.15, 0.30)	0.67 (0.62, 0.72)	0.78 (0.67, 0.89)	0.45 (0.33, 0.57)	0.04 (0.00, 0.08)	0.99 (0.97, 1.00)	0.03 (0.00, 0.07)	0.77 (0.73, 0.82)	0.50 (0.10, 0.90)	0.27 (0.00, 0.67)
	ECL (BDC)	0.35 (0.30, 0.40)	0.94 (0.91, 0.96)	0.29 (0.23, 0.34)	0.69 (0.65, 0.72)	0.78 (0.71, 0.85)	0.47 (0.39, 0.55)	0.30 (0.22, 0.37)	0.89 (0.85, 0.93)	0.19 (0.11, 0.27)	0.66 (0.61, 0.71)	0.64 (0.53, 0.75)	0.30 (0.18, 0.42)	0.13 (0.05, 0.20)	0.98 (0.96, 1.00)	0.11 (0.03, 0.18)	0.79 (0.74, 0.83)	0.62 (0.39, 0.86)	0.41 (0.17, 0.65)
	LIPS LoIdx (DRI)	0.62 (0.56, 0.67)	0.85 (0.82, 0.88)	0.47 (0.40, 0.53)	0.77 (0.74, 0.81)	0.73 (0.67, 0.78)	0.50 (0.43, 0.56)	0.63 (0.55, 0.70)	0.81 (0.76, 0.86)	0.44 (0.35, 0.53)	0.77 (0.72, 0.82)	0.68 (0.60, 0.76)	0.45 (0.36, 0.54)	0.26 (0.16, 0.36)	0.94 (0.91, 0.97)	0.20 (0.10, 0.30)	0.81 (0.76, 0.85)	0.56 (0.39, 0.72)	0.36 (0.20, 0.53)
	LIPS HiIdx (DRI)	0.62 (0.57, 0.68)	0.86 (0.83, 0.89)	0.48 (0.42, 0.54)	0.78 (0.74, 0.81)	0.74 (0.69, 0.80)	0.52 (0.45, 0.58)	0.60 (0.53, 0.68)	0.81 (0.77, 0.86)	0.42 (0.33, 0.51)	0.76 (0.71, 0.81)	0.68 (0.60, 0.76)	0.44 (0.35, 0.53)	0.24 (0.14, 0.33)	0.94 (0.92, 0.97)	0.18 (0.08, 0.28)	0.80 (0.76, 0.85)	0.56 (0.39, 0.73)	0.37 (0.19, 0.54)
	LIPS HiStd (DRI)	0.58 (0.53, 0.64)	0.86 (0.83, 0.89)	0.44 (0.38, 0.51)	0.76 (0.72, 0.80)	0.74 (0.68, 0.79)	0.49 (0.43, 0.56)	0.57 (0.49, 0.64)	0.87 (0.83, 0.91)	0.43 (0.35, 0.52)	0.75 (0.70, 0.80)	0.74 (0.66, 0.82)	0.49 (0.40, 0.58)	0.20 (0.11, 0.29)	0.96 (0.94, 0.99)	0.16 (0.07, 0.25)	0.80 (0.75, 0.84)	0.62 (0.43, 0.82)	0.42 (0.23, 0.62)
	ADAP (Enable)	0.52 (0.46, 0.57)	0.87 (0.84, 0.90)	0.39 (0.32, 0.45)	0.73 (0.70, 0.77)	0.72 (0.66, 0.78)	0.45 (0.38, 0.52)	0.39 (0.31, 0.47)	0.87 (0.83, 0.91)	0.26 (0.17, 0.35)	0.69 (0.63, 0.74)	0.66 (0.56, 0.76)	0.34 (0.24, 0.45)	0.12 (0.05, 0.19)	0.99 (0.97, 1.00)	0.10 (0.03, 0.18)	0.79 (0.74, 0.83)	0.75 (0.51, 0.99)	0.54 (0.29, 0.78)
	ECL (MSD)	0.15 (0.11, 0.19)	0.97 (0.95, 0.98)	0.12 (0.07, 0.16)	0.63 (0.60, 0.67)	0.74 (0.63, 0.85)	0.38 (0.26, 0.49)	0.13 (0.07, 0.18)	0.95 (0.93, 0.98)	0.08 (0.02, 0.14)	0.62 (0.57, 0.67)	0.65 (0.48, 0.81)	0.27 (0.09, 0.44)	0.11 (0.04, 0.17)	0.95 (0.92, 0.98)	0.05 (0.00, 0.13)	0.78 (0.73, 0.82)	0.38 (0.17, 0.59)	0.16 (0.00, 0.37)
	RBA (tGADA) & RBA (IA2βA) & LIPS (pIAA) (IDR)	0.28 (0.23, 0.33)	0.96 (0.94, 0.98)	0.24 (0.19, 0.29)	0.67 (0.64, 0.71)	0.82 (0.75, 0.89)	0.49 (0.41, 0.57)	0.19 (0.13, 0.26)	0.97 (0.95, 0.99)	0.17 (0.10, 0.23)	0.65 (0.60, 0.70)	0.82 (0.69, 0.94)	0.46 (0.33, 0.60)	0.01 (0.00, 0.04)	1.00 (1.00, 1.00)	0.01 (0.00, 0.04)	0.77 (0.72, 0.81)	1.00 (1.00, 1.00)	0.77 (0.72, 0.81)
GADA & IA2A	RBA	0.38 (0.32, 0.43)	0.92 (0.90, 0.95)	0.30 (0.24, 0.36)	0.69 (0.66, 0.73)	0.76 (0.69, 0.83)	0.46 (0.38, 0.53)	0.47 (0.39, 0.54)	0.84 (0.80, 0.89)	0.31 (0.22, 0.40)	0.71 (0.65, 0.76)	0.66 (0.57, 0.75)	0.37 (0.27, 0.47)	0.26 (0.16, 0.36)	0.93 (0.90, 0.96)	0.19 (0.09, 0.30)	0.80 (0.76, 0.85)	0.54 (0.38, 0.70)	0.35 (0.18, 0.51)

IA Combination	Assay	Age 0-9						Age 10-19						Age 20+					
		Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
& ZnT8A	ECL (BDC)	0.47 (0.41, 0.53)	0.89 (0.87, 0.92)	0.36 (0.30, 0.43)	0.72 (0.68, 0.76)	0.74 (0.68, 0.80)	0.46 (0.39, 0.53)	0.58 (0.50, 0.66)	0.80 (0.75, 0.85)	0.38 (0.29, 0.47)	0.74 (0.69, 0.80)	0.66 (0.58, 0.74)	0.40 (0.31, 0.50)	0.32 (0.22, 0.43)	0.88 (0.85, 0.92)	0.21 (0.10, 0.32)	0.81 (0.76, 0.86)	0.46 (0.33, 0.60)	0.27 (0.13, 0.41)
	LIPS (DRI)	0.58 (0.52, 0.63)	0.85 (0.82, 0.88)	0.43 (0.36, 0.49)	0.76 (0.72, 0.79)	0.72 (0.66, 0.77)	0.47 (0.40, 0.54)	0.73 (0.66, 0.80)	0.77 (0.72, 0.83)	0.50 (0.42, 0.59)	0.81 (0.76, 0.86)	0.68 (0.61, 0.75)	0.49 (0.41, 0.58)	0.38 (0.27, 0.49)	0.87 (0.82, 0.91)	0.25 (0.13, 0.36)	0.82 (0.78, 0.87)	0.46 (0.34, 0.58)	0.28 (0.15, 0.41)
	ADAP (Enable)	0.39 (0.33, 0.44)	0.89 (0.86, 0.92)	0.28 (0.22, 0.34)	0.69 (0.65, 0.73)	0.70 (0.63, 0.77)	0.39 (0.31, 0.47)	0.40 (0.32, 0.47)	0.88 (0.84, 0.92)	0.27 (0.19, 0.36)	0.69 (0.64, 0.74)	0.68 (0.58, 0.77)	0.37 (0.26, 0.47)	0.21 (0.12, 0.30)	0.94 (0.91, 0.97)	0.15 (0.05, 0.24)	0.80 (0.75, 0.84)	0.52 (0.34, 0.69)	0.31 (0.13, 0.49)
	ECL (MSD)	0.44 (0.38, 0.49)	0.90 (0.87, 0.93)	0.34 (0.28, 0.40)	0.71 (0.67, 0.75)	0.74 (0.68, 0.80)	0.45 (0.38, 0.52)	0.53 (0.45, 0.61)	0.81 (0.76, 0.86)	0.34 (0.25, 0.43)	0.72 (0.67, 0.78)	0.65 (0.56, 0.73)	0.37 (0.27, 0.47)	0.30 (0.20, 0.41)	0.88 (0.84, 0.92)	0.18 (0.07, 0.29)	0.81 (0.76, 0.85)	0.43 (0.29, 0.56)	0.23 (0.09, 0.37)
GADA & IAA & ZnT8A	RBA	0.32 (0.26, 0.37)	0.91 (0.89, 0.94)	0.23 (0.17, 0.29)	0.67 (0.64, 0.71)	0.71 (0.63, 0.78)	0.38 (0.30, 0.46)	0.28 (0.21, 0.35)	0.93 (0.90, 0.97)	0.21 (0.13, 0.29)	0.66 (0.61, 0.71)	0.73 (0.62, 0.85)	0.40 (0.27, 0.52)	0.05 (0.00, 0.10)	0.98 (0.97, 1.00)	0.04 (0.00, 0.09)	0.77 (0.73, 0.82)	0.50 (0.15, 0.85)	0.27 (0.00, 0.62)
	ECL (BDC)	0.36 (0.31, 0.42)	0.90 (0.88, 0.93)	0.27 (0.21, 0.33)	0.68 (0.65, 0.72)	0.71 (0.64, 0.78)	0.40 (0.32, 0.48)	0.31 (0.24, 0.39)	0.90 (0.86, 0.94)	0.21 (0.13, 0.29)	0.67 (0.62, 0.72)	0.67 (0.56, 0.77)	0.33 (0.22, 0.45)	0.13 (0.05, 0.20)	0.98 (0.96, 1.00)	0.11 (0.03, 0.19)	0.79 (0.74, 0.83)	0.67 (0.43, 0.91)	0.45 (0.21, 0.70)
	LIPS LoIdx (DRI)	0.62 (0.57, 0.68)	0.79 (0.75, 0.83)	0.41 (0.35, 0.48)	0.76 (0.73, 0.80)	0.66 (0.61, 0.72)	0.42 (0.36, 0.49)	0.64 (0.56, 0.71)	0.77 (0.71, 0.82)	0.40 (0.31, 0.49)	0.76 (0.71, 0.82)	0.64 (0.56, 0.71)	0.40 (0.31, 0.49)	0.24 (0.14, 0.33)	0.92 (0.88, 0.95)	0.15 (0.05, 0.25)	0.80 (0.75, 0.85)	0.46 (0.31, 0.62)	0.26 (0.10, 0.42)
	LIPS HiIdx (DRI)	0.63 (0.57, 0.68)	0.81 (0.77, 0.84)	0.44 (0.37, 0.50)	0.77 (0.73, 0.81)	0.68 (0.63, 0.74)	0.45 (0.38, 0.52)	0.59 (0.51, 0.67)	0.78 (0.73, 0.83)	0.37 (0.28, 0.46)	0.74 (0.69, 0.80)	0.64 (0.56, 0.71)	0.38 (0.28, 0.47)	0.22 (0.13, 0.32)	0.92 (0.89, 0.96)	0.15 (0.05, 0.25)	0.80 (0.75, 0.84)	0.47 (0.31, 0.64)	0.27 (0.10, 0.44)
	LIPS HiStd (DRI)	0.59 (0.54, 0.65)	0.82 (0.79, 0.86)	0.41 (0.35, 0.48)	0.75 (0.72, 0.79)	0.68 (0.63, 0.74)	0.44 (0.37, 0.50)	0.56 (0.48, 0.64)	0.84 (0.79, 0.88)	0.40 (0.30, 0.49)	0.74 (0.69, 0.80)	0.69 (0.61, 0.77)	0.43 (0.34, 0.53)	0.20 (0.11, 0.29)	0.95 (0.93, 0.98)	0.15 (0.06, 0.24)	0.80 (0.75, 0.84)	0.56 (0.37, 0.74)	0.35 (0.16, 0.55)
	ADAP (Enable)	0.34 (0.28, 0.39)	0.91 (0.88, 0.93)	0.24 (0.19, 0.30)	0.68 (0.64, 0.71)	0.71 (0.63, 0.78)	0.38 (0.30, 0.46)	0.27 (0.20, 0.34)	0.93 (0.90, 0.96)	0.20 (0.12, 0.28)	0.66 (0.61, 0.71)	0.72 (0.60, 0.83)	0.38 (0.25, 0.50)	0.06 (0.01, 0.12)	1.00 (0.99, 1.00)	0.06 (0.01, 0.12)	0.78 (0.73, 0.82)	0.83 (0.54, 1.00)	0.61 (0.31, 0.91)

IA Combination	Assay	Age 0-9						Age 10-19						Age 20+					
		Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
	ECL (MSD)	0.17 (0.12, 0.21)	0.94 (0.92, 0.96)	0.11 (0.06, 0.16)	0.63 (0.60, 0.67)	0.65 (0.55, 0.76)	0.29 (0.18, 0.40)	0.12 (0.07, 0.17)	0.93 (0.89, 0.96)	0.05 (0.00, 0.11)	0.62 (0.57, 0.67)	0.51 (0.35, 0.67)	0.13 (0.00, 0.30)	0.16 (0.08, 0.24)	0.93 (0.90, 0.96)	0.09 (0.00, 0.18)	0.79 (0.74, 0.83)	0.41 (0.23, 0.59)	0.20 (0.01, 0.38)
IA2A & IAA & ZnT8A	RBA	0.33 (0.27, 0.38)	0.95 (0.93, 0.97)	0.28 (0.22, 0.34)	0.68 (0.65, 0.72)	0.82 (0.75, 0.89)	0.50 (0.43, 0.58)	0.27 (0.20, 0.34)	0.96 (0.94, 0.99)	0.23 (0.16, 0.31)	0.67 (0.62, 0.72)	0.83 (0.72, 0.93)	0.50 (0.38, 0.61)	0.03 (0.00, 0.06)	0.99 (0.98, 1.00)	0.02 (0.00, 0.06)	0.77 (0.72, 0.82)	0.50 (0.01, 0.99)	0.27 (0.00, 0.76)
	ECL (BDC)	0.32 (0.27, 0.37)	0.94 (0.91, 0.96)	0.26 (0.20, 0.31)	0.68 (0.64, 0.71)	0.77 (0.69, 0.84)	0.44 (0.36, 0.53)	0.28 (0.21, 0.35)	0.92 (0.88, 0.95)	0.20 (0.12, 0.28)	0.66 (0.61, 0.71)	0.69 (0.58, 0.80)	0.35 (0.23, 0.48)	0.10 (0.04, 0.17)	0.98 (0.96, 1.00)	0.08 (0.01, 0.15)	0.78 (0.74, 0.83)	0.57 (0.31, 0.83)	0.35 (0.09, 0.62)
	LIPS LoIdx (DRI)	0.58 (0.53, 0.64)	0.85 (0.82, 0.88)	0.43 (0.37, 0.50)	0.76 (0.72, 0.79)	0.72 (0.66, 0.78)	0.48 (0.41, 0.54)	0.62 (0.54, 0.69)	0.84 (0.79, 0.89)	0.46 (0.37, 0.54)	0.77 (0.72, 0.82)	0.72 (0.64, 0.79)	0.49 (0.39, 0.58)	0.20 (0.11, 0.29)	0.94 (0.92, 0.97)	0.14 (0.05, 0.24)	0.80 (0.75, 0.84)	0.52 (0.34, 0.70)	0.31 (0.13, 0.50)
	LIPS HiIdx (DRI)	0.59 (0.53, 0.64)	0.87 (0.84, 0.90)	0.46 (0.39, 0.52)	0.76 (0.73, 0.80)	0.74 (0.69, 0.80)	0.51 (0.44, 0.57)	0.60 (0.53, 0.68)	0.84 (0.80, 0.89)	0.45 (0.36, 0.54)	0.76 (0.71, 0.82)	0.72 (0.64, 0.79)	0.48 (0.39, 0.57)	0.18 (0.10, 0.27)	0.95 (0.93, 0.98)	0.14 (0.05, 0.23)	0.79 (0.75, 0.84)	0.54 (0.35, 0.73)	0.33 (0.14, 0.53)
	LIPS HiStd (DRI)	0.55 (0.49, 0.60)	0.87 (0.84, 0.90)	0.42 (0.36, 0.48)	0.75 (0.71, 0.78)	0.73 (0.68, 0.79)	0.48 (0.41, 0.55)	0.56 (0.48, 0.64)	0.89 (0.85, 0.93)	0.45 (0.36, 0.54)	0.76 (0.71, 0.81)	0.77 (0.69, 0.84)	0.52 (0.43, 0.61)	0.16 (0.08, 0.24)	0.97 (0.95, 0.99)	0.13 (0.04, 0.21)	0.79 (0.75, 0.84)	0.60 (0.39, 0.81)	0.39 (0.17, 0.61)
	ADAP (Enable)	0.33 (0.28, 0.38)	0.92 (0.89, 0.94)	0.25 (0.19, 0.31)	0.68 (0.64, 0.71)	0.72 (0.65, 0.80)	0.40 (0.32, 0.48)	0.23 (0.16, 0.29)	0.94 (0.91, 0.97)	0.17 (0.10, 0.24)	0.65 (0.60, 0.70)	0.72 (0.60, 0.84)	0.37 (0.24, 0.50)	0.05 (0.00, 0.10)	1.00 (1.00, 1.00)	0.05 (0.00, 0.10)	0.78 (0.73, 0.82)	1.00 (1.00, 1.00)	0.78 (0.73, 0.82)
	ECL (MSD)	0.15 (0.11, 0.19)	0.97 (0.95, 0.98)	0.12 (0.07, 0.16)	0.63 (0.60, 0.67)	0.75 (0.64, 0.86)	0.38 (0.27, 0.50)	0.13 (0.07, 0.18)	0.95 (0.93, 0.98)	0.08 (0.02, 0.14)	0.62 (0.57, 0.67)	0.65 (0.48, 0.81)	0.27 (0.09, 0.44)	0.09 (0.03, 0.16)	0.94 (0.92, 0.97)	0.04 (0.00, 0.11)	0.78 (0.73, 0.82)	0.33 (0.13, 0.53)	0.11 (0.00, 0.32)
GADA & IA2A & IAA & ZnT8A	RBA	0.26 (0.22, 0.31)	0.96 (0.94, 0.98)	0.22 (0.17, 0.28)	0.67 (0.63, 0.70)	0.81 (0.73, 0.89)	0.48 (0.39, 0.56)	0.24 (0.17, 0.31)	0.96 (0.94, 0.99)	0.20 (0.13, 0.27)	0.66 (0.61, 0.71)	0.81 (0.70, 0.92)	0.47 (0.34, 0.59)	0.03 (0.00, 0.06)	0.99 (0.98, 1.00)	0.02 (0.00, 0.06)	0.77 (0.72, 0.82)	0.50 (0.01, 0.99)	0.27 (0.00, 0.76)
	ECL (BDC)	0.29 (0.24, 0.34)	0.94 (0.92, 0.96)	0.23 (0.18, 0.29)	0.67 (0.63, 0.71)	0.77 (0.69, 0.84)	0.44 (0.35, 0.52)	0.26 (0.20, 0.33)	0.92 (0.88, 0.95)	0.18 (0.11, 0.26)	0.66 (0.61, 0.71)	0.68 (0.56, 0.79)	0.33 (0.21, 0.46)	0.10 (0.04, 0.17)	0.98 (0.96, 1.00)	0.08 (0.01, 0.15)	0.78 (0.74, 0.83)	0.62 (0.35, 0.88)	0.40 (0.13, 0.67)

IA Combination	Assay	Age 0-9						Age 10-19						Age 20+					
		Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI	Sensitivity	Specificity	Youden's J	NPV	PPV	PSI
	LIPS LoIdx (DRI)	0.53 (0.47, 0.59)	0.87 (0.84, 0.90)	0.40 (0.33, 0.46)	0.74 (0.70, 0.77)	0.72 (0.66, 0.78)	0.46 (0.39, 0.53)	0.59 (0.51, 0.67)	0.84 (0.79, 0.89)	0.43 (0.34, 0.52)	0.76 (0.71, 0.81)	0.71 (0.63, 0.78)	0.47 (0.37, 0.56)	0.20 (0.11, 0.29)	0.94 (0.92, 0.97)	0.14 (0.05, 0.24)	0.80 (0.75, 0.84)	0.52 (0.34, 0.70)	0.31 (0.13, 0.50)
	LIPS HiIdx (DRI)	0.53 (0.48, 0.59)	0.88 (0.85, 0.91)	0.41 (0.35, 0.48)	0.74 (0.71, 0.78)	0.74 (0.69, 0.80)	0.49 (0.42, 0.55)	0.57 (0.49, 0.64)	0.84 (0.80, 0.89)	0.41 (0.32, 0.50)	0.75 (0.70, 0.80)	0.70 (0.62, 0.78)	0.45 (0.36, 0.55)	0.18 (0.10, 0.27)	0.95 (0.93, 0.98)	0.14 (0.05, 0.23)	0.79 (0.75, 0.84)	0.54 (0.35, 0.73)	0.33 (0.14, 0.53)
	LIPS HiStd (DRI)	0.50 (0.44, 0.56)	0.88 (0.85, 0.91)	0.38 (0.32, 0.45)	0.73 (0.69, 0.77)	0.74 (0.68, 0.80)	0.47 (0.40, 0.54)	0.53 (0.46, 0.61)	0.89 (0.85, 0.93)	0.42 (0.34, 0.51)	0.74 (0.69, 0.80)	0.76 (0.68, 0.84)	0.50 (0.41, 0.60)	0.16 (0.08, 0.24)	0.97 (0.95, 0.99)	0.13 (0.04, 0.21)	0.79 (0.75, 0.84)	0.60 (0.39, 0.81)	0.39 (0.17, 0.61)
	ADAP (Enable)	0.30 (0.25, 0.35)	0.93 (0.90, 0.95)	0.23 (0.17, 0.28)	0.67 (0.63, 0.71)	0.72 (0.65, 0.80)	0.39 (0.31, 0.48)	0.22 (0.16, 0.28)	0.94 (0.91, 0.97)	0.16 (0.09, 0.23)	0.65 (0.60, 0.70)	0.71 (0.59, 0.84)	0.36 (0.23, 0.50)	0.05 (0.00, 0.10)	1.00 (1.00, 1.00)	0.05 (0.00, 0.10)	0.78 (0.73, 0.82)	1.00 (1.00, 1.00)	0.78 (0.73, 0.82)
	ECL (MSD)	0.14 (0.10, 0.18)	0.97 (0.95, 0.98)	0.11 (0.07, 0.15)	0.63 (0.60, 0.67)	0.74 (0.63, 0.85)	0.37 (0.26, 0.49)	0.12 (0.07, 0.17)	0.95 (0.93, 0.98)	0.07 (0.02, 0.13)	0.62 (0.57, 0.67)	0.63 (0.46, 0.81)	0.26 (0.08, 0.44)	0.09 (0.03, 0.16)	0.95 (0.93, 0.98)	0.04 (0.00, 0.11)	0.78 (0.73, 0.82)	0.37 (0.15, 0.59)	0.15 (0.00, 0.37)

Figure S1. Glutamic acid decarboxylase autoantibodies (GADA) assay correlations.

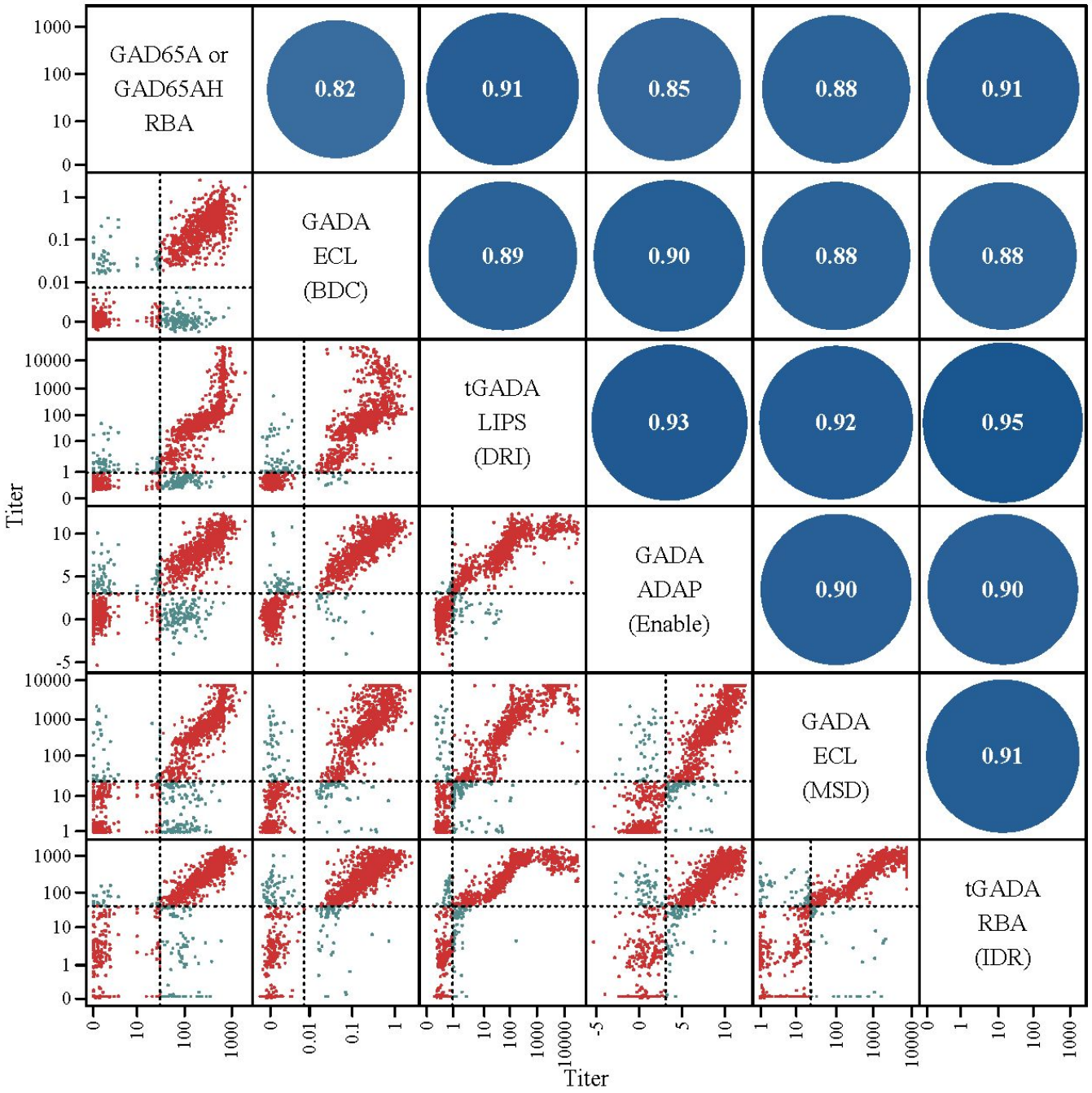


Figure S2. ICA512 (IA2A) autoantibodies assay correlations.

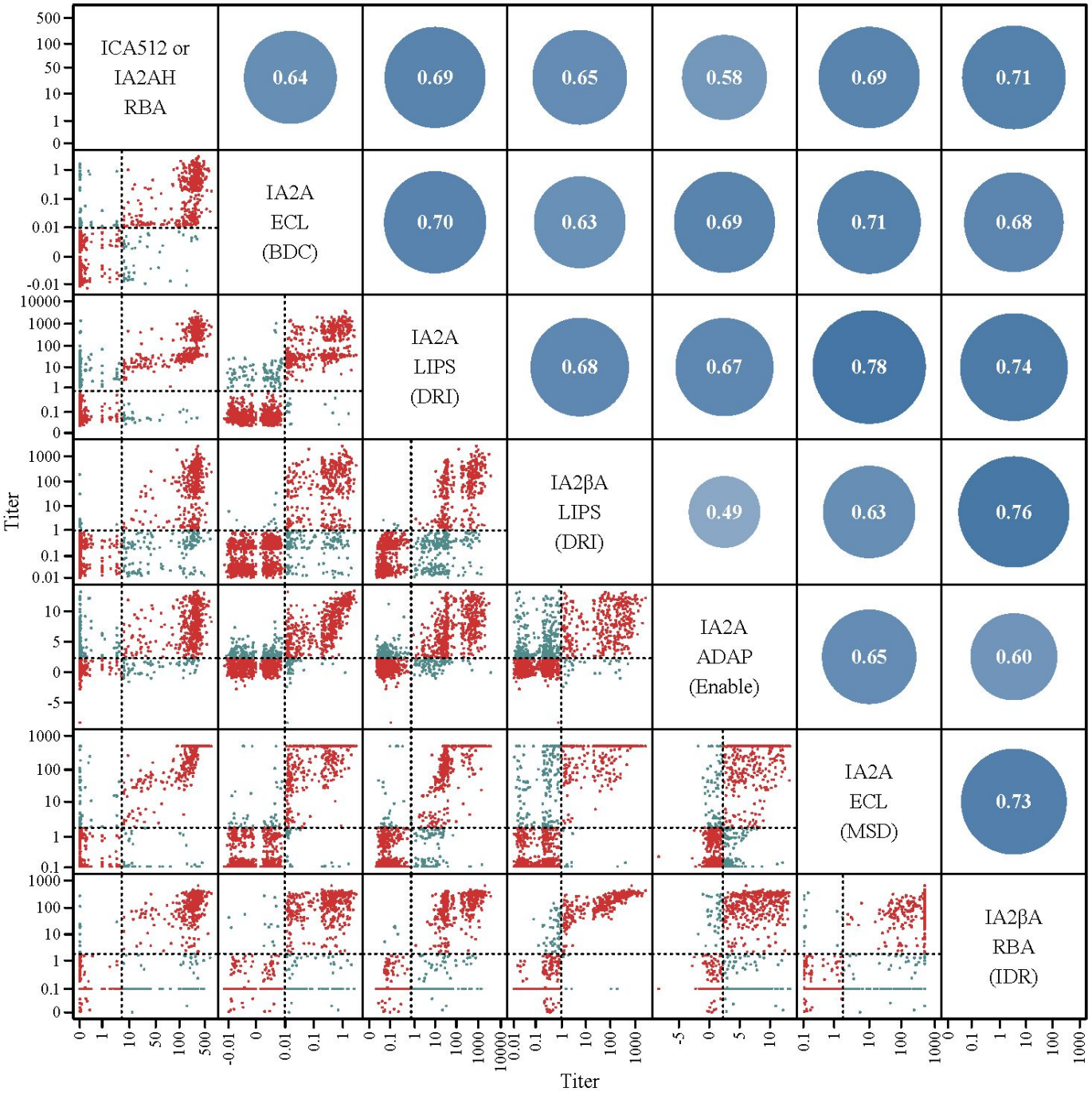


Figure S3. Insulin autoantibodies (IAA) assay correlations.

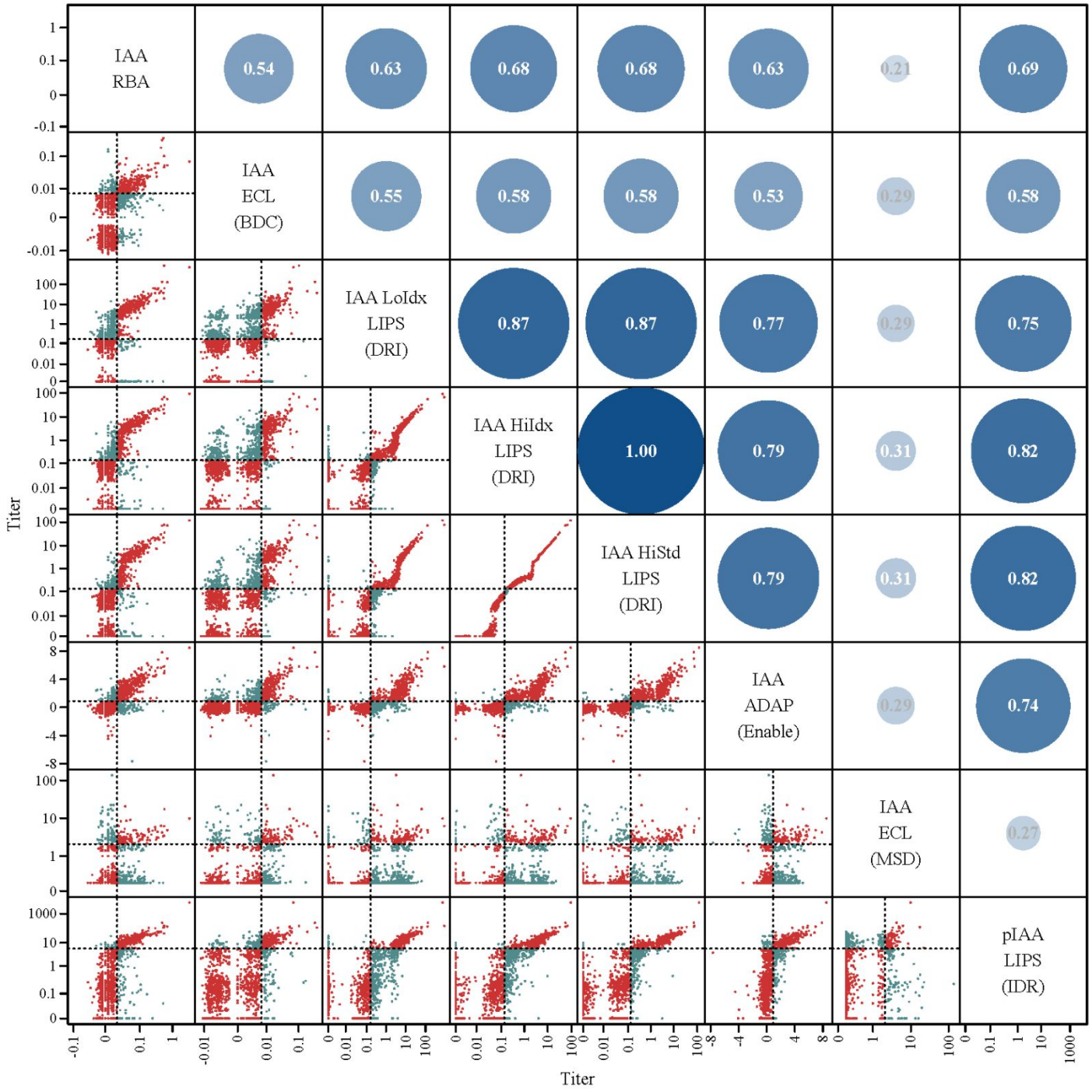


Figure S4. ZNT8 autoantibodies (ZnT8A) assay correlations.

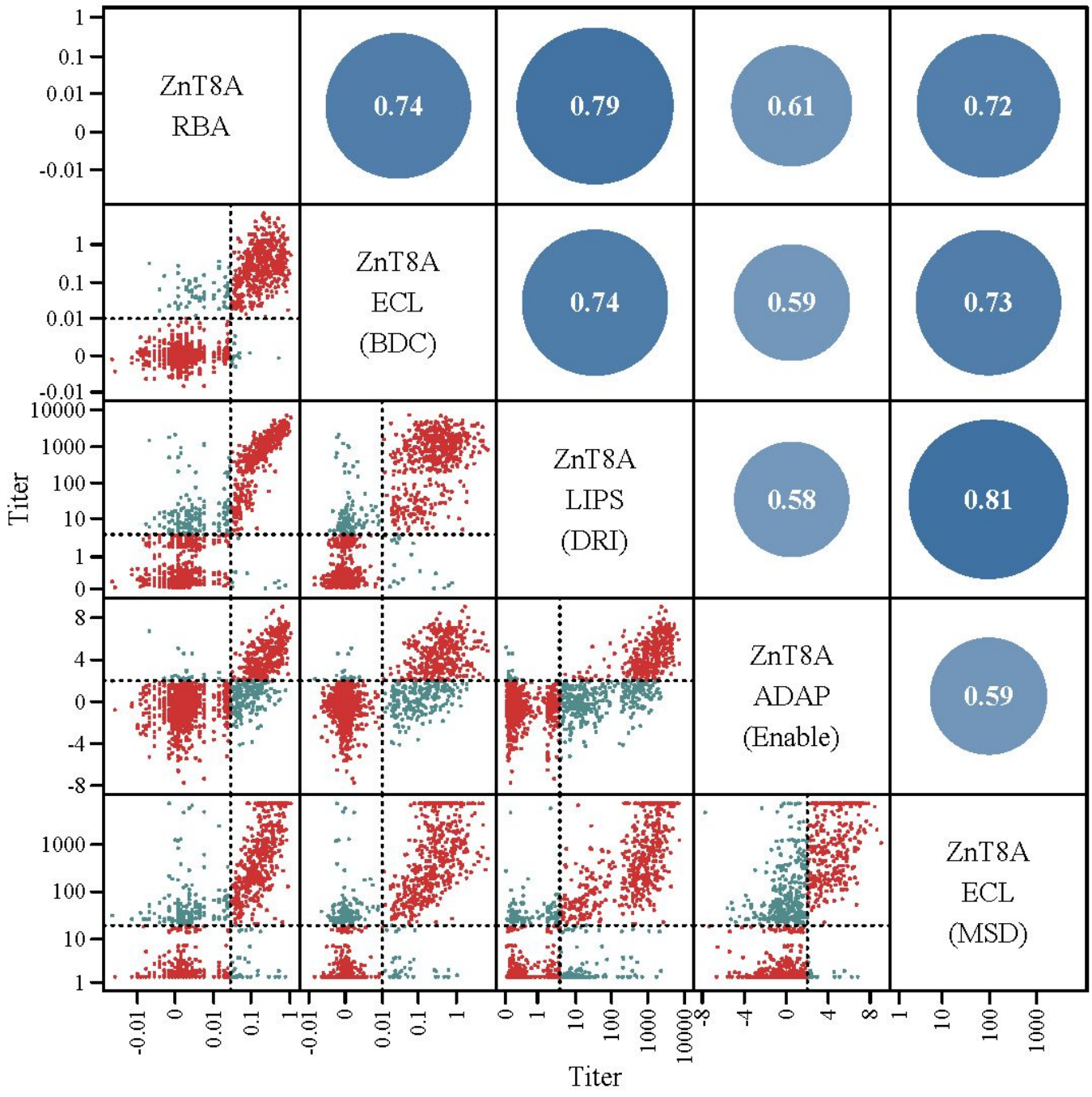


Figure S5. Percent of samples that agree across all assays by autoantibody.

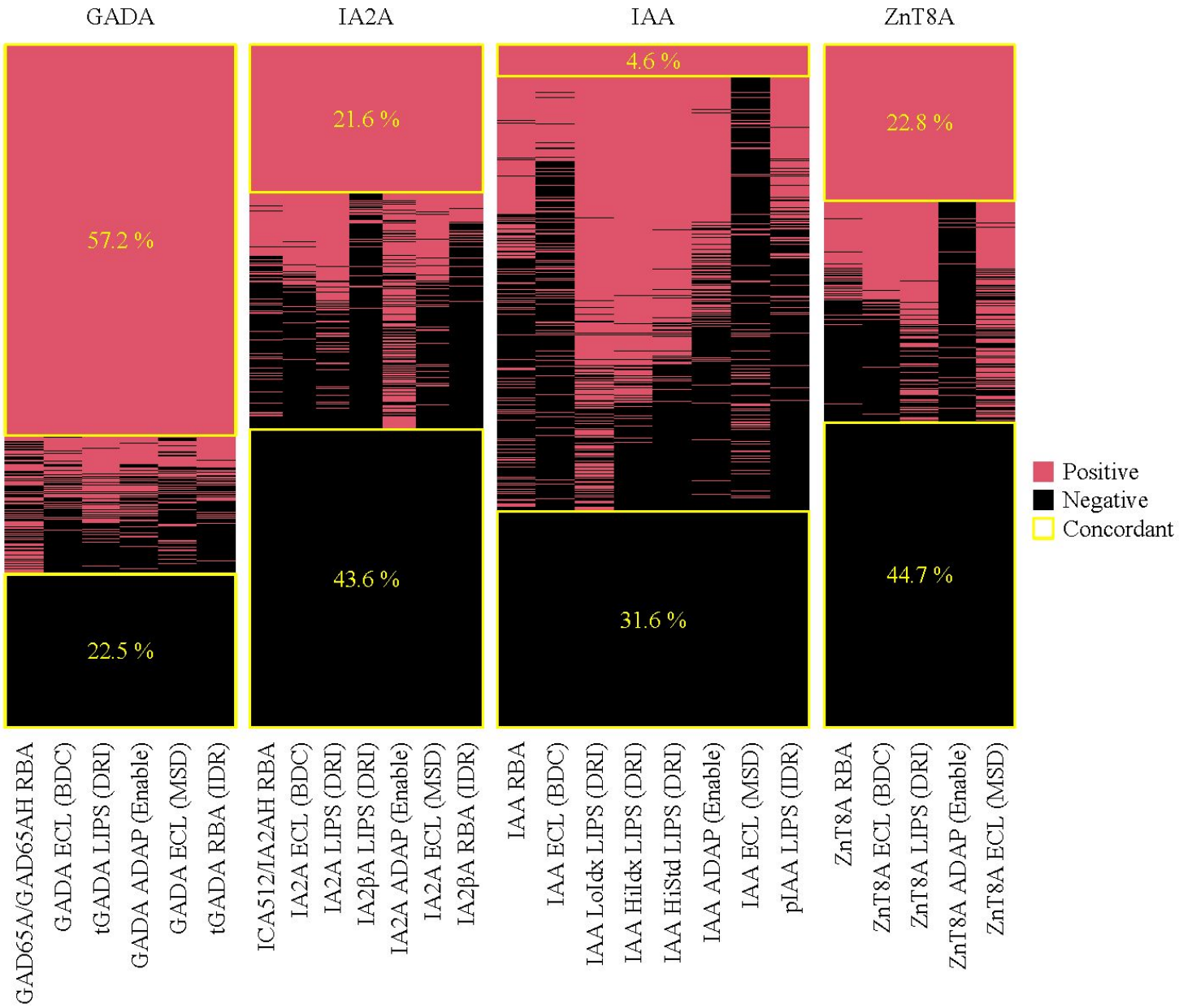


Figure S6. GADA titer Receiver Operating Curve (ROC) for 5-year T1D by assay. Parameters listed are Sensitivity (SEN), Specificity (SPE), Youden Index (YOU), and area under the curve (AUC). Thresholds listed are assay specific.

Assay	Threshold (+)	SEN (95% CI)	SPE (95% CI)	YOU (95% CI)	AUC (95% CI)
GAD65A/GAD65AH RBA	20	0.81 (0.78,0.85)	0.39 (0.36,0.42)	0.20 (0.16,0.25)	0.63 (0.61,0.66)
GADA ECL (BDC)	0.01	0.88 (0.85,0.91)	0.48 (0.45,0.51)	0.36 (0.32,0.40)	0.69 (0.66,0.71)
tGADA LIPS (DRI)	0.8761	0.91 (0.89,0.94)	0.44 (0.41,0.47)	0.35 (0.31,0.39)	0.68 (0.65,0.70)
GADA ADAP (Enable)	3.1	0.90 (0.88,0.93)	0.48 (0.44,0.51)	0.38 (0.34,0.42)	0.68 (0.65,0.70)
GADA ECL (MSD)	37.4	0.82 (0.79,0.85)	0.47 (0.44,0.50)	0.29 (0.24,0.33)	0.66 (0.64,0.69)
tGADA RBA (IDR)	30	0.84 (0.80,0.87)	0.46 (0.43,0.49)	0.29 (0.25,0.34)	0.67 (0.64,0.70)

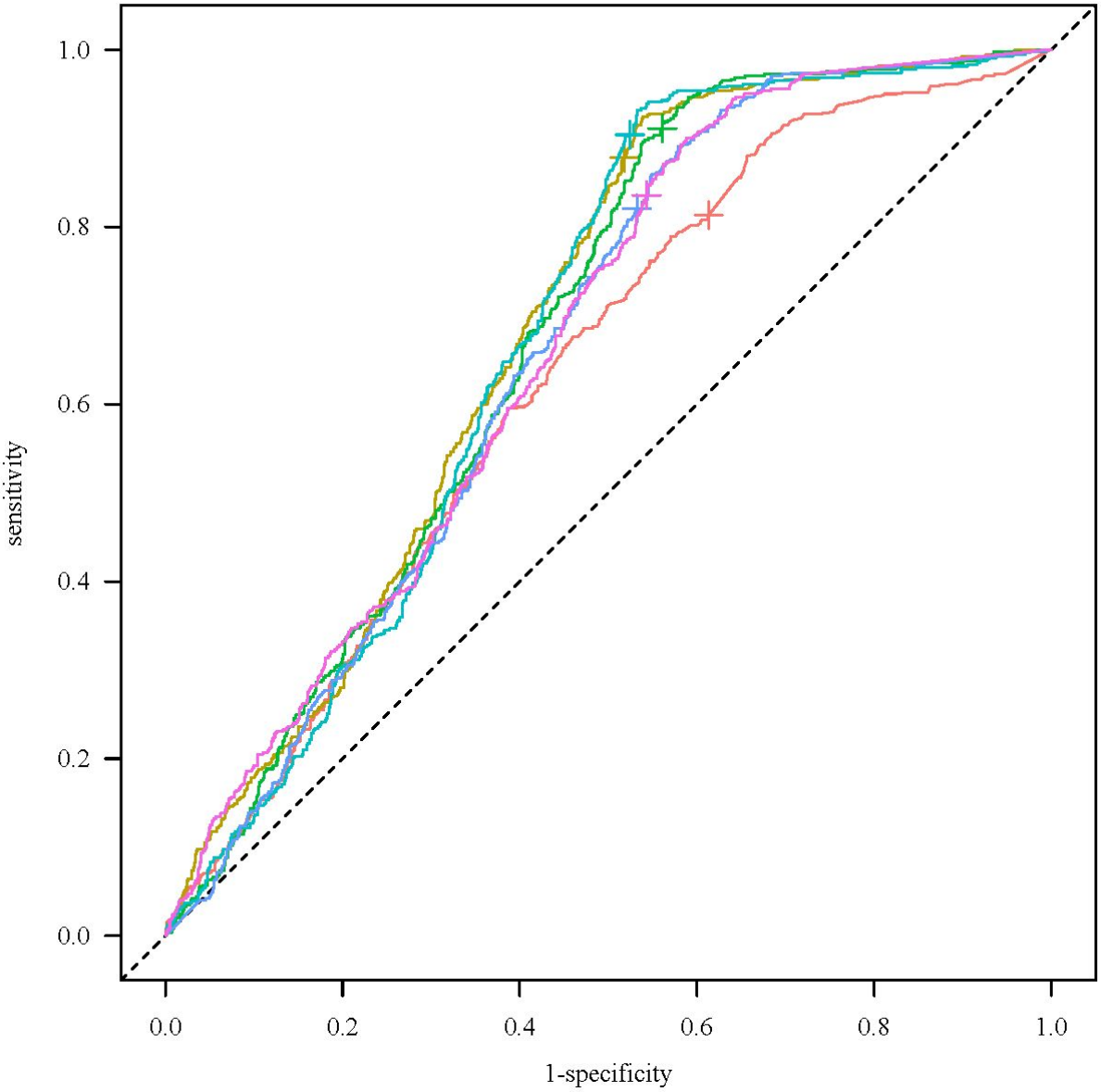


Figure S7. IA2A titer Receiver Operating Curve (ROC) for 5-year T1D by assay. Parameters listed are Sensitivity (SEN), Specificity (SPE), Youden Index (YOU), and area under the curve (AUC). Thresholds listed are assay specific.

Assay	Threshold (+)	SEN (95% CI)	SPE (95% CI)	YOU (95% CI)	AUC (95% CI)
ICA512/IA2AH RBA	5	0.60 (0.56,0.64)	0.82 (0.80,0.85)	0.43 (0.38,0.48)	0.73 (0.70,0.76)
IA2A ECL (BDC)	0.01	0.65 (0.61,0.69)	0.82 (0.80,0.85)	0.47 (0.42,0.52)	0.74 (0.71,0.77)
IA2A LIPS (DRI)	0.9793	0.73 (0.70,0.77)	0.78 (0.75,0.81)	0.51 (0.47,0.56)	0.80 (0.78,0.83)
IA2βA LIPS (DRI)	0.9793	0.48 (0.44,0.52)	0.89 (0.87,0.91)	0.37 (0.32,0.41)	0.70 (0.67,0.73)
IA2A ADAP (Enable)	2.3	0.70 (0.66,0.74)	0.67 (0.64,0.70)	0.38 (0.33,0.42)	0.74 (0.72,0.77)
IA2A ECL (MSD)	2.5	0.65 (0.61,0.69)	0.80 (0.78,0.83)	0.46 (0.41,0.50)	0.77 (0.75,0.80)
IA2βA RBA (IDR)	3	0.54 (0.49,0.58)	0.87 (0.85,0.89)	0.41 (0.36,0.46)	0.72 (0.69,0.74)

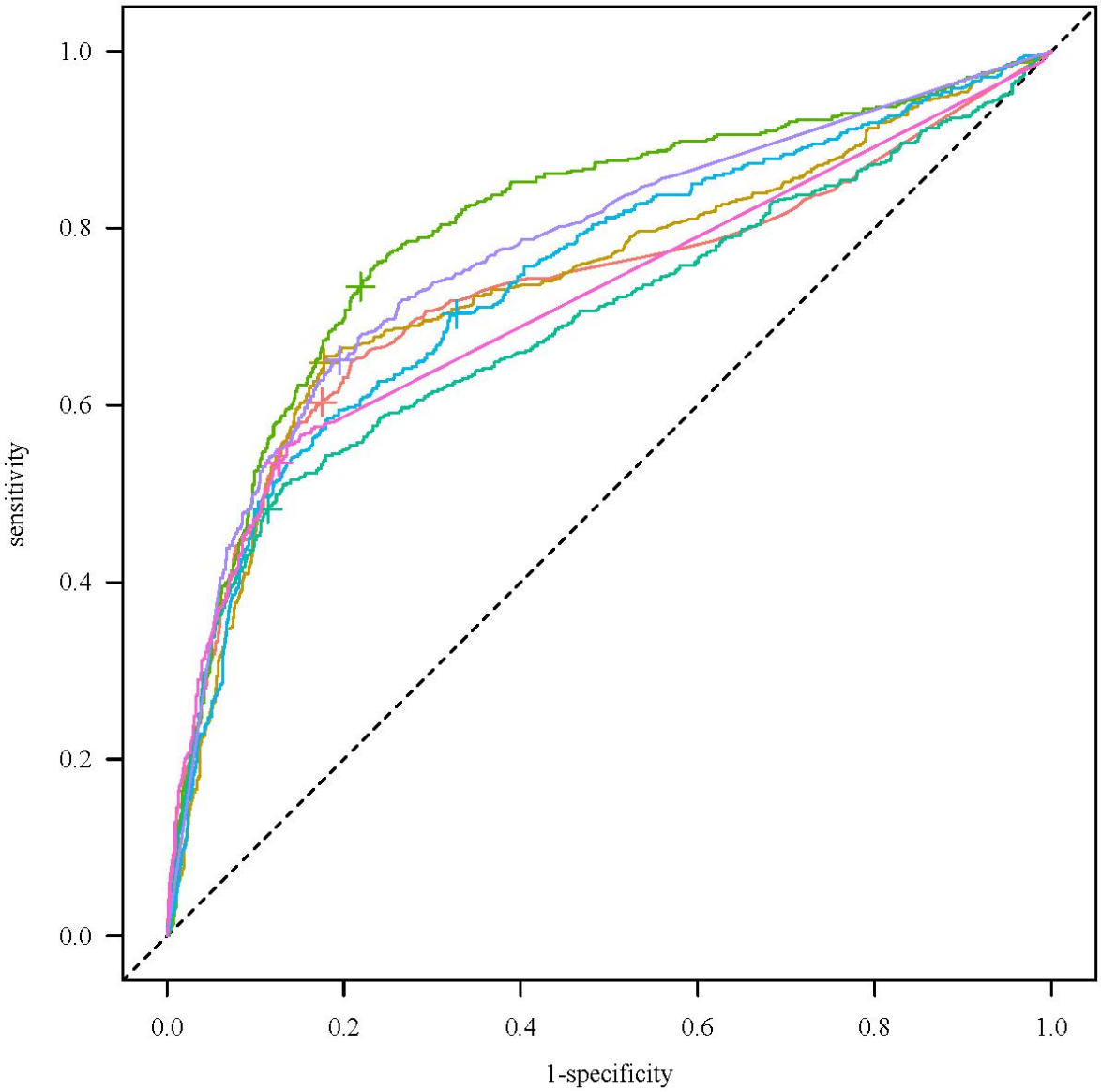


Figure S8. IAA titer Receiver Operating Curve (ROC) for 5-year T1D by assay. Parameters listed are Sensitivity (SEN), Specificity (SPE), Youden Index (YOU), and area under the curve (AUC). Thresholds listed are assay specific.

Assay	Threshold (+)	SEN (95% CI)	SPE (95% CI)	YOU (95% CI)	AUC (95% CI)
IAA RBA	0.01	0.50 (0.45,0.54)	0.74 (0.71,0.77)	0.24 (0.19,0.29)	0.66 (0.63,0.69)
IAA ECL (BDC)	0.006	0.43 (0.39,0.47)	0.88 (0.86,0.90)	0.31 (0.26,0.35)	0.70 (0.67,0.72)
IAA LIPS LoIdx (DRI)	0.16	0.77 (0.74,0.81)	0.64 (0.61,0.67)	0.41 (0.37,0.46)	0.75 (0.72,0.77)
IAA LIPS HiIdx (DRI)	0.1417	0.70 (0.66,0.74)	0.70 (0.67,0.73)	0.40 (0.36,0.45)	0.75 (0.72,0.77)
IAA LIPS HiStd (DRI)	0.2361	0.80 (0.77,0.83)	0.56 (0.53,0.59)	0.36 (0.31,0.41)	0.74 (0.71,0.76)
IAA ADAP (Enable)	0.9	0.60 (0.55,0.64)	0.79 (0.76,0.81)	0.38 (0.34,0.43)	0.74 (0.72,0.77)
IAA ECL (MSD)	1.3	0.19 (0.15,0.22)	0.90 (0.88,0.92)	0.08 (0.05,0.12)	0.63 (0.60,0.65)
pIAA LIPS (IDR)	4	0.43 (0.39,0.47)	0.84 (0.82,0.86)	0.27 (0.22,0.32)	0.72 (0.69,0.75)

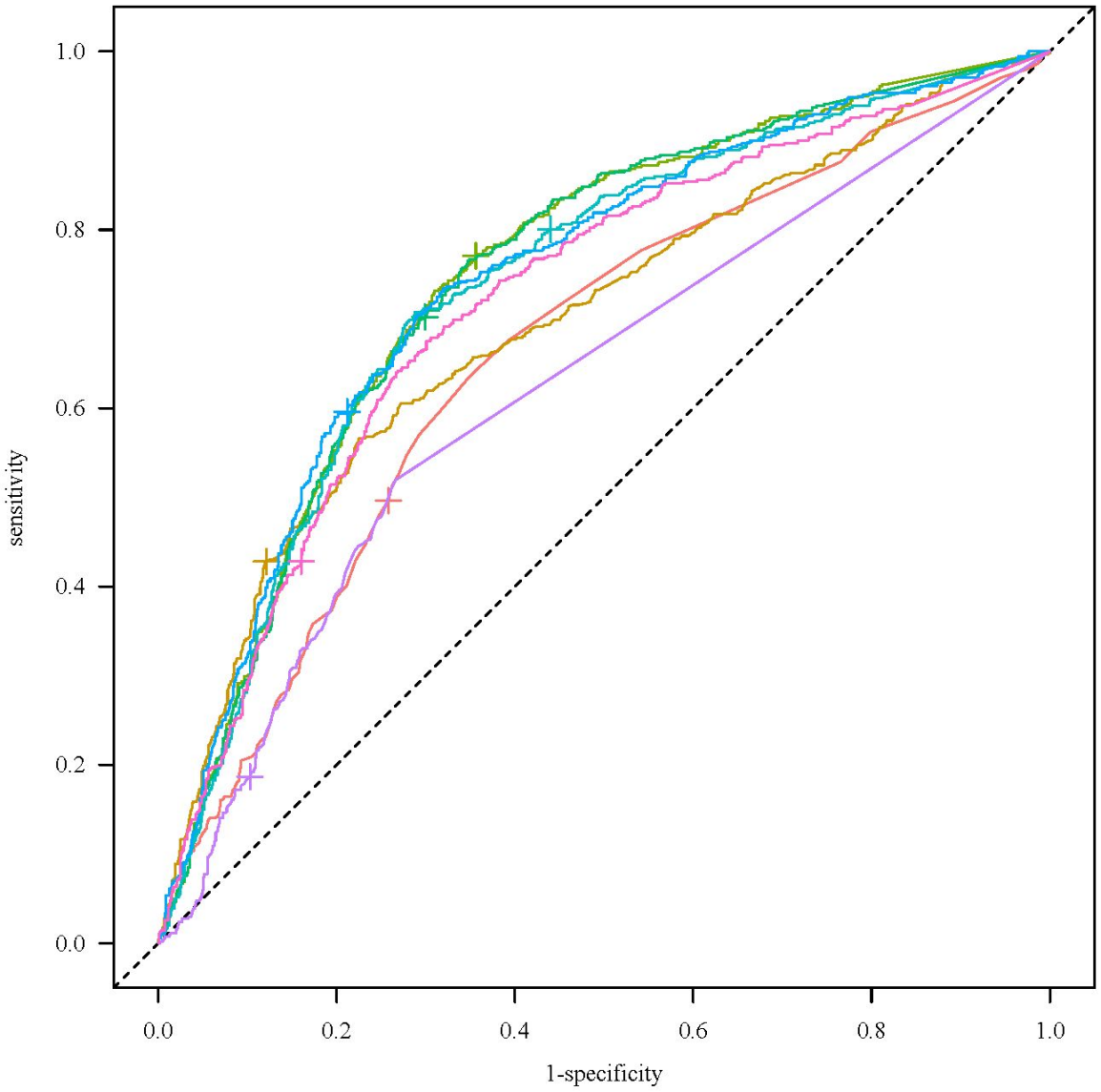
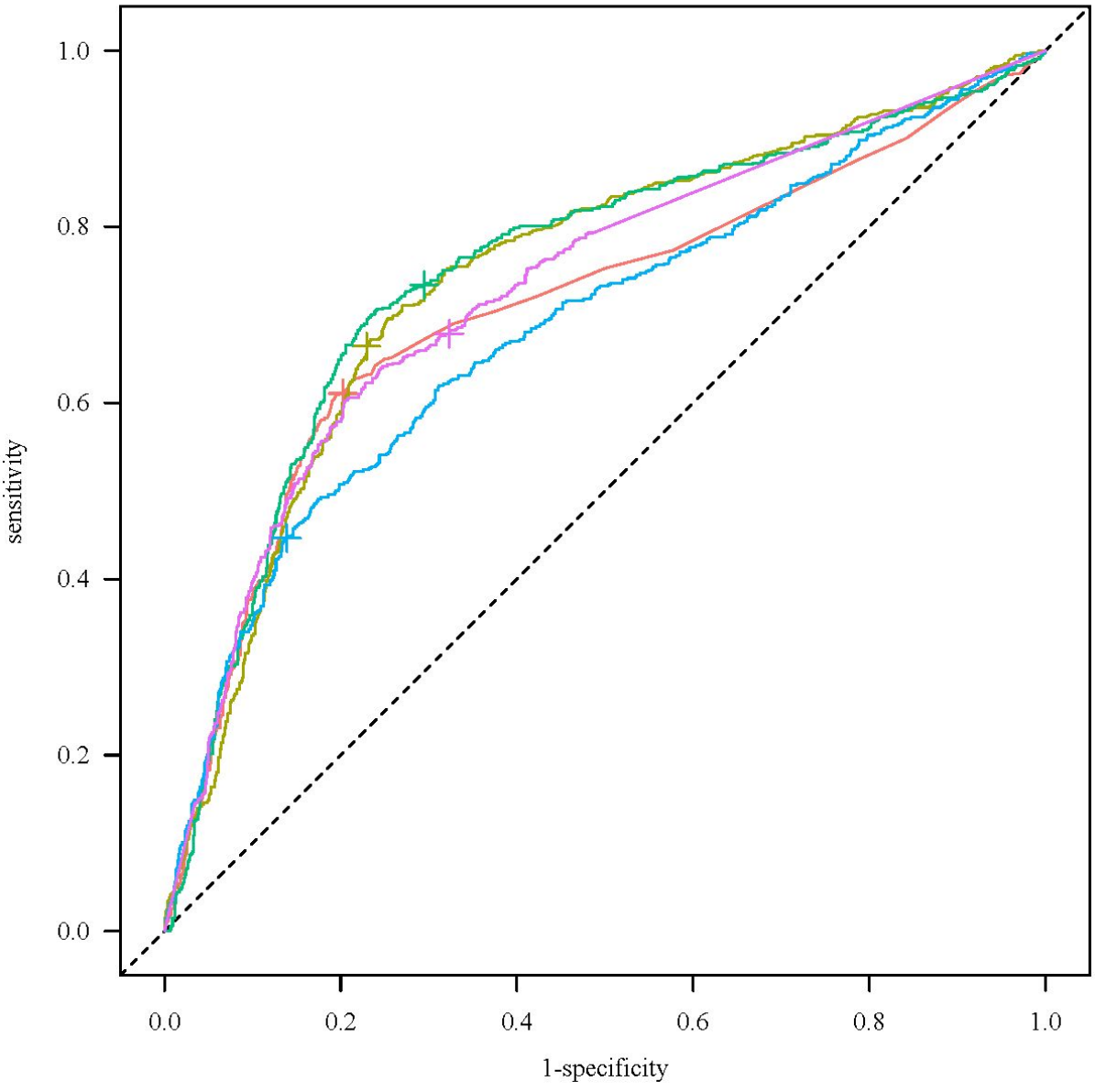


Figure S9. ZnT8A titer Receiver Operating Curve (ROC) for 5-year T1D by assay. Parameters listed are Sensitivity (SEN), Specificity (SPE), Youden Index (YOU), and area under the curve (AUC). Thresholds listed are assay specific.

Assay	Threshold (+)	SEN (95% CI)	SPE (95% CI)	YOU (95% CI)	AUC (95% CI)
ZnT8A RBA	0.02	0.61 (0.57,0.65)	0.80 (0.77,0.82)	0.41 (0.36,0.46)	0.71 (0.68,0.74)
ZnT8A ECL (BDC)	0.01	0.66 (0.62,0.70)	0.77 (0.74,0.80)	0.43 (0.39,0.48)	0.74 (0.72,0.77)
ZnT8A LIPS (DRI)	2.5091	0.73 (0.70,0.77)	0.71 (0.68,0.73)	0.44 (0.39,0.49)	0.75 (0.72,0.78)
ZnT8A ADAP (Enable)	2	0.45 (0.40,0.49)	0.86 (0.84,0.88)	0.31 (0.26,0.35)	0.69 (0.66,0.71)
ZnT8A ECL (IDR)	12.5	0.68 (0.64,0.72)	0.68 (0.65,0.71)	0.36 (0.31,0.40)	0.73 (0.70,0.76)



ONLINE APPENDIX. Type 1 Diabetes TrialNet Study Group.
Personnel as of 4/28/2023.

Steering Committee: Kevan Herold (Yale University), Mark Anderson (University of California, San Francisco), Mark A. Atkinson (University of Florida), Todd Brusko (University of Florida), Jane Buckner (Benaroya Research Institute), Mark Clements (The Children's Mercy Hospital), Peter G. Colman (Walter & Eliza Hall Institute of Medical Research), Mark Daniels (Children's Hospital of Orange County), Linda DiMeglio (Indiana University), Carmella Evans-Molina (Indiana University), Jason Gaglia (Joslin Diabetes Center), Stephen E. Gitelman (University of California, San Francisco), Robin Goland (Columbia University), Peter Gottlieb (Barbara Davis Center for Childhood Diabetes), Michael Haller (University of Florida), Carla J. Greenbaum (Benaroya Research Institute), Martin Hessner (Medical College of Wisconsin), Jeffrey P. Krischer (University of South Florida), Megan Levings (University of British Columbia), Ingrid Libman (University of Pittsburgh), Peter Linsley (Benaroya Research Institute), Alice Long (Benaroya Research Institute), Sandra Lord (Benaroya Research Institute), Wayne Moore (The Children's Mercy Hospital), Antoinette Moran (University of Minnesota), Andrew Muir (Emory Children's Center), Priya Prahalad (Stanford University), William Russell (Vanderbilt Eskind Diabetes Clinic), Jennifer Sherr (Yale University), Lisa Spain (National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK]), Andrea Steck (Barbara Davis Center for Childhood Diabetes), John Wentworth (Walter & Eliza Hall Institute of Medical Research), Diane Wherrett (University of Toronto), Perrin White (University of Texas Southwestern), Darrell M. Wilson (Stanford University), William Winter (University of Florida).
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Executive Committee: Kevan Herold (Yale University), Linda DiMeglio (Indiana University), Carla J. Greenbaum (Benaroya Research Institute), Jeffrey P. Krischer (University of South Florida), Ellen Leschek (National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK]), Lisa Spain (National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK]).

Past Members: Katarzyna Bourcier (National Institute of Allergy and Infectious Diseases [NIAID]), Richard Insel (JDRF), John Ridge (National Institute of Allergy and Infectious Disease [NIAID]), Jay S. Skyler (University of Miami).

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Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria: Peter Colman, John M. Wentworth, Candice Breen, Marika Bjorasen, Spiros Fourlanos, Leonard Harrison, Felicity Healy, Leanne Redl.

Yale University, New Haven, Connecticut: Jennifer Sherr, Kevan Herold, Lori Carria, Jeanine May, William Tamborlane, Eileen Tichy, Stuart Weinzimer, Kate Weyman.