Simultaneous measurement of GAD$_{65}$, IA-2 and ZnT8 autoantibodies in an ELISA platform and correlation of data with type 1 Diabetes Mellitus patients.

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Introduction
Type 1 DM is an organ-specific autoimmune condition, which occurs when the beta cells of the islet of Langerhans are destroyed by various autoantibodies which leads to a reduction in or no insulin production. These antibodies include the following: islet cell autoantibodies (ICA), glutamic acid decarboxylase (GAD$_{65}$), insulinoma-associated-2 (IA-2) and zinc transporter 8 (ZnT8).

The appearance of these autoantibodies occurs in an order, the initial development is ICA or GAD$_{65}$ then IA-2 and ZnT8 (University of Bristol, no date). These can appear at early stage (6 months of age) and around 90% of newly diagnosed patients are positive for at least one of these autoantibodies. Having more autoantibodies increases the probability of having type 1 DM.

The aim of the study was to evaluate the 3 Screen ICA ELISA by performing inter and intra assay variation, linearity and ROC analysis.

Method
Samples from 3 groups (positive diabetic samples, disease and negative controls) were tested using the RSR ELISA Kit. Samples were tested in duplicate using 25μL volume and incubated at 2-8°C for 20 hours overnight. The plate was washed the following day, leaving any bound autoantibodies from the sera to GAD$_{65}$, IA-2 and ZnT8 antigens coated in the wells. Subsequently, 100μL of the cold reconstituted 3 Screen-Biotin was added into each well (except the blank) then incubated at 2-8°C for 1 hour. The bound Biotin was determined with the use of diluted Streptavidin Peroxidase (SA-POD). Subsequently, 100μL of 3,3′,5,5′-tetramethyl-benzidine substrate (TMB) was added into each well and the plate was incubated in the dark for at least 20 minutes at room temperature. Following this, 100μL of the stop solution was added and the plate was read on an ELISA plate reader within 10 minutes.

Results
In the positive diabetic group, 48 out of 50 samples (96%) gave positive results with 2 discrepancies using 5μL/mL as the cut-off. The disease control group demonstrated 90% specificity using 5μL/mL as the cut-off and 92% specificity when using 6 μL/mL as the cut-off.

The negative control group demonstrated 95% specificity using 5μL/mL as the cut-off with 2 discrepancies, 98% specificity using 6μL/mL as the cut-off with 1 discrepant result, and 100% specificity using 8μL/mL as the cut-off.

Discussion
Simultaneous measurement of GAD$_{65}$, IA-2 and ZnT8 autoantibodies in an ELISA platform helps in saving valuable staff time and resources. Any negative samples will not be required to be tested for the individual autoantibodies and this will result in improved turnaround time for diabetes antibodies testing in the clinical laboratory.

References

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