nPOD
Network for Pancreatic Organ Donors with Diabetes

GAD ANTIBODY PROTOCOL

1 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to outline procedures for using the modified KRONUS® GAD Autoantibody ELISA kit.

2 SCOPE

This SOP will be applied to all autoantibody screening performed by screening labs in partnership with the nPOD project.

3 RESPONSIBILITIES

3.1 Managers and supervisors are responsible for making sure that technicians are properly trained and equipment and facility are maintained in good working order.
3.2 Laboratory personnel are responsible for reading and understanding this SOP and related documents and to perform these tasks in accordance with the SOPs. They are responsible for following clinical laboratory and tissue banking best practices.

4 EQUIPMENT and MATERIALS

The materials, equipment and forms listed in the following list are recommendations only and alternative products as suitable may be substituted for the site specific task or procedure.

KRONUS GAD autoantibody ELISA kit
Microtiter plate reader (with proper filter), set at 450nm or 405nm
Pipettors calibrated to 25 and 100 ul
Graduated cylinders
Deionized or distilled water
ELISA plate shaker or orbital rotator (500 rpm)
Wash bottles or automatic plate washing system

5 SAFETY

5.1 Use universal safety precautions when handling human samples and personal protective equipment (e.g., face mask with shield, gloves, lab coat or apron).

6 PROCEDURE

6.1 Calculate the number of individual ELISA plate wells needed for the assay. Allow all the reagents supplied, including the pouch containing the plate wells, to reach room temperature, at least 30 minutes. Remove the number of strip wells required and fit them firmly into the frame provided. Kit calibrators and positive control (duplicate wells for each) must be included in each assay run. Calculate the volume of streptavidin-peroxidase (SA-POD) needed for the assay.

6.2 Pipette 25 ul of calibrators (in duplicate) into suitable wells of the coated strips and similarly for the serum samples and kit positive and negative controls.

6.3 Cover the frame and incubate on an ELISA plate shaker (shaking at 500 rpm) at room temperature for 1 hour. During this 1 hour incubation, reconstitute the GAD65-biotin, dilute the required amount of SA-POD, and dilute the concentrated wash. See instructions in the package insert.

6.4 After the first incubation period, aspirate or shake out the samples from the wells and wash each well with dilute washing solution three times. After the final wash, remove any excess liquid by gently tapping the inverted plate on an absorbent material.

6.5 Pipette 100 ul of reconstituted GAD65-biotin into each well (a repeating Eppendorf type pipette is preferred). Cover the frame and incubate on an ELISA plate shaker (shaking at 500 rpm) at room temperature for 1 hour.

6.6 Repeat the washing procedure as described in 6.4 above, inverting and tapping the plate gently after the final wash.

6.7 Pipette 100 ul of reconstituted SA-POD into each well (a repeating Eppendorf type pipette is preferred). Cover the frame and incubate on an ELISA plate shaker (shaking at 500 rpm) at room temperature for 20 minutes.

6.8 After this incubation, aspirate or shake out the contents of the wells and wash each well with dilute washing solution three times and once with deionized water (water wash step is not required if an automated plate washer is used). Invert and tap as before.

6.9 Pipette 100 ul of peroxidase substrate (TMB) into each well (a repeating Eppendorf type pipette is preferred). Cover the frame and incubate for 20 minutes at room temperature in the dark without shaking during which time a blue color will develop.

6.10 Stop the substrate reaction by addition of 100 ul of stop solution to each well (a repeating Eppendorf type pipette is preferred). Shake the plates for 5 seconds at about 500 shakes per minute. This will cause the blue color to turn yellow.

6.10.1 It is important to ensure that the substrate incubation time (i.e., time from addition of substrate to addition of stop solution) is the same for each well.

6.11 Within 5 min after the addition of stop solution to the plate wells, read their absorbance at 405 nm and then again at 450 nm using an ELISA plate reader blanked against a well containing 100 ul of substrate plus 100 ul of stop solution. Plot a calibration curve with absorbance at 450 nm or 405 nm on the vertical axis and calibrator concentration on a log10 scale on the horizontal axis and read off the concentration of GAD65 antibody in the test sera and positive control.

6.12 For purposes of nPOD, a reading greater than the second calibrator, i.e., >18 IU/ml, will be considered positive.

6.12.1 Should this value change, all participants will be alerted individually and this document will be updated.

7 REFERENCES

7.1 Eisenbarth, G.S. and McCulloch, D.K. (2008). Pathogenesis of type 1 diabetes mellitus.

7.2 Bonifacio, E., Yu, L., Williams, A.K., Eisenbarth, G.S., Bingley, P.J., Marcovina, S.M., Adler, K., Ziegler, A.G., Mueller, P.W., Schatz, D.A., Krischer, J.P., Steffes, M.W. & Akolkar, B. (2010). Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for national institute of diabetes and digestive kidney diseases consortia. Journal of Clinical Endocrinology & Metabolism, 95:3360-7.

8 REVISION HISTORY

Version	Date	Revision
0	03/08/12	Added a reference. AW

Prepared by	CliveWasserfall		
Approved by	Mark Atkinson		
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