Laboratory Procedure Manual

Analyte: Tissue Transglutaminase Assay (IgA)

Matrix: Serum
Method: IgA (ELISA)
Method No:

Revised:

as performed by: Mayo Clinic
Rochester, Minnesota

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Important Information for Users

The Mayo Clinic periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

File Name	Variable Name	SAS Label
TGEMA_H	LBXTTG	Tissue Transglutaminase

1. Summary of Test Principle and Clinical Relevance

An Enzyme-Linked Immunoabsorbant Assay (ELISA) for the semi-quantitative detection of IgA antibodies to tissue transglutaminase (endomysium) in human serum, Microwells are pre-coated with recombinant human tTG antigen. The calibrators, controls and patient samples are added to the wells and autoantibodies recognizing the tTG antigen bind during first incubation. Unbound proteins are removed during washing and purified peroxidase conjugate is added which binds to the captured human autoantibody; excess is washed. TMB substrate is added which gives a blue reaction product; intensity is proportional to the concentration of the autoantibody in the sample. Phosphoric acid is added to stop the reaction which gives the yellow color. Plate is read at 450 nm.

2. SAFETY PRECAUTIONS

Eye protection, face shields or stationary Plexiglas shields must be used whenever there is a potential for splashing, spraying or splattering of blood or body fluids to mucous membranes of the eyes, nose or mouth.

Additional PPE is required when handling hazardous chemicals. In addition to wearing lab coats, gloves and eye protection, hazardous chemicals should be handled in a chemical fume hood with the hood sash pulled down to the approved working height.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

A. Each shipment of specimens received from the NHANES mobile unit arrives with a corresponding transmittal sheet and an electronic version of the shipping/resulting file. The file structure is determined by NHANES and is described in the National Health and Nutrition Examination Survey (NHANES) Contract Laboratory Manual.

B. After the testing is completed results are transferred to the laboratory server system, which is backed up daily.

C. QC results are transferred to an Excel file using laboratory-developed software. This file calculates the QC statistics, plots Levey-Jennings charts, displays relevant instrument flags, tracks reagent lots and recent calibrations. QC results are reviewed prior to resulting samples.

D. Sample results are transferred to an Excel file using laboratory-developed software that enters results after matching sample identifiers from the instrument file with those provided in the NHANES shipping/resulting file. This Excel file is formatted to match the NHANES shipping/resulting file and the program uses the conventions outlined in the NHANES Contract Laboratory Manual.

E. Data entry is checked for errors.

F. After the testing has also been completed, resulted, and checked, the result file is transmitted electronically to NHANES. Electronic and hard copies of the files are kept in the laboratory.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

A. No special instructions, such as fasting or special diets, are required.

B. Serum is the preferred sample.

C. The appropriate amount of serum/plasma is dispensed into a Nunc cryovial or other plastic screw-capped freezing vial labeled with the participant's ID. Blood spots are collected through finger or heal prick onto the underside of prepared filter blot pads (provided by the lab) as instructed.

D. Serum and plasma specimens collected in the field should be frozen, and then shipped on dry ice by overnight mail. Once received, specimens should be stored at –20°C until analyzed. Residual specimens are refrozen at -20 °C. Blood spot specimens must be permitted to completely dry before placing them into individual zip-lock bags. They should be shipped at ambient temperature within one week of collection.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. PREPARATION OF REAGENTS, CALIBRATORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

Reagents:

Human Transglutaminase Coated Wells: 12 x 8 break apart well strips coated with recombinant tTG.

Transglutaminase IgA Calibrators: 5 vials each containing 1 mL of diluted human serum with the following concentrations of anti- tTG IgA autoantibody: 100, 33, 11.1, 3.7, 1.23 U/mL.

Transglutaminase IgA Positive Control: 1 vial containing 1 mL of diluted human serum. Expected value given on QC certificate.

Transglutaminase Negative Control: 1 vial containing 1 mL of diluted human serum. Expected value given on QC certificate.

Type III Sample Diluent: 2 bottles containing 50 mL of buffer for sample dilution; colored yellow.

Type III Wash Buffer: 1 bottle containing 50 mL of a 20-fold concentrated buffer for washing wells.

Transglutaminase IgA Conjugate: 1 bottle containing 12 mL of purified peroxidase; colored green.

TMB Substrate: 1 bottle containing 14 mL TMB substrate.

Stop Solution: 1 bottle containing 14 mL of 3M phosphoric acid.

Equipment:

DSX

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

The assay is calibrated in U/mL against an arbitrary reference calibrator, as no internationally recognized reference preparation is currently available.

The calibration curve can be plotted automatically or manually by plotting the anti-tTG IgA autoantibody concentration on the log scale against the OD on the linear scale for each calibrator:

Automatic- use appropriately validated software, and curve fit that best fits data.

Manual- using log/linear graph paper draw a smooth curve through the points (not a straight line or point to point).

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preliminaries

ALL REAGENTS MUST BE BROUGHT TO ROOM TEMPERATURE PRIOR TO BEGINNING THE ASSAY.

Click Revelation icon to start operation of the ELISA DSX.

Turn on the DSX, Load # of samples to be run into sample rack.

Choose proper procedure tTG IgA and follow instructions for assay.

Load reagents and controls/calibrators.

Prepare wash.

B. Instrument Operation (see operator's manual for details).

C. Recording of Data

The ELISA assay is very sensitive and is capable of detecting even small differences in sample populations. The values shown under calculation of results are suggested values only. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and sample population according to their own established procedures.

Results can be obtained by opening the C-drive, program files, dynex, revelation, text files. Find the desired file and highlight, right click and copy file. Make sure that the memory stick is in the computer. Open the removable disk drive and paste as this will paste a copy of the text file into the memory stick.

The text file can be saved into the Murray network drive and placed into the ttg results folder.

D. Replacement and Periodic Maintenance of Key Components

Periodic Maintenance to be performed by the manufacturer's service engineer.

E. Calculations

The following ranges are used as a guide and are as follows

Negative	<4.0 U/mL
Weak Positive	4-10 U/mL
Positive	>10 U/mL

The above normal range was determined on serum from 200 normal adult blood donors. One sample was confirmed to contain anti tTG IgA antibodies and was excluded from this determination.

9. REPORTABLE RANGE OF TEST RESULTS

Negative, positive, and indeterminate values are in the reportable range.

10. QUALITY CONTROL (QC) PROCEDURES

TTG ELISA positive and negative control and calibrators should be run with every batch of samples to ensure that all reagents and procedures are performing properly. Values for positive and negative controls should be in the ranges specified on QC certificate. Curve shape should be similar to calibration curve on QC certificate.

1) With each tTG run
   a) In order for the assay to be valid the calibrators and positive/ negative controls must be included in every run; the values obtained for the controls should be in the ranges specified on the QC certificate; the curve shape should be similar to the calibration curve shown on the QC certificate.
   b) The positive and negative controls are tracked and plotted with help by our statisticians. These plots are discussed at every meeting to ensure the controls obtained values are coming out as expected.
   c) With help by our statisticians an automated job was set up to compile an up-to-date binomial control chart The control chart plots the percentage of sero-positive samples tested each month. Both upper and lower control limits are set at 3 standard deviations for that particular month.
   d) When performing controls with each run: If any calibrator/ control should fail and the obtained value is out of the specified range on the QC certificate or the curve shape differs from the curve shown on the QC certificate corrective action steps are taken to correct the problem.
   e) A visual inspection is also completed with each run. The microwells of a sample with an increased result will be yellow in color. The higher the intensity of the yellow color of the microwell the higher the tTG result. This also allows the tech to inspect the plate for any bubbles that might have occurred.
2) Corrective Action Steps
   a) Check the event log by going to view and then view event log. This will show if there were any mechanical errors by the DSX.
   b) Check the calibrators and positive and negative controls to be sure the obtained values came out as expected on the QC certificate. Also check the curve to be sure it looks like the curve on the QC certificate.
   c) Call The Binding Site to see if there have been any issues with the lot number used.
   d) Call The Binding Site and fax over a copy of the plate file to have them take a look and see what the issue may have been.

e) If there have not been any issues with the kit lot number retest using the same lot number. If there has been reported issues to The Binding Site in regards to the lot number use a different lot number and check into sending the defective kits back to The Binding Site.

All CDC testing must have the 2 QC samples ran with the batch. This includes the positive and negative sample.
The ranges for each sample will be figured by testing each sample 20 times and then calculating the mean and standard deviation of each. The range of each QC sample would then be the mean plus or minus 3 standard deviations.

A new pool information sheet needs to be filled out for every new lot number. Open Lab65 QC pool excel sheet. Pool name, variable name, and pool type information remain the same. Add information for lot no., instrument, method, fixed mean, fixed SD, max reps, from run date, and to run date (from the previous lot #). The new Lab65 QC pool excel sheet is then submitted to CDC using FileZilla as described in SOP # XXXXXX.

If a QC sample does not come out as expected or if QC samples were not ran with the batch then the run is automatically rejected and all samples are re-tested.

If the QC samples continue to fail then contact INOVA to check into kit failure or recalls. Contact Dr. Lacher from CDC for additional help on this subject.

CDC will send excel files to document the QC samples. Once received from CDC complete and submit to CDC using FileZilla as described in SOP # XXXXXX.
For the QC files from CDC fill in the QC value and QC run date information only.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

If the run is declared "out of control", the system (instrument, calibration standards, etc.) is investigated to determine the root of the problem before any results are released. Consult with the supervisor for appropriate actions.

If a QC sample does not come out as expected or if QC samples were not ran with the batch then the run is automatically rejected and all samples are re-tested.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

The tissue transglutaminase kit is used to aid in diagnosis. A positive result suggests certain diseases that must be confirmed by clinical findings and other serological tests.

The results obtained are not diagnostic proof of the presence or absence of disease.

The use of the assay and normal range (result interpretation) has not been established for pediatric samples.

A negative result may be due to IgA deficiency and does not rule out celiac disease.

13. REFERENCE RANGES (NORMAL VALUES)

A negative value is considered normal.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable to this procedure.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens are keep frozen until ready for testing. After testing the samples are kept frozen until they are returned to the NCHS biorespository.

16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

If the analytical system fails, the specimens should be refrigerated at 4-8^{o}C until the analytical system is restored. If long-term interruption is anticipated, specimens are refrozen at –20^{o}C or lower.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable to this procedure.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Standard record-keeping means (e.g., electronic, data files, laboratory notebook) are used to track specimens. Records are maintained indefinitely. Specimens are retained at the laboratory for at least one year, and then may be placed in archival storage at the CDC facility in Lawrenceville, GA. Only numerical identifiers are used. All personal identifiers are kept masked and available only to the project coordinator in order to safeguard confidentiality.

19. Summary Statistics and QC Graphs

Qualitative assays are assays with a positive, negative or indeterminate result. Since the controls do not generate quantitative values, plots are not generated for quality control purposes.

References

None