DIAGNOSTIC AUTOMATION, INC.

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IVD	See external label	2°C-8°C	Σ=96 tests	REF Cat # 1103-11

Toxoplasma IgA

Cat # 1103-11

Test	**Toxoplasma Gondii IgA ELISA**
Method	**ELISA: Enzyme Linked Immunosorbent Assay**
Principle	**Indirect ELISA : Antigen Coated Plate**
Detection Range	**Qualitative: Positive & Negative Control**
Sample	**5µL**
Specificity	**100%**
Sensitivity	**94%**
Total Time	**~90 min**
Shelf Life	**12-14 months**

* *Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

NAME AND INTENDED USE

The Diagnostic Automation ELISA, Toxoplasma IgA is intended for use in the detection of IgA to Toxoplasma gondii.

SUMMARY AND EXPLANATION OF THE TEST

Toxoplasmosis is caused by the intracellular parasite Toxoplasma gondii and may be contracted by consuming contaminated meat or by contact with cat feces containing oocysts. In adolescence and adulthood, most infections are subclinical. However, if a pregnant woman contracts toxoplasmosis, it may be passed through the placenta to the fetus, resulting in congenital toxoplasmosis, which is a cause of mortality and malformation. Asymptomatic infants may develop anomalies later in life. The antibodies present to Toxoplasma gondii may be of the IgA, IgM and IgG isotypes. The physiological function of IgA and its clinical implication is still unclear. The DIAGNOSTIC AUTOMATION ELISA Toxoplasma IgA is an accurate and sensitive serologic method to detect Toxoplasma antibody IgA isotype.

PRINCIPLE OF THE TEST

Purified Toxoplasma gondii antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the Toxoplasma gondii IgA specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgA specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

MATERIALS PROVIDED

1. Microwell Strips: purified Toxoplasma antigen coated wells	(12X8 wells)
2. Absorbent Solution: Black Cap	1 Vial (22 ml)
3. Calibrator: Factor value (f) stated on label. Red Cap	1 Vial (150µL)
4. Negative Control: Range Stated on Label. Natural Cap	1 Vial (150µL)
5. Positive control: Range Stated on label. Green Cap	1 Vial (150µL)
6. Washing Concentrate 10X. White Cap	1 bottle (100 ml)
7. Enzyme Conjugate: Red color solution	1 Vial (12 ml)
8. TMB Chromogenic Substrate: Amber bottle	1 Vial (12 ml)
9. Stop Solution	1 Vail (12 ml)

STORAGE AND STABILITY

1. Store the kit at 2 - 8 oC.
2. Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested and found nonreactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as a integral unit. The components of different lots should not be mixed.
4. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2 - 8 oC for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

PREPARATION FOR ASSAY

1. Prepare 1x washing buffer.
   Prepare washing buffer by adding distilled or deionized water to 10x wash concentrate to a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20-25 oC) and gently mix.

ASSAY PROCEDURE

1. Place desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding 5 µl of the samples, negative control, positive control, and calibrator to 200 µl of absorbent solution. Mix well.
3. Dispense 100 µl of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µl absorbent solution in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
4. Remove liquid from all wells and repeat washing three times with washing buffer.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
7. Dispense 100 µl of TMB Chromogenic Substrate to each well and incubate for 30 minutes at room temperature.
8. Add 100 µl of 2 N HCl to stop reaction.
   Make sure there are no air bubbles in each well before reading
9. Read O.D. at 450 nm with a microwell reader.

CALCULATION OF RESULTS

1. To obtain cut –off value: Multiply the OD 450 of calibrator by factor (f) printed on the label of Calibrator.
2. Calculate the IgA index of each determination by dividing the OD value of cut-off.

Note: This factor (f) is a variable for each kit.

For example: If factor (f) value on label = 0.35 calculated cut off value equal 1.798 X 0.35 = 0.63

Sample	OD 450	Mean OD 450	Calculated cut –off value (B)	Index A/B	Interpretation
Calibrator f = 0.35	1.806 1.790	1.798	0.63		
Positive Control	1.643 1.662	1.653		2.62	Positive
Negative Control	0.023 0.022	0.023		0.04	Negative
Patient Sample 1	1.318 1.339	1.359		2.16	Positive
Patient Sample 2	0.206 0.212	0.209		0.33	Negative

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.
2. If the O.D. value of the Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The Toxo A Index for Negative and Positive Control should be in the range stated on the labels.

INTERPRETATION

Negative: Toxo A Index less than 0.90 are negative for IgA antibody to T. gondii.

Equivocal: Toxo A Index between 0.91-0.99 are equivocal. Sample should be retested.

Positive: Toxo A Index of 1.00 or greater are positive for IgA antibody to T. gondii.

EXPECTED VALUES

216 serum specimens from random, asymptomatic blood donors were tested with DIAGNOSTIC AUTOMATION ELISA Toxoplasma IgA. Of the 216 specimens, 22 were found to be positive (10.2 %) and 194 were found to be negative (89.9 %). For another set of 49 serum specimens from random and asymptomatic blood donors were also tested, of 49 specimens, 2 were found to be positive (4.1 %) and 47 were found to be negative (95.9 %). Of these 2 IgA positive samples were found to be IgG positive also. Prevalence may vary depending on a variety of factors such as geographical location, age, socioeconomic status, race, type of test employed, specimen collection and handling procedures, clinical and epidemiological history.

PERFORMANCE CHARACTERISTICS

Precision:

The precision of the assay was evaluated by testing three different sera of eight replicates over 3 days. The intra-assay and inter-assay C.V. are summarized below:

	Negative	Low Positive	Positive
Intra-assay	7.4%	6.2%	5.3%
Inter-assay	10.6%	7.9%	6.5

LIMITATIONS OF THE PROCEDURE

1. To prevent false negative results caused by the presence of specific IgG in some specimens, reagents provided in this kit has been formulated to resolve these interferences.
2. Lipemic, hemolyzed, icteric or heat inactivated sera may cause erroneous results.
3. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.
4. The physiological function of IgA and its clinical implication is still unclear.

REFERENCE

1. Turune, H.J., P.O. Leinikke, and K. M. Saari. Demonstration of Intraocular Synthesis of Immunoglobulin G Toxoplasma Antibodies for Specific diagnosis of Toxoplasmic Chorioretinitis by Enzyme Immunoassay. J. Clin. Microbiol. 17:988-992, 1983.
2. Lin, T.M., S.P. Halbert and G.R. O'Connor. Standardized Quantitative Enzyme-linked Immunoassay for Antibodies to Toxoplasma Gondii. J. Clin. Microbiol. Vol.11, 6:675-681, 1980.
3. Roller, A., A. Bartlett and D.E. Bidwell. Enzyme Immunoassay with Special Reference ELISA Technique. J. Clin. Path. 31:507-520, 1987.
4. Voller, A., D.E. Bidwell, A. Bartlett, D.G. Flick, M. Perkins and B. Oladshin. A Microplate Enzyme-immunoassay for Toxoplasma Antibodies. J. Clin. Path. 29:150-153, 1976.

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