



AccuDiag™ Toxoplasma IgM (Toxo IgM) ELISA Kit

REF 1102-P1

IVD See External Label 2°C 96 Tests

Toxoplasma IgM (Toxo IgM) ELISA	
Principle	Indirect ELISA
Detection	Qualitative
Sample	10 µL serum/plasma
Incubation Time	75 minutes
Sensitivity	100%
Specificity	100%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation/Cortez Diagnostics, Inc. (DAI) *Toxoplasma gondii* (Toxo) IgM Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the presumptive qualitative detection of IgM antibody to *Toxoplasma gondii gondii* in human serum for the presumptive diagnosis of acute, recent, or reactive *Toxoplasma gondii gondii* infection. Testing of patient sera must be performed in conjunction with an anti-*Toxoplasma gondii gondii* IgG antibody assay. This product is not FDA cleared (approved) for use in testing (i.e., screening) blood or plasma donors. The assay's performance has not been established for screening of prenatal women or newborns. **High complexity test.**

SIGNIFICANCE AND SUMMARY

Toxoplasma gondii is a coccidian parasite initially isolated in 1908 from a North African rodent -- the *gondii*. Since then, the organism has been found in many species of birds, reptiles and mammals.¹

Man is infected with *Toxoplasma gondii* from various suspected sources: ingestion of infected meat, especially mutton and pork, or ingestion of soil contaminated by oocyst from domestic and feral cats.² Transmission by organ transplant, transfusion or activation of quiescent infections is also documented. Congenital Toxoplasmosis is a disease with an extraordinarily wide range of manifestations; so wide in fact, that it must be considered in the differential diagnosis of nearly all types of obscure illness occurring during infancy.³

Because symptoms are sometimes nonspecific (i.e., anemia, splenomegaly, jaundice, fever, hepatomegaly, adenopathy and vomiting), congenital Toxoplasmosis is easily misdiagnosed on clinical grounds, even in sick infants who have the generalized form of the disease.⁴ Toxoplasmosis must also be considered in the differential diagnosis in any immunosuppressed patient who has clinical or laboratory evidence of damage to the central nervous system.⁵ This organism is one of the most common latent infectious agents of man throughout the world.⁶ In acquired Toxoplasmosis, levels of IgM antibody are generally detectable very early in the infection and peak within one or two months after clinical onset. They typically remain detectable for only a few weeks⁷ but can persist for as long as 2 years.⁵

The detection of IgM specific antibody can be of major importance in the diagnosis of congenital Toxoplasmosis in the neonate, because IgM class antibodies do not cross the placental barrier.⁵ It is also helpful in differentiating recently acquired (acute) toxoplasmosis from chronic infection.

The sensitivity, specificity, and reproducibility of enzyme-linked immunoassays is comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, hemagglutination and radioimmunoassay.^{8, 9, 10}

ASSAY PRINCIPLE

Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials (e.g., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgM globulin conjugated with horseradish peroxidase which will bind to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H₂SO₄, the contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader.^{10, 11, 12, 13}

SPECIMEN COLLECTION & PREPARATION

1. Handle all blood and serum as if capable of transmitting infectious agents.¹⁵



- Optimal performance of the kit depends upon the use of fresh serum samples (clear, non-hemolyzed, non-lipemic, non-icteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture.¹⁶ Early separation from the clot prevents hemolysis of serum.
- Store serum between 2° and 8°C if testing will take place within two days. If specimens are to be kept for longer periods, store at -20°C or colder. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.
- The NCCLS provides recommendations for storing blood specimens (Approved Standard Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).¹⁶

REAGENTS

Materials provided with the kit

Materials provided with the test kit

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

- Purified *Toxoplasma gondii* antigen coated microassay plate:** 96 wells, configured in twelve 1x8 strips stored in a foil pouch with desiccant. Allow the wells to equilibrate to room temperature (21°-25°C) in the pouch to protect from condensation. When stored at 2-8°C, coated strips are stable until the labeled expiration date. (one plate)
- Calibrator:** Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature and other testing conditions. (one vial, 0.4 mL)*
- Positive Control:** Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Positive Control is utilized to control the positive range of the assay. (one vial, 0.4 mL)*
- Negative Control:** Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Negative Control is utilized to control the negative range of the assay (one vial, 0.4 mL)*
- Horseradish-peroxidase (HRP) Conjugate:** Ready to use. Goat anti-human IgM, containing ProClin® (0.1%) and gentamicin as preservatives. (one bottle, 16 mL)
- Serum Diluent Plus:** Ready for use. Contains goat/sheep anti-human IgG for serum absorption to remove competing IgG, with protein stabilizers and ProClin® (0.1%) as a preservative. (two bottles, 45 mL each)
- Wash Buffer Type I (20X concentrate):** dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-20 and ProClin® (0.1%) as a preservative. (one bottle, 50 mL)
- Chromogen/Substrate Solution Type I:** Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (one bottle, 15 mL)
- Stop Solution:** Ready to use, contains a 1N H₂SO₄ solution. (one bottle, 15 mL)

*Note: serum vials may contain excess volume

Materials required but not provided

- Wash bottle, automated or semi-automated microwell plate washing system.

- Micropipettes, including multichannel, capable of accurately delivering 10-200 µL volumes (less than 3% CV).
- One liter graduated cylinder.
- Paper towels.
- Test tube for serum dilution.
- Reagent reservoirs for multichannel pipettes.
- Pipette tips.
- Distilled or deionized water (dH₂O), CAP (College of American Pathology) Type 1 or equivalent.^{18, 19}
- Timer capable of measuring to an accuracy of +/- 1 second (0 – 60 minutes).
- Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH₂O).
- Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

Note: Use only clean, dry glassware.

REAGENT PREPARATION

- All reagents must be removed from refrigeration and allowed to come to room temperature before use (21° to 25°C). Return all reagents to refrigerator promptly after use.
- All samples and controls should be vortexed before use.
- Dilute 50 mL of the 20X Wash Buffer Type I to 1 L with distilled and/or deionized H₂O. Mix well.

ASSAY PROCEDURE

- Place the desired number of strips into a microwell frame. Allow four (4) Control/ Calibrator determinations (one Negative Control, two Calibrators and one Positive Control) per run. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control/ Calibrator configurations. Return unused strips to the sealable bag with desiccant, seal and immediately refrigerate.

Example Configuration:

Plate Location	Sample Description	Plate Location	Sample Description
1A	RB	2A	Patient #4
1B	NC	2B	Patient #5
1C	Cal	2C	Patient #6
1D	Cal	2D	Patient #7
1E	PC	2E	Patient #8
1F	Patient #1	2F	Patient #9
1G	Patient #2	2G	Patient #10
1H	Patient #3	2H	Patient #11

RB = Reagent Blank - Well without serum addition run with all reagents. Used to blank reader.

NC = Negative Control

Cal = Calibrator

PC = Positive Control

- Dilute test sera, Calibrator and Control sera 1:81 (e.g., 10 µL + 800 µL) in Serum Diluent Plus. (For manual dilutions it is suggested to dispense the Serum Diluent into the test tube first and then add the patient serum). Mix well (Vortexing recommended).



- To individual wells add 100 µL of diluted patient sera, Calibrator and Control sera. Add 100 µL of Serum Diluent Plus to the reagent blank well. Check software and reader requirements of the correct reagent blank well configuration.
- Incubate each well at room temperature (21° to 25°C) for **30 minutes +/- 2 minutes**.
- Aspirate or shake out liquid from all wells. Using semi-automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out to remove all liquid. Repeat the wash procedure two times (for a total of three washes) for semi-automated equipment or four times (for a total of five washes) for automated equipment. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

****IMPORTANT NOTE: Regarding steps 5 and 8 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi - automated or automated equipment set to deliver a volume to completely fill each well (250-300 µL) is recommended. A total of five (5) washes may be necessary with automated equipment. Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.**

- Add 100 µL Conjugate to each well, including the reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
- Incubate each well **30 minutes +/- 2 minutes** at room temperature (21° to 25° C).
- Repeat wash as described in Step 5**.
- Add 100 µL Chromogen/Substrate solution (TMB) solution to each well, including reagent blank well, maintaining a constant rate of addition across the plate.
- Incubate each well at room temperature (21° to 25°C) for **15 minutes +/- 2 minutes**.
- Stop reaction by addition of 100 µL of Stop Solution (1N H₂SO₄) following the same order of Chromogen/Substrate addition, including reagent blank well. Tap the plate gently along the outsides to mix contents of the wells. The plate may be held up to one (1) hour after addition of the Stop Solution before reading.
- The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650. The instrument should be blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the reagent blank is ≥ 0.150, the run must be repeated. Blank the reader on the reagent blank well and then continue to read the entire plate. Dispose of used plates after readings have been obtained.

SERUM TREATMENT

Solid phase immunoassays for the detection of virus-specific IgM are known to be sensitive to interfering factors. The goat/sheep anti-human IgG in the Serum Diluent Plus diminishes competing virus-specific IgG, which would be responsible for false negative reactions. False positives are similarly minimized by removing the IgG, thus neutralizing the bound rheumatoid factor in the samples.

RESULTS

- Mean Calibrator O.D. (Optical Density) - Calculate the mean O.D. value for the Calibrator from the two Calibrator determinations.
- Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined by Diagnostic Automation/Cortez Diagnostics, Inc. for each lot of kits. The Correction Factor is printed on the Calibrator vial.

- Cutoff Calibrator Value - The Cutoff Calibrator Value for each assay is determined by multiplying the Correction Factor by the mean Calibrator O.D. determined in Step 1.
- ISR Value - Calculate an Immune Status Ratio (ISR) for each specimen by dividing the specimen O.D. Value by the Cutoff Calibrator Value determined in Step 3.

Example: O.D.s obtained for Calibrator = 0.38, 0.42, 0.40
 Mean O.D. for Calibrator = 0.40
 Correction Factor = 0.50
 Cutoff Calibrator Value = 0.50 x 0.40 = 0.20
 O.D. obtained for patient sera = 0.60
 ISR Value = 0.60/0.20 = 3.00

ANALYSIS

ISR Value	Results
≤0.90	Negative
0.91-1.09	Equivocal
≥1.10	Positive

The patients' ISR (Immune Status Ratio) values are interpreted as follows

Anti-T. IgM Result	Anti-T. IgG Result	Report/Interpretation
Negative ≤ 0.90	Negative ≤ 0.90	It is presumed the patient has not been infected with and is not undergoing an acute infection with <i>Toxoplasma gondii</i> . If symptoms persist submit a new specimen within three weeks.
Negative ≤ 0.90	Positive ≥ 1.10	From this testing it cannot be determined whether the patient is or is not undergoing a reactivated <i>Toxoplasma gondii</i> infection. It appears the patient has been previously infected with <i>Toxoplasma gondii</i> . Infection occurred more than one year ago.
Negative ≤ 0.90	Equivocal 0.91-1.09	Obtain a new specimen for further testing. Patient may not be undergoing an acute infection with <i>Toxoplasma gondii</i> . Determining whether the patient has been previously infected with <i>Toxoplasma gondii</i> is not possible.
Equivocal 0.91-1.09	Negative ≤ 0.90	Obtain a new specimen for determination of IgM antibodies to <i>Toxoplasma gondii</i> . It cannot be determined if the patient is undergoing an acute <i>Toxoplasma gondii</i> infection. It appears the patient has not been previously infected with <i>Toxoplasma gondii</i> . If the new specimen result is positive or equivocal for IgM antibodies, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Equivocal 0.91-1.09	Positive ≥ 1.10	Obtain a new specimen for determination of IgM antibodies to <i>Toxoplasma gondii</i> . It cannot be determined if the patient is undergoing or has undergone an acute <i>Toxoplasma gondii</i> infection. It appears the patient has been previously infected with <i>Toxoplasma gondii</i> . If the new specimen is equivocal or positive for IgM antibodies, the specimen should be sent to a reference



Anti-T. IgM Result	Anti-T. IgG Result	Report/Interpretation
		laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Equivocal 0.91-1.09	Equivocal 0.91-1.09	Obtain a new specimen for further testing. It cannot be determined if the patient is undergoing an acute infection or has been previously infected with <i>Toxoplasma gondii</i> . If the new specimen result is equivocal or positive for IgM antibodies, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Positive ≥ 1.10	Negative ≤ 0.90	Obtain a new specimen for further testing. The patient may or may not be acutely infected with <i>Toxoplasma gondii</i> . Since the IgG antibodies to <i>Toxoplasma gondii</i> are negative, the specimen may have been obtained too early in the disease process for an accurate determination. Retest the new specimen with a different anti- <i>Toxoplasma gondii</i> IgM assay. If the new specimen result is still positive for IgM antibodies, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Positive ≥ 1.10	Positive ≥ 1.10	The patient may or may not be acutely infected with <i>Toxoplasma gondii</i> . Obtain a new specimen for further testing. Since the IgG antibodies to <i>Toxoplasma gondii</i> are positive, it appears the patient may be acutely infected with <i>Toxoplasma gondii</i> . The new specimen should be repeated with a different anti- <i>Toxoplasma gondii</i> IgM assay. If the new specimen is still positive for IgM and IgG antibodies to <i>Toxoplasma gondii</i> , the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Positive ≥ 1.10	Equivocal 0.91-1.09	It cannot be determined if the patient is acutely infected with <i>Toxoplasma gondii</i> . Obtain a new specimen for further testing. Determining whether the patient has been previously infected with <i>Toxoplasma gondii</i> is not possible. The specimen may have been collected too early during the disease process for an accurate determination. Retest the new specimen with a different anti- <i>Toxoplasma gondii</i> IgM assay. If the new specimen result is still positive for antibodies to IgM and the IgG is positive/negative/equivocal for antibodies to <i>Toxoplasma gondii</i> the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.

- The magnitude of the measured result, above the cutoff, is not indicative of the total amount of antibody present.
- If the specimen was collected too early in the course of the disease IgM antibodies may not be detectable. IgM antibodies also may not be detectable during a reactivation of infection.
- The cutoff for the assay was determined by assaying known positive and negative sera and choosing a cutoff that gave the best fit for relative sensitivity and specificity.

QUALITY CONTROL

For the assay to be considered valid the following conditions must be met:

- Calibrator and Controls must be run with each test run.
- Reagent blank (when read against air blank) must be < 0.150 Absorbance (A) at 450 nm.
- Negative Control must be ≤ 0.250 A at 450 nm (when read against reagent blank).
- Each Calibrator must be ≥ 0.300 A at 450 nm (when read against reagent blank).
- Positive Control must be ≥ 0.250 A at 450 nm (when read against reagent blank).
- The ISR for the Positive and Negative Controls should be in their respective ranges printed on the vial labels. If the Control values are not within their respective ranges, the test should be considered invalid and the test should be repeated.
- Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
- Refer to NCCLS C24A for guidance on appropriate Quality Control practices.¹⁷
- If above criteria are not met on repeat testing, contact Diagnostic Automation/Cortez Diagnostics, Inc.

EXPECTED RANGES OF VALUES

IgM antibodies appear in the first week of infection and usually peak within a month. Although uncommon, low levels of IgM may persist for one year or longer.¹⁴ The annual incidence rates are reported to vary in different geographical areas from 0.1% - 0.9%.²⁰

A total of 108 random serum specimens collected from US Blood Centers in the Northeast were tested to establish the expected values in a population with no known clinically apparent *Toxoplasma gondii* infection. Table 1-A summarizes the distribution of Diagnostic Automation/Cortez Diagnostics, Inc. Toxo IgM (Catalog # 1102-1) Assay ISR values observed for the population.

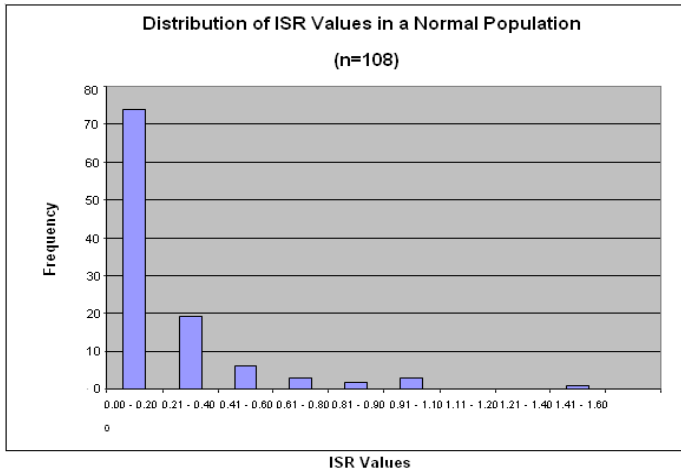
Table 1-A
Distribution of DAI Toxo IgM (Catalog # 1102-1)
Assay ISR Values From 108 Individuals

DAI <i>Toxoplasma gondii</i> IgM Assay ISR Range	Number of Specimens	Percent of Total
0.00-0.20	74	68.5%
0.21-0.40	19	17.6%
0.41-0.60	6	5.6%
0.61-0.80	3	2.8%
0.81-0.90	2	1.9%
0.91-1.10	3	2.8%
1.11-1.20	0	0.0%



DAI <i>Toxoplasma gondii</i> IgM Assay ISR Range	Number of Specimens	Percent of Total
1.21-1.40	0	0.0%
1.41-1.60	1 ^a	0.9%

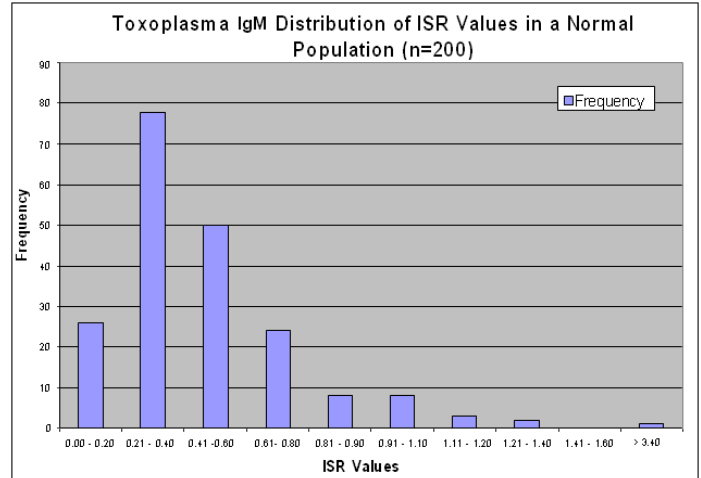
^a This sample was positive and all other samples were negative when tested by other commercially available ELISA.



A total of 200 random serum samples collected from US blood centers; 100 from blood centers in California and 100 from blood centers on the east coast were tested to establish the expected values in a population with no known clinically apparent *Toxoplasma* infection. Table 1-B summarizes the distribution of Diagnostic Automation/Cortez Diagnostics, Inc. (Catalog # 1102-1) assay ISR Values observed for the population.

Table 1-B
Distribution of DAI Toxo IgM (Catalog #1102-1)
Assay ISR Values From 200 Individuals

DAI <i>Toxoplasma gondii</i> IgM Assay ISR Range	Number of Specimens	Percent of Total
0.00-0.20	26	13%
0.21-0.40	78	39%
0.41-0.60	50	25%
0.61-0.80	24	12%
0.81-0.90	8	4%
0.91-1.10	8	4%
1.11-1.20	3	1.5%
1.21-1.40	2	1%
1.41-1.60	0	0%
> 3.41	1	0.5%



To establish the expected values for individuals suspected of recent clinically apparent *Toxoplasma gondii* infection, 69 serum samples obtained from clinical reference laboratories and brokerage houses were assayed by the Diagnostic Automation/Cortez Diagnostics, Inc. Toxo IgM (Catalog #1102-1) Assay. The results are summarized in Table 2.

Table 2
Distribution of DAI Toxo IgM (Catalog #1102-1)
Assay ISR Values From 69 Samples Suspected of Recent Clinically Apparent *Toxoplasma gondii* Infection

DAI <i>Toxoplasma gondii</i> IgM Assay ISR Range	Number of Specimens	Percent of Total
0.00-0.50	1 ^a	1.4%
0.51-0.90	7 ^b	10.1%
0.91-1.10	8 ^c	11.6%
1.11-1.50	21 ^d	30.4%
1.51-2.00	24 ^e	34.8%
2.01-2.50	6 ^f	8.7%
2.51-3.00	1 ^g	1.4%
3.01-3.50	1 ^g	1.4%
3.51-4.00	0	0.0%

^a Sample negative when tested by other commercially available ELISA.

^b 6 samples were negative and 1 equivocal when tested by other commercially available ELISA.

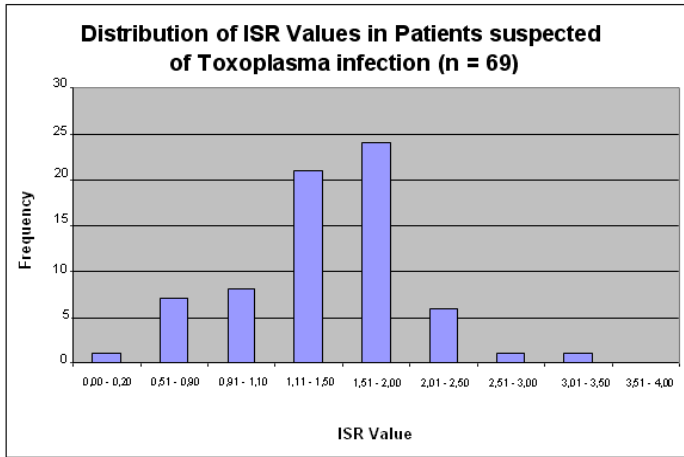
^c 1 sample was positive, 6 equivocal and 1 negative when tested by other commercially available ELISA.

^d 18 samples were positive and 3 equivocal when tested by other commercially available ELISA.

^e 22 samples were positive, 1 equivocal and 1 negative when tested by other commercially available ELISA.

^f 4 samples were positive and 2 equivocal when tested by other commercially available ELISA.

^g Both samples were positive when tested by other commercially available ELISA.



For the clinical significance of ISR values, please refer to the "Results" section of this insert.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

The Diagnostic Automation/Cortez Diagnostics, Inc. Toxo IgM ELISA (Catalog # 1102-1) was evaluated in comparison to a commercially available Toxo IgM ELISA. The study population of 177 samples was comprised of randomly collected sera from apparently healthy ambulatory donors as well as positive samples from independent clinical laboratories in the Northeastern U.S. Discordant samples were refereed by further testing with a commercially available IFA kit for detecting IgM antibodies to *Toxoplasma gondii*. 18 samples remained equivocal or unresolved among the 3 test systems and were not used in the calculations of sensitivity and specificity. The results are presented in Table 3:

Table 3
Relative Sensitivity & Specificity
DAI ELISA

ELISA		(+)	(-)	Relative Sensitivity	Relative Specificity
or	(+)	45	0	100%	97.4%
Referee	(-)	3	111		

95% confidence intervals - 93.5-100%* 94.4-100%

The 95% confidence intervals were calculated using the normal method.
*This confidence interval was calculated using one false negative.

Please be advised that "relative" refers to comparison of this assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with disease absence or presence. No judgment can be made on the comparison assay's accuracy to predict disease. Due to the apparent low prevalence of anti-*Toxoplasma gondii* IgM in the United States, the specimens used for establishing this assay's performance characteristics may not be representative of the user's population. With very low prevalence analytes, there is the increased possibility that a positive result is truly a false positive, reducing the assay's positive predictive value.

AGREEMENT

A study was performed to document the agreement between the DAI *Toxoplasma gondii* IgM ELISA (Catalog # 1102-1) and DAI *Toxoplasma gondii* IgM ELISA (Catalog # 1102-1). The study included 296 specimens consisting of samples from a random normal population, first trimester prenatal samples and known positive and negative samples. The results are presented in Table 4:

Table 4

		Toxoplasma IgM ELISA		
		+	-	Eq
Toxoplasma IgM ELISA	+	9	0	0
	-	0	284	0
	Eq	0	2	1

Agreement = 294/296 = 99.32%

PRECISION

A study was performed to document typical assay precision with the Diagnostic Automation/Cortez Diagnostics, Inc. *Toxoplasma gondii* IgM ELISA Kit (Catalog # 1102-1). The mean, SD, and % CV, were calculated for Intra- and Inter-assay precision.

Intra-Assay Precision

Table 5 presents a summary of the results of the six (6) samples individually pipetted in groups of ten (10) in a single assay.

Table 5
Intra-Assay Precision for DAI Toxo IgM ELISA

	n	Mean ISR	Std Dev	% CV
Serum 1	10	1.10	0.05	1.5%
Serum 2	10	1.98	0.12	6.0%
Serum 3	10	1.58	0.05	3.4%
Serum 4	10	1.56	0.09	6.0%
Serum 5	10	0.22	0.01	4.4%
Serum 6	10	0.19	0.01	3.2%

Inter-Assay Precision

Table 6 presents the summary of the Inter-Assay precision data determined by replicate testing of six (6) samples individually pipetted in groups of 10 in three separate assays.

Table 6
Inter-Assay Precision for DAI Toxo IgM

	n	Assay 1	Assay 2	Assay 3	Mean ISR	Std Dev	%CV
Serum 1	30	1.36	1.10	1.13	1.20	0.15	12.3%
Serum 2	30	1.99	1.98	1.97	1.98	0.18	9.1%
Serum 3	30	1.98	1.58	1.67	1.75	0.20	11.3%
Serum 4	30	1.78	1.56	1.53	1.62	0.13	8.0%
Serum 5	30	0.26	0.22	0.21	0.23	0.02	10.1%
Serum 6	30	0.24	0.19	0.20	0.21	0.02	11.5%

CROSS-REACTIVITY

Method

A study was performed to assess possible interference by antinuclear antibodies (ANA) and rheumatoid factor (RF) with the Diagnostic Automation/Cortez Diagnostics, Inc. Toxo IgM ELISA test kit (Catalog#1102-1). Nineteen (19) samples, negative for *Toxoplasma gondii* by a commercially available ELISA, which tested positive by other commercially available kits for



ANA (11) or RF (8) were assayed on the Diagnostic Automation/Cortez Diagnostics, Inc. Toxo IgM ELISA kit.

Results

Negative Toxo IgM ELISA test results in all samples indicate an absence of interference by samples containing ANA or RF.

LIMITATIONS OF THE ASSAY

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
2. The results of ELISA immunoassays performed on serum from immunosuppressed patients must be interpreted with caution.
3. Samples that remain equivocal after repeat testing should be retested by an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken.
4. The absence of detectable IgM antibody does not rule out the possibility of recent or current infection.
5. Specific IgG may compete with the IgM for sites and may result in a false negative. Conversely, rheumatoid factor in the presence of specific IgG may result in a false positive reaction.^{15, 16} The Serum Diluent Plus Solution diminishes competing virus-specific IgG and minimizes rheumatoid factor interference in samples. Studies indicate that the maximum amount of IgG which can be removed by the kit Serum Diluent Plus Solution is in excess of the expected high end of the normal range for IgG > 1380mg/dL. The highest titer of RF + tested (1:2560;1000 IU/mL) did not adversely affect the performance of the assay.
6. Some antinuclear antibodies have been found to cause a false positive reaction on some ELISA tests.
7. It is strongly recommended that neonate's and mother's serum samples be tested in parallel. The presence of IgM antibody in the neonate's serum can be considered indicative of congenital infection only if there has not been placental leakage. Additionally, if the infant has a congenital infection, the IgM antibody (and IgG antibody) level may persist or rise, whereas if the source of the antibody is maternal, the neonate's antibody level will drop in parallel to the half-life of that immunoglobulin.
8. Results of this test should be interpreted by the physician in the light of other clinical findings and diagnostic procedures.
9. This test is not intended for the determination of immune status. It is intended for the determination of a person's antibody response to indicate active infection to Toxo and not as an indication of immunity.
10. Assay performance characteristics have not been established with specimens from patients with documented Toxoplasma infection nor with matrices other than serum.

STORAGE CONDITIONS

1. Store unopened kit between 2° and 8°C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened micro assay plates must be stored between 2° and 8°C. Unused strips must be immediately resealed in a sealable bag with desiccant and returned to storage between 2° and 8°C.
3. Store HRP Conjugate between 2° and 8°C.
4. Store the Calibrator, Positive and Negative Controls between 2° and 8°C.
5. Store Serum Diluent Plus and 20X Wash Buffer Type 1 between 2° and 8°C.

6. Store the Chromogen/Substrate Solutions Type 1 between 2° and 8°C. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells.
7. Store 1X (dilute) Wash Buffer Type 1 at room temperature (21° to 25°C) for up to 5 days or up to 1 week between 2° and 8°C.

Note: If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit. Refer to package label for expiration date. Precautions were taken in the manufacture of this product to protect the reagents from contamination and bacteriostatic agents have been added to the liquid reagents. Care should be exercised to protect the reagents in this kit from contamination. Do not use if evidence of microbial contamination or precipitation is present.

PRECAUTIONS

1. For *in vitro* diagnostic use.
2. The human serum components used in the preparation of the Controls and Calibrator in this kit have been tested by an FDA approved method for the presence of antibodies to human immunodeficiency virus1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen and found negative. Because no test method can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
3. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.¹⁵
4. The components in this kit have been quality control tested as a Master Lot unit. Do not mix components from different lot numbers except Chromogen/Substrate Solution Type I, Stop Solution, Wash Buffer Type I. Do not mix with components from other manufacturers.
5. Do not use reagents beyond the stated expiration date marked on the package label.
6. All reagents must be at room temperature (21° to 25°C) before running assay. Remove only the volume of reagents that is needed. **Do not pour reagents back into vials as reagent contamination may occur.**
7. Before opening Control and Calibrator vials, tap firmly on the benchtop to ensure that all liquid is at the bottom of the vial.
8. Use only distilled or deionized water and clean glassware.
9. Do not let wells dry during assay; add reagents immediately after completing wash steps.
10. Avoid cross-contamination of reagents. Wash hands before and after handling reagents. **Cross-contamination of reagents and/or samples could cause false results.**
11. If washing steps are performed manually, wells are to be washed three times. Up to five wash cycles may be necessary if a washing manifold or automated equipment is used.
12. **Sodium azide inhibits Conjugate activity. Clean pipette tips must be used for the Conjugate addition so that sodium azide is not carried over from other reagents.** It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing, flush drains with water to minimize build-up of metal azide compounds.
13. It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing, flush drains with water to minimize build-up of metal azide compounds.
14. Never pipette by mouth or allow reagents or patient sample to come into contact with skin. Reagents containing ProClin®, sodium azide, and TMB may be irritating. Avoid contact with skin and eyes. In case of contact, flush with plenty of water.



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I M M U N O D I A G N O S T I C S

15. If a sodium hypochlorite (bleach) solution is being used as a disinfectant, do not expose to work area during actual test procedure because of potential interference with enzyme activity.
16. Avoid contact of Stop Solution (1N sulfuric acid) with skin or eyes. If contact occurs, immediately flush area with water.
17. **Caution:** Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite (bleach) solution to avoid formation of poisonous gas. Recommend disposing of reacted, stopped plates in biohazard bags. See Precaution 3.
18. The concentrations of anti-Toxoplasma gondii in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

The safety data sheet is available upon request.



WARNING

Serum diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300®, a biological preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

H317: May cause an allergic skin reaction.

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P302+P352: IF ON SKIN: Wash with plenty of soap and water.

P333+P313: If skin irritation or rash occurs: Get medical advice/attention.

P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

WARNING

Serum Diluent and Controls contain < 0.1% sodium azide.

H302: Harmful if swallowed.

P264: Wash thoroughly with plenty of soap and water after handling.

P270: Do not eat, drink or smoke when using this product.

P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.

P330: If swallowed, rinse mouth.

P501: Dispose of contents/container in to accordance to local, regional, national and international regulations.


REFERENCES

1. Feldman, H.A. 1974. Toxoplasmosis: An Overview. Bull. N.Y. Acad. Med. 50:110-127.
2. Jacobs, L. 1974. Toxoplasma gondii: Parasitology and Transmission. Bull. N.Y. Acad. Med. 50:128-145.
3. Eichenwald, H.F. 1959. A study of Congenital Toxoplasmosis with Particular Emphasis on Clinical Manifestations, Sequelae and Therapy. In: Human Toxoplasmosis. Si, J.C., ed. Vol. 2. Mansard, Copenhagen.
4. Alford, C.A., Jr., S. Stagno, and D.W. Reynolds. 1974. Congenital Toxoplasmosis: Clinical, Laboratory, and Therapeutic Considerations, with Special Reference to Subclinical Disease. Bull. N.Y. Acad. Med. 50:160-181.
5. Remington, J.S. 1974. Toxoplasmosis In the Adult. Bull. N.Y. Acad. Med. 50:211-227.
6. Beattie, C.P. 1967 Toxoplasmosis. In: Recent Advances in Medical Microbiology. Waterson, A.P., ed. pp 318-351.
7. Fraser, K.B., P.V. Shiro Daria, and C.F. Sandford. 1971. Fluorescent Staining and Human IgM. Br. Med. J. 3:707.

8. Walls, K.W. 1978. Serodiagnosis of Toxoplasmosis. Lab. Mgmt. January: 26-31.
9. Bakerman, S. 1980. Enzyme Immunoassays. Lab. Mgmt. August:21-29.
10. Engvall, E., K. Jonsson, and P. Perlman. 1971. Enzyme-Linked Immunosorbent Assay (ELISA) Quantitative Assay of Immunoglobulin G. Immunochemistry. 8:871-874.
11. Engvall, E. and P. Perlman. 1971. Enzyme-Linked Immunosorbent Assay, ELISA. In: Protides of the Biological Fluids. H. Peeters, ed. Proceedings of the Nineteenth Colloquium, Brugge Oxford. Pergamon Press. pp 553-556.
12. Engvall, E., K. Jonsson, and P. Perlman. 1971. Enzyme-Linked Immunosorbent Assay. II. Quantitative Assay of Protein Antigen, Immunoglobulin-G, By Means of Enzyme- Labelled Antigen and Antibody-Coated tubes. Biochem. Biophys. Acta. 251: 427-434.
13. Van Weeman, B. K. and A.H.W.M. Schuur. 1971. Immunoassay Using Antigen-Enzyme Conjugates. FEBS Letter. 15:232- 235.
14. Engvall, E. and P. Perlman. 1972. Enzyme-Linked Immunosorbent Assay, ELISA. III. Quantitation of Specific Antibodies by Enzyme-Labeled Anti-Immunoglobulins in Antigen- Coated Tubes. J. Immunol. 109: 129-135.
15. CDC(NIH) Interagency Working Group. 1993. Biosafety in Microbiological and Biomedical Laboratories. 3rd Edition. U. S. Dept. of Health and Human Services, Public Health Service. pp 18-24.
16. National Committee for Clinical Laboratory Standards. 1990. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture Approved Standard. NCCLS Publication H18-A.
17. NCCLS. 1991. National Committee for Clinical Laboratory Standard. Internal Quality Control Testing: Principles & Definition. NCCLS Publication C24- A.
18. <http://www.cap.org/html/ftpdirectory/checklistftp.html>. 1998. Laboratory General – CAP (College of American Pathology) Checklist (April 1998). pp 28-32.
19. NCCLS. 1997. National Committee for Clinical Laboratory Standard. Preparation and Testing of Reagent Water in the Clinical Laboratory. NCCLS Publication C3- A3.
20. Feldman, H. A. 1982. Epidemiology of Toxoplasma gondii Infections. Epidem. Rev. 4:204-213.

MANUFACTURER AND BRAND DETAILS

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Date Adopted	2023-11
Brand Name	AccuDiag™
REF 1102-P1	AccuDiag™ - Toxoplasma IgM (Toxo IgM) ELISA

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