Toxoplasma M (Toxo M)

CE 0088

Assay for the Detection of IgM Antibodies to *Toxoplasma gondii*

Assay Summary

Sample Type	***Serum, Heparinized Plasma, EDTA Plasma***
Sample Volume	***10 µL***
Calibrator	***Toxo M***
Sensitivity and Assay Range	***0.10–40.00 Index***

Contents

REF	*Contents*	*Number of Tests*
05303018 (120153)	1 ReadyPack® primary reagent pack containing ADVIA Centaur® Toxo M Lite Reagent and Solid Phase	50
	ADVIA Centaur Toxo M Master Curve card	
	1 vial Toxo M Low Calibrator CAL L	
	1 vial Toxo M High Calibrator CAL H	
	ADVIA Centaur Toxo M Calibrator Assigned Value card	

For a definition of symbols used in product labeling, refer to *Understanding the Symbols* in Appendix D.

Intended Use

The ADVIA Centaur Toxoplasma M assay is an IgM antibody capture microparticle direct chemiluminometric *in vitro* diagnostic immunoassay intended for the qualitative detection of IgM antibodies to *Toxoplasma gondii* in serum or plasma (EDTA, heparin) using the ADVIA Centaur and ADVIA Centaur XP systems.

The ADVIA Centaur Toxoplasma M assay is used to measure IgM antibody against *T. gondii* which is presumptive of an acute, recent, or reactivated toxoplasma infection. Any measurement of IgM antibody to *T. gondii* must be performed in conjunction with the determination of IgG antibody to *T. gondii*.

WARNING: The detection of toxoplasma IgM in a given specimen, as determined by assays from different manufacturers, can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the toxoplasma IgM assay used. Values obtained with different assay methods cannot be used interchangeably. The reported IgM level cannot be correlated to an endpoint titer.

This assay is not intended for use in screening blood, plasma, or tissue donors. The effectiveness of this assay for use in screening blood, plasma, or tissue donors has not been established.

Materials Required but Not Provided

REF	*Description*	*Contents*
03089388 (120383)	ADVIA Centaur Toxo M quality control material	2 x 1.5 mL Negative Control CONTROL -
		2 x 1.5 mL Positive Control CONTROL +
		Expected Value card
01137199 (112351)	ADVIA Centaur Wash 1 WASH 1	2 x 1500 mL/pack
or 03773025	ADVIA Centaur Wash 1 WASH 1 *	2 x 2500 mL/pack

*for use with systems with 2500 mL capacity

Summary and Explanation of the Test

Toxoplasma gondii is an intracellular parasitic protozoan that affects birds and mammals, with cats being the primary host. Infection is typically spread by eating raw or undercooked meat containing cysts or by coming in contact with oocyst-infected cat feces. Climate, dietary customs, and presence of cats influence the prevalence of *T. gondii* which can vary considerably by geographical location and age. In healthy immunocompetent individuals, infections are usually asymptomatic or subclinical. If toxoplasmosis is diagnosed during the early stages of infection, the disease can be treated effectively with antibiotic therapy.

In pregnant women, *T. gondii* infection poses a potential threat to the fetus. The risk of a pregnant woman passing infection to the fetus is approximately 25% in the first trimester and increases to approximately 65% in the third trimester.[1] The earlier in the pregnancy that the mother is infected the greater the potential severity of congenital toxoplasmosis. If the fetus becomes infected, the infant may have symptoms such as lymphadenopathy, chorioretinitis, microcephaly and cerebral calcifications.

In immunosuppressed populations, such as cancer patients undergoing chemotherapy, transplants recipients, and AIDS patients, *T. gondii* has emerged as an important opportunistic pathogen leading to severe or fatal infections.[2,3] The immunosuppressed state of these patients is thought to allow reactivation of a latent infection,[3] and these patients may present symptoms such as headaches, confusion, fever, and focal neurological deficits.

Because isolation of the organism is difficult, toxoplasma IgM assays have been used in conjunction with clinical information in the diagnosis of *T. gondii* infection. In toxoplasma infected patients, the IgM antibody against *T. gondii* increases during acute infection but may be present for many months. A confirmed positive IgM anti-*T. gondii* test result is presumptive of a current or recent infection. In reactivated infections in immunosuppressed individuals, an IgM immunoresponse has not been demonstrated.[4] The presence of IgG antibodies against *T. gondii* indicates that the individual has had a past infection, but the level of reactivity does not indicate how recently the infection occurred.

Assay Principle

The ADVIA Centaur Toxoplasma M assay is an immunoglobulin class-capture sandwich immunoassay using direct, chemiluminometric technology. The anti-human IgM_{μ} monoclonal antibody is covalently coupled to paramagnetic particles in the Solid Phase. In the Lite Reagent, the *T. gondii* antigen is complexed with an anti-p30 monoclonal labeled with acridinium ester. Antibody-antigen complexes will form if toxoplasma IgM is present in the sample.

The system automatically performs the following actions:

- Dispenses 10 µL of sample into a cuvette.
- Dispenses 340 µL of Solid Phase and incubates the mixture for 18 minutes at 37°C.
- Separates the Solid Phase from the mixture and aspirates the unbound reagent.
- Washes the cuvette with Wash 1.
- Dispenses 200 µL Lite Reagent and incubates the mixture for 18 minutes at 37°C.
- Separates the Solid Phase from the mixture and aspirates the unbound reagent.
- Washes the cuvette with Wash 1.
- Dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction.
- Reports results according to the selected option, as described in the system operating instructions or in the online help system.

A direct relationship exists between the amount of toxoplasma IgM activity present in the patient sample and the amount of relative light units (RLUs) detected by the system. A result of reactive (positive) or nonreactive (negative) is determined using an Index Value. Refer to *Interpretation of Results* for a description of the Index Value.

Specimen Collection and Handling

Serum, heparinized plasma, or EDTA plasma are the recommended sample types for this assay. A study of 8 specimens across the dynamic range of the assay and 10 specimens near the cut-off demonstrated that the index values in all matrices yielded equivalent performance.

- Do not use heat inactivated specimens.
- The performance of the ADVIA Centaur Toxoplasma M assay has not been established with cord blood, neonatal specimens, immunosuppressed specimens, cadaver specimens, or body fluids other than serum or plasma such as saliva, urine, amniotic, or pleural fluids.
- Handle all samples as if capable of transmitting disease.
- Test samples as soon as possible after collecting. Store samples at 2° to 8°C if not tested immediately.
- Store specimens at 2° to 8°C up to 7 days. Specimens may be stored on the clot.
- Freeze samples, devoid of red blood cells, at or below -20°C for longer storage. Do not store in frost-free freezer. When weakly positive samples and negative samples were subject to 3 freeze/thaw cycles, no qualitative differences were observed.
- Package and label samples for shipment in compliance with applicable federal and international regulations covering the transport of clinical samples and etiological agents. Store samples at 2° to 8°C upon arrival. If shipment is expected to exceed 7 days, ship specimens frozen.

Before placing samples on the system, ensure that samples have the following characteristics:

- Samples are free of fibrin or other particulate matter. Remove particulates by filtration or centrifugation at 1000 x g for 10 to 15 minutes.
- Samples are free of bubbles.

Reagents

Store the reagents upright at 2–8°C.
Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to Appendix C, *Handling Reagents*.

Reagent Pack	Reagent	Volume	Ingredients	Storage	Stability
ADVIA Centaur Toxo M ReadyPack primary reagent pack	Lite Reagent	10.0 mL/ reagent pack	partially purified *T. gondii* antigen (~3 µg/mL) complexed with a mouse anti-*T.gondii* p30 monoclonal antibody labeled with acridinium ester in protein buffer with surfactant and preservatives	2–8°C	Until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
	Solid Phase	17.0 mL/ reagent pack	mouse anti-human IgMµ monoclonal antibody (~24 µg/mL) covalently coupled to paramagnetic particles in protein buffer with surfactant and preservatives	2–8°C	Until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*

Reagent Pack	Reagent	Volume	Ingredients	Storage	Stability
Toxo M calibrator vials	Calibrators	600 µL / vial	defibrinated recalcified processed human plasma positive for toxoplasma IgM antibodies with preservatives	2–8°C	Until the expiration date on the vial or onboard–8 hours.
Toxo M quality control material vials*	Controls	1.5 mL/ vial	defibrinated processed human plasma negative and positive for toxoplasma IgM antibodies with preservatives	2–8°C	Until the expiration date on the vial or onboard–8 hours.
ADVIA Centaur WASH 1 *	Wash 1	1500 mL/ pack	phosphate buffered saline with sodium azide (< 0.1%) and surfactant	2–25°C	Until the expiration date on the vial or onboard–1 month.
ADVIA Centaur WASH 1 *	Wash 1	2500 mL/ pack	phosphate buffered saline with sodium azide (< 0.1%) and surfactant	2–25°C	Until the expiration date on the vial or onboard–1 month.

* See *Materials Required but Not Provided.*

CAUTION! POTENTIAL BIOHAZARD: Contains human source material. While each human serum or plasma donor unit used in the manufacture of this product was tested by FDA-approved methods and found nonreactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV), and antibody to HIV-1/2, all products manufactured using human source material should be handled as potentially infectious. Because no test method can offer complete assurance that hepatitis B or C viruses, HIV, or other infectious agents are absent, these products should be handled according to established good laboratory practices.[5-7]

CAUTION: This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

NOTE: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal in a drain is in compliance with federal, state, and local requirements.

For *in vitro* diagnostic use.

Loading Reagents

Ensure that the system has sufficient primary reagent packs. For detailed information about preparing the system, refer to the system operating instructions or to the online help system.

Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to Appendix C, *Handling Reagents*.

Load the ReadyPack primary reagent packs in the primary reagent compartment using the arrows on the packs as a placement guide. The system automatically mixes the primary reagent packs to maintain homogeneous suspension of the reagents. For detailed information about loading reagents, refer to the system operating instructions or to the online help system.

NOTE: The Low and High Calibrators provided in this kit are matched to the ReadyPack primary reagent pack. Do not mix calibrator lots with different lots of reagent packs.

Onboard Stability and Calibration Interval

Additionally, the ADVIA Centaur Toxoplasma M assay requires a two-point calibration:

Onboard Stability	Calibration Interval
28 days	14 days

- when changing lot numbers of primary reagent packs
- when replacing system components

- when quality control results are repeatedly out of range

NOTE:

- Discard reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

Calibration

Using Barcode Labels

NOTE: Calibrator barcode labels are lot-number specific. Do not use barcode labels from one lot of calibrators with any other lot of calibrators.

Use the ADVIA Centaur Toxoplasma M Calibrator barcode labels to identify the Low and High Calibrator sample cups when performing the ADVIA Centaur Toxoplasma M assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing a Calibration

Each lot of calibrators contains a Calibrator Assigned Value card to facilitate entering the calibration values on the system. Enter the values using the barcode scanner or the keyboard.

NOTE: This procedure uses calibrator volumes sufficient to measure each calibrator in duplicate.

1. Schedule the calibrators to the worklist.
2. Label two sample cups with calibrator barcode labels: one for the low and another for the high.

NOTE: Each drop from the calibrator vial is approximately 50 µL.

3. Gently mix the Low and High Calibrators and dispense at least 3 to 4 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any calibrator remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh calibrators.

Quality Control

For quality control of the ADVIA Centaur Toxoplasma M assay, use ADVIA Centaur Toxoplasma M quality control material. Refer to the Expected Value card for the suggested expected values specific for the lot number of the positive and negative controls. Additional controls may be tested according to the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. Please refer to NCCLS C24-A for guidance on quality control practices.

Using Barcode Labels

Control barcode labels are lot-number specific. Do not use barcode labels from one lot of controls with any other lot of controls.

Use the ADVIA Centaur Toxoplasma M quality control barcode labels to identify the positive and negative sample cups when performing the ADVIA Centaur Toxoplasma M assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends, as a minimum requirement, two levels of quality control material should be assayed on each day that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

NOTE: This procedure uses control volumes sufficient to measure each control in duplicate.

1. Schedule the quality control samples to the worklist.
2. Label two sample cups with quality control barcode labels: one for the positive and another for the negative.

NOTE: Each drop from the control vial is approximately 50 µL.

3. Gently mix the quality control materials and dispense at least 3 to 4 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any quality control materials remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials.

Taking Corrective Action

If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical support provider or distributor for assistance.

Sample Volume

This assay requires 10 µL of sample for a single determination. This volume does not include the unusable volume in the sample container or the additional volume required when performing duplicates or other tests on the same sample. For detailed information about determining the minimum required volume, refer to *Sample Volume Requirements* in the *ADVIA Centaur Reference Manual.*

Assay Procedure

For detailed procedural information, refer to the system operating instructions or to the online help system.

Procedural Notes

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

Interpretation of Results

For detailed information about how the system calculates results, refer to the system operating instructions or to the online help system.

The cutoff for the ADVIA Centaur Toxoplasma M assay was established by running a known population of 1295 samples using the ADVIA Centaur Toxoplasma M assay and adjusting the cutoff Index value between known positives and non-positives to 1.0. The cutoff for the ADVIA Centaur Toxoplasma M assay was verified based on results of Receiver-Operator characteristics (ROC) Curve generated from the results of the clinical studies and commercially available panels.

The system reports toxoplasma IgM antibody results as an Index value and as reactive (positive) or nonreactive (negative). A cutoff of 1.0 Index Value is used to distinguish positive from negative samples:

- Samples with a calculated value of less than 0.9 Index are considered negative.
- Samples with a calculated value between 0.9 and 0.99 Index are considered equivocal.
- Samples with a calculated value greater than or equal to 1.0 Index are considered positive.
- Sample results are invalid and must be repeated if the controls are out of range.

The magnitude of the measured result above the cutoff is not indicative of the total amount of antibody present in the sample.

The detection of toxoplasma IgM in a given specimen, as determined by assays from different manufacturers, can vary due to differences in assay methods and reagent specificity.

When interpreting results from the ADVIA Centaur Toxoplasma M assay, use the following table:

Anti-T. gondii IgM Result	*Anti-T. gondii* IgG Result	Interpretation
Negative	Negative	It is presumed the patient has not been infected with and is not undergoing an acute infection with *T. gondii*. If symptoms persist, submit a new specimen within 3 weeks.
Negative	Positive	From this testing it cannot be determined whether the patient is or is not undergoing a reactivated *T. gondii* infection. It appears the patient has been previously infected with *T. gondii*. Infection occurred more than 1 year ago.
Negative	Equivocal	Obtain a new specimen for further testing. Patient may not be undergoing an acute infection with *T. gondii*. Determining whether the patient has been previously infected with *T. gondii* is not possible.
Equivocal	Negative	Obtain a new specimen for determination of IgM antibodies to *T. gondii*. It cannot be determined if the patient is undergoing an acute *T. gondii* infection. It appears the patient has not been previously infected with *T. gondii*. 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.

Anti-T. gondii IgM Result	Anti-T. gondii IgG Result	Interpretation
Equivocal	Positive	Obtain a new specimen for determination of IgM antibodies to *T. gondii*. It cannot be determined if the patient is undergoing or has undergone an acute *T. gondii* infection. It appears the patient has been previously infected with *T. gondii*. 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	Equivocal	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 *T. gondii*. 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.
Positive	Negative	Obtain a new specimen for further testing. The patient may or may not be acutely infected with *T. gondii*. Since the IgG antibodies to *T. gondii* 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-*T. gondii* 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	Positive	The patient may or may not be acutely infected with *T. gondii*. Obtain a new specimen for further testing. Since the IgG antibodies to *T. gondii* are positive, it appears the patient may be acutely infected with *T. gondii*. The new specimen should be repeated with a different anti-*T. gondii* IgM assay. If the new specimen result is still positive for IgM and IgG antibodies to *T. gondii*, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Positive	Equivocal	It cannot be determined if the patient is acutely infected with *T. gondii*. Obtain a new specimen for further testing. Determining whether the patient has been previously infected with *T. gondii* 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-*T. gondii* IgM assay. If the new specimen result is still positive for IgM and the IgG is positive/negative/equivocal for antibodies to *T. gondii*, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.

Limitations

The following information pertains to limitations of the assay:

- Testing should not be performed as a screening procedure for the general population. The predictive value of a positive or negative serologic result depends on the pretest likelihood of toxoplasmosis being present. Testing should only be done when clinical evidence suggests the diagnosis of toxoplasmosis.
- The ADVIA Centaur Toxoplasma M assay is limited to the detection of IgM antibodies to *T. gondii* in human serum or plasma.
- Do not use heat inactivated specimens.
- The performance of the ADVIA Centaur Toxoplasma M assay has not been established with cord blood, neonatal specimens, immunosuppressed specimens, cadaver specimens or body fluids other than serum or plasma, such as saliva, urine, amniotic, or pleural fluids.
- Patient specimens collected very early during the acute phase of infection may contain toxoplasma IgM levels below the cutoff of the ADVIA Centaur Toxoplasma M assay. Additionally, diagnosis of a recent infection should not be made based on a single sampling because IgM antibodies to *T. gondii* may persist in serum many months after infection.[8] The presence of IgM antibodies to *T. gondii* is not diagnostic for recent infection.
- Because toxoplasma IgM may remain in serum for many months after infection, use caution in interpreting results from patients who may have received blood transfusions or infusion of other blood products within the past several months.

- In geographic regions that have an apparent low prevalence of toxoplasma IgM in asymptomatic populations, the positive predictive value of any assay is reduced due to the increased possibility that a positive result is actually falsely positive.[9] As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.[10]
- Human anti-mouse antibodies (HAMA) or heterophile antibodies may be present in samples from individuals exposed to mouse or animal immunoglobulins from natural sources or as part of disease therapies. These antibodies may interfere with the ADVIA Centaur Toxoplasma M assay and give falsely positive or falsely negative results.[11] These samples should not be tested.

Following the NCCLS guidelines[12], toxoplasma IgM positive and negative samples were tested for interference as shown in the following table:

Serum specimens that are . . .	*Demonstrate ≤ 10% change in results . . .*
hemolyzed	up to 500 mg/dL of hemoglobin
lipemic	up to 1000 mg/dL of triglycerides
icteric	up to 40 mg/dL of conjugated bilirubin up to 20 mg/dL of unconjugated bilirubin
hypo/hyperproteinemia	between 3–12 g/dL of protein

There was no change in clinical interpretation of results.

Expected Values

The incidence of toxoplasmosis varies considerably by the geographic location and age of patient. The following have been reported in the literature:[13]

Location	*Seroprevalance Rate*
Europe	
France, Italy	50–85%, by region
Germany	20–72%, by region
United Kingdom	20%
Japan	24%
Africa	20–65%, by country
S. America	36–82%, by country
N. America	8–38%, by region

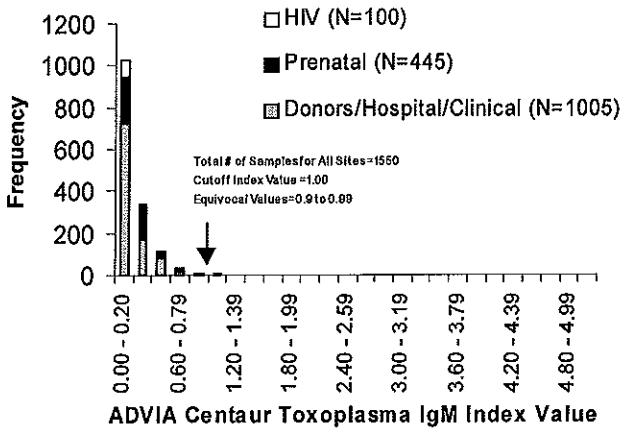
In this study, the toxoplasma IgM seropositive rate for serum samples obtained in the U.S. from asymptomatic pregnant women, hospital patients, blood donors, and HIV positive patients was determined to be 1.2%.

The distribution of ADVIA Centaur Toxoplasma M seropositive rates observed in this study are summarized in the following table:

Population	*N*	*Positive*
Pregnant women	445	7 (1.6%)
Hospital patients	465	9 (1.9%)
Blood donors	540	4 (0.7%)
HIV positive patients	100	0 (0.0%)
Total	1550	20 (1.2%)

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.[10]

ADVIA Centaur Toxoplasma IgM: Observed Values for Various Sample Populations



Performance Characteristics

Sensitivity and Specificity

Relative Agreement

The performance of the ADVIA Centaur Toxoplasma M assay was determined by testing a total of 1800 samples at three U.S. sites. The ADVIA Centaur Toxoplasma M results were compared to test results generated on a commercially available, automated toxoplasma IgM EIA. Clinical studies were performed at two sites in Massachusetts and one site in Virginia. Three hundred fifteen fresh and 1485 frozen serum samples were used. Samples were obtained from the mid-Atlantic and Midwest regions of the United States as well as Germany and included the following populations: prenatal (N=445), asymptomatic blood donors (N=540), asymptomatic hospital patients (N=465), and patients with confirmed toxoplasma IgM positive status. Of the 1800 specimens tested, 2 were equivocal by the ADVIA Centaur Toxoplasma M assay. Discordant results were found on 15 specimens. Further testing was done on the discordant samples using other commercially available tests for toxoplasma IgM.

Relative Sensitivity

Using the alternative method, 256 tested positive for toxoplasma IgM antibody. Of the specimens that tested positive, 0 were equivocal, 254 were positive, and 2 were negative using the ADVIA Centaur Toxoplasma M assay. The relative sensitivity was 99.2%.

Relative Specificity

Using the alternative method, 1542 tested negative for toxoplasma IgM antibody. Of the specimens that tested negative, 2 were equivocal, 13 were positive, and 1527 were negative using the ADVIA Centaur Toxoplasma M assay. The relative specificity was 99.0%.

NOTE: Samples giving equivocal results were not included in the calculation of relative sensitivity and relative specificity, but were included in calculating the relative agreement.

Relative Sensitivity, Specificity, and Agreement

Site	*N*	*Relative Sensitivity (%)*	*Relative Specificity (%)*	*Relative Agreement (%)*
1	1086	99.4 (154/155)	99.6 (926/930)	99.5 (1080/1086)
2	350	98.0 (50/51)	99.3 (294/296)	98.3 (344/350)
3	364	100.0 (50/50)	97.5 (307/314)	98.1 (357/364)
Total	1800	99.2 (254/256)	99.2 (1527/1540)	98.9 (1781/1800)

NOTE: *Relative* refers to a direct comparison of ADVIA Centaur Toxoplasma M results to that of a similar assay. No attempt has been made to correlate with disease presence or absence, and no judgement can be made regarding the predicate assay's accuracy to predict toxoplasma disease.

		Predicate Toxoplasma IgM			
		Positive	Equivocal	Negative	Total
ADVIA Centaur Toxoplasma M	Positive	254	1	13	268
	Equivocal	0	0	2	2
	Negative	2	1	1527	1530
	Total	256	2	1542	1800

Relative Sensitivity = 99.2% (254/256) 95% Confidence Limits 97.21–99.91
Relative Specificity = 99.2% (1527/1540) 95% Confidence Limits 98.56–99.55
Relative Agreement = 98.9% (1781/1796) 95% Confidence Limits 98.36–99.36

Consensus Testing

Further analysis of the 15 specimens with discordant results was performed using another commercially available EIA for toxoplasma IgM. Of the 13 specimens that were positive by ADVIA Centaur Toxoplasma M assay and negative by predicate EIA, 2 were positive, 1 was equivocal, and 10 were negative by consensus analysis. Of the 2 specimens that were positive by ADVIA Centaur Toxoplasma M assay and negative by predicate EIA, 1 was negative and 1 was positive by consensus analysis.

CDC Panel

A characterized toxoplasma serology panel obtained from the Centers for Disease Control (CDC) was tested. The testing was performed to provide additional information about the performance of the ADVIA Centaur Toxoplasma M assay with a masked characterized serum panel. This does not imply an endorsement of the assay by the CDC.

The panel consisted of 32 true positives, 3 serial dilutions of true positives, and 65 true negatives. The ADVIA Centaur Toxoplasma M assay correctly identified the 32 true positives, identified 2 of the 3 serial dilutions as positive, and 63 of the 65 true negatives. The ADVIA Centaur Toxoplasma M assay had 98% total agreement with the CDC results. Of the results obtained by the ADVIA Centaur Toxoplasma M assay, there was 100% agreement with the positive specimens and 97% agreement with the negative specimens.

Evaluation of Potential Interfering Agents

The ADVIA Centaur Toxoplasma M assay was evaluated for potential cross reactivity/ interference with 113 disease-state samples. Potential cross-reactive viral antibodies and disease-state specimens were tested with two lots of ADVIA Centaur Toxoplasma M reagents. The toxoplasma IgM status of the specimens was verified using alternative EIAs. The following table outlines the results obtained using the ADVIA Centaur Toxoplasma M assay and alternative method:

			Toxoplasma IgM Positive		
Sample Type	Matrix	Number Tested	ADVIA Centaur Lot 8010	ADVIA Centaur Lot 8011	Alternate EIA
Anti-mitochondria antibody (AMA)	Serum	10	0	0	0
Anti-nuclear antibody (ANA)	Serum	10	1	2	0
Human anti-mouse antibody (HAMA)	Plasma	10	0	0	0
Multiple IgM Myeloma	Serum	10	0	0	0
Rheumatoid factor (RF)	Serum	10	0	0	0
anti-Cytomegalovirus (CMV) IgM	Plasma	10	0	0	0
anti-Epstein Barr (EBV) IgM	Serum	10	0	0	0
anti-Herpes Simplex (HSV) IgM	Serum	9	0	0	0

			Toxoplasma IgM Positive		
Sample Type	Matrix	Number Tested	ADVIA Centaur Lot 8010	ADVIA Centaur Lot 8011	Alternate EIA
anti-Measles (Rubeola) IgM	Serum	10	1	1	1
anti-Parvovirus B19 IgM	Serum	10	0	0	0
anti-Syphilis IgM	Serum	9	0	0	0
anti-Varicella Zoster (VZV) IgM	Serum	5	0	0	0

Matrix Evaluation

Ten samples with Index values near the cutoff were evaluated in matrices of serum, EDTA, and heparin. Samples were run in replicates of four and mean values compared with serum mean values. There were no qualitative differences observed in interpretative results.

Sample ID	Matrix	Index Value	% Recovery from Control
1	EDTA	1.3	92
	Heparin	1.2	86
	Serum	1.4	100
2	EDTA	1.7	130
	Heparin	1.8	138
	Serum	1.3	100
3	EDTA	1.9	146
	Heparin	1.8	138
	Serum	1.3	100
4	EDTA	1.6	100
	Heparin	1.7	106
	Serum	1.6	100
5	EDTA	1.6	100
	Heparin	1.5	94
	Serum	1.6	100
6	EDTA	2.1	124
	Heparin	2.3	135
	Serum	1.7	100
7	EDTA	1.8	113
	Heparin	1.9	119
	Serum	1.6	100
8	EDTA	2.8	97
	Heparin	2.8	97
	Serum	2.9	100
9	EDTA	2.4	109
	Heparin	2.3	105
	Serum	2.2	100
10	EDTA	1.6	94
	Heparin	1.6	94
	Serum	1.5	100
Mean	EDTA		111
Mean	Heparin		111

Precision

Reproducibility of the ADVIA Centaur Toxoplasma M assay was determined as described in NCCLS protocol EP5-T2.[14] An eight-member panel was assayed two times in two separate daily runs, over a period of 20 days (n = 80). The following results were obtained using one reagent lot and a stored calibration curve:

Panel Member	N	Index	Within-run SD	Within-run % CV	Total* SD	Total* % CV
Negative Control	80	0.12	0.10	8.84	0.011	9.53
Positive Control	80	2.57	0.076	2.94	0.114	4.44
1	80	0.18	0.022	12.16	0.023	12.72
2	80	0.40	0.022	5.51	0.029	7.18
3	80	0.73	0.035	4.88	0.045	6.22
4	80	1.39	0.052	3.77	0.077	5.57
5	80	4.26	0.168	3.95	0.203	4.78
6	80	8.91	0.302	3.39	0.455	5.11

* Includes within-run and run-to-run.

System reproducibility was determined by testing a 7 member panel with 2 reagent lots including 5 instruments and 3 sites over multiple days. The panel was assayed 3 times in each of 25 runs. The following results were obtained:

Panel Member	N	Index	Within-run SD	Within-run % CV	Total† SD	Total† % CV
Negative Control	165	0.11	0.02	NA*	0.02	NA
Positive Control	165	2.13	0.07	3.31	0.09	4.01
1	165	0.10	0.00	2.19	0.00	2.90
2	165	1.11	0.07	6.51	0.08	6.96
3	165	1.65	0.05	2.99	0.07	4.15
4	165	2.81	0.17	6.14	0.19	6.89
5	165	3.74	0.12	3.12	0.15	3.92
6	150	5.67	0.19	3.30	0.22	3.81
7	163	8.28	0.26	3.16	0.35	4.21

* Not applicable.

† Includes within-run and run-to-run.

The reproducibility was also calculated for each individual site with each panel member. The following results were obtained:

Toxoplasma IgM Precision Summary by Site

Sample ID	Site	N	Mean Index	Within-run SD	Within-run % CV	Total† SD	Total† % CV
Negative	1	135	0.11	0.01	*NA	0.02	NA
	2	15	0.10	0.01	NA	0.01	NA
	3	15	0.10	0.00	NA	0.00	NA
Positive	1	135	2.12	0.07	3.2	0.07	3.5
	2	15	2.15	0.07	3.1	0.11	5.1
	3	15	2.17	0.09	4.2	0.14	6.3
1	1	135	0.10	0.00	NA	0.00	NA
	2	15	0.10	0.00	NA	0.00	NA
	3	15	0.10	0.00	NA	0.00	NA
2	1	135	1.07	0.08	7.3	0.08	7.7
	2	15	1.32	0.03	2.5	0.03	2.5
	3	15	1.17	0.03	2.8	0.05	4.5

Sample ID	Site	N	Mean Index	Within-run SD	Within-run % CV	Total† SD	Total† % CV
3	1	135	1.62	0.05	3.1	0.06	4.0
	2	15	1.88	0.05	2.4	0.08	4.1
	3	15	1.76	0.04	2.5	0.09	5.4
4	1	135	2.77	0.18	6.5	0.20	7.3
	2	15	3.14	0.16	5.0	0.17	5.4
	3	15	2.80	0.08	2.7	0.11	3.9
5	1	135	3.65	0.11	3.1	0.15	4.0
	2	15	4.34	0.10	2.4	0.11	2.6
	3	15	3.89	0.15	3.9	0.17	4.3
6	1	135	5.58	0.16	2.9	0.20	3.6
	2	15	6.53	0.31	4.8	0.31	4.8
7	1	133	8.04	0.26	3.2	0.31	3.9
	2	15	9.88	0.27	2.7	0.28	2.9
	3	15	8.83	0.30	3.4	0.62	7.1

* Not applicable.

† Includes within-run and run-to-run.

Standardization

The ADVIA Centaur Toxoplasma M assay standardization is based upon the relative clinical agreement with commercially available Toxoplasma M assays (see Performance Characteristics). Assigned values for calibrations and controls are traceable to this standardization.

Technical Assistance

For customer support, contact your local technical support provider or distributor.

www.siemens.com/diagnostics

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