Toxoplasma G (Toxo G)

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

Assay Summary

Sample Type	**Serum, Heparinized Plasma, EDTA Plasma**
Sample Volume	**10 µL**
Calibrator	**Toxo G**
Sensitivity and Assay Range	**0.5–700 IU/mL**

Contents

REF	Contents	Number of Tests
04520287 (120154)	1 ReadyPack® primary reagent pack containing ADVIA Centaur® Toxo G Lite Reagent and Solid Phase	100
	ADVIA Centaur and ADVIA Centaur CP Toxo G Master Curve card	
	1 vial Toxo G Low Calibrator CAL L	
	1 vial Toxo G High Calibrator CAL H	
	ADVIA Centaur and ADVIA Centaur CP Toxo G Calibrator Assigned Value cards	

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

Intended Use

The ADVIA Centaur Toxoplasma G assay is an IgG-antibody capture microparticle direct *in vitro* diagnostic immunoassay intended for the quantitative and qualitative detection of IgG antibodies to the *Toxoplasma gondii* parasite in human serum or plasma (EDTA, heparin) using the ADVIA Centaur and ADVIA Centaur XP systems. The measurement of Toxoplasma IgG may be used to aid in the assessment of a patient's immunological response from individuals including women of childbearing age. This assay may be utilized with an IgM Toxoplasma result to determine recent serological response to Toxoplasma.

WARNING: The use of the ADVIA Centaur Toxoplasma G assay to diagnose recent infection by testing acute and convalescent samples is not recommended. The calculated values for toxoplasma IgG 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 IgG assay used. Values obtained with different assay methods cannot be used interchangeably.

This assay has not been cleared or approved by the FDA for the screening of blood or plasma donors. Testing should not be performed as a screening procedure for the general population.

Materials Required but Not Provided

REF	Description	Contents
06317233 (120382)	ADVIA Centaur Toxo G quality control material	2 x 2.7 mL Negative Control CONTROL -
		2 x 2.7 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.

The use of toxoplasma IgG assays has been shown to be a reliable method for establishing serological status and evaluating susceptibility to *T. gondii* infection. The presence of IgG antibodies indicates that the individual has been infected with toxoplasma in the past, but the level of reactivity does not indicate how recently the infection occurred. In the majority of AIDS patients, the IgG response to primary *T. gondii* infection often lacks a significant rise in IgG titers.[4]

Assay Principle

The ADVIA Centaur Toxoplasma G assay is an immunoglobulin class-capture sandwich immunoassay using direct, chemiluminometric technology. The anti-human IgG_{Fc} monoclonal antibody is covalently coupled to paramagnetic particles in the Solid Phase. In the Lite Reagent, the purified *T. gondii* antigen is bound to an anti-p30 monoclonal antibody ($F(ab)_2$ fragment) labeled with acridinium ester. Antibody-antigen complexes will form if toxoplasma IgG is present in the sample.

The system automatically performs the following actions:

- Dispenses 10 µL of sample into a cuvette.
- Dispenses 250 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 100 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 IgG 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 a clinical cutoff value of 10 IU/mL. Refer to Interpretation of Results for a description.

Specimen Collection and Handling

Serum, heparinized plasma, or EDTA plasma are the recommended sample types for this assay. Do not use heat inactivated specimens. The performance of the ADVIA Centaur Toxoplasma G assay has not been established with cord blood, neonatal 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 serum 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. Serum samples maintained at room temperature up to 7 days or refrigerated up to 14 days demonstrated no qualitative differences. Store samples at 2° to 8°C upon arrival. If shipment is expected to exceed 7 days, ship specimens frozen.
- Handle plasma specimens as per Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) H18-A3, *Procedures for the Handling and Processing of Blood Specimens*.[5]

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 G ReadyPack primary reagent pack	Lite Reagent	10.0 mL/ reagent pack	purified *T. gondii* p30 antigen (~0.75 µg/mL) complexed with mouse anti-p30 monoclonal antibody ($F(ab)_2$ fragment) 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	25.0 mL/ reagent pack	mouse anti-human IgG_{Fc} monoclonal antibody (~0.3 mg/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.
Toxo G calibrator vials	Calibrators	1.0 mL/ vial	processed defibrinated human plasma positive for toxoplasma IgG antibodies with preservatives	2–8°C	Until the expiration date on the vial or onboard–8 hours.
Toxo G quality control material vials*	Controls	2.7 mL/ vial	processed defibrinated human plasma negative and positive for toxoplasma IgG 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.[6-8]

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

Onboard Stability	Calibration Interval
28 days	14 days

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

- 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 Bar-code Labels

Calibrator bar-code labels are lot-number specific. Do not use bar-code labels from one lot of calibrators with any other lot of calibrators.

Use the ADVIA Centaur Toxoplasma G Calibrator bar-code labels to identify the Low and High Calibrator sample cups when performing the ADVIA Centaur Toxoplasma G assay. Place the bar-code 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 bar-code scanner or the keyboard.

Perform the calibration procedure using the following steps:

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 bar-code 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 G assay, use ADVIA Centaur Toxoplasma G 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 my be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. Choose quality control material that has a composition similar to or identical with the sample matrix under analysis. Please refer to CLSI C24-A2, *Statistical Quality Control for Quantitative Measurements: Principles and Definitions*[9], for guidance on quality control practices.

Using Bar-code Labels

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

Use the ADVIA Centaur Toxoplasma G quality control bar-code labels to identify the positive and negative sample cups when performing the ADVIA Centaur Toxoplasma G assay. Place the bar-code 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.

Perform the quality control procedure using the following steps:

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 bar-code 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 of the ADVIA Centaur Toxoplasma G assay was established by running a known population of 1718 samples on the ADVIA Centaur Toxoplasma G assay and adjusting the cutoff IU/mL between known positives and non-positives. The cutoff was verified based on results of the Receiver-Operator Characteristics (ROC) Curve generated from the results of the clinical studies and commercially available panels.

The system reports toxoplasma IgG antibody results in IU/mL and as reactive (positive) or nonreactive (negative). A Cutoff Value of 10.0 IU/mL is used to distinguish positive from negative samples:

- Samples with a calculated value of less than 6.4 IU/mL are considered negative.
- Samples with a calculated value between 6.4 and 9.9 IU/mL are equivocal.

- Samples with a calculated value greater than or equal to 10.0 IU/mL 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 magnitude of the reported IgG level cannot be correlated to an endpoint titer.

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

The following table may be used when interpreting Toxoplasma G results:

Anti-*T. gondii* IgG Result	Anti-*T. gondii* IgM Result	Interpretation
Negative	Negative	It is presumed that 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.
Positive	Negative	From these results, it cannot be determined whether the patient is or is not undergoing a reactivated *T. gondii* infection. It appears that the patient has been previously infected with *T. gondii*. Infection may have occurred more than one year ago. If the individual has not previously tested positive for *T. gondii* antibodies, confirm the result with a reference laboratory with experience in the diagnosis of toxoplasmosis.
Equivocal	Negative	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.
Negative	Equivocal	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 that 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.
Positive	Equivocal	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 that the patient may have 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 and/or IgG antibodies to *T. gondii*, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Negative	Positive	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 Toxoplasma IgM antibodies, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.

Anti-*T. gondii* IgG Result	Anti-*T. gondii* IgM Result	Interpretation
Positive	Positive	The patient may or may not be acutely infected with *T. gondii*. Obtain a new specimen for further testing. Since the IgG and IgM 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* IgG and IgM assay. If the new specimen is still positive for IgG and IgM antibodies to *T. gondii*, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Equivocal	Positive	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 and IgG 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:

The performance of the ADVIA Centaur Toxoplasma G assay has not been established with cord blood, neonatal specimens, immunosuppressed populations, cadaver specimens, or body fluids other than serum or plasma, such as saliva, urine, amniotic, or pleural fluids. The performance characteristics of this assay are limited to detection of IgG antibodies to *T. gondii* using human serum or heparinized or EDTA plasma.

The ADVIA Centaur Toxoplasma G assay is limited to the detection of IgG antibodies to *T. gondii* in human serum or plasma.

Specimens collected in the early stages of infection may have IgG levels that are classified as negative.

Do not use heat inactivated specimens.

The use of the assay to diagnose recent infection by testing acute and convalescent samples has not been validated.

Because toxoplasma IgG 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. The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.

In geographic regions that have an apparent low prevalence of toxoplasma IgG 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.[10] As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.[11]

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 G assay and give falsely positive or falsely negative results.[12] These samples should not be tested.

The presence of anti-nuclear antibodies (ANA) and anti-mitochondrial antibodies (AMA) in samples from patients with autoimmune syndrome may interfere with the ADVIA Centaur Toxoplasma G assay and give falsely positive or falsely negative results. These samples should not be tested.

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.

Following the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) guidelines[13], toxoplasma IgG 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 60 mg/dL of conjugated bilirubin up to 40 mg/dL of unconjugated bilirubin
hypo/hyperproteinemia	between 3 to 12 g/dL of protein

There was no change in clinical interpretation of results.

For patient samples measuring at or around the cutoff value of 10 to 17 IU/mL, significantly elevated or increasing concentrations of total IgG may change a positive qualitative interpretation to equivocal, or rarely, may result in a negative interpretation.

Expected Values

The prevalence of IgG antibody to *T. gondii* varies considerably by geographic location and the age of the patient. The following seroprevalence rates for various populations have been reported in the literature:

Location	Seroprevalence 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

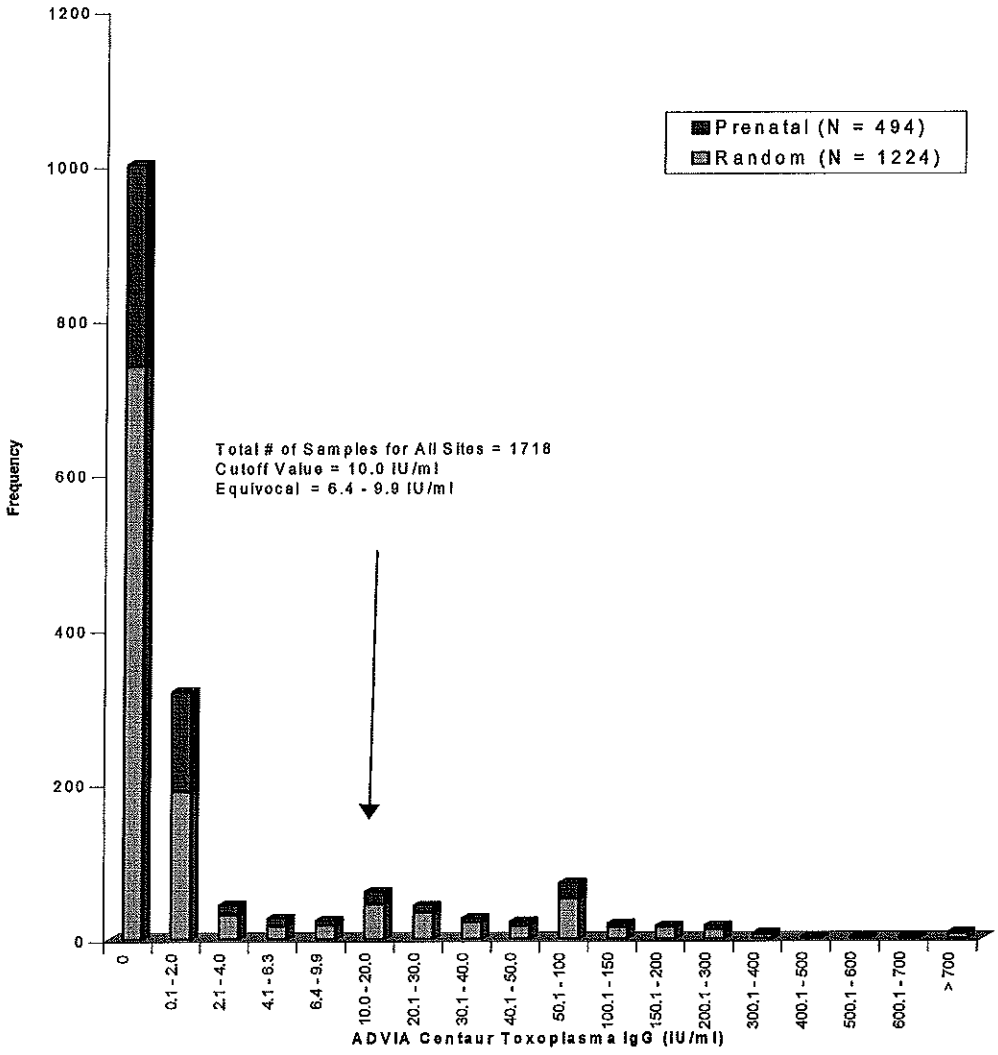
In clinical trials, the seropositive rates for IgG antibody to *T. gondii* of serum samples obtained in the U.S. from pregnant women (N = 494) and low risk and healthy individuals (N = 1224) were 15.0% and 18.6%, respectively.

The distribution of ADVIA Centaur Toxoplasma G classifications observed in the clinical trials are summarized below:

Population	N	Positive
Pregnant women	494	74 (15.0%)
Random Hospital/Clinical patients	1224	228 (18.6%)
Total	1718	302 (17.6%)

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.[11]

ADVIA Centaur Toxoplasma G: Observed Values for Various Sample Populations



Performance Characteristics

Percent Agreement

The performance of the ADVIA Centaur Toxoplasma G assay was determined by testing a total of 1804 samples at three U.S. sites. The ADVIA Centaur results were compared to test results generated on a commercially available, automated toxoplasma IgG EIA. Five hundred fresh samples and 1304 frozen samples from the mid-Atlantic and Midwest regions of the United States were used. The samples included the following populations: prenatal (N = 494), asymptomatic blood donors (N = 418), asymptomatic hospital patients (N = 806), and 86 patients with confirmed toxoplasma IgG positive status. Positive status was determined utilizing a commercial EIA method. Of the 1804 specimens tested, 39 were equivocal by either the ADVIA Centaur or the predicate EIA. Discordant results were found on 32 specimens which were further evaluated using other commercially available tests for toxoplasma IgG.

Positive Percent Agreement

Using the alternative method, 388 tested positive for toxoplasma IgG antibody. Of the specimens that tested positive, 12 were equivocal, 363 were positive, and 13 were negative using the ADVIA Centaur Toxoplasma G assay. The Positive Percent Agreement was 96.5%.

Negative Percent Agreement

Using the alternative method, 1400 tested negative for toxoplasma IgG antibody. Of the specimens that tested negative, 11 were equivocal, 19 were positive, and 1370 were negative using the ADVIA Centaur Toxoplasma G assay. The Negative Percent Agreement was 98.6%.

NOTE: Samples giving equivocal results were not included in the calculation of positive percent agreement, negative percent agreement, and total percent agreement.

Percent Agreement Before Resolution of Discordant Samples

Site	N	Positive Percent Agreement (%)	Negative Percent Agreement(%)	Total Percent Agreement (%)
1	804	99.5 (210/211)	98.4 (568/577)	98.7 (778/788)
2	500	94.7 (89/94)	97.7 (384/393)	97.1 (473/487)
3	500	90.1 (64/71)	99.8 (418/419)	98.4 (482/490)
Total	1804	96.5 (363/376)	98.6 (1370/1389)	96.1 (1734/1804)

NOTE: *Percent Agreement* refers to a direct comparison of ADVIA Centaur Toxoplasma G 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 G EIA			
		Positive	Equivocal	Negative	Total
ADVIA Centaur Toxoplasma G	Positive	363	6	19	388
	Equivocal	12	1	11	24
	Negative	13	9	1370	1392
	Total	388	16	1400	1804

Positive Percent Agreement = 96.5% (363/376), 95% CI (Confidence Interval) = 94.16–98.15
Negative Percent Agreement = 98.6% (1370/1389), 95% CI = 97.9–99.2
Total Percent Agreement = 96.1% (1734/1804), 95% CI = 95.12–96.96

Consensus Testing

Further analysis of the 32 specimens with discordant results was performed using another commercially available test for toxoplasma IgG. Upon retest in duplicate, two ADVIA Centaur positive specimens were equivocal. Of the thirteen specimens that were negative by ADVIA Centaur and positive by EIA, three were equivocal and three were negative by consensus testing. Seven specimen interpretations remained unchanged. Of the seventeen specimens that were positive by ADVIA Centaur and negative by EIA, six were equivocal and three were positive by consensus testing. Eight specimen interpretations remained unchanged.

CDC Panel

A characterized CDC Toxoplasma 1998 Human Serum Panel was obtained from the Centers for Disease Control (CDC) and tested with the ADVIA Centaur Toxoplasma G assay. Testing was performed to provide additional information about the performance of the ADVIA Centaur Toxoplasma G assay with a masked characterized panel. Results were submitted to the CDC for their interpretation. This does not imply an endorsement of the assay by the CDC.

The panel consisted of 70 positive and 30 negative specimens as defined by the Dye Test. Of the 70 positives, ADVIA Centaur identified 68 as positive and 2 as equivocal. The two equivocal specimens were aliquots of the same sample. Of the 30 negatives, ADVIA Centaur identified 30 as negative. The ADVIA Centaur Toxoplasma G assay had 98% total agreement with the CDC results. Of the results obtained by the ADVIA Centaur Toxoplasma G assay, there was 97% agreement with the positive specimens and 100% agreement with the negative specimens.

Evaluation of Potentially Interfering Agents

The ADVIA Centaur Toxoplasma G assay was evaluated for potential cross reactivity/ interference with 128 viral antibodies and disease state specimens. The negative toxoplasma IgG status of the specimens was verified using alternative EIAs. The following table outlines the results obtained on the ADVIA Centaur Toxoplasma G assay.

			ADVIA Centaur Toxoplasma G Results		
Sample Type	Matrix	Number Tested	Negative	Equivocal	Positive
Anti-Mitochondrial Antibodies (AMA)	Serum	10	6	1	3
Anti-Nuclear Antibodies (ANA)	Serum	8	8	0	0
Epstein Barr Virus (EBV) IgG	Serum	5	5	0	0
Flu Vaccine	Serum	11	10	0	0
Heterophilic/HAMA	Plasma	10	10	0	0
Herpes Simplex Virus (HSV) IgG	Serum	10	10	0	0
Measles (Rubeola) IgG	Serum	14	14	0	0
Multiple Myeloma (MM) IgG	Serum	10	9	1	0
Parvovirus B19 IgG	Serum & Plasma	10	10	0	0
Rheumatoid Factor (RF)	Serum & Plasma	12	11	1	0
Syphilis	Serum	9	8	0	1
Varicella Zoster (VZV) IgG	Serum	11	10	1	0
Cytomegalovirus (CMV) IgG	Serum & Plasma	8	8	0	0

Precision

Reproducibility of the ADVIA Centaur Toxoplasma G assay was determined as described in CLSI protocol EP5-A2.[14] A 5-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	Mean Concentration (IU/mL)	Within-run SD	Within-run %CV	Total† SD	Total† %CV
Negative Control	80	0.37	0.06	NA*	0.09	NA
Positive Control	80	27.54	0.89	3.2	0.98	3.6
1	80	1.67	0.09	5.2	0.12	7.3
2	80	8.22	0.14	1.7	0.33	4.0
3	80	20.00	0.27	1.3	0.65	3.3

* Not applicable.

† Includes within-run and run-to-run.

System reproducibility was determined by testing a 6 member panel with 3 reagent lots including 5 instruments and 3 sites over multiple days. The panel was assayed three times in each of 40 runs. The following results were obtained:

Panel Member	N	Mean Concentration (IU/mL)	Within-run SD	Within-run %CV	Total† SD	Total† %CV
Negative Control	210	0.20	0.23	NA*	0.25	NA
Positive Control	210	29.91	0.61	2.05	0.75	2.51
1	120	18.32	0.44	2.42	0.53	2.91
2	120	45.02	0.84	1.86	1.12	2.50
3	120	50.57	0.96	1.89	1.15	2.27
4	120	123.71	7.84	6.33	7.88	6.37

* Not applicable.

† Includes within-run and run-to-run.

Standardization

The ADVIA Centaur Toxoplasma G assay standardization is traceable to World Health Organization (WHO) 3rd International standard for human anti-toxoplasma serum (TOXM). A comparison over the range of 0 to 500 IU/mL using four lots of reagents gave the following correlation:

ADVIA Centaur Toxoplasma G = 1.02 (WHO) + 10.7 IU/mL

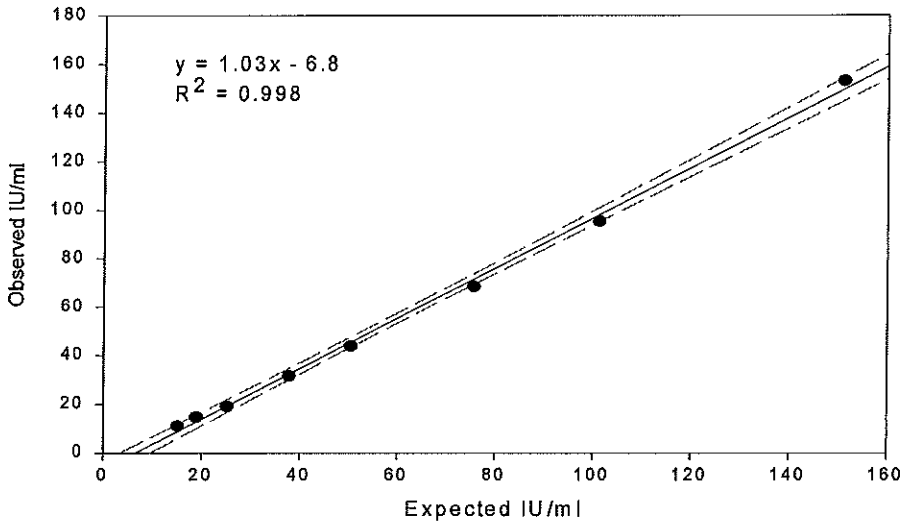
r = 0.99

Assigned values for calibrators and controls are traceable to this standardization.

Linearity

Nine specimens in serum, EDTA, and heparin, and two serum pools with anti *T. gondii* titers were independently diluted. Linear regression demonstrated linearity throughout the range of the assay. A representative sample is shown with slope, y-intercept, correlation coefficient, and 95% confidence limits.

ADVIA Centaur Toxoplasma G: Linearity Study
Sample F099 (Serum Redtop) diluted in Negative Serum Pool
(With 95% Confidence Limits)



Technical Assistance

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

www.siemens.com/diagnostics

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