

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

A. 510(k) Number:

K102681

B. Purpose for Submission:

To seek clearance for modification of the ADVIA Centaur Toxoplasma G assay (K012183)

C. Measurand:

Toxoplasma IgG antibodies

D. Type of Test:

Immunoglobulin Class-Capture Chemiluminescence Immunoassay

E. Applicant:

Siemens Healthcare Diagnostics, Inc.

F. Proprietary and Established Names:

ADVIA Centaur Toxoplasma G (Toxo G) assay

G. Regulatory Information:

1. Regulation section: 21CFR 866.3780; Toxoplasma gondii Serological Reagents
2. Classification: Class: II
3. Product code: LGD; Enzyme Linked Immunosorbent Assay, Toxoplasma gondii
4. Panel: 83, Microbiology

H. Intended Use:

1. Intended use(s):

The ADVIA Centaur Toxoplasma IgG 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 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.

2. Indication(s) for use:

The ADVIA Centaur Toxoplasma IgG 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 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.

3. Special conditions for use statement(s):

The use of the ADVIA Centaur Toxoplasma IgG 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.

4. Special instrument requirements:

ADVIA Centaur System

I. Device Description:

The modified ADVIA Centaur Toxo G Assay is comprised of the following:

- ADVIA Centaur Toxo G Lite Reagent (10 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 a cridinium ester in protein buffer with surfactant and preservatives
- ADVIA Centaur Toxo G 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
- ADVIA Centaur Toxo G Calibrators (1.0 mL/vial)
 - processed defibrinated human plasma positive for toxoplasma IgG antibodies with preservatives
- ADVIA Centaur Toxo G Quality Control Material (2.7 mL/vial)
 - processed defibrinated human plasma negative and positive for toxoplasma IgG antibodies with preservatives

J. Substantial Equivalence Information:

1. Predicate device name(s):

ADVIA Centaur Toxo G Assay

1. Predicate 510(k) number(s):

K012183

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	K102681	K012183
Intended Use	The ADVIA Centaur Toxoplasma IgG assay is an IgG antibody capture microparticle direct <i>in vitro</i> diagnostic immunoassay intended for the quantitative and qualitative detection of IgG antibodies to the <i>Toxoplasma gondii</i> parasite in human serum or plasma (EDTA, heparin) using the ADVIA Centaur 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.	Same
Sample Type	Serum, Heparinized Plasma, EDTA Plasma	Same
Sample Volume	10 µL	Same
Assay Range	0.5–700 IU/mL	Same
Performance Characteristics	Sensitivity: 96.5% Specificity: 98.6%	Same

Differences		
Item	Device	Predicate
	K102681	K012183
Lite Reagent Conjugate p30 Ag Concentration	1.5 µg/mL	0.75 µg/mL
Lite Reagent Conjugate Loading Ratio	30:1	18:1
Lite Reagent Antibody Format	Whole IgG	F(ab) ₂ Fragment recognizing same epitope as current assay
Mouse IgG Concentration	Lite Reagent Buffer: 800 mg/L Solid Phase Buffer: none	Lite Reagent Buffer: 400 mg/L Solid Phase Buffer: 400 mg/L
PEG8000 in Solid Phase	None	20 g/L
Tween 20 in Solid Phase	None	5 g/L

K. Standard/Guidance Document Referenced (if applicable):

Not applicable

L. Test Principle:

The ADVIA Centaur Toxoplasma G assay is an immunoglobulin class-capture sandwich immunoassay using direct, chemiluminescent 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision

Assay imprecision (within run and total) of the Toxo G assay was evaluated on ADVIA Centaur. A 20-day precision study (as per CLSI EP5-A2) was performed using two (2) reagent pilot lots (Pilot 1 and Pilot 2) with respective calibrators and controls. Samples included three in-house Medical Decision Pools and two controls (i.e. Negative and Positive), and two runs per day were performed.

The acceptance criteria, as defined by the sponsor, were as follows:

Within-Run CV:

- 15 to 30 IU/mL – ≤ 4.0%
- 50 to 175 IU/mL – ≤ 6.0%
- > 250 IU/mL – ≤ 6.0%

Total CV:

- 15 to 30 IU/mL – ≤ 8.0%
- 50 to 175 IU/mL – ≤ 10.0%
- > 250 IU/mL – ≤ 10.0%

Results were as follows:

Pilot Lot 1 (Pooled)

n	Mean (IU/mL)	Within Run CV	Total CV
160	28.07	2.6	3.7
160	10.96	3,2	4.1
160	120.5	2.6	4.6
160	455.3	2.5	4.8

Pilot Lot 2 (Pooled)

n	Mean (IU/mL)	Within Run CV	Total CV
160	28.07	2.7	3.4
160	10.96	3,7	4.6
160	120.5	2.5	3.7
160	455.3	2.9	3.9

These results met the acceptance criteria and are acceptable.

b. Linearity/assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Stability:

Assay performance was assessed for control recovery of the “Rocked/Pierced” reagent packs stored on-system relative to day zero recovery. A fresh reagent pack was used as the control for each time point and it was compared with the Rocked/Pierced and Rocked/Sealed reagent packs started on Day 0. A lot-specific Master Curve was used. The study was completed on (2) ADVIA Centaurs. Adjusted SD (ADSD) is calculated from Total SD (TSD) and Within Run SD (WRSD) from the precision claims in the IFU. The ADSD is given by the following equation where n is the number of replicates per run:

$$\text{Adjusted SD (ADSD)} = \text{SQRT} \{(\text{TSD}^2 - [(n-1)/n] * \text{WRSD}^2)\}$$

Acceptance criteria were Positive control recoveries for rocked pierced packs $\pm 10.0\%$ of Day 0. The minimum targets are 28 days for onboard stability and 14 days for calibration interval, as is claimed for the current Toxo G assay.

Results were as follows:

Pilot Lot 1 on System AR - All positive control recoveries were within 10% of Day 0 value up to (and including) Day 30, with a 14-day calibration interval

Pilot Lot 2 on System AR - All positive control recoveries were within 10% of Day 0 value up to (and including) Day 30, with a 14-day calibration interval.

These results met the acceptance criteria and are acceptable.

d. Detection limit:

During initial development of the ADVIA Centaur Toxo G (K012183), the clinical cut-off of the assay was established to be 10 IU/mL. The assay was standardized and calibrators were value assigned in order to meet this cut-off. The device modifications did not impact standardization or calibrator value assignments, and did not alter method parameters (i.e. instructions to the instrument to run assay). Therefore, the cutoff of the Toxo G assay continues to be 10 IU/mL.

e. Analytical specificity:

Interference:

The following substances were spiked into Toxo IgG nonreactive and reactive specimens:

- Human IgG (16 mg/mL)
- Human IgG (25 mg/mL)
- Low Protein (3.5 g/dL)
- High Protein (12 g/dL)
- Hemoglobin (500 mg/dL)
- Conjugated Bilirubin (40 mg/dL)
- Unconjugated Bilirubin (60 mg/dL)
- Triglycerides (1000 mg/dL)

Nine samples per interferent were tested for nonreactive specimens, and 27 samples per interferent were tested for reactive specimens. Testing was performed by measuring samples with and without spiked interferent, as calculated by the following equation:

$$\% \text{ Interference} = \text{Spiked/Unspiked} \times 100\%$$

Testing was performed using Pilot Lot 3 of the revised version of the Toxo G assay, and Lot 68 of the current assay. For Toxo IgG reactive samples, the current and revised assay versions were compared by calculating the difference in percent interference using both methods.

The acceptance criteria were as follows:

Toxo IgG nonreactive samples - No change in interpretation from the current assay.

Toxo IgG reactive samples - Less than an average of 10% difference between the current and revised ADVIA Centaur Toxo G assay.

Results were as follows:

Substance	% Matching Current Assay Results (Nonreactive Samples)	Average % Difference from Current Assay (Reactive Samples)
Human IgG (16 mg/mL)	100	2.8
Human IgG (25 mg/mL)	100	4.1
Low Protein (3.5 g/dL)	100	3.4
High Protein (12 g/dL)	100	0.8
Hemoglobin (500 mg/dL)	100	1.4
Conjugated Bilirubin (40 mg/dL)	100	3.0
Unconjugated Bilirubin (60 mg/dL)	100	0.9
Triglycerides (1000 mg/dL)	100	1.5

These results met the acceptance criteria and are acceptable.

Cross-Reactivity:

The following cross-reacting samples were tested:

- ANA
- AMA
- HAMA
- RF

Ten samples per cross-reacting substance were tested on one lot of the current Toxo G assay and three lots of the revised assay. Diagnostic interpretations, based on testing results, were compared across all lots.

The acceptance criterion, as defined by the sponsor, was that there was no change in interpretation from the current assay. These results met the acceptance criteria and are acceptable.

Results of Cross-Reactivity Study

Sample Type	Number of Samples	% matching current assay results
ANA	10	100
AMA	10	100
HAMA	10	100
RF	10	100

f. Assay cut-off:

The device modification did not change the assay cut-off of 0.5 IU/mL (Determined in K012183).

2. Comparison studies:

a. Method comparison with predicate device:

A total of 892 samples were tested by single replicate with the revised ToxoG assay (samples were split across three lots) and the current ToxoG assay (Lot 061068). Samples that were discrepant between the current and revised ToxoG assays were tested further using the VIDAS Toxo IgG assay.

Acceptance criteria, as defined by the sponsor, were that results must be greater or equal to the upper 95% confidence interval limits for percent agreement, as claimed for the current Toxo G assay.

- Positive Agreement of the methods $\geq 98.15\%$
- Equivocal Agreement of the methods – no specification
- Negative Agreement of the methods $\geq 99.2\%$
- Total Agreement of the methods $\geq 96.96\%$

Results were as follows:

Predicate (K012183)	Modified Proposed (K102681)		
	Negative	Positive	Equivocal
Negative	684	0	2*
Positive	0	200	1**
Equivocal	0	0	5

- Positive Agreement = 99.5% (200/201)
- Equivocal Agreement = 100% (5/5)

Negative Agreement = 99.7% (684/686)
Total Agreement = 99.7% (889/892)

- * both samples negative on VIDAS Toxo IgG assay
- ** sample equivocal on VIDAS Toxo IgG assay

These results met the acceptance criteria and are acceptable.

CDC Panel Testing:

A panel of 100 human specimens was obtained from CDC to include samples which were characterized by the CDC as Toxoplasma negative, Toxoplasma-specific IgG positive but IgM negative, Toxoplasma-specific IgG and IgM positive and Toxoplasma-specific IgG and IgA positive. These specimens have varying reactivity and 70 are positive and 30 negatives. All 100 CDC samples were tested with Verification Lot 1 of the revised Toxo G assay and Lot 061165 of the current Toxo G assay.

Acceptance criteria were as follows:

Revised ToxoG Assay vs. Current ToxoG Assay - Total Agreement \geq 98%

Revised ToxoG Assay vs. CDC - Positive Agreement \geq 97% Negative Agreement \geq 100%

Results were as follows:

The Total Agreement between the current and revised assays = 100% (100/100)

Positive Agreement vs CDC = 97% (68/70)

Negative Agreement vs CDC = 100% (30/30)

These results met the acceptance criteria and are acceptable.

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 by Site

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)

Overall Percent Agreement

Advia Centaur Toxoplasma G	Predicate Toxoplasma G EIA			Total
	Positive	Equivocal	Negative	
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

b. *Matrix comparison:*
Not applicable

3. Clinical studies:

Not applicable

a. *Clinical Sensitivity:*

Not applicable

b. *Clinical specificity:*

Not applicable

c. *Other clinical supportive data (when a. and b. are not applicable):*

Not applicable

4. Clinical cut-off:

10 IU/mL (traceable to the WHO 3rd International Standard)

5. Expected values/Reference range:

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:

Prevalence of IgG antibody to *T. gondii*

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.

N. Instrument Name:

ADVIA Centaur System

O. System Descriptions:

Instrument Description is documented in K012183. Subsequent modifications to the instrument have been reviewed and approved in numerous PMA submissions.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:

Not applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.