510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K233932

B Applicant

Abbott Laboratories

C Proprietary and Established Names

Alinity i Toxo IgM

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LGD	Class II	21 CFR 866.3780 - Toxoplasma Gondii Serological Reagents	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

Clearance of a new device

B Measurand:

IgM antibody to *Toxoplasma gondii*

C Type of Test:

Chemiluminescent Microparticle Immunoassay (CMIA)

III Intended Use/Indications for Use:

A Intended Use(s):

The Alinity i Toxo IgM assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of IgM antibodies to Toxoplasma gondii in human serum, serum separator, and plasma tubes (lithium heparin, lithium heparin separator, and tripotassium EDTA) on the Alinity i system.

The Alinity i Toxo IgM assay is to be used as an aid in the diagnosis of acute or recent Toxoplasma gondii infection in suspected individuals including women of child-bearing age. It is recommended that the assay be performed in conjunction with a Toxoplasma gondii IgG assay.

The Alinity i Toxo IgM assay has not been cleared for use in screening blood, plasma, or tissue donors.

B Indication(s) for Use:

NA

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

Performance has not been established for the use of bodily fluids other than human serum or plasma.

D Special Instrument Requirements:

Alinity i system

IV Device/System Characteristics:

A Device Description:

The Alinity i system is a fully automated immunoassay analyzer using chemiluminescent microparticle immunoassay (CMIA) technology. The Alinity i Toxo IgM immunoassay requires the use of Alinity i Toxo IgM Reagent Kit (cartridges, microparticles, and conjugate), Alinity i Toxo IgM Calibrator, and the Alinity i Toxo IgM Controls (positive and negative). The Alinity i Toxo IgM immunoassay components, reagent kit, calibrator and controls are packaged separately and designed to be used on the Alinity i system.

B Principle of Operation:

The Alinity i Toxo IgM assay is an automated, two-step immunoassay for the qualitative detection of IgM antibodies to *Toxoplasma gondii* in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Pre-diluted sample and anti-human IgM murine monoclonal antibody coated paramagnetic microparticles are combined and incubated. Together with IgM antibodies of other specificities, anti-Toxo specific IgM present in the sample binds to the anti-human IgM murine monoclonal

antibody coated microparticles, forming an antibody-antibody complex. The mixture is washed. A conjugate complex consisting of an acridinium-labeled anti-Toxo p30 antigen murine monoclonal F(ab')2 fragment and native *Toxoplasma gondii* lysate, containing the p30 antigen, is added to create a reaction mixture and incubated. This conjugate complex is bound by anti-Toxo specific IgM that has been captured by the anti-human IgM murine monoclonal antibody coated microparticles, forming an antibody-antibody-conjugate complex. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as a relative light unit (RLU). There is a direct relationship between the amount of anti-Toxo IgM in the sample and the RLU detected by the system optics. The presence or absence of anti-Toxo IgM in the sample is determined by comparing the chemiluminescent RLU in the reaction to the cutoff RLU determined from an active calibration.

The cutoff is 1.00 S/CO. The Alinity i system automatically calculates the signal-to-cutoff (S/CO) ratios, and interprets the results as presented in **Table 1**.

Table 1: Alinity i Toxo IgM Results Interpretation

S/CO	Instrument Interpretation	Retest Procedure
< 0.83 S/CO	Nonreactive for IgM antibodies to *Toxoplasma gondii*	Individuals with such results are presumed not to be recently infected with *Toxoplasma gondii* and susceptible to acute infection. No retest is required.
0.83 to < 1.00 S/CO	Grayzone/Equivocal	Specimens that are considered grayzone/equivocal may contain low levels of anti-Toxo IgM. It is recommended to take a second sample within a reasonable period of time (e.g. 2 weeks) and to repeat Alinity i Toxo IgM testing.
≥ 1.00 S/CO	Reactive for IgM antibodies to *Toxoplasma gondii*	Individuals that are considered reactive for anti-Toxo IgM are presumed to have an acute or recent infection with *Toxoplasma gondii*. No retest is required.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Vidas Toxoplasma gondii IgM Asssay

B Predicate 510(k) Number(s):
K923166

C Comparison with Predicate(s):

Device & Predicate Device(s):	Predicate K923166	Candidate Test K233932
Device Trade Name	VIDAS TOXO IgM Assay	Alinity i Toxo IgM
General Device Characteristic Similarities		
Intended Use/Indications For Use	The VIDAS TOXO IgM (TXM) assay is intended for use on the instruments of the VIDAS family (VITEK ImmunoDiagnostic Assay System) as an automated enzyme-linked fluorescent immunoassay (ELFA) for the presumptive qualitative detection of anti-Toxoplasma gondii IgM antibodies in human serum, as an aid in the diagnosis of acute, recent, or reactivated Toxoplasma gondii infection. This assay must be performed in conjunction with an anti-Toxoplasma gondii lgG antibody assay. VIDAS TOXO IgM (TXM) assay performance has not been established for prenatal screening or newborn testing. This assay has not been cleared by the FDA for blood/plasma donor screening.	The Alinity i Toxo IgM assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of IgM antibodies to *Toxoplasma gondii* in human serum, serum separator, and plasma tubes (lithium heparin, lithium heparin separator, and tripotassium EDTA) on the Alinity i system. The Alinity i Toxo IgM assay is to be used as an aid in the diagnosis of acute or recent *Toxoplasma gondii* infection in suspected individuals including women of child-bearing age. It is recommended that the assay be performed in conjunction with a *Toxoplasma gondii* IgG assay. The Alinity i Toxo IgM assay has not been cleared for use in screening blood, plasma, or tissue donors.
Calibrator(s)	1 Calibrator	1 Calibrator
Control(s)	2 (Negative and Positive)	2 (Negative and Positive)
General Device Characteristic Differences		

Antigen and Antibody Used	• Anti-Toxoplasma p30 antigen antibody (murine, monoclonal) and native *Toxoplasma gondii* lysate • Anti-human IgM murine monoclonal antibody	• Immunocomplex of *T. gondii* antigen (RH Sabin strain) • Mouse monoclonal anti-P30 antibodies
Type of Specimen	Serum	Serum and Plasma
Methodology	Enzyme-linked fluorescent immunoassay	Chemiluminescent microparticle immunoassay
Interpretation of Results	Negative: < 0.55 Test Value Equivocal: ≥ 0.55 to < 0.65 Test Value Positive: ≥ 0.65 Test Value	Nonreactive: < 0.83 S/CO Grayzone/Equivocal: 0.83 to < 1.00 S/CO Reactive: ≥ 1.00 S/CO
Components	Solid Phase Receptacle (SPR) – SPR coated goat anti-μ chain antibodies Reagent Strip – Strip consists of 10 wells covered with labeled, foil seal. The wells contain the various reagents required for the assay including: • Sample diluent: 300 µL of TRIS buffered saline (0.05 mol/L, pH 7.4) with protein and chemical stabilizers and 1 g/L sodium azide. • Pre-wash: 600 µL of TRIS buffered saline (0.05 mol/L, pH 7.4) with protein and chemical stabilizers and 1 g/L sodium azide. • Wash buffer: 600 µL of TRIS buffered saline (0.05 mol/L, pH 7.4) with protein and chemical stabilizers and 1 g/L sodium azide. • Conjugate: 400 µL of immunocomplex of *T. gondii* antigen (RH Sabin strain) grown in mice (9) and mouse	Microparticles – Anti-human IgM (murine, monoclonal) antibody coated microparticles in TRIS buffer with protein (bovine and goat) stabilizers, and detergent. Minimum concentration: 0.08 % solids. Preservatives: antimicrobial agents. Conjugate – Conjugate complex consisting of acridinium-labeled anti-Toxoplasma p30 antigen antibody (murine, monoclonal) and native *Toxoplasma gondii* lysate in phosphate buffer with protein (bovine) stabilizer, and detergent. Minimum concentration: 25 µg/mL. Preservative: sodium azide.

	monoclonal anti-P30 antibodies conjugated to alkaline phosphatase with gentamycin 0.02% and 0.9 g/L sodium azide. • Reading cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/L) + diethanolamine (DEA) (0.62 mol/L or 6.6%, pH 9.2) + 1 g/L sodium azide (300 µL).	
Calibration Storage	14 days	Maximum of 30 days

VI Standards/Guidance Documents Referenced:

Standards

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline–Third Edition
- CLSI EP07 3rd ed., Interference Testing in Clinical Chemistry
- CLSI EP37 1st ed., Supplemental Tables for Interference Testing in Clinical Chemistry
- CLSI EP12-A2, User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline— Second Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Within-Laboratory Precision: A 20-day within-laboratory precision study was conducted using 3 lots of the Alinity i Toxo IgM reagents, 3 lots of the Alinity i Toxo IgM Calibrator, 3 lots of the Alinity i Toxo IgM Controls, and 1 Alinity i system. Two controls and 4 recalcified human plasma samples (representing serum matrix) were tested in 3 replicates at 2 separate times per day over 20 days using 3 reagent lot/calibrator lot combinations, where a unique reagent lot and a unique calibrator lot are paired. The within laboratory precision data summary is shown in **Table 2**.

Table 2: Alinity i Toxo IgM Assay Within-Laboratory Precision

Sample ID	n	Mean (S/CO)	Repeatability (Within-Run)		Between-Run		Between-Day		Between-Lot [a]		Overall Within Laboratory [b]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control	360	0.14	0.012	NA[c]	0.000	NA[c]	0.004	NA[c]	0.008	NA[c]	0.014	NA[c]
Positive Control	360	2.72	0.077	2.8	0.034	1.3	0.031	1.2	0.051	1.9	0.104	3.8
1	358 [d]	0.14	0.013	NA[c]	0.000	NA[c]	0.005	NA[c]	0.006	NA[c]	0.015	NA[c]
2	360	0.81	0.028	NA[c]	0.006	NA[c]	0.009	NA[c]	0.027	NA[c]	0.040	NA[c]
3	359 [d]	1.34	0.047	3.5	0.000	0.0	0.007	0.5	0.042	3.2	0.064	4.7
4	359 [d]	2.54	0.078	3.1	0.000	0.0	0.023	0.9	0.073	2.9	0.109	4.3

[a] Alinity i Toxo IgM reagent lot and Alinity i Toxo IgM calibrator lot are confounded, and the confounding effect is represented by between-lot.
[b] Overall within-laboratory variability contains repeatability (within-run), between-run, between-day, and between-lot variance components.
[c] Not applicable
[d] In cases where n < 360, replicate(s) were excluded due to an instrument error and no results were reported.

Reproducibility Study (multi-site precision): A 5-day reproducibility study was conducted at 3 US sites, using the same samples panel used in the within-laboratory precision study, in addition to one positive and one negative controls. Four replicates per sample were evaluated in 2 runs per day over 5 days. The testing was performed using 3 lots of Alinity i Toxo IgM Reagents, 2 lots of Alinity i Toxo IgM Calibrators, and 1 lot of Alinity i Toxo IgM Controls at each of the 3 testing sites. The reproducibility data summary is shown in **Table 3**.

Table 3: Alinity i Toxo IgM Assay Reproducibility

Sample ID	n	Mean S/CO	Repeatability		Between-Run		Between-Day		Between-Site		Between-Lot [a]		Reproducibility[b]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control	360	0.13	0.013	NA[c]	0.002	NA[c]	0.001	NA[c]	0.009	NA[c]	0.008	NA[c]	0.018	NA[c]
Positive Control	360	2.62	0.074	2.8	0.031	1.2	0.022	0.8	0.056	2.1	0.075	2.9	0.132	5.1
1	360	0.11	0.013	NA[c]	0.000	NA[c]	0.004	NA[c]	0.006	NA[c]	0.008	NA[c]	0.017	NA[c]
2	360	0.76	0.030	NA[c]	0.009	NA[c]	0.006	NA[c]	0.000	NA[c]	0.023	NA[c]	0.041	NA[c]
3	360	1.30	0.042	3.2	0.017	1.3	0.000	0.0	0.016	1.3	0.040	3.1	0.067	5.1
4	360	2.49	0.077	3.1	0.032	1.3	0.019	0.8	0.059	2.4	0.080	3.2	0.136	5.5

[a] Alinity i Toxo IgM reagent lot and Alinity i Toxo IgM calibrator lot are confounded, and the confounding effect is represented by between-lot.
[b] Reproducibility contains repeatability, between-run, between-day, between-lot, between-site, and site-lot interaction variance components.
[c] Not applicable

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:

a. Potential Cross-Reactivity: Potential cross-reactivity for the Alinity i Toxo IgM assay was determined by testing a total of 177 serum samples from individuals with other medical conditions unrelated to Toxoplasmosis infection, in addition to samples from individuals with High titer *Toxoplasma gondii* IgG antibodies. Out of 10 Rheumatoid Factor specimens, one resulted in a false reactive result with the Alinity i Toxo IgM assay.

Table 4: Alinity i Toxo IgM Assay Cross-Reactivity study

Potential Cross reactants	N	Number of Alinity i Toxo IgM False Reactive Results
Anti-dsDNA antibodies	10	0
Anti-Nuclear Antibodies (ANA)	10	0
Cytomegalovirus (IgM)	10	0
Epstein-Barr virus (IgM)	9	0
Herpes simplex virus types 1/2 (IgG/IgM)	18	0
Human anti-mouse antibody (HAMA)	10	0
Hyper IgG	10	0
Hyper IgM	10	0
Influenza vaccine recipients	10	0
Measles (IgM)	10	0
Parvo-B19 virus (IgG)	6	0
Parvo-B19 virus (IgM)	4	0
Rheumatoid Factor (RF)	10	1[a]
Rubella (IgM)	10	0
Samples from Immunocompromised patients	10	0
Syphilis	10	0
Toxoplasmosis high Titer IgG	10	0
Varicella Zoster Virus	10	0
TOTAL	**177**	**1**

[a] One out of 10 RF specimens was falsely reactive with the Alinity i Toxo IgM assay.

b. Potentially Interfering Endogenous Substances: The Alinity i Toxo IgM assay was evaluated for potential interference caused by endogenous substances. Each substance was evaluated using samples containing anti-Toxo IgM at the target ranges of 0.60 to 0.99 S/CO and 1.00 to 2.00 S/CO. No significant interference was observed at the following concentrations:

Table 5: Endogenous Interfering Substances Evaluated

Substance	Concentrations tested
Unconjugated bilirubin	40 mg/dL
Conjugated bilirubin	40 mg/dL
Hemoglobin	1000 mg/dL
Triglycerides	3000 mg/dL
Total protein (high)	150 g/L

c. Potentially Interfering, Other Conditions: The Alinity i Toxo IgM assay was evaluated for potential interference caused by HAMA and RF using samples containing anti-Toxo IgM at the following target range: 1.00 to 1.40 S/CO. No significant interference was observed at the following concentrations:

Table 6: Potentially Interfering Other Conditions Evaluated

Substance	Concentrations tested
Human anti-mouse antibody (HAMA)	800 ng/mL
Rheumatoid Factor (RF)	200 IU/mL

d. Potentially Interfering Drugs and Other Substances: The Alinity i Toxo IgM assay was evaluated for potential interference caused by exogenous substances using samples containing anti-Toxo IgM at the target ranges of 0.60 to 0.99 S/CO and 1.00 to 2.00 S/CO. No significant interference was observed at the following concentrations:

Table 7: Exogenous Interfering Substances Evaluated

Substance	Concentrations tested
Ascorbic Acid	300 mg/L
Atovaquone	120 mg/L
Beta Carotene	6 mg/L
Biotin	4250 ng/mL
Clindamycin	5.1 mg/dL
Folic Acid	100 nmol/L
Pyrimethamine	15 mg/L
Spiramycine	4.2 mg/L
Sulfadiazine	25.5 mg/dL
Sulfamethoxazole	210 mg/dL
Trimethoprim	4.2 mg/dL

4. Assay Reportable Range:

   Not Applicable.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Not Applicable.

6. Detection Limit:

   Not Applicable.

7. Assay Cut-Off:

   The cut-off for the Alinity i Toxo IgM assay was established using samples characterized with a commercially available anti-Toxo IgM assay. A total of 1219 samples (1053 anti-Toxo IgM nonreactive samples, and 166 anti-Toxo IgM reactive samples) were included. A receiver-operating characteristic analysis supports a cut-off of 1.00 S/CO (with a grayzone from 0.83 to 1.00 S/CO).

B Comparison Studies:

1. Method Comparison:

Clinical Agreement:

A clinical study was conducted to evaluate the clinical performance of the Alinity i Toxo IgM assay. To evaluate the percent agreement between the Alinity i Toxo IgM assay and an FDA-cleared, commercially available anti-Toxo IgM assay with samples collected from two populations. Population 1 was comprised of 897 consecutively collected remnant specimens sent to a laboratory for anti-Toxo IgM testing including specimens collected in the US (n = 169) and outside of the US (n = 710), and Population 2 was comprised of 207 consecutively collected remnant specimens from pregnant women sent to a laboratory for anti-Toxo IgM testing in the US.

Demographic information for specimens collected in the US from Population 1 and 2 is shown in the **Table 8** below (n=376).

Table 8: Subject Demographics (US specimens)

Specimen	Age	Female (n)	Male (n)	Unknown (n)	Total (n)
Population 1 (n=**169**)	≤ 5 years	2	2	0	**4**
	6 to 21 years	5	7	0	**12**
	22 to 59 years	75	36	1	**112**
	≥ 60 years	20	19	0	**39**
	Unknown	0	2	0	**2**
	Total	**102**	**66**	**1**	**169**
Population 2 (n=**207**)	≤ 5 years	0	0	0	**0**
	6 to 21 years	8	0	0	**8**
	22 to 59 years	196	0	0	**196**
	≥ 60 years	0	0	0	**0**
	Unknown	3	0	0	**3**
	Total	**207**	**0**	**0**	**207**
Total	≤ 5 years	**2**	**2**	**0**	**4**
	6 to 21 years	**13**	**7**	**0**	**20**
	22 to 59 years	**271**	**36**	**1**	**308**
	≥ 60 years	**20**	**19**	**0**	**39**
	Unknown	**3**	**2**	**0**	**5**
	Total	**309**	**66**	**1**	**376**

Positive percent agreement (PPA) and negative percent agreement (NPA) between the Alinity I Toxo IgM assay and an FDA-cleared was calculated for each population separately and are shown in **Tables 9** and **10** below.

Table 9: Alinity i Toxo IgM Clinical Performance - **Population 1** (n= 897)

Alinity i Toxo IgM	Comparator		
	Positive	**Equivocal**	**Negative**
Reactive	150	16	11
Grayzone/Equivocal	1	1	14
Nonreactive	6	1	697
Total	157	18	722
PPA[a]	94.94% (150/158)		95%CI= 90.33% to 97.41%
NPA[a]	94.44% (697/738)		95% CI= 92.55% to 95.88%

[a]The 95% CI for PPA and NPA were estimated using the Wilson score method.

Table 10: Alinity i Toxo IgM Clinical Performance - **Population 2**[b] (n=234)

Alinity i Toxo IgM	Comparator		
	Positive	**Equivocal**	**Negative**
Reactive	18	0	0
Grayzone/Equivocal	0	0	0
Nonreactive	1	0	215
Total	19	0	215
PPA[a]	94.74% (18/19)		95% CI= 75.36% to 99.06%
NPA[a]	100.00% (215/215)		95% CI= 98.24% to 100.00%

[a]The 95% CI for PPA and NPA were estimated using the Wilson score method.
[b]Twenty seven specimens from Population 1 were from pregnant females and therefore, were also included in the Population 2.

CDC Panel study:
The Centers for Disease Control and Prevention (CDC) *Toxoplasma* 1998 Human Serum Panel was tested using the Alinity i Toxo IgM assay. The Alinity i Toxo IgM assay results were submitted to the CDC for data analysis and for the result interpretation for each sample.

The panel consisted of 32 true positive Toxoplasma specimens and 65 true negative Toxoplasma specimens. The Alinity i Toxo IgM assay reported the 32 positive specimens as reactive and the 65 negative specimens, as nonreactive. The positive percent agreement

(PPA) was 100% with a 95% confidence interval (CI) of 89.28% to 100.00%. The negative percent agreement (NPA) was 100% with a 95% CI of 94.42% to 100%.

The results are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply endorsement of the assay by the CDC.

2. Matrix Equivalency:

A study was performed to evaluate whether specific blood collection tube types are suitable for use with the Alinity i Toxo IgM assay.

The matrix collection tube type equivalency study was conducted including 43 donors of reactive (20 donors) and nonreactive (23 donors) samples in 5 types of blood collection tubes; serum, serum separator, lithium heparin plasma, lithium heparin plasma (separator tube), and K3 EDTA plasma for use with the Alinity i Toxo IgM assay. Data was analyzed using regression comparing numerical S/CO value results of all matrices to serum.
All of the blood collection tube types tested are acceptable for use with the Alinity i Toxo IgM assay.

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable

2. Clinical Specificity:

Not applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

Not applicable

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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