510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K162678

B. Purpose for Submission:

To obtain substantial equivalence for the Elecsys Toxo IgM assay and Elecsys Toxo IgM PreciControl set

C. Measurand:

Toxoplasma IgM antibody

D. Type of Test:

Immunoglobulin Class-Capture Chemiluminescence Immunoassay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys Toxo IgM, Elecsys Toxo IgM PreciControl

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3780 - *Toxoplasma gondii* serological reagents

2. Classification:

   Class II

3. Product code:

   LGD; enzyme linked immunoabsorbent assay, *Toxoplasma gondii*
   JJX; single (specified) analyte controls (assayed and unassayed)

4. Panel:

83 Microbiology

H. Intended Use:

1. Intended use(s):

   Elecsys Toxo IgM Immunoassay

   The Elecsys Toxo IgM immunoassay is for the in vitro qualitative detection of IgM antibodies to *Toxoplasma gondii* in human serum and plasma. This assay may be used as an aid in the diagnosis of an acute or recent Toxoplasmosis infection in suspected patients and pregnant women. Patient testing must be performed in conjunction with an anti-*Toxoplasma gondii* IgG antibody assay.

   The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immuno-assay analyzers. This assay has not been cleared by the FDA for blood/plasma donor screening.

   PreciControl Toxo IgM

   PreciControl Toxo IgM is used for quality control of the Elecsys Toxo IgM immunoassay on the Elecsys and cobas e immunoassay analyzers.

2. Indication(s) for use:

   Same as intended use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Elecsys 2010 and cobas e 411 analyzers

I. Device Description:

The Elecsys Toxo IgM is a two-step sandwich immunoassay with streptavidin microparticles and electrochemiluminescence detection. In the first incubation, 10 µL of sample is automatically prediluted 1:20 with Elecsys Diluent Universal, with *T. gondii*-specific recombinant antigen labeled complex then added. Anti-Toxo IgM antibodies present in the sample react with the labeled *T. gondii*-specific recombinant antigen. Biotinylated monoclonal IgM specific antibodies and streptavidin-coated microparticles are subsequently added. The complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then

induces chemiluminescent emission which is measured by a photomultiplier. Results are determined automatically by the Elecsys software by comparing the electrochemiluminescence signal obtained from the reaction. The assay can be run on either the Elecsys 2010 of the cobas e 411 analyzers platforms.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   VIDAS *Toxoplasma Gondii* IgM Assay (K923166)

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

   K923166

3. Comparison with predicate:

Similarities		
Item	Device	Predicate (K923166)
Intended Use	Elecsys Toxo IgM Immunoassay: The Elecsys Toxo IgM immunoassay is for the in vitro qualitative detection of IgM antibodies to *Toxoplasma gondii* in human serum and plasma. This assay may be used as an aid in the diagnosis of an acute or recent Toxoplasmosis infection in suspected patients and pregnant women. Patient testing must be performed in conjunction with an anti-*Toxoplasma gondii* IgG antibody assay. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immuno-assay analyzers. This assay has not been cleared by the FDA for blood/plasma donor screening. PreciControl Toxo IgM: PreciControl Toxo IgM is used for quality control of the Elecsys	The VIDAS TOXO IgM (TXM) assay is intended for use with a VJDAS (Vitek ImmunoDiagnostic Assay System) instrument 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 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.

Similarities		
Item	Device	Predicate (K923166)
	Toxo IgM immunoassay on the Elecsys and cobas e immunoassay analyzers.	
Calibrator	Included with the kit	Same
Assay type	Sandwich ELISA	Same
Instrument Platform	Fully automated system	Same

Differences		
Item	Device	Predicate (K923166)
Assay Protocol	Electrochemiluminescence Immunoassay (ECLIA)	Enzyme-linked fluorescent immunoassay (ELFA)
Sample type	Serum, lithium heparin plasma, potassium EDTA plasma and sodium citrate plasma	Serum
Calibrators	Two	One

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

- EP05-A2, Evaluation of Precision Performance of Quality Measurement Methods; Approved Guideline - Second Edition 2004
- EP17-A, Protocols For the Determination of the Limits of Detection and Limits of Quantitation

L. Test Principle:

Electrochemiluminescence immunoassay

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was determined at one sites using Elecsys reagents, three human sera pools and two controls. Testing was performed in accordance with the Clinical and Laboratory Standards Institute Document EP5-A with the following modification: Samples were tested six times daily for 10 days (n = 60 data points per panel member). The following results were obtained and summarized below in Table 1 and were determined to be acceptable.

Table 1. Precision

			Repeatibility		**Between-run**		**Intermediate precision**	
Sample	N	Mean [s/co]	SD [s/co]	CV (%)	SD [s/co]	CV (%)	SD [s/co]	CV (%)
PC Toxo IgM 1	60	0.146	0.004	2.5	0.003	1.9	0.005	3.2
PC Toxo IgM 2	60	1.61	0.037	2.3	0.045	2.8	0.059	3.6
Serum Pool 1	60	0.147	0.003	2.3	0.005	3.1	0.006	3.9
Serum Pool 2	60	2.06	0.075	3.7	0.031	1.5	0.082	4.0
Serum Pool 3	60	3.49	0.172	4.9	0.060	1.7	0.183	5.2

Reproducibility

Representative performance data on the analyzers are given below.

Reproducibility was determined at two sites using Elecsys reagents using three human sera panels. Testing was performed in accordance with the Clinical and Laboratory Standards Institute Document EP5-A with the following modification: Samples were tested six 6 times daily for 10 days at each site (n = 60 data points per site). The following results were obtained and summarized below in Table 2.

Table 2. Reproducibility

			Repeatibility		**Between-run**		**Intermediate precision**		**Between-site**		**Total precision**	
Serum Pool	N	Mean [s/co]	SD [s/co]	CV (%)	SD [s/co]	CV (%)	SD [s/co]	CV (%)	SD [s/co]	CV (%)	SD [s/co]	CV (%)
Pool 1	120	0.161	0.003	1.9	0.006	3.5	0.006	4.0	0.021	13.0	0.022	13.6
Pool 2	120	2.01	0.065	3.3	0.03	1.5	0.072	3.6	0.067	3.3	0.098	4.9
Pool 3	120	3.37	0.135	4.0	0.073	2.2	0.153	4.6	0.174	5.2	0.232	6.9

b. Linearity/assay reportable range:

Not applicable – This assay is qualitative

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

Traceability:

There is no international standard available for measuring anti-Toxoplasma IgM in serum; therefore, calibrators are traceable to an in-house reference preparation.

Calibrators
Calibration of an Elecsys Toxo IgM reagent lot is recommended every 28 days (1 month). Elecsys Toxo IgM reagent kits can be stored on-board the analyzers for up to 2 weeks. A new calibration of a kit kept on-board is recommended every 7 days

Stability:

Reagents
Reagent stability was determined by testing five human samples and two control samples. The results support the claim of reagent stability for 12 weeks of storage after first opening at 2-8°C (not on board). Reagents are only stable on-board the analyzer up to 84 hours total time. The real-time data currently available supports a shelf-life claim of 14 months at 2-8°C for unopened reagents.

Calibrators
Calibrator stability was determined by testing the calibrators in duplicate once after first opening and once again after 8 weeks without loss of signal. The results support the claim of calibrator stability for 8 weeks of storage after first opening at 2-8°C (not on board). Calibrators are also stable beyond 5 hrs. when tested every hour on board the analyzer at 20-25°C. The data provided supports a 5 hr. on-board stability claim. The real-time data currently available supports a shelf-life claim of 14 months at 2-8°C without opening.

Controls
The PreciControl Toxo IgM is stable at 2-8°C after first opening for up to 8 weeks. The PreciControl Toxo IgM is stable for 5 h under on-board conditions. The real-time data currently available supports a shelf-life claim of 14 months.

Samples
Samples are stable are for 3 days at 15-25°C, 3 weeks at 2-8°C and 3 months at -20°C.

Expected values:

- Calibrator 1 is manufactured to have a target Index value range of 400 - 2500.
- Calibrator 2 is manufactured to have a target Index value range of 4500 - 35000.
- The negative control is manufactured to have a target Index value of 0.18 COI*
- The positive control is manufactured to have a target Index value of 2.0 COI*.

*cutoff index (COI) is calculated as a ratio of sample signal over cutoff. The target Index value range for each control is reported in the rackpack or PerciControl kit to make sure that the correct target values are used.

d. Detection limit:

Not applicable – This assay is qualitative

e. Analytical specificity:

Cross reactivity:

The cross-reactivity study for the Elecsys Toxo IgM assay was designed to evaluate potential interference from the presence of potentially cross-reactive antibodies or substances that may cause symptoms similar to or that may mimic Toxoplasmosis infection. Only samples that were sero-positive for the cross reactant and sero-negative for *Toxoplasma gondii* IgM by a commercially available Toxoplasma lgM assay were used to test for potentially cross-reactive organisms. The results are summarized in table 3. below.

Table 3. Cross Reactivity Data

Cross- reactant	**# of Toxo IgM Elecsys reactants / Total Tested**
AMA*	1/15
ANA	0/26
Chlamydia	0/5
CMV	0/13
EBV**	1/10
Gonorrhea	0/5
HAV	0/10
HBV**	1/21
HCV	0/12
HIV	0/13
HSV	0/9
Influenza	0/16
Malaria**,***	2/25
Parvo B19	0/11
Rubella***	1/10
Syphilis	0/5
TPHA	0/4
VZV	0/8

*Contains a false positive result
** Equivocal results were counted as positive
AMA Anti-mitochondria antibodies.
ANA Antinuclear antibodies
TPHA Treponema pallidum human antibodies

High Dose Hook Effect (false negative):

High-dose hook effect was evaluated by testing three samples with *Toxoplasma gondii* lgM levels out-of-range > 64 COI (i.e., 64 x minimum positive value). In this study, three samples with very high Toxo IgM levels were serially diluted with negative sample to cover the entire assay range. The neat sample and dilutions were tested using one lot on one instrument. The signal obtained was plotted versus the dilution. The samples resulted in calculated concentration values above the measuring range with no sample misclassification, indicating no hook effect was observed.

Interference

Testing was performed to determine whether the presence of endogenous or exogenous substances (compounds) may interfere with assay results. Matched sample pools (3 negative and 3 positive) were tested neat and spiked with the respective interferent. Acceptance criteria were defined as the % change in signal must not be more than + 20% and no change the qualitative result. The assay is unaffected at the concentration for each substance listed in Table 4 below on the Elecsys Toxo IgM assay.

Table 4. Endogenous Interference Results

Compound	**Concentration**
Bilirubin	< 684 µmol/L or < 40 mg/dL
Hemoglobin	< 1.24 mmol/L or < 2 g/dL
Intralipid	< 2000 mg/dL
Biotin	< 246 nmol/L or < 60 ng/mL

f. Assay cut-off:

The cut-off for the Elecsys Toxo IgM assay was established by the use of 900 clinical samples from which a preliminary cutoff was set by ROC analysis. The results of the internal and external testing to evaluate the performance of the Elecsys Toxo IgM assay demonstrate that the cutoff of 1.0 COI is appropriate to obtain good sensitivity and specificity.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Studies were conducted to evaluate the suitability of the following 5 types of blood collection tubes; serum/gel separation tubes, lithium heparin plasma, K_2-EDTA plasma, K_3-EDTA plasma and sodium citrate plasma. A minimum of 49 sample pairs were drawn into serum and plasma collection tubes. The recovery of the analyte in the presence of the anti-coagulant was compared to serum. The results indicate there was no difference between any of the plasma types when compared to serum.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

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

Comparative testing:
Clinical performance of the Elecsys Toxo IgM assay was evaluated in three studies (two prospective and one retrospective) and with a CDC panel. These studies were conducted to compare the performance of the subject assay to an FDA-cleared predicate device. Overall, there were 601 samples and 97 panel members tested in these studies which comprised of samples from individuals who were sent to the laboratory for *T gondii IgM* testing, or were from a retrospective collection. The demographics of the patients vary widely and will be described with each pooled data set.

Study 1

A total of 440 prospective specimens were obtained from US reference laboratory. All specimens represented samples for whom anti-Toxo testing had been ordered for clinical routine testing. The patient population consisted of 162 males and 278 females (158 were from pregnant women). The patient's age ranged from 2 to 86 years. The performance of the Elecsys Toxo IgM immunoassay, relative to an FDA cleared method is presented in the Table 5 below.

Table 5. US prospectively collected (overall patient population)

Comparator Toxo IgM Method					
		Positive	**Equivocal**	**Negative**	**Total**
Elecsys Toxo IgM *	Reactive	2	0	2	4
	Equivocal	0	0	4	4
	Non-reactive	0	1	431	432
	Total	2	1	437	440

*The Elecsys IgM assay calls positive results "reactive" and negative results "non-reactive"

Agreement Classification	**Numerator/ Denominator**	**Percent Agreement**	**95 % Confidence Interval**
Positive agreement*	2/3	66.7	9.43 - 99.2
Negative agreement*	431/437	98.6	97.0- 99.4

*For agreement calculation, equivocal results are counted as discrepant against the Elecsys Toxo IgM.

Study 2

A total of 60 retrospective samples were obtained from a US commercial sample vendor. The patient population consisted of 4 males and 56 females (50 were from pregnant women). All specimens represented samples from whom anti-Toxo testing had been ordered per clinical routine. The performance of the Elecsys Toxo IgM immunoassay, relative to an FDA cleared method is presented in Table 6 below.

Table 6. US retrospectively collected (overall patient population)

Comparator Toxo IgM Method					
		Positive	**Equivocal**	**Negative**	**Total**
Elecsys Toxo IgM	Reactive	0	0	0	0
	Equivocal	0	0	1	1
	Non-reactive	0	0	59	59
	Total	0	0	60	60

Agreement Classification	**Numerator/ Denominator**	**Percent Agreement**	**95 % Confidence Interval**
Positive agreement	0/0	NA	NA
Negative agreement*	59/60	98.3	91.1 - 100

*For agreement calculation, the equivocal result was counted as discrepant against the Elecsys Toxo IgM

Study 3

One-hundred one (101) samples collected prospectively were obtained from the general population in Europe under suspect of Toxoplasmosis infection consistin of 5 males and 96 females with an age range of 22 to 69 years. The performance of the Elecsys Toxo IgM immunoassay, relative to an FDA cleared method is presented in Table 7 below.

Table 7. EU Prospectively collected (overall patient population)

Comparator Toxo IgM Method					
		Positive	**Equivocal**	**Negative**	**Total**
Elecsys Toxo IgM	Reactive	81	1	5	87
	Equivocal	0	1	1	2
	Non-reactive	4	1	7	12
	Total	85	3	13	101

Agreement Classification	**Numerator/ Denominator**	**Percent Agreement**	**95 % Confidence Interval**
Positive agreement*	81/86	94.2	87.0 - 98.1
Negative agreement*	7/14	50.0	23.0 - 77.0

*For agreement calculation, equivocal results are counted as discrepant results against the Elecsys Toxo IgM. In case of unanimously equivocal samples, samples were not used for agreement calculation.

Of the 101 prospectively collected European specimens described above, 87 were from pregnant women. The agreement results are shown in Table 8 below.

Table 8. EU prospectively collected (pregnant women)

Reference Toxo IgM Method					
Elecsys Toxo IgM		**Positive**	**Equivocal**	**Negative**	**Total**
	Reactive	68	0	5	73
	Equivocal	0	1	1	2
	Non-reactive	4	1	7	12
	Total	72	2	13	87

Agreement Classification	**Numerator/ Denominator**	**Percent Agreement**	**95 % Confidence Interval**
Positive agreement*	68/73	93.2	84.7 - 97.7
Negative agreement*	7/13	53.9	25.1 - 80.8

*For agreement calculation, equivocal results are counted as discrepant results against the Elecsys Toxo IgM. In case of unanimously equivocal samples, samples were not used for agreement calculation.

CDC Panel study:
A panel of 97 samples (32 known positive and 65 known negative) was obtained through the US Centers for Disease Control and Prevention (CDC) and was tested for Toxo IgM on the Elecsys 2010 analyzer. The results are presented as agreement with the known results as additional information on the performance of this assay with a masked, characterized serum panel. This does not imply an endorsement of the assay by the CDC. The results of the CDC panel testing are sumerized below in Table 9.

Table 9. CDC Panel results

	ElecsysToxo IgM Percent Agreement (n/N)	**95 % Confidence interval**
CDC positives	91 % (29/32)	75 - 98 %
CDC negatives	98 % (64/65)	92 - 100 %

4. Clinical cut-off**:**

Non-reactive: < 0.8 COI

Equivocal: ≥ 0.8 - < 1.0 COI

Reactive: ≥ 1.0 COI

Samples with a cutoff index < 0.8 are non-reactive in the Elecsys Toxo IgM assay.

Samples with a cutoff index between ≥ 0.8 and < 1.0 are considered equivocal. The sample should be retested. In case the result is still equivocal, a second sample should be tested e.g. within 2-3 weeks. Samples with a cutoff index ≥ 1.0 are reactive in the Elecsys Toxo IgM assay.

5. Expected values/Reference range:

Expected value range will be provided

N. Proposed Labeling:

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

O. Conclusion:

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