

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

K073390

**B. Purpose for Submission:**

Clearance of new device

**C. Measurand:**

Rubella-specific IgG in human serum

**D. Type of Test:**

Chemiluminescence immunoassay (CLIA)

**E. Applicant:**

DiaSorin Inc.

**F. Proprietary and Established Names:**

DiaSorin LIAISON<sup>®</sup> Rubella IgG Assay

DiaSorin LIAISON<sup>®</sup> Rubella IgG Tri-Controls

**G. Regulatory Information:**

1. Regulation section:

21CFR §866.3510, Rubella virus serological reagents

2. Classification:

Class II

3. Product code:

LSD, JJX (Other serological assays, rubella and single analyte controls)

4. Panel:

Microbiology (83)

## H. Intended Use:

1. Intended use(s):

The LIAISON<sup>®</sup> Rubella IgG uses chemiluminescence immunoassay (CLIA) technology on the LIAISON<sup>®</sup> Analyzer for the qualitative determination of IgG antibodies to rubella virus in human serum specimens. It is intended for use as an aid in the determination of immune status to rubella in individuals including pregnant women.

The performance of this device has not been established for cord blood, neonatal samples, or for any matrices other than human serum. Likewise, performance has not been established for population(s) of immunocompromised or immunosuppressed individuals.

The LIAISON<sup>®</sup> Rubella IgG Tri-Control kit is intended for use as assayed quality control samples to monitor the performance of the LIAISON<sup>®</sup> Rubella IgG assay.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

For professional use only

4. Special instrument requirements:

LIAISON<sup>®</sup> Analyzer, Model 15790. Included in cleared: K032844, K033426, K040120, K040290, K052499, K060204, K061247, K061820, K062473

## I. Device Description:

The LIAISON<sup>®</sup> Rubella IgG assay is an indirect chemiluminescence immunoassay (CLIA) for qualitative determination of IgG specific to rubella virus. The test uses a first incubation to allow rubella antibodies from the samples to bind to magnetic particles (solid phase) coated with rubella antigen. A second incubation facilitates the reaction of an isoluminol-antibody conjugate (using mouse monoclonal antibody to human IgG) with the rubella IgG bound to the solid phase. Detection is performed through chemiluminescence reaction once the starter reagents are added. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU), and correlated to detection of rubella antibody from the sample. The assay is calibrated by means of two calibrators (cleared with this submission) that are traceable to the 1<sup>st</sup> WHO standard reference material for Rubella IgG (RUBI-1-94). The test results are reported as index values with an associated interpretation of “Negative”, “Equivocal”, or “Positive” for the

presence of anti-rubella IgG at the cut-off value of 1.0, which corresponds to 10 International Units/mL (IU/mL) (equivocal zone is set greater than index 0.9 and less than index 1.0 IU/mL). The test results are to be used in conjunction with other clinical information and history to suggest immune status versus rubella virus.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

Bayer Diagnostics ADVIA Centaur Rubella IgG assay.

2. Predicate K number(s):

K003412.

3. Comparison with predicate:

<b>Similarities</b>		
Item	Device	Predicate
Intended Use	The LIAISON <sup>®</sup> Rubella IgG uses chemiluminescence immunoassay (CLIA) technology on the LIAISON <sup>®</sup> Analyzer for the qualitative determination of IgG antibodies to rubella virus in human serum specimens. It is intended for use as an aid in the determination of immune status to rubella in individuals including pregnant women. The performance of this device has not been established for cord blood, neonatal samples, or for any matrices other than human serum. Likewise, performance has not been established for population(s) of immunocompromised or immunosuppressed individuals	The ADVIA <sup>®</sup> Centaur <sup>™</sup> Rubella IgG assay is an IgG antibody capture microparticle direct chemiluminometric immunoassay for the quantitative and qualitative detection of IgG antibodies to rubella virus in human serum or plasma (EDTA, heparin) as an aid in the assessment of immune status to rubella in individuals including women of childbearing age
Sample handling/processing	Automated	Automated
Antigen Used	Inactivated rubella viral particle (strain HPV 77)	Inactivated rubella virus (strain HPV 77)
Indications for Use	Use as an aid in the determination of immune status to rubella in individuals including pregnant women	Aid in the assessment of immune status to rubella in individuals including women of childbearing age
Measurement System	Photomultiplier (flash chemiluminescence reader)	Photomultiplier (flash chemiluminescence reader)
Reagent Storage	On-board or in refrigerator	On-board or in refrigerator
Calibration	Two-point verification of stored 10-point master curve. Traceable to Rubi-1-94	Two point calibration. Traceable to Rubi-1-94
CDC Rubella Panel evaluation	Yes	Yes
CLSI Standards Used	I/L6, EP5	I/L6, EP5

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Sample Type	Serum	Serum, EDTA plasma or heparin plasma
Basic Principle	Indirect Chemiluminescence Immunoassay	Direct Chemiluminometric technology
Controls	Three (negative, low positive, and positive)	Two (negative and positive)
Detector	Mouse monoclonal anti-human IgG	Anti-human IgG <sub>FC</sub> monoclonal antibody
Cut-off	1 index	10 IU/mL
Equivocal Zone	0.9 – 1.0 Index	5.0 – 9.9 IU/mL
Unit of Measure	Index	IU/mL
Reportable Range	Not defined	0.2 – 500 IU/mL
Linearity	Not established. Qualitative assay	Quantitative and qualitative assay

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

CLSI I/L6-A, “Detection and Quantitation of Rubella IgG Antibody: Evaluation and Performance Criteria for Multiple Component Test Products, Specimen Handling, and Use of the Test Products in the Clinical Laboratory”.

Guidance for Off-the-Shelf Software Use in Medical Devices. Cybersecurity for Networked Medical Devices Containing Off-the-Shelf (OTS) Software.

Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices.

CLSI EP7, “Interference Testing in Clinical Chemistry”

CLSI EP5-A2, “Evaluation of Precision Performance of Clinical Chemistry Devices”

CLSI EP15-A2, “User Verification of Performance for Precision and Trueness”

**L. Test Principle:**

All assay steps (with the exception of magnetic particle resuspension) and incubations are performed by the LIAISON<sup>®</sup> Analyzer. The principal components of the test are magnetic particles (solid phase) coated with Rubella antigen and a conjugate of mouse monoclonal antibody to human IgG linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, Rubella virus antibodies present in the calibrators, specimens or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with Rubella virus IgG already bound to the solid phase. After each incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount

of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU).

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

*Precision:*

Assay precision performance was established at DiaSorin in a 20-day study following protocol outlined in CLSI document, EP5-A2. A coded panel comprised of 12 frozen repository samples was prepared by DiaSorin and tested in the LIAISON® Rubella IgG assay. The panel contained samples prepared to represent negative levels, low to mid positive analyte levels and moderate to high positive levels. All panel members were divided into aliquots and stored frozen prior to testing. The coded panel was tested in four replicates per run for twenty runs. The results are summarized in the following table.

Sample ID#	N	Mean (Index)	Within-run		Between run		Overall	
			sd	%CV	sd	%CV	sd	%CV
Neg Ctl	80	<<0.1	NA	NA	NA	NA	NA	NA
Low Pos Ctl	80	1.63	0.08	5.12	0.14	8.40	0.16	9.6
Hi Pos Ctl	80	14	1.75	13.1	1.34	9.66	2.17	15.7
R01	80	0.68	0.03	4.06	0.04	5.23	0.04	6.3
R02	80	0.61	0.02	3.32	0.03	5.07	0.04	5.9
R03	80	0.90	0.03	3.64	0.04	4.74	0.05	5.9
R04	80	1.07	0.04	4.19	0.05	4.99	0.07	6.5
R05	80	0.88	0.03	3.42	0.04	5.09	0.05	6.0
R06	80	1.16	0.05	4.52	0.10	8.38	0.11	9.4
R07	80	2.4	0.11	4.85	0.21	9.13	0.23	9.9
R08	80	2.5	0.17	7.05	0.24	9.64	0.29	11.5
R09	80	3.1	0.19	6.20	0.27	8.68	0.32	10.3
R10	80	3.2	0.21	6.52	0.30	9.45	0.36	10.9
R11	80	15	1.37	9.26	1.69	11.3	2.14	13.9
R12	80	24	2.21	10.2	2.99	12.8	3.65	15.3

*Reproducibility:*

An assay reproducibility study was conducted at two external U.S. laboratories and at DiaSorin, in a five-day study according to CLSI document EP15-A2. The study included 3 different kit lots and the same coded panel as described in the twenty day study. The same coded panel was tested at all three sites, in four replicates per run for five runs. The results are summarized in the following table.

sample ID#	N	mean Index	within run SD	within run %CV	between run SD	between run %CV	between site SD	between site CV	overall sd	overall %CV
Neg Ctl	60	<<0.1	NA	NA	NA	NA	NA	NA	NA	NA
Low Pos Ctl	60	1.50	0.06	4.02	0.12	4.23	0.12	8.37	0.13	8.78
Hi Pos Ctl	60	12.6	1.0	8.17	1.30	6.04	1.28	17.0	1.64	13.09
R01	60	0.67	0.02	3.56	0.03	4.27	0.02	16.8	0.04	5.70
R02	60	0.59	0.02	3.02	0.04	4.90	0.02	12.6	0.04	6.90
R03	60	0.90	0.02	2.51	0.06	6.12	0.02	4.21	0.06	6.73
R04	60	1.06	0.04	4.15	0.09	6.37	0.05	1.12	0.10	9.07
R05	60	0.88	0.03	3.21	0.06	6.50	0.02	6.47	0.07	7.53
R06	60	1.12	0.03	2.77	0.09	6.44	0.06	6.83	0.10	8.59
R07	60	2.3	0.1	3.36	0.22	4.14	0.20	10.3	0.22	9.54
R08	60	2.4	0.1	4.03	0.26	7.52	0.17	8.02	0.27	11.07
R09	60	3.1	0.1	4.77	0.26	7.68	0.15	9.56	2.90	9.46
R10	60	3.2	0.2	5.87	0.28	7.82	0.10	8.05	0.33	10.45
R11	60	14.3	1.0	7.05	2.03	11.32	1.19	18.6	2.2	15.62
R12	60	21.7	1.4	6.56	2.24	6.79	1.95	18.8	2.66	12.25

*Quality Control:*

The LIAISON® Rubella IgG procedure suggests the running of controls once per day of use. The LIAISON® Rubella IgG Controls that consists of one negative, one low-positive and one high-positive control were tested in duplicate with every run at the clinical sites.

*b. Linearity/assay reportable range:*

N/A. The study does not present a linearity study, nor does it claim a range of quantitative results.

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

*Traceability:*

Concordance of the assay with the WHO International Standard was evaluated using serial dilutions of the standard preparation. The dilutions were tested with two lots of in the LIAISON® Rubella IgG.

The cut-off (=1 Index) calculated by interpolation on Index doses corresponds to 32400 RLUs. This RLU value calculated by interpolation on the WHO curve is 9.42 IU/mL.

Dilutions	Expected index	Actual index (mean)	Expected WHO dose (IU/mL)
1:8	20.0	20.4	200
1:16	10.0	10.1	100
1:32	5.0	4.7	50
1:64	2.5	2.2	25

1:128	1.3	1.32	12.5
1:256	0.63	0.67	6.25
1:512	0.31	0.24	3.13

Linear regression between WHO doses (IU/mL) vs. Index:

Linear regression	Lot 1	Lot 2
R square	0.9987	0.9982
Intercept	0.1081	0.1542
Slope	0.1086	0.0962
Result at WHO cut off	0.98 Index	0.81 Index

*Stability: Kit and Controls:*

*Storage:*

In accordance with recognized standard EN 13640:2001, studies were performed in which three kit lots were tested after storing in conditions different from those recommended (2-8°C) to simulate the stress of transport. A coded panel was tested in parallel by the stressed kit and not-stressed kits stored at +2-8°C, according to the package insert. Controls were tested by stressing one control lot at the specific conditions and testing it in parallel with non-stressed controls.

The performed tests were considered satisfactory when compared to the acceptable criteria to support the following claims:

Storage of Reagent Integral kit and controls up to five days at +30°C.

The recommendation in the package insert is that the reagent integral should not be frozen.

Storage of Reagent Integral kit and controls for 24 hrs at +37°C.

*On-board stability:*

On-board stability was evaluated after simulating the storage conditions usually found in user laboratories. The study was performed to evaluate the on board stability of the Reagent Integral. Reagent Integrals stored (after opening) in the refrigerator at 2-8°C or 'on board' the LIAISON<sup>®</sup> Analyzer. Stability testing was performed in accordance with the recognized standard EN 13640:2001.

Two Reagents Integrals (of the same kit lot) were opened, seals removed and calibrated. One was then left on board the instrument, in the refrigerated area (12-19°C), for 63 days, while the other was stored in the refrigerator at 2-8°C. At regular intervals, the on board kit performance was evaluated in parallel with the kit stored at 2-8°C by testing the coded panel according to the package insert's instructions for use. Calibration was performed every four weeks. The obtained data demonstrated that, opened LIAISON<sup>®</sup> Rubella IgG Reagent Integrals are stable for at least 63 days when left on-board the LIAISON<sup>®</sup> Analyzer. The claim of eight (8) weeks on-board stability is stated in the package insert.

*Calibration Stability:*

One lot of kit was used and Reagent Integrals were opened and seals removed. The calibration was performed, the coded panel was tested, and then the Integral was left on board the instrument in the refrigerated area (12-19°C).

Every 7 days the same samples were tested, according to the package insert's instructions for use, using the opened Integral and the initial assay calibration. This study continued for 35 days.

Calibration stability was considered acceptable because tested coded panel were correctly classified. Therefore the %CV of the sample results calculated across days is comparable to the acceptance criteria.

A second test was performed using an opened Integral and stored in the refrigerator at 2-8°C. The %CVs of the sample results calculated across days are comparable to the acceptance criteria.

Calibration is considered stable for a minimum of 28 days. The claim of four (4) weeks calibration stability is stated in the package insert.

*Open Controls Stability:*

One lot of LIAISON<sup>®</sup> Rubella IgG Tri-Controls was used in this study. One of each level of control vial was opened, tested and stored during a 35 day period. Every 7 days, the opened vials and a freshly opened vial were tested in parallel using opened and stored on board Integrals.

Index values were calculated with calibration at time zero. Open control stability was considered acceptable because %CV of results calculated across days is comparable to the expected between run precision and no significant differences on doses were observed between already opened vials and freshly opened ones, therefore the controls are considered stable for 28 days. The claim of four (4) weeks opened vial stability is stated in the Instruction for Use.

*Freeze/Thaw Study:*

A dedicated fresh sera panel was collected and tested the same morning after collection. The specimens were classified as follows: two high negative samples; four borderline samples; four low positive samples; two moderate to high positive samples. Each sample was cycled through five freeze-thaws and aliquots of sample representing each freeze-thaw cycle were re-tested in a single run with one LIAISON<sup>®</sup> Rubella IgG kit lot. The %CV on doses of the sample results was calculated across freeze/thaw cycles and found to be consistent with the between-run precision. No sample showed any trend that might suggest the influence of the freezing process on the assay result.

*d. Detection limit:*

See *Assay cut-off* below.

*e. Analytical specificity:*

*Cross-reactivity:*

The cross-reactivity study for the LIAISON<sup>®</sup> Rubella IgG assay was designed to evaluate potential interference from other organisms that may cause symptoms similar to Rubella virus infection (VZV, Measles, Mumps), other organisms that may cause infectious disease (HAV, HBV, HCV, HIV, CMV, HSV, EBV, *Toxoplasma gondii*, Parvovirus, *Treponema pallidum*) and from other conditions that may result from atypical immune system activity (hypergammaglobulin, antinuclear autoantibodies, Rheumatoid Factor). Samples for these studies were pre-screened with another commercially available Rubella IgG assay. If found negative for Rubella IgG antibodies the samples were used to study potential cross-reactivity. The presence of IgG antibodies to the potential cross-reactants in the samples was confirmed using FDA-cleared assays.

<b>Organism/condition</b>	<b>Number of Expected Negative Samples</b>	<b>LIAISON<sup>®</sup> Positive or equivocal Result</b>
anti-HAV	59	0/59
CMV IgG	58	0/58
HBsAg	3	0/3
HSV ½ IgG	67	0/67
Toxoplasma IgG	12	0/12
VCA IgG	70	0/70
VZV IgG	50	0/50
Measles IgG	3	0/3
Mumps IgG	2	0/2
Anti-HCV	3	0/3
Anti-HIV 1/2	2	0/2
Parvovirus IgG	2	0/2
γ-globulin	3	0/3
ANA	11	0/11
RF	1	0/1
<i>Treponema pallidum</i>	1	0/1

No positive result was found for the specimens when tested by LIAISON<sup>®</sup> Rubella IgG. Assessment of potential cross-reactivity due to circulating HAMA was not established. The user is responsible for establishing cross-reactivity performance with these antibodies.

*Interference:*

The DiaSorin LIAISON<sup>®</sup> Rubella IgG assay was evaluated for interference according to CLSI Document EP7. A panel of 12 samples with rubella Index values ranging from 0.61 to 19.4 were tested with two levels each of hemoglobin (500 and 1000 mg/dL), bilirubin (10 and 20 mg/dL) and triglycerides (500 and 3000 mg/dL). None of the interferents at the levels

tested produced a significant change in the qualitative results of the assay.  
 The matched spiked and unspiked samples were tested in a single run.  
 The raw data for the interference study are presented in the following table:

	Expected Value	Hb 500 mg/dL	Hb 100 mg/dL	bil. 10 mg/dL	bil. 20 mg/dL	Tr 500 mg/dL	Tr 3000 mg/dL			
ID SAMPLE	Index							Mean	St Dev	CV%
RG260607-R01	0.62	0.55	0.52	0.59	0.6	0.55	0.66	0.58	0.05	8%
RG260607-R02	0.61	0.61	0.6	0.6	0.61	0.59	0.61	0.6	0	1%
RG260607-R03	0.85	0.82	0.82	0.84	0.86	0.84	0.84	0.84	0	2%
RG260607-R04	0.93	0.92	0.89	0.92	0.95	0.96	0.96	0.93	0	3%
RG260607-R05	0.82	0.82	0.81	0.81	0.81	0.79	0.8	0.81	0	1%
RG260607-R06	1.17	1.16	1.12	1.16	1.16	1.1	1.16	1.15	0	2%
RG260607-R07	2.3	2.3	2.2	2.5	2.3	2.3	2.4	2.3	0.1	4%
RG260607-R08	2.4	2.4	2.5	2.3	2.5	2.4	2.5	2.4	0.1	3%
RG260607-R09	3.0	3.1	2.9	3.0	3.0	3.1	3.1	3.0	0.1	2%
RG260607-R10	3.0	3.0	3.0	3.1	3.1	3.2	3.3	3.1	0.1	4%
RG260607-R11	13.1	15	13.5	13.2	14.0	13.5	10.6	13.3	1.3	10%
RG260607-R12	19.4	18.7	19.3	19	20.3	19.6	19.9	19.5	0.5	3%

The paired-T test was applied to each potentially interfering substance study and showed no significant difference in terms of Index among samples both for negative and positive specimens.

Human Serum Albumin:

The spiking of 12 samples with serum albumin (up to 1.7 g/dL) showed no significant difference in terms of Index among samples when comparing results from the original samples with those after the addition of serum albumin in order to reach the higher level.

*f. Assay cut-off:*

The cut-off for the LIAISON® Rubella IgG assay (index = 1) was set at 10 IU/mL based on the 1st International Standard for anti-Rubella Immunoglobulin, Human (1997) in accordance to the CLSI guideline I/LA6-A (1997). In order to determine the accuracy of the assay's cutoff, an analysis of the cumulative frequency distributions (ROC curve) was performed, according to the CLSI guidance document GP10-A, on the LIAISON® Rubella IgG assay results for the 1989 positive and 90 negative samples to assess the assay's cutoff. The relevant cumulative frequency distribution graph was generated by calculating relative class frequencies of the expected positive results from the expected negative sample results and comparing their patterns to visualize if the assay was designed to guarantee the required cutoff value.

The assay's cut-off was set in accordance to the CLSI Guideline and evaluated on the observed results to guarantee the best levels of specificity, without compromising the sensitivity. The analysis of the data determined that the cut-

off at an Index value of 1.0 guaranteed the best sensitivity and specificity balance. A grey zone of - 10% of the cutoff was selected due to observed assay imprecision. Therefore, in the LIAISON® Rubella IgG assay, a sample is defined as positive if the Index value is equal to or greater than 1.0, and defined as negative if the Index value is lower than 0.90. Samples with results between 0.90 and 1.0 are classified as equivocal.

*WHO Calibration:*

The international preparation (i.e. RUBI-1-94 - NIBSC 1st International Standard for anti-Rubella Immunoglobulin, Human - 1997) was used to prepare a dilution curve in serum matrix to cover the assay range. The points were tested in three replicates each for three days, 2 runs per day by two different lots. The analytical sensitivity was based on a dilution curve plotted with the mean of each standard point, and on this curve the cut-off dose was calculated by interpolation. A linear regression of the LIAISON® Rubella IgG result versus the calculated WHO standard concentration of each dilution was used to determine the assay result at the cut-off. The mean cut-off level corresponds to a level of the WHO 1st International Standard of 9.42 IU/mL. Regression analysis of these results with the calculated WHO rubella IgG concentration was evaluated. Linear regression of WHO doses (IU/mL) vs. Index:

<b>Linear regression</b>	<b>Lot 1</b>	<b>Lot 2</b>
R square	0.9987	0.9982
Intercept	0.1081	0.1542
Slope	0.1086	0.0962
Result at WHO cut off	0.98 Index	0.81 Index

2. Comparison studies:

a. *Method comparison with predicate device:*

*Performance Requirements:* All discordant equivocal results were tallied against the investigational device. A consensus approach, with the possibility of using two out of three FDA cleared predicates to reach consensus for comparison was implemented. The “Routine/General Population Cohort” and the “Pregnant Women Cohort” were segregated as distinctive target populations to assess performance of the test. According to special controls requirements a 95% point estimate for positive and negative agreement was requested on each of the populations, as well as the need to satisfy the requirements of containing 50 low positive, 50 high positive and 100 negative specimens in the whole study.

The performance of the LIAISON® Rubella IgG assay was determined by percent agreement among negative samples and percent agreement among

positive samples, with reference method in specific populations. The relevant 95% confidence limits were computed by applying the exact method.

All specimens were tested for the presence of Rubella IgG antibodies using the LIAISON<sup>®</sup> Rubella IgG assay and a predicate. Three FDA cleared devices were chosen as the additional methods to test the predicate device equivocal samples. The classification of samples equivocal by the predicate device was reassigned based on the consensus of the combined 2 out of 3 results from the three ELISA results, i.e. a sample with concordant result by at least two of the three methods was defined following the consensus. If the results by the three methods were either discordant among themselves or equivocal by consensus, the sample was classified as equivocal.

Specimens that were equivocal by both, the LIAISON<sup>®</sup> Rubella IgG assay and the consensus from the three ELISA tests were not included in the percent agreement calculation. Positive or negative results from the LIAISON<sup>®</sup> Rubella IgG assay were considered as non-agreements in the calculation of percent positive agreement and percent negative agreement when the corresponding comparator method result was equivocal. Comparison method: compared number of samples positive on both tests to sum of all positive samples on the reference method + samples equivocal on the comparator method and negative on the LIAISON<sup>®</sup> Rubella IgG. Compared number of samples negative on both tests to sum of all negative samples on the reference method + samples equivocal on the comparator method and positive on the LIAISON<sup>®</sup> Rubella IgG.

*b. Matrix comparison:*

N/A

3. Clinical studies:

*a. Clinical Sensitivity:*

N/A

*b. Clinical specificity:*

N/A

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

Percent positive and percent negative agreement with a comparator method was determined as specified under Comparison Studies (see above).

In this submission, the use of the terms “Routine Cohort” and “General Population Cohort” is interchangeable, and corresponds to all specimens from

individuals referred to the lab for rubella testing, with the exception of pregnant women. These populations were separated because of current medical practice for rubella referrals, and since pregnancy may have an effect on the results of this IgG test.

A total of 2806 prospectively collected frozen specimens were tested in the study using four different US testing sites. Of these, 2608 were from non-selected subjects: 2159 subjects sent to the laboratory for routine rubella IgG testing and 449 pregnant women. Samples were divided randomly and the testing sites were blinded to samples' populations and comparator results prior to LIAISON® testing. Given the lack of statistical power to properly assess the pregnant women negative agreement an unbiased analysis of 100 pre-selected samples was performed as shown below. Of the 2,806 specimens, 198 negative pre-selected subjects were tested: 98 subjects sent to the laboratory for routine Rubella IgG testing and 100 pregnant women tested for Rubella antibodies as part of their routine pre-natal care. (All samples were collected in the U.S. except for the 100 pre-selected negative pregnant women, which were a population from Italy.) The rubella antibody testing performed by the laboratories was used to define the samples as negative. All of the specimens were tested for the presence of Rubella IgG antibodies using the LIAISON® Rubella IgG assay and a FDA cleared rubella chemiluminescence test kit. All tests were performed in a blinded fashion to the testing site, not knowing the expected results and mixing together prospective specimens with pre-selected negative samples. Three FDA cleared devices were chosen as the additional methods to test the predicate device equivocal samples. No equivocal results were found in the initial predicate test of the 100 pregnant women, hence there was no need for consensus result to be performed on this cohort. The following tables compare the results obtained for the LIAISON® Rubella IgG assay and commercially available Rubella tests.

Additional pre-selected negative Pregnant Women study: The original pregnant women study contained 217 specimens and showed a negative agreement of 66.6% (2/3). Unable to ascertain performance based on this result, an additional study with negative pregnant women (at least 100, tested together with at least a 20% of the total number being positive results (100 negatives + X positives) was requested. The sponsor collected 100 pregnant retrospective specimens from 2 sites in Italy, and an additional 232 prospective pregnant specimens (unknown rubella status) from US. All of them were tested together in a blinded fashion. The prospective specimens were subsequently presented pooled with the original pregnant cohort; the retrospective pre-selected negatives were presented in a separate table (see below).

**Prospective Non-selected Subjects**

<b>LIAISON® Rubella IgG Results</b>	<b>Routine Samples Consensus Result<sup>a</sup></b>				<b>Pregnant Women Samples Consensus Result<sup>a</sup></b>			
	Pos.	Equiv.	Neg.	Total	Pos.	Equiv.	Neg.	Total
<b>Pos.</b>	1985	2	3	1990	416	1	2	419
<b>Equiv.</b>	11	0	4	15	4	1	2	7
<b>Neg.</b>	40	6	108	154	13	1	9	23
<b>Total</b>	2036	8	115	2159	433	3	13	449

	<b>% Agreement</b>	<b>95% CI<sup>b</sup></b>	<b>% Agreement</b>	<b>95% CI<sup>b</sup></b>
<b>Positive</b>	<b>97.2% (1984/2042)</b>	<b>96.5 – 97.7%</b>	<b>95.8% (416/434)</b>	<b>93.9 - 97.3%</b>
<b>Negative</b>	<b>92.3% (108/117)</b>	<b>87.0 – 95.9%</b>	<b>64.3% (9/14)<sup>c</sup></b>	<b>39.1 – 84.7%</b>

<sup>a</sup> 2 out of 3 rule using 3 additional FDA cleared assays performed on initial testing equivocal only

<sup>b</sup> Confidence Interval

<sup>c</sup> Number of samples too low to reliably calculate % negative agreement-Additional study with pre-selected negative samples was performed.

**Pre-selected Negative Subjects**

<b>LIAISON® Rubella IgG Results</b>	<b>Routine Samples Consensus Result<sup>a</sup></b>				<b>Pregnant Women Samples</b>			
	Pos.	Equiv.	Neg.	Total	Pos.	Equiv.	Neg.	Total
<b>Pos.</b>	9	0	0	9	0	0	0	0
<b>Equiv.</b>	0	0	0	0	0	0	0	0
<b>Neg.</b>	3	1	85	89	1	0	99	100
<b>Total</b>	12	1	85	98	1	0	99	100

	<b>% Agreement</b>	<b>95% CI</b>	<b>% Agreement</b>	<b>95% CI</b>
<b>Positive</b>	<b>69% (9/13)<sup>b</sup></b>	<b>42.8 – 88.7%</b>	<b>N/A (0/1)</b>	<b>N/A</b>
<b>Negative</b>	<b>100% (85/85)</b>	<b>89.6 – 98.0%</b>	<b>100% (99/99)</b>	<b>97.0 – 100.0%</b>

<sup>a</sup> 2 out of 3 rule using 3 additional FDA cleared assays performed on initial testing equivocal results only

<sup>b</sup> Number of samples too low to reliably calculate % negative agreement

4. Clinical cut-off:

The clinical cut-off for immunity to infection with rubella virus has been determined to be 10 IU/mL, as published in NCCLS I/L6-A, “Detection and Quantitation of Rubella IgG Antibody: Evaluation and Performance Criteria for Multiple Component Test Products, Specimen Handling, and Use of the Test Products in the Clinical Laboratory”.

5. Expected values/Reference range:

*Reference Range:*

below 0.9	Negative
between 0.9 and 1.0	Equivocal
equal to or above 1.0	Positive

Index units. (Index 1 corresponds to 10 IU/mL)

*Expected Values*

The LIAISON<sup>®</sup> Rubella IgG assay was tested with prospectively collected specimens from U.S. subjects routinely sent to the laboratory for rubella IgG testing (n=2159) and from pregnant women (n=449) to evaluate the assay’s performance in these populations. Of the 2159 samples sent to the laboratory for routine rubella IgG testing, 91.7% were positive. Of the 449 pregnant women samples, 96.4% were positive. The distribution of results for IgG antibodies to rubella in these populations as determined by the LIAISON<sup>®</sup> Rubella IgG assay is summarized as follows.

**Prospectively-collected Samples from Subjects sent to the Laboratory for Rubella IgG Testing:**

	<b>N</b>	<b>Negative</b>	<b>Equivocal</b>	<b>Positive</b>	<b>Prevalence</b>
<b>Total</b>	2159	154	15	1990	91.7%
<b>Gender</b>					
<b>Female</b>	1848	127	14	1707	92.4%
<b>Male</b>	310	27	1	282	91.8%
<b>Unknown</b>	1	0	0	1	100%
<b>Age (years)</b>					
<b>&lt; 20</b>	285	24	1	260	91.2%
<b>20 – 39</b>	1427	101	13	1313	92.0%
<b>40 – 59</b>	381	28	2	353	92.6%
<b>&gt;60</b>	61	1	1	59	96.7%
<b>Unknown</b>	5	0	0	5	100%

**Preselected Samples from Pregnant Women**

	<b>N</b>	<b>Negative</b>	<b>Equivocal</b>	<b>Positive</b>	<b>Prevalence</b>
<b>Total</b>	449	13	3	433	96.4%
<b>Age (years)</b>					
<b>&lt;20</b>	66	2	1	63	95.4%
<b>20 – 29</b>	259	8	2	249	96.1%
<b>30 – 39</b>	117	3	0	114	97.4%
<b>&gt;40</b>	6	0	0	6	100%
<b>Unknown</b>	1	0	0	1	100%

**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.