510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K150358

B. Purpose for Submission:

The purpose of this submission is to show that the Gold Standard Diagnostics AIX1000 Rapid Plasma Reagin (RPR) Automated Test System (which consists of the Gold Standard Diagnostics RPR reagents and the AIX1000® Analyzer) is substantially equivalent to the Arlington Scientific Inc. (ASI) RPR Card Test for syphilis on the ASiManager-AT Analyzer.

C. Measurand:

Serum antibodies (cardiolipin and lecithin) against rapid plasma reagin

D. Type of Test:

Non-treponemal macroscopic flocculation test

E. Applicant:

Gold Standard Diagnostics (GSD)

F. Proprietary and Established Names:

Proprietary Name: Gold Standard Diagnostics AIX1000 Rapid Plasma Reagin (RPR) Automated Test System

Common Name: Rapid Plasma Reagin (RPR) Test

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3820, *Treponema pallidum* nontreponemal test reagents

2. Classification:

Class II

3. Product codes:

GMQ

4. Panel:

Microbiology (83)

H. Intended Use:

1. Intended use(s):

The Gold Standard Diagnostics AIX1000 Rapid Plasma Reagin (RPR) Automated Test System is a non-treponemal flocculation test that can qualitatively determine the presence of reagin antibodies in human serum. It may be used to aid in the diagnosis of syphilis when used in conjunction with supplemental treponemal laboratory tests and other clinical information. This test may also be used to detect non-treponemal antibodies in samples serially diluted to establish titer information. This test is not intended for screening blood or tissue donors.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

Prescription use only

4. Special instrument requirements:

The GSD AIX1000 RPR Automated Test System uses the AIX1000 Analyzer. The AIX instrument automates sample preparation and results interpretation.

I. Device Description:

The GSD AIX1000 RPR Automated Test System is a non-treponemal test for the qualitative determination of reagin antibodies in human serum to aid in the diagnosis of syphilis. The test is also used to detect non-treponemal antibodies in samples serially diluted to establish titer information. The system consists of the AIX1000 Analyzer and RPR test reagents. The AIX1000 Analyzer delivers serum from collection tubes into test wells. After the antigen suspension is added, the test wells are then incubated while being shaken. An onboard camera is used to create a high resolution image. This image is analyzed by the proprietary software algorithm to interpret the results.

The RPR test reagents consist of a reactive control, a non-reactive control, and the antigen suspended in a carbon solution. When the antigen is mixed with sera, if antibodies are present, they will bind to the antigen and form black flocculants due to the presence of carbon particles. If no antibodies are present, then the carbon particles remain evenly distributed.

The antigen used in the GSD AIX1000 RPR Automated Test System is a modified VDRL carbon antigen. The formulation is the same as that established by the Centers for Disease Control and Prevention (CDC)[1] containing 0.03% cardiolipin, 0.9% cholesterol, and 0.21% lecithin.

The kit also includes untreated sterile 48 well reaction plates, a reactive control, and a non-reactive control.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   ASI RPR Card Test for syphilis on the ASiManager-AT Analyzer

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

   K111356

3. Comparison with predicate:

Similarities		
Item	**Subject Device: Gold Standard Diagnostics AIX1000 Rapid Plasma Reagin (RPR) Automated Test System (K150358)**	**Predicate Device: Arlington Scientific Inc. (ASI) RPR Card Test for syphilis on the ASiManager-AT Analyzer (K111356)**
Intended Use	The Gold Standard Diagnostics AIX1000 Rapid Plasma Reagin (RPR) Automated Test System is a non-treponemal flocculation test that can qualitatively determine the presence of reagin antibodies in human serum. It may be used to aid in the diagnosis of syphilis when used in conjunction with supplemental treponemal laboratory tests and other clinical information. This test may also be used to detect non-treponemal antibodies in samples serially diluted to establish titer information.	The ASiManger-AT is intended to be used as an integrated digital particle analyzer to objectively interpret the ASI RPR Card Test for syphilis. The ASiManger-AT is designed to provide standardized test interpretation, an initial predictive titer analysis, and provides for storage, retrieval and transmittal of the test results. It is intended to be acquired, possessed and used only by healthcare professionals. For *in vitro* Diagnostic Use

[1] Kennedy, E.J. and Creighton, E.T. Venereal Disease Research Laboratory (VDRL) Slide Test. Syphilis Manual, Chapter 8. 1998. http://www.cdc.gov/std/syphilis/manual-1998/CHAPT8.pdf

Similarities		
Item	**<u>Subject Device:</u> Gold Standard Diagnostics AIX1000 Rapid Plasma Reagin (RPR) Automated Test System (K150358)**	**<u>Predicate Device:</u> Arlington Scientific Inc. (ASI) RPR Card Test for syphilis on the ASiManager-AT Analyzer (K111356)**
	This test is not intended for screening blood or tissue donors.	Only, not intended for screening blood and tissue donors.
Assay Format	Reports qualitative results and titer of non-treponemal antibodies in serially diluted samples	Same
Technology	Flocculation test	Same
Antigen	Modified VDRL carbon antigen	Same
Reported Results	Reactive, non-reactive, titer results	Same
Interpretation	Automated	Same
Differences		
Item	**<u>Subject Device:</u> Cepheid Xpert TV Assay (K151565)**	**<u>Predicate Device:</u> Gen-Probe APTIMA *Trichomonas vaginalis* Assay (K122062)**
Sample Processing	Automated	Manual
Sample Matrix	Serum	Serum or Plasma
Controls	Reactive and non-reactive	Reactive, weak reactive, non-reactive

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

1. CLSI EP7-A2, *Interference Testing in Clinical Chemistry, Approved Guideline* – Second Edition; 2004.

L. Test Principle:

This is a non-treponemal macroscopic flocculation test that uses image capture and analysis to detect the presence of reagin. When reagin antibodies are present in a sample, they bind to their lipid antigens. Charcoal particles added to the solution co-agglutinate with these complexes and form black clumps that are macroscopically visible.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

a. Precision/Reproducibility:

Precision

The within-laboratory precision study was conducted in-house with clinical samples diluted at the following concentrations: low RPR reactivity (<1:8), moderately reactive (1:16), reactive (1:64), highly reactive (1:128), and non-reactive serum (the highly reactive sample was a pooled sample while all the other samples were individual patient sera). Each concentration level was tested in replicates of nine. These nine replicates were spread across five panels (as shown in Table 1 below) that were tested every day for five consecutive days by one operator using one instrument (9 replicates x 5 days = 45 measurements for each concentration tested). The sample panels were masked and randomized. Reactive and non-reactive controls were run each day of testing.

Table 1 – Panel Member Randomization Configuration (Each Cell Denotes a Single Test Replicate)

Panel ID	Sample # 1	Sample # 2	Sample # 3	Sample # 4	Sample # 5	Sample # 6	Sample # 7	Sample # 8	Sample # 9
Panel I	N	MR	MR	N	HR	LR	R	HR	LR
Panel II	MR	HR	N	R	LR	N	MR	LR	N
Panel III	LR	N	R	R	N	MR	LR	R	HR
Panel IV	HR	LR	MR	R	HR	HR	N	LR	MR
Panel V	R	N	LR	MR	HR	R	MR	R	HR

LR = Low RPR Reactivity (< 1:8); MR = Moderately Reactive (1:16); R = Reactive (1:64), HR = Highly Reactive (1:256); and N = Non-Reactive

The acceptance criteria for this study was 95% agreement within +/- 1 dilution for each panel member with a lower bound of the two-sided confidence interval of 90% or greater. Results of the highest dilution ("end point titer results") detected by the GSD AIX1000 RPR Automated Test System are shown below in Table 2.

Table 2 – Results from In-House Precision Study

	End Point Titer Results										
Sample Reactivity	**Non-reactive**	**Neat**	**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**	**≥1:256**	**% Agreement within ± 1 titer (95% C.I.)**
Non-reactive	**45**	0	0	0	0	0	0	0	0	0	100% (93.6% - 100%)
Low Reactive (1:4)	0	0	**2**	**38**	**5**	0	0	0	0	0	100% (93.6% - 100%)
Moderate Reactive (1:16)	0	0	0	0	**27**	**18**	0	0	0	0	100% (93.6% - 100%)

Reactive (1:64)	0	0	0	0	0	**1**	**25**	**15**	**4**	0	97.8% (88.2% - 99.9%)
High Reactive (1:128)	0	0	0	0	0	0	0	**19**	**19**	**7**	100% (93.6% - 100%)
Reactive control	0	**5**	0	0	0	0	0	0	0	0	100% (54.9% – 100%)
Non-reactive control	**5**	0	0	0	0	0	0	0	0	0	100% (54.9% – 100%)

The data presented in Table 2 demonstrates acceptable precision when multiple samples of various concentrations are run on the GSD AIX1000 RPR Automated Test System by a single operator on a single instrument over multiple days.

Reproducibility

To investigate operator-to-operator and instrument-to-instrument variability, six operators, three instruments, and two runs were tested each day over five consecutive days as outlined in the testing schedule below (3 instruments x 6 operators x 2 runs per day x 5 days = 180 observations per panel).

Reproducibility Study Testing Schedule

Operator & Instrument ID	**Day 1**		**Day 2**		**Day 3**		**Day 4**		**Day 5**	
	Run 1	**Run 2**	**Run 1**	**Run 2**	**Run 1**	**Run 2**	**Run 1**	**Run 2**	**Run 1**	**Run 2**
Operator 1, Instrument 1	Panel I		Panel II		Panel III		Panel IV		Panel V	
Operator 2, Instrument 1		Panel II		Panel III		Panel IV		Panel V		Panel I
Operator 3, Instrument 2	Panel III		Panel IV		Panel V		Panel I		Panel II	
Operator 4, Instrument 2		Panel IV		Panel V		Panel I		Panel II		Panel III
Operator 5, Instrument 3	Panel V		Panel I		Panel II		Panel III		Panel IV	
Operator 6, Instrument 3		Panel I		Panel II		Panel III		Panel IV		Panel V

Each operator tested the five sample panels described in Table 1 above. The identity of panel members were masked and randomized. A reactive and a non-reactive control were run on each day of testing. The acceptance criteria for this study was 95% agreement within +/- 1 dilution for each panel member with a lower bound of the two-sided confidence interval of 90% or greater. Results are shown below in Table 3.

Table 3 –Reproducibility Study Results

	End Point Titer Results										
Sample Reactivity	**Non-reactive**	**Neat**	**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**	**≥1:256**	**% Agreement within ± 1 titer (95% C.I.)**
Non-reactive	**54**	0	0	0	0	0	0	0	0	0	100% (94.5% - 100%)
Low Reactive (1:4)	0	0	**0**	**23**	**31**	0	0	0	0	0	100% (94.5% - 100%)
Moderate Reactive (1:16)	0	0	0	0	**7**	**42**	**5**	0	0	0	100% (94.5% - 100%)
Reactive (1:64)	0	0	0	0	0	**0**	**42**	**12**	**0**	0	100% (94.5% - 100%)
High Reactive (1:128)	0	0	0	0	0	0	**1**	**28**	**20**	**5**	98.1% (90.1% - 99.9%)
Reactive control	0	**30**	0	0	0	0	0	0	0	0	100% (90.5% – 100%)
Non-reactive control	**30**	0	0	0	0	0	0	0	0	0	100% (90.5% – 100%)

The results agreements (within ± 1 titer) between runs, between days, between operators, and between instruments are summarized in Table 4 below.

Table 4 - Sources of Variability in the Reproducibility Study

Sample Reactivity	Between-Runs	Between-Days	Between-Operators	Between-Instruments
Non-Reactive Serum	100%	100%	100%	100%
Low RPR Reactivity	100%	100%	100%	100%
Moderately Reactive (1:16)	100%	100%	100%	100%
Reactive (1:64)	100%	97.8%	100%	100%
Highly Reactive (1:128)	100%	100%	98.1%	100%

The data presented in Tables 3 and 4 demonstrate acceptable reproducibility between runs, days, operators, and instruments.

b. Linearity/assay reportable range:

N/A

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

External Controls

The kit contains an external control set, consisting of a non-reactive control (human serum) and a reactive control (human serum reactive for syphilis). The controls are preserved with sodium azide (1mg/ml).

Shipping and Storage Stability

1. Fresh vs. Frozen

A fresh vs. frozen study was conducted to demonstrate that freezing does not alter the performance of human serum samples in the GSD AIX1000 RPR Automated Test System. In this study, 65 RPR non-reactive serum samples were collected within 52 hours from bleed time, refrigerated, and delivered to GSD. Sixty (60) of the RPR non-reactive samples were spiked to create RPR reactive samples. Each concentration was spiked using a single RPR reactive patient sample (e.g., the five 1:8 samples were created using five individual RPR non-reactive samples spiked with one RPR reactive sample).The 65 member panel consisted of 40 samples (40/65 = 61.5%) having low reactivity (20 samples at 1:2 and 20 samples at 1:4), five samples having a titer of 1:8, five samples having a titer of 1:16, five samples having a titer of 1:32, five samples having a titer of 1:64, and five non-reactive samples.

All "fresh" testing was conducted on samples tested within 72 hours from bleed time. All "frozen" testing was conducted on samples that had been stored at -20°C for 24 hours and then thawed at room temperature. The qualitative (non-titer) results from frozen samples were compared with the qualitative results from the fresh samples. Results are summarized in Table 5.

Table 5 – Results from the Fresh vs. Frozen Study

Concentration Level	Percent Agreement	95% Confidence Interval
Non-reactive	5/5 = 100%	54.9% - 100%
Low reactivity (1:2)	20/20 = 100%	96.1% - 100%
Low reactivity (1:4)	20/20 = 100%	96.1% - 100%
Moderately reactive (1:8)	5/5 = 100%	54.9% - 100%
Moderately reactive (1:16)	5/5 = 100%	54.9% - 100%
Reactive (1:32)	5/5 = 100%	54.9% - 100%
Reactive (1:62)	5/5 = 100%	54.9% - 100%

These results indicate that it is acceptable to use frozen serum samples as part of the clinical evaluation for K150358 as their performance was similar to that of freshly collected human serum.

2. Long and Short Term Sample Storage

The conditions claimed for specimen transport and storage were evaluated for human serum tested with the GSD AIX1000 RPR Automated Test System. In this study, RPR non-reactive serum samples were collected within 52 hours from bleed time, refrigerated, and delivered to GSD. The RPR non-reactive samples were spiked with individual RPR reactive samples to create a four-member panel of low reactivity samples (two samples with a titer of 1:2 and two samples with a titer of 1:4). These four samples were tested "fresh" (within 72 hours from bleed time) and then split into two groups that were stored at different conditions (short term storage at 2-8°C or long term storage at -20°C). For each of the four panel members, three aliquots were evaluated per storage condition.

- Samples stored at 2-8°C were tested at selected time points up to 11 days (t = 0, 4, 7, and 11 days).
- Samples stored at -20°C were tested at selected time points up to 18 days (t = 0, 4, 11, and 18 days).

The qualitative (non-titer) results were compared to the results at day 0 ("fresh"). Results are included in Tables 6 and 7.

Table 6 – Results from the Short Term Sample Stability Study

Short Term (2-8°C)		
Time Point	**Percent Agreement**	**95% Confidence Interval**
Day 0	4/4 = 100%	47.3% - 100%
Day 4	4/4 = 100%	47.3% - 100%
Day 7	4/4 = 100%	47.3% - 100%
Day 11	4/4 = 100%	47.3% - 100%

Table 7 – Results from the Long Term Sample Stability Study

Long Term (-20°C)		
Time Point	**Percent Agreement**	**95% Confidence Interval**
Day 0	4/4 = 100%	47.3% - 100%
Day 4	4/4 = 100%	47.3% - 100%
Day 11	4/4 = 100%	47.3% - 100%
Day 18	4/4 = 100%	47.3% - 100%

The results of this study support the following claims for specimen shipping and storage:

- Human serum stored at refrigerated at 2-8°C for 7 days.
- Human serum stored frozen at -20°C for 14 days.

3. Multiple Freeze Thaw Cycles

Stability after multiple freeze thaw cycles was evaluated for human serum tested with the GSD AIX1000 RPR Automated Test System. In this study, RPR non-reactive serum samples were collected within 52 hours from bleed time, refrigerated, and delivered to GSD. The RPR non-

reactive samples were spiked to create an 80 member panel at the following concentrations: 20 samples each at titer levels 1:2 and 1:4, 10 samples each at titer levels 1:8, 1:16, 1:32, and 1:64, and 10 non-reactive samples. Each concentration was spiked using one RPR reactive sample (e.g., the ten 1:8 samples were created using ten individual RPR non-reactive samples spiked with one RPR reactive sample).

All "fresh" testing was conducted on samples tested within 72 hours from bleed time. All "frozen" testing was conducted on samples that had been stored at -20°C for 24 hours and then thawed at room temperature. This cycle was repeated twice more for a total of three freeze thaw cycles. For each freeze-thaw cycle an aliquot was removed and tested. The qualitative (non-titer) results of each freeze thaw cycle were compared with the freshly tested results. Results are included in Table 8.

Table 8 – Results from the Multiple Freeze Thaw Stability Study

Freeze-thaw Cycle	Percent Agreement	95% Confidence Interval
1	90/90 = 100%	96.7% - 100%
2	90/90 = 100%	96.7% - 100%
3	90/90 = 100%	96.7% - 100%

These results support a claim of stability after two freeze thaw cycles.

d. Detection limit:

N/A

e. Analytical specificity:

Cross Reactivity

This study was conducted to evaluate potential cross reactivity in the GSD AIX1000 RPR Automated Test System when non-target antibodies are present (e.g., due to infection or autoimmune disease). A panel of antibodies from 17 different conditions (10 viral, 3 bacterial, and 4 autoimmune conditions) was obtained from serum brokers who confirmed the presence of each disease marker. For each condition, 10-16 individual patient samples were tested. Reactive and non-reactive controls were run on each day of testing. Results are summarized in Table 9 below.

Table 9 - Cross Reactivity

Antibody Source	Number Tested	Number Reactive
Rubella	10	0
Varicella Zoster Virus (VZV)	10	0
Human Immunodeficiency Virus (HIV)	10	0
Hepatitis B	16	0
Hepatitis C	11	0
Epstein Barr Virus (EBV)	10	0

Herpes Simplex Virus (HSV) Type 1	10	0
Herpes Simplex Virus (HSV) Type 2	10	0
Cytomegalovirus (CMV)	11	0
Heterophile Antibodies*	10	0
Toxoplasma gondii	10	0
Leptospira biflexa	10	0
Borrelia burgdorferi	10	0
Systemic Lupus Erythematosus (SLE)	10	0
Rheumatoid Arthritis	10	0
Scleroderma	10	0
Primary Anti-Phospholipid Syndrome	16	0

*Heterophiles samples were tested for infectious mononucleosis (EBV and un-related non-EBV heterophile antibodies).

Interfering Substances

An interfering substance study was conducted to examine if substances that may be present in serum at high concentrations would affect the performance of the GSD AIX1000 RPR Automated Test System. The panel consisted of seven endogenous substances and two prescription drugs that could be used to treat syphilis patients. Five samples, one non-reactive and four reactive samples from four individual patients (with titers of 1:2, 1:4, 1:16, and 1:64), were obtained from a serum broker and were tested in the presence (interferents spiked in-house at the concentration described in Table 6 below) or absence of interferents. The qualitative (non-titer) result was recorded for each sample. The concentrations selected were recommended in CLSI EP7-A2 document. Reactive and non-reactive controls were run on each day of testing. For all interfering substances tested, the RPR reactive samples remained reactive and RPR non-reactive samples remained non-reactive, therefore, the tested substances did not affect the performance of the GSD AIX1000 RPR Automated Test System. Results are shown in Table 10.

Table 10 - Potentially Interfering Substances in Serum

Substance	Concentration	Interference
Hemoglobin	20 g/dL	None Observed
Bilirubin (unconjugated)	15 mg/dL	None Observed
Cholesterol	250 mg/dL	None Observed
Albumin	5 g/dL	None Observed
Gamma Globulin	60 mg/dL	None Observed
Glucose	120 mg/dL	None Observed
Triglyceride	500 mg/dL	None Observed
Antibiotic (Cephalexin)	337 umol/L	None Observed
Antibiotic (Tetracycline)	34 umol/L	None Observed

Carry-over

The purpose of the carry-over study was to uncover the presence of contamination in negative specimens due to carry-over of RPR antibodies during sample processing on the GSD AIX1000

RPR Automated Test System. The study was conducted over three consecutive days on a single AIX1000 Analyzer. One reactive (1:64), one highly reactive (1:128) and two negative samples were tested over five runs. The samples used were from individual patients (not pooled). The qualitative (non-titer) result was recorded for each sample. Highly reactive samples were alternated with non-reactive samples 96 times per run. All 480 replicates of the negative samples were reported as non-reactive, therefore, no evidence of carry-over was observed.

f. Assay cut-off:

No numerical value is given by the GSD AIX1000 RPR Automated Test System. The instrument captures an image and the assay software uses an interpretation algorithm to analyze it. The samples used to validate the assay interpretation algorithm were purchased; reactive samples obtained from serum brokers and non-reactive samples obtained from a clinical laboratory. The samples obtained from the serum brokers contained different concentration (titers) of antibodies. The samples from the clinical laboratory were samples routinely submitted for syphilis test. In all, 560 samples (280 reactive and 280 non-reactive) were used to demonstrate that the algorithm correctly identified RPR reactive and non-reactive samples.

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison was based on the results from the GSD AIX1000 RPR Automated Test System (which consists of the GSD RPR reagents and the AIX1000 Analyzer) compared to the ASI RPR Card Test for syphilis on the ASiManager-AT Analyzer. The testing description and data are listed below in the Clinical Studies section.

b. Matrix comparison:

N/A

3. Clinical studies:

a. Clinical Sensitivity and Specificity

Clinical Studies

i. Prospectively Collected Samples

Prospective sample collection was conducted at two geographically distinct (Southeastern and Western United States) reference laboratories that received samples from local clinics, hospitals, and doctor's offices. Testing was conducted at three sites (one in house and two locations that represented the intended use sites for the GSD AIX1000 RPR Automated Test System). For all testing sites, reactive and non-reactive controls were run on each day of testing. All 765 serum samples were collected prospectively from patient samples with a physician's order to perform syphilis testing. Samples were stored frozen at -20°C for a maximum of 5 months before testing.

All samples that were shipped were transported and stored frozen until testing. All sites performed their own comparator testing.

All prospectively collected samples were "de-identified," therefore only pregnancy and HIV status were recorded. No information regarding gender, age, syphilis stage, or antibiotic use was available.

Seven hundred and sixty five (765) serum samples were tested on both the GSD AIX1000 RPR Automated Test System and the comparator device (a commercially available FDA cleared RPR assay). The initial tests resulted in 26 invalid results (invalid rate = 26/765 = 3.4% with 95% CI: 2.33%-4.93%). All 26 samples were re-tested and gave non-reactive results. The results for the prospectively collected clinical samples are summarized in Tables 11 and 12 below.

Table 11 - Performance of Prospectively Collected Samples (Non-Treponemal Comparator)

Prospective Samples		**Comparator Device**		
		Reactive	Non-reactive	**Total**
GSD AIX1000 RPR Test System	Reactive	21	1*	22
	Non-reactive	1*	742	743
	Total	22	743	765

*The two discrepant samples were tested on a third FDA cleared RPR assay. Both samples were non-reactive on the third RPR assay.

The positive percent agreement and negative percent agreement of the GSD AIX1000 RPR Automated Test System with the comparator device (along with their 95% confidence intervals) are 95.5% (95%CI 77.2% - 99.9%) and 99.9% (95% CI 99.3% - 100%), respectively.

To further investigate the serologic status of the non-treponemal antibody positive samples (NT+), the samples that gave a reactive result either by the GSD AIX1000 RPR Automated Test System or by the comparator device were further tested on an FDA cleared treponemal (TP) assay. Of the 21 samples that were non-treponemal antibody reactive on both the GSD AIX1000 RPR Automated Test System and on the comparator device, only 18 (18/21 = 85.7%) had enough volume for further testing; all 18 samples were positive for TP antibodies. The one sample that was NT+ on the GSD AIX1000 Automated Test System and RPR non-reactive (NT-) on the comparator device was negative for TP antibodies. The one sample that was NT- on the GSD AIX1000 Automated Test System and NT+ on the comparator device was negative for TP antibodies. The 742 samples that were concordant non-reactive with the test device and the comparator device did not receive further TP testing (742/765 = 97.0%).

ii. Retrospectively Collected Samples

In addition, 2,246 retrospectively collected samples from patients referred for syphilis testing were tested on the GSD AIX1000 RPR Automated Test System and on the comparator device. The samples were obtained from two geographically distinct reference laboratories that received samples from local clinics, hospitals, and doctor's offices. The samples were collected between January 2005 and July 2014 (the collection dates for 195 samples were not disclosed) and stored at -20°C until the time of testing. Samples included 607 men and 666 women 10 to 98 years of

age (mean = 35 years). The age range was known for 2,021 of the samples; the gender and age of the remaining samples were not disclosed. No information regarding syphilis stage or antibiotic use was available. All samples were tested in-house by a single operator. Reactive and non-reactive controls were run on each day of testing. The initial tests resulted in six invalid results (percent invalid = 6/2,246 = 0.27%). All six samples were re-tested and gave one reactive and five non-reactive results. The results are summarized in Table 13 below.

Table 13 – Performance of Retrospectively Collected Samples (Non-Treponemal Comparator)

Retrospective Samples		**Comparator Device**		
		Reactive	Non-reactive	**Total**
GSD AIX1000 RPR Test System	Reactive	556	15*	571
	Non-reactive	16*	1659	1675
	Total	572	1674	2246

*The 31 discrepant samples were tested on a third FDA cleared RPR assay. Of the 16 GSD non-reactive and comparator device reactive samples, the third RPR assay called 12 reactive and 4 non-reactive. Of the 15 GSD reactive and comparator device non-reactive samples, the third RPR assay called 11 reactive and 4 non-reactive.

The positive percent agreement and negative percent agreement of the GSD AIX1000 RPR Automated Test System with the comparator device (along with their 95% confidence intervals) are 97.2% (95%CI 95.5% - 98.4%) and 99.1% (95%CI 98.5% - 99.5%), respectively.

To further investigate the serologic status of the non-treponemal antibody positive samples (NT+), the samples that gave a reactive result either by the GSD AIX1000 RPR Automated Test System or the comparator device were further tested on an FDA cleared treponemal (TP) assay. Of the 556 samples that were non-treponemal antibody reactive on both the GSD AIX1000 RPR Automated Test System and on the comparator device, only 404 had enough volume for further TP testing (404/556 = 72.7%). Of the 15 samples that were NT+ on the GSD AIX1000 RPR Automated Test System and RPR non-reactive (NT-) on the comparator device, only three had enough volume for further TP testing (3/15 = 20%). Of the 16 samples that were NT- on the GSD AIX1000 RPR Automated Test System and NT+ on the comparator device, only nine had enough volume for further TP testing (9/16 = 56.3%). A total of 416 samples that were reactive by either the test device or the comparator device received further TP testing. Samples that were concordant non-reactive with the test device and the comparator device did not receive further TP testing. The results of this testing is included in Table 14 below.

Table 14 - Retrospectively Collected Samples (Serologic Status)

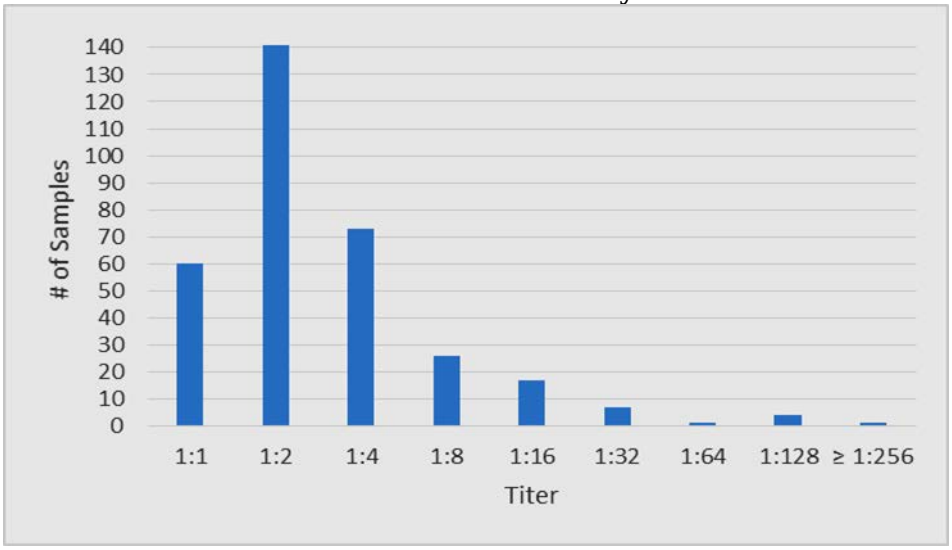
		Comparator Device NT + / Trep +	**Comparator Device NT + / Trep -**	**Comparator Device NT - / Trep +**	**Comparator Device NT - / Trep -**
GSD AIX1000 NT Assay Result	Reactive	366	38	1	2
	Non-Reactive	5	4	N/A*	N/A*

* Samples with concordant non-reactive results by the comparator device and the GSD AIX1000 RPR Automated Test System did not receive further TP testing.

Three hundred thirty (330) of the retrospective collected samples were tested for titer level (330/587 samples collected = 56.2%). The frequency distribution of titer results from samples

that are RPR reactive on the GSD AIX1000 RPR Automated Test System is shown in Figure 1 below.

Figure 1 - Distribution of Titer Results from Samples Designated as RPR Reactive on the GSD AIX1000 RPR Automated Test System.



iii. Retrospectively Collected Samples from Special Populations

Pregnant Women

In addition, 250 samples that were non-reactive for non-treponemal antibodies (NT-) were retrospectively collected from pregnant women at one site (Southeastern United States). The age of these women ranged from 15-44 years old (median = 29 years old) for 163 samples (the age of the remaining samples were not disclosed). The samples were collected between July 2012 and August 2013 (the collection dates for 25 samples were not disclosed) and stored at -20°C until the time of testing. To create non-treponemal antibody reactive (NT+) samples, sera from 30 individual pregnant women were collected and spiked with a pool created by combining highly reactive RPR positive samples.

Sera from 30 pregnant women were obtained and were tested on an FDA cleared Human Chorionic Gonadotropin (HCG) test to confirm the pregnancy status. All sera gave a positive HCG result. The 30 sera were then spiked with a pool of highly reactive (1:128 and 1:64) RPR positive samples. No more than 10% of the volume from the sera of pregnant women was supplanted by spiking. The spiked sera were tested again on the HCG test to confirm a positive result.

These samples were tested on the GSD AIX1000 RPR Automated Test System and on the comparator device. All samples were tested in-house by a single operator. The identity of the samples was masked. For all testing sites, reactive and non-reactive controls were tested with the assay on each day of testing. No invalid results were obtained. The results are summarized in Table 15 below.

Table 15 – Performance in Pregnant Women (Non-Treponemal Comparator)

Pregnant Women		Comparator Device		
		Reactive	Non-reactive	**Total**
GSD AIX1000 RPR Test System	Reactive	30	0	30
	Non-reactive	0	250	250
	Total	30	250	280

The positive percent agreement and negative percent agreement of the GSD AIX1000 RPR Automated Test System with the comparator device (along with their 95% confidence intervals) are 100% (95%CI 90.5% - 100%) and 100% (95%CI 98.8% - 100%), respectively.

HIV Positive Individuals

In addition, 250 samples that were non-reactive for non-treponemal antibodies (NT-) and 30 samples that were reactive for non-treponemal antibodies (NT+) were retrospectively collected from HIV positive individuals at four sites (one Southeastern, one Mid-Western, and two Western States). The age ranged from 19-60 years old (median = 41 years). Sixteen (16) women and 71 men were included in this group (the age and gender of the other samples were not disclosed). The samples were collected between February 2012 and June 2015 (the collection dates for 156 samples were not disclosed) and stored at -20°C until the time of testing.

These samples were tested on the GSD AIX1000 RPR Automated Test System and the comparator device. All samples were tested in-house by a single operator. The identity of the samples was masked and the samples from HIV positive individuals were randomized with samples collected from HIV negative individuals. For all testing sites, reactive and non-reactive controls were tested with the assay on each day of testing. No invalid results were obtained. The results are summarized in Table 16 below:

Table 16 – Performance in HIV Positive Individuals (Non-Treponemal Comparator)

HIV Positive		Comparator Device		
		Reactive	Non-reactive	**Total**
GSD AIX1000 RPR Test System	Reactive	30	0	30
	Non-reactive	0	250	250
	Total	30	250	280

The positive percent agreement and negative percent agreement of the GSD AIX1000 RPR Automated Test System with the comparator device (along with their 95% confidence intervals) are 100% (95%CI 90.5% - 100%) and 100% (95%CI 98.8% - 100%), respectively.

Apparently Healthy Individuals

To determine the percentage of RPR reactivity with the GSD AIX1000 RPR Automated Test System in a population of apparently healthy individuals, 100 serum samples prospectively collected from healthy individuals not at risk for syphilis and for whom a syphilis test had not been ordered (samples were submitted to the source laboratories for routine chemistry testing) were tested with the GSD AIX1000 RPR Automated Test System. All 100 samples were non-reactive with the GSD AIX1000 RPR Automated Test System.

The percentage of RPR reactivity with the GSD AIX1000 RPR Automated Test system in the 765 prospective serum samples collected from two geographically distinct regions of the United States from patients with a physician's order to perform syphilis testing, 2.9% (22/765) were reactive with the GSD AIX1000 RPR Automated Test System.

Correlation with Clinically Diagnosed Syphilis Sera – Various Stages

A panel of sera samples collected from patients clinically positive for syphilis at various stages of the disease was purchased from the University of Washington. The sera consisted of treated and untreated samples at the primary, secondary, and latent stages of syphilis. The age, gender, and collection dates for the samples were not disclosed. The primary syphilis samples given were characterized by documented genital lesion with positive dark field microscopy (if performed) and with reactive treponemal test. The secondary syphilis samples were characterized by documented rash or mucous patches or candylomata lata with reactive treponemal test. And the latent syphilis samples were characterized by having reactive treponemal and non-treponemal test with a non-reactive non-treponemal test for more than a year or for an unknown duration of infection.

The sera were tested on both the GSD AIX1000 RPR Automated Test System and on the comparator device. The sample panel members were masked and the order of testing was randomized. There were no invalid results reported for any of the samples tested. The results are summarized in Table 17 below.

Table 17 – Performance with Clinically Diagnosed Sera (Non-Treponemal Comparator)

	GSD AIX1000 RPR Test System and Comparator Device Results			
Clinical Diagnosis	**# Reactive***	**# Non-reactive***	**% Agreement**	**95% C.I.**
Primary Treated	13	0	100%	79.4% - 100%
Primary Untreated	12	0	100%	77.9% - 100%
Secondary Treated	25	0	100%	88.7% - 100%
Secondary Untreated	25	0	100%	88.7% - 100%
Latent Treated	25	0	100%	88.7% - 100%
Latent Untreated	25	0	100%	88.7% - 100%

*Note: The results of the sample population tested may not be consisted with what has been reported in the literature. It is important to perform follow-up testing on patients suspected of having syphilis.

4. Clinical cut-off**:**

   N/A

5. Expected values/Reference range:

   N/A

N. Instrument Name:

AIX1000 Analyzer

O. System Description:

The AIX1000 is a combination device with a single intended use. The instrument is intended to be used as general purpose laboratory equipment which is labeled or promoted for a specific medical use. The instrument is intended to duplicate manual analytical procedures of a flocculation test by automating all necessary procedural steps. The instrument alone is intended to perform as an 'accessory' which is intended to be used with a device to enable that device to be used in accordance with its intended purpose. The GSD RPR Test is flocculation test kit intended to be used with the AIX 1000 Automated RPR Processor. The kit is intended to be a consumable *in vitro* diagnostic device for the instrument. The complete system (instrument and test kit) is labeled and promoted by GSD for this specific medical use. The System is a qualitative non-treponemal flocculation test to aid in the diagnosis of syphilis using human serum. This test detects non-treponemal antibodies in samples serially diluted to establish their titer information. This test is not intended for screening blood or tissue donors.

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ____X____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes ____X____ or No _____

2. Software:

   The AIX1000 system includes a software package that is required in order for the AIX1000 instrument to operate. The necessary software applications are: AIX1000 Server, AIX1000 System Settings, and AIX1000 Instrument GUI (Graphical User Interface).

   The AIX1000 System Settings software is used to configure the AIX1000 Server (these two software components must always be installed together on the same computer). The AIX1000 Server controls the configurations of each GUI to which it is connected (in the GUI settings the user specifies which Server each GUI is connected to). All software components are pre-installed on the computer included with the AIX1000 instrument.

   FDA has reviewed applicant's Hazard Analysis and software development processes for

this line of product types:

Yes ___X_____ or No _____

3. Specimen Identification:

   Specimens are identified by scanning a barcode or by manual entry.

4. Specimen Sampling and Handling:

   Sample processing is automated by the AIX1000. The AIX1000 is a fully automated microtiter plate processor that is able to completely perform sample processing steps, including dilutions, dispenses, and incubations.

5. Calibration:

   Daily, weekly and monthly calibration and maintenance is required by the end user. These actions include instrument priming, instrument alignment, camera alignment, wash pump calibration, light intensity and camera integration time.

6. Quality Control:

   Quality control is addressed by external reactive and non-reactive controls that are provided with the Gold Standard RPR Assay.

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

N/A

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.