QA RELEASED

AIX1000

Rapid Plasma Reagin (RPR)

Automated Test System

REF **GSD01-1600**

REF **GSD34-1600-R**

FOR IN VITRO DIAGNOSTIC USE ONLY

℞ only
FOR PRESCRIPTION USE ONLY

CE

DAVIS CA, 95618 USA
TEL: 530-759-8000 FAX: 530-759-8012

Contents

1.0 Intended Use 2
2.0 Summary and Explanation 2
3.0 Test Principle 2
4.0 Materials 2
5.0 Storage and Shelf Life of the Test kit and the Ready to Use Components 3
6.0 Precautions and Warnings 3
6.1 Procedural Precautions 3
6.2 Personal Safety 3
6.3 Incident reporting 4
7.0 Materials Required but Not Supplied 4
8.0 Test Procedure 4
8.1 Samples 4
8.2 Preparation of Reagents 4
8.3 Test Procedure 5
9.0 Test Evaluation 6
9.1 Assay Controls 6
9.2 Results Interpretation 7
9.3 Limitations 7
10.0 Performance Data 8
10.1 Prospectively Collected Samples 8
10.2 Retrospectively Collected Samples 8
10.3 Retrospectively Collected Samples from Special Populations 11
10.4 Correlation with Clinically Diagnosed Syphilis Sera – Various Stages 12
10.5 Precision 12
10.6 Reproducibility 13
10.7 Cross Reactivity 14
10.8 Interfering Substances 15
10.9 Carryover 15
11.0 References 15
12.0 Symbols Glossary 16
13.0 Device Manufacturer 17
14.0 UK Responsible Person 17
15.0 Revision History 17

1.0 Intended Use

The Gold Standard Diagnostics (GSD) 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.

2.0 Summary and Explanation

Serologic tests are an important aid in the clinical diagnosis of syphilis, a sexually transmitted disease that occurs in individuals infected with the spirochete *Treponema pallidum (T. pallidum*). Serologic tests for syphilis are divided into two groups: treponemal tests which detect antibodies that are specific to *T. pallidum* and non-treponemal tests which detect non-specific, anti-lipid antibodies (reagin). The detection of reagin has been the basis for the development of a reagent utilizing extracts of various tissue components, namely cardiolipin, lecithin and cholesterol (1-4).

Serologic testing for syphilis consists of an algorithm approach. The traditional algorithm is to screen with a non-treponemal assay and then confirm with a treponemal test. The reverse algorithm involves using an automated treponemal assay followed by a non-treponemal test.

3.0 Test Principle

The GSD AIX1000 RPR Automated Test System consists of the GSD RPR reagents and the GSD AIX1000 Agglutination Analyzer (AIX1000). The AIX1000 delivers the serum in test wells followed by an antigen suspension. The test wells are then incubated while being shaken. An onboard camera creates a high resolution image. The image is then analyzed by the proprietary software algorithm to produce a result. For a reactive serum, black flocculants are formed due to the presence of the carbon particles. For a non-reactive serum, no antibodies are present, and the carbon particles remain evenly distributed. The reactive sera can then be titered using the AIX1000.

4.0 Materials

GSD01-1600: Gold Standard Diagnostics Rapid Plasma Reagin (RPR) Test System x 480 tests (Store at 2-8°C)

- REF GSD01-1600-W Microtiterplates (Pack of 5 x 48-well microtiter plates). 2 units.
- REF GSD01-1600-AS Antigen suspension - 22 mL (ready to use, modified VDRL carbon antigen preserved with sodium azide (1 mg/mL)
- REF GSD01-1600-NC RPR non-reactive control – 0.35mL (ready to use, human serum preserved with sodium azide (1 mg/mL). GREEN CAP
- REF GSD01-1600-PC RPR reactive control – 0.35 mL (ready to use, human serum reactive for syphilis preserved with sodium azide (1 mg/mL). RED CAP

GSD34-1600: GSD Rapid Plasma Reagin (RPR) Test System 34x Bulk Pack

REF GSD34-1600-R Gold Standard Diagnostics RPR Reagent Pack (Store at 2-8°C)

- REF GSD01-1600-AS Antigen suspension – 34 x of 22 mL (ready to use, modified VDRL carbon antigen preserved with sodium azide (1 mg/mL)
- REF GSD01-1600-NC RPR non-reactive control – 34 x 0.35mL (ready to use, human serum preserved with sodium azide (1 mg/mL). GREEN CAP
- REF GSD01-1600-PC RPR reactive control – 34 x 0.35 mL (ready to use, human serum reactive for syphilis preserved with sodium azide (1 mg/mL). RED CAP

REF GSD-1600-W-CASE Microtiterplates (pack of 85 x 48-well microtiter plates). 4 units.

Sold separately:

- REF GSD05-1600-W GSD RPR Microtiterplate - 5 pack sleeve (48-well microtiter plates), 5 plates
- REF GSD-1600-W-CASE Microtiterplates (48-well microtiter plates), 85 plates

5.0 Storage and Shelf Life of the Test kit and the Ready to Use Components

1. The Gold Standard Diagnostics Rapid Plasma Reagin (RPR) Test System and Gold Standard Diagnostics RPR Reagent Pack are shipped at ambient temperature. Store the test kit/any unopened components at 2-8°C. The expiration date of each component is shown on its respective label. The entire kit expires on the earliest expiration date of any of the components.
2. Components, either unopened or opened, may not be used after the expiry date printed on the label of each component.
3. Once opened, the reagents are good for a 90 day period, if kept refrigerated between runs.
4. Do not freeze the antigen suspension. Do not store the antigen suspension in plastic bottles, as this will reduce the shelf life of the product. Do not pour unused portion into another bottle.

6.0 Precautions and Warnings

6.1 Procedural Precautions

1. Do not exchange assay specific components between kits having different lot numbers.
2. Do not reuse microtiter plate wells that have already been used for samples or controls.
3. All reagents must be mixed well before use, and any bubbles formed must be removed from reagent bottles before starting the test.
4. Avoid microbial or any other contamination of reagents.
5. Do not run manually. This is an automated test. The results must be interpreted using the automated image capture and software interpretation algorithm.
6. Ensure that the stir bar has been cleaned by rinsing with Deionized (DI) water and dried with a clean non-abrasive wipe prior to use.
7. **WARNING:** Please note that, although option to retry a sample (due to insufficient sample volume) is available in the AIX1000 software, this feature has not been evaluated and should not be used with the GSD AIX1000 RPR Automated Test System.

6.2 Personal Safety

CAUTION: Handle reagents with care; potentially infectious material. This product includes materials prepared from human serum or plasma that have been tested and found to be non-reactive for antibodies to HIV-1 and HIV-2, HIV-1 RNA or HIV-1 Ag, antibody to hepatitis C virus (HCV) as well as for hepatitis B surface antigen (HBsAg). However, as it is not possible to offer complete assurance that infectious agents are not present, all materials of human origin should be handled as though they might contain potentially infectious agents.

1. All such materials should be handled with caution and treated as being potentially infectious.
2. The antigen suspension and the controls contain sodium azide as a preservative. Sodium azide has the following precautions:
   - Harmful if swallowed.
   - Do not breathe fumes.
   - Do not empty into drains. This material may exhibit characteristics of USA federal EPA Resource Conservation and Recovery Act (RCRA) hazardous waste requiring specific disposal requirements. Check state and local regulations as they may differ from federal disposal regulations. Institutions outside of the United States should check their country's hazardous waste disposal requirements.
   - Wear suitable protective clothing, gloves and eye/face protection.

3. Use disposable gloves and handle all materials used in the assay including samples, waste solution, and reaction wells, cautiously, as though capable of transmitting infectious agents. Consult a physician immediately in the event that contaminated materials are ingested or come in contact with open lacerations, lesions, or other breaks in the skin.

6.3 Incident reporting

NOTICE: Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

7.0 Materials Required but Not Supplied

1. Stir bar (Magnetic Micro, PTFE fluoropolymer covered); required to mix Antigen Suspension. Clean with DI water and store clean, dry stir bar at room temperature between runs. REF 6266
2. Saline solution pH 6.0-7.5; required to prepare low and high titer dilutions of reactive samples. Store according to laboratory guidelines.
3. Serum non-reactive for syphilis diluted 1:50 in saline solution pH 6.0-7.5; required to prepare high titer dilutions of reactive samples. Store according to laboratory guidelines.
4. 96 well flat-bottom microtiter plates; required to prepare high titer dilutions of reactive samples. REF 260836
5. Phosphate Buffered Saline (PBS) solution; required as system wash buffer. REF PBS47
6. Decontamination (Decon) Solution (0.5 M HCL); required for probe needle decontamination. REF HCL050
7. DI Water; required for daily shutdown maintenance priming and cleaning of stir bar.
8. Alcohol wipes; required for daily startup maintenance cleaning of instrument backlights.
9. AIX1000 Instrument. REF 00400

8.0 Test Procedure

8.1 Samples

1. Specimen collection
   Human serum should be used. Blood should be collected by normal venipuncture technique and handled with the proper precautions.
2. Specimen handling and storage
   Fresh specimens may be stored for up to seven days at 2 to 8°C if free of microbial contamination. If longer storage is required, specimens should be stored frozen at -20°C or below for 14 days. Conditions that favor microbial growth should be avoided. A maximum of two freeze thaw cycles can be used if necessary.
   It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.
   Specimens must be equilibrated to room temperature before testing begins. At least 0.3ml of serum is required to perform a screen and titer test.

8.2 Preparation of Reagents

1. Reagents and samples must be equilibrated to room temperature before beginning the test.
2. Store reagents at 2 to 8°C when not in use.
3. Ensure that the Antigen Suspension is shaken well before use.
4. A clean stir bar must be added to the Antigen Suspension to ensure a well-mixed solution. Be sure to place a stir bar into the bottle prior to use. A magnetic micro, PTFE fluoropolymer covered stir bar is recommended.

Note: Proper maintenance of the stir bar must be performed to avoid possible contamination and possible adverse effects on sample results. The stir bar must be removed from the bottle at the end of each testing day or when a new Antigen Suspension bottle is placed in the instrument. Following removal, the stir bar must be rinsed with DI water and dried with a clean non-abrasive wipe, prior to its next use.

8.3 Test Procedure

The test is to be run with the AIX1000. Refer to the AIX1000 User's Manual for more detailed instructions.

1. Place sufficient PBS buffer solution in the Wash 1 bottle position next to the instrument.
2. Turn on the AIX1000 and attached computer. Open the AIX1000 Instrument Manager software, log in, and wait for instrument to complete homing/initialization.
3. Perform daily startup maintenance as prompted by the software: Prime for 10 cycles with wash buffer and clean backlights.

Note: Steps 4-16 differ slightly based on the version of AIX1000 software being used.

If using AIX1000 1.5.4.2 or earlier:

4. Navigate to the **Samples** tab.
5. Click **Add Samples**. Follow pop up window instructions to select control scheme (No Controls, Controls only on first plate, or Controls on all plates) and add samples to the worklist, while placing samples in the sample racks inside the instrument.
6. Use the radio buttons to select an action for each sample (some samples may already be automatically selected for their next action). Choices include **screen** action (qualitative) and **titer** actions. The titer actions include a **low titer** (1:2, 1:4, 1:8, and 1:16) and a **high titer** (1:16, 1:32, 1:64, 1:128 and 1:256).
7. Navigate to the **Microtiter Plates** tab.
8. Verify that all desired controls/samples appear in the MTP layout.
9. Place the appropriate number of reaction plates (and predilution plate, if required) onto the MTP carrier inside the instrument, as indicated on the screen.
10. Navigate to the **Racks** tab.
11. Load reagents and controls in their appropriate locations as indicated on the screen; use appropriate reagent adapters, and check for/remove any bubbles from reagent/control bottles.
12. Verify the placement of all loaded samples, removing any bubbles from sample tubes.
13. Navigate to the **Worklist** tab.
14. Type a name for the worklist in the **Name** field.
15. Click **Lot #** and enter lot number and expiration date of the kit being used (if not already entered).
16. Press **Start** and check reagent and control volumes and MTP placement, as indicated by the **Reagent Loading Wizard** (if wizard is enabled). Continue with step 17 below.

If using AIX1000 2.0.3.0 or later:

4. Navigate to the **Worklist** tab.
5. Type a name for the worklist in the **Name** field.
6. Click **Add Samples**. Follow pop up window instructions to select control scheme (No Controls, Controls only on first plate, or Controls on all plates) and add samples to the worklist, while placing samples in the sample racks inside the instrument.
7. Use the radio buttons to select an action for each sample (some samples may already be automatically selected for their next action). Choices include **screen** action (qualitative) and **titer** actions. The titer actions include a **low titer** (1:2, 1:4, 1:8, and 1:16) and a **high titer** (1:16, 1:32, 1:64, 1:128 and 1:256).
8. Navigate to the **Microtiter Plates** tab.
9. Verify that all desired controls/samples appear in the MTP layout.

10. Place the appropriate number of reaction plates (and predilution plate, if required) onto the MTP carrier inside the instrument, as indicated on the screen.
11. Navigate to the **Racks** tab.
12. Load reagents and controls in their appropriate locations as indicated on the screen; use appropriate reagent adapters, and check for/remove any bubbles from reagent/control bottles.
13. Verify the placement of all loaded samples, removing any bubbles from sample tubes.
14. Navigate to the **Home** tab.
15. Click **Lot #** and enter lot number and expiration date of the kit being used (if not already entered).
16. Press **Start** and check reagent and control volumes and MTP placement, as indicated by the **Worklist Loading Wizard** (if wizard is enabled). Continue with step 17 below.

17. Close the lid and allow the worklist to run to completion.
18. Click **OK** to stop the alarm (if enabled) when worklist is completed.
19. Remove stir bar from Antigen Suspension bottle as prompted, and clean with DI water.
20. Navigate to the **Evaluation** tab.
21. Select a worklist from the dropdown menu. Once selected, all controls (if included in run) and samples from the selected worklist are listed on the left in the Controls and Samples list and one of several worklist views is displayed on the right.
22. Use the icons in the top right corner to toggle between the different worklist views (summary, image, titer, or grid view). Select a well to view by clicking on its image or ID. Selected well is indicated by a white box around the image (grid view) and highlighted ID in the Controls and Samples list. The result of the selected well is displayed in the red bar at the top of tab. Well result is also indicated by color/symbol in the Controls and Samples list. Green indicates non-reactive, red indicates reactive, white indicates skipped or invalid sample well, blue indicates endpoint titer.
23. Use the filter on the bottom left to view only selected groups of samples.
24. Visually review all well images/sample results; use the change and next action options as appropriate.
25. Send results to LIS if desired (if LIS connectivity has been correctly configured).
26. Display/export/save worklist and/or sample reports as desired.
27. Remove all reagents and samples from inside the instrument and close the lid.
28. If completed worklist is the final run of the day, place sufficient DI water in the Wash 1 bottle position next to the instrument. Click the **Power** button on the Title bar to shut down the GUI and instrument.
29. Perform daily shutdown maintenance as prompted by the software: Prime for 50 cycles with DI water and empty waste bottle.

9.0 Test Evaluation

9.1 Assay Controls

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and each laboratory's standard Quality Control Procedure. **At a minimum, the controls provided with the assay should be included in each day of testing.** If controls do not yield the expected response, the assay results should be considered invalid and the assay repeated.

9.2 Results Interpretation

The AIX1000 RPR Automated Test System will automatically generate the results. All possible results and their interpretation are listed below:

AIX1000 RPR Automated Test System Result	Interpretation
Reactive	Indicates that the sample tested contains RPR antibodies.
Non-reactive	Indicates that the sample tested either does not contain RPR antibodies or that it contains RPR antibodies at concentrations below the detectable limits.
Invalid	Retest sample.
Skipped	Indicates that the sample tested had insufficient volume during the test. Retest the sample with sufficient volume.
Titer (e.g. 1:2; 1:4; 1:8; 1:16; 1:32; 1:64; 1:128; 1:256)	Indicates the highest dilution that showed a reactive result.

9.3 Limitations

1. Test results are intended to aid in diagnosis only. As with all serological tests for syphilis, results should always be interpreted in conjunction with additional treponemal serologic test results, the patient's clinical symptoms, medical history, and other clinical and/or laboratory findings to produce a diagnosis of syphilis by disease stage.
2. A reactive test result is not diagnostic of syphilis without additional serologic testing and a full clinical evaluation.
3. The test is not intended for screening blood, plasma, or tissue donors.
4. Results in samples from immunosuppressed patients or from patients with disorders leading to immunosuppression should be interpreted with caution.
5. Specimens with reactive non-treponemal results should be further tested by serial dilution endpoint titration to establish a base line from which changes in titer can be determined.
6. Reactive non-treponemal test results should be followed up with treponemal antibody testing.
7. The use of plasma specimens to establish a baseline from which changes in titer can be determined has not been evaluated.
8. The appearance of reactive assay results may be affected by wide fluctuations in room temperature.
9. With cardiolipin type antigens, biological false reactive results have been reported in diseases or conditions such as infectious mononucleosis, pregnancy, leprosy, malaria, intravenous drug users, autoimmune disease, lupus erythematosus, vaccinia, viral pneumonia, and in people who have been recently immunized.
10. Pinta, yaws, bejel and other treponemal diseases may produce reactive results with non-treponemal tests.
11. A prozone reaction may occur. In a prozone reaction, reactivity with an undiluted sample is inhibited due to high antibody concentrations. The prozone phenomenon may be suspected when an undiluted specimen produces only a weakly reactive result. Therefore, all undiluted specimens producing reactive results should be tested by using the titer actions. In addition, a specimen should be tested for the prozone phenomenon when the clinician suspects syphilis but the undiluted non-treponemal test result is nonreactive.
12. Turbid or hemolyzed samples are not acceptable for this test.
13. Non-treponemal test titers of persons treated in latent or late stages of syphilis or who have become reinfected do not decrease as rapidly as do those from persons in the early stages of their first infection. In fact, these patients may remain "serofast," retaining a low-level reactive titer for life.
14. Factors such as sample degradation, debris, and contaminants may result in false results.
15. The performance of the GSD AIX1000 RPR Automated Test System has not been evaluated for cerebrospinal fluid (CSF).
16. Do not use heated samples with the GSD AIX1000 RPR Automated Test System.

17. Detailed information, such as time between treatments and historical RPR titers, was not available for the Clinically Diagnosed Syphilis Sera from various stages (Section 11.4).

10.0 Performance Data

10.1 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 external controls were tested with the assay 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 (-20°C) for a maximum of five 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 was recorded. No information regarding gender, age, syphilis stage, or antibiotic use was available.

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

Prospective Samples		**Comparator Device**		
		Reactive	Non-reactive	**Total**
GSD AIX1000 RPR Automated 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% (C.I. 77.2% - 99.9%) and 99.9% (C.I. 99.3% - 100%), respectively.

To further investigate the serologic status of the non-treponemal antibody reactive 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 non-treponemal 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%).

10.2 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 sample brokers who collect from multiple sites across the United States. The samples were collected between January 2005 and July 2014 and stored at -20°C until the time of

testing. Samples included 607 men and 666 women ranging in age from 10 to 98 years (mean = 35 years. The gender and age of the remaining samples were not disclosed). All samples were tested in-house by a single operator. Reactive and non-reactive controls were tested with the assay on each day of testing. The initial tests resulted in six invalid results (invalid range of 0.27% with 95% CI: 0.12% - 0.58%). All six samples were re-tested and gave one reactive and five non-reactive results. The results are summarized below:

Retrospective Samples		**Comparator Device**		
		Reactive	Non-reactive	**Total**
GSD AIX1000 RPR Automated 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 AIX1000 RPR Automated Test System reactive and comparator device non-reactive samples, the third RPR assay called 11 reactive and 4 non-reactive.

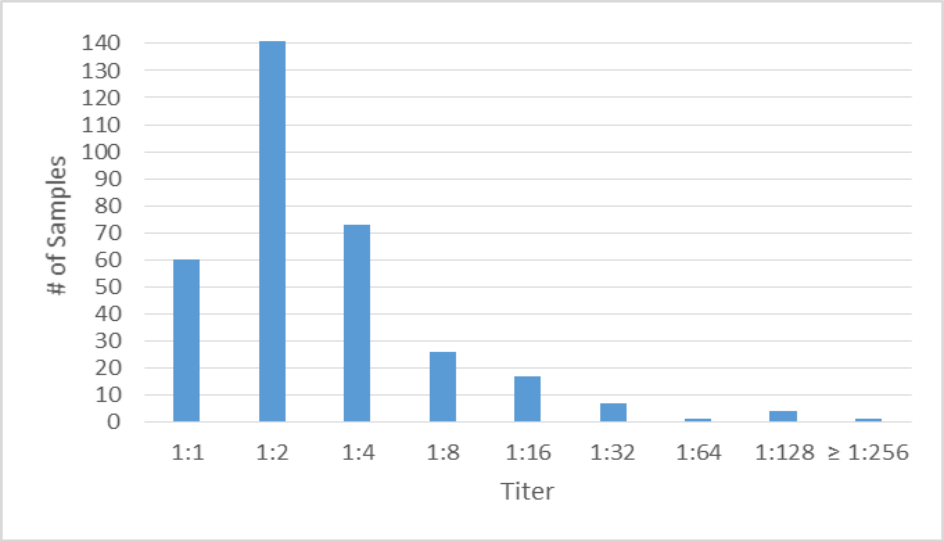
The positive percent agreement and negative percent agreement of the GSD AIX1000 RPR Automated Test System with the comparator device (along with their 95% confidence intervals) are 97.2% (C.I. 95.5% - 98.4%) and 99.1% (C.I. 98.5% - 99.5%), respectively.

To further investigate the serologic status of the non-treponemal antibody reactive 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 Rapid RPR Automated Test System and non-reactive for non-treponemal antibodies (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 are summarized below:

		Comparator Device NT + / Trep +	**Comparator Device NT + / Trep -**	**Comparator Device NT - / Trep +**	**Comparator Device NT - / Trep -**
GSD AIX1000 RPR Automated Test System	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 (330/587 samples collected = 56.2%). The frequency distribution of titer results from samples that were RPR reactive on the GSD AIX1000 RPR Automated Test System is shown in the figure below:



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

10.3 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). The samples were collected between July 2012 and August 2013 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 samples. No more than 10% of the volume from the sera of pregnant women was supplanted by spiking.

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. Reactive and non-reactive controls were tested with the assay on each day of testing. No invalid results were obtained. The results are summarized below:

Pregnant Women		**Comparator Device**		
		Reactive	Non-reactive	**Total**
GSD AIX1000 RPR Automated 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% (C.I. 90.5% - 100%) and 100% (C.I. 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.

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. Reactive and non-reactive controls were tested with the assay on each day of testing. No invalid results were obtained. The results are summarized below:

HIV Positive		**Comparator Device**		
		Reactive	Non-reactive	**Total**
GSD AIX1000 RPR Automated 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% (C.I. 90.5% - 100%) and 100% (C.I. 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.

For reference, based on data from the prospective study in 10.1, of 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.

10.4 Correlation with Clinically Diagnosed Syphilis Sera – Various Stages

A sample panel of sera collected from patients clinically positive for syphilis at various stages of the disease were purchased. 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 condyloma lata with reactive treponemal test. 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 in any of the tests. The results are summarized below.

	GSD AIX1000 RPR Automated 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.

10.5 Precision

To test the precision of the GSD AIX1000 RPR Automated Test System, a within-lab precision study was conducted. This study was conducted in-house with clinical samples at the following RPR concentrations: a low reactive (<1:8), a moderately reactive (1:16), a reactive (1:64), a highly reactive (1:128), and a 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 that were tested every day for five consecutive days by one operator using one instrument. The sample panels were masked and randomized. Reactive and non-reactive controls were run on each day of testing. The results are summarized below.

			Endpoint Titer Results								
Sample Reactivity	**Non-reactive**	**Reactive**	**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**									100% (54.9% – 100%)
Non-reactive control	**5**	0									100% (54.9% – 100%)

10.6 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.

Each operator tested the five sample panels (described in the Precision section above). The sample panels were masked and randomized. Reactive and non-reactive controls were run on each day of testing. The results are summarized below:

			Endpoint Titer Results								
Sample Reactivity	**Non-reactive**	**Reactive**	**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**									100% (90.5% – 100%)
Non-reactive control	**30**	0									100% (90.5% – 100%)

The sample agreement (within ± 1 titer) for between-runs, between-days, between-operators, and between-instruments are summarized below:

Sample Reactivity	Between-Runs	Between-Days	Between-Operators	Between-Instruments
Non-reactive	100%	100%	100%	100%
Low Reactive (1:4)	100%	100%	100%	100%
Moderate Reactive (1:16)	100%	100%	100%	100%
Reactive (1:64)	100%	97.8%	100%	100%
High Reactive (1:128)	100%	100%	98.1%	100%

10.7 Cross Reactivity

The study was conducted to evaluate potential cross reactivity from different disease conditions. 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 respective disease marker. The samples were tested on the GSD AIX1000 RPR Automated Test System. Each condition tested 10-16 individual patient samples. Reactive and non-reactive controls were run on each day of testing. The results are summarized below:

Positive For	Number Tested	Number Reactive
Rubella	10	0
Vericella 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).

10.8 Interfering Substances

The effect of potential interfering substances on samples using the GSD AIX1000 RPR Automated Test System was evaluated. 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 (with a concentration of 1:2, 1:4, 1:16, and 1:64 from four individual patients), were obtained from a serum broker and were tested in the presence (interferents spiked in-house at the concentrations described below) or absence of interferents. The qualitative (non-titer) result was recorded for each sample. The concentrations selected were recommended in the Clinical and Laboratory Standards Institute standard document CLSI EP7-A2. Reactive and non-reactive controls were run on each day of testing. For all substances, the RPR samples remained reactive, therefore the tested substances did not affect the performance of the GSD AIX1000 RPR Automated Test System.

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

10.9 Carryover

The purpose of the carryover study was to uncover the presence of contamination in non-reactive specimens due to carryover 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 instrument. One reactive (1:64), one highly reactive (1:128) and two non-reactive 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 (reactive 1 with non-reactive 1; reactive 1 with non-reactive 2- tested twice; reactive 2 with non-reactive 1; and reactive 2 with non-reactive 2) 96 times per run. All 480 replicates of the non-reactive samples were reported as non-reactive, therefore, no evidence of carryover was observed.

11.0 References

1. Peeling R.W., and Hook E.W. J. Pathol. 208(2): 224-234. 2006.
2. Larsen S., et. Al. Washington: American Public Health Association, 1990. 9th ed.
3. Tomizawa T. and Kasamatsu S. J. Med. Sci. Biol. 19, 305-308, 1966.
4. Van der Sluis, JJ. Genitourin Med. 38, 413-419, 1992.
5. Kim Y.A., et. Al. Journal of Korean Medical Science. 3(1): 13-17, 1988.
6. Centers for disease Control and Prevention. 2011 Sexually Transmitted Disease Surveillance. Syphilis – Reported Cases by Stage of Infection, United States.

12.0 Symbols Glossary

Symbol	Title	Explanation	Reference	Reference number
	Manufacturer	Indicates the medical device manufacturer	ISO 15223-1	5.1.1
	Authorized representative in the European Community/ European Union	Indicates the authorized representative in the European Community/ European Union	ISO 15223-1	5.1.2
	Use-by date	Indicates the date after which the medical device is not to be used	ISO 15223-1	5.1.4
	Batch code/lot number	Indicates the manufacturer's batch code so that the batch or lot can be identified	ISO 15223-1	5.1.5
	Catalogue number	Indicates the manufacturer's catalogue number so that a specific medical device can be identified	ISO 15223-1	5.1.6
	Importer	Indicates the entity importing the medical device into the locale	ISO 15223-1	5.1.8
	Temperature limit	Indicates the temperature limits to which the medical device can be safely exposed	ISO 15223-1	5.3.7
	Do not re-use	Indicates a medical device that is intended for one single use only	ISO 15223-1	5.4.2
	Consult instructions for use	Indicates the need for the user to consult the instructions for use	ISO 15223-1	5.4.3
	In vitro diagnostic medical device	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device	ISO 15223-1	5.5.1
	Contains sufficient for <*n*> tests	Indicates the total number of tests that can be performed with the medical device	ISO 15223-1	5.5.5
℞ only	Rx only	Indicates a prescription in vitro diagnostic product	21 CFR §809.10	-
	CE marking of conformity	Indicates CE marking of conformity	Regulation (EU) 2017/746, Annex V	-
	UKCA (UK Conformity Assessed) marking	Indicates UKCA marking of conformity	UK MDR 2002	-

13.0 Device Manufacturer

Company Name	Gold Standard Diagnostics Corp.
Company Address	2795 2nd St Ste 300, Davis, CA 95618
Country	USA
Phone	530-759-8000
Fax	530-759-8012
Website	www.gsdx.us

14.0 UK Responsible Person

Company Name	LAUNCH DIAGNOSTICS LIMITED
Company Address	Ash House, Ash Road New Ash Green, Longfield Kent, DA3 8JD
Country	England

15.0 Revision History

Revision	Date	Change
K	10/28/2022	Added sections 6.3, 12.0, 15.0, 16.0; added "℞ only" (cover); added sentence about shipping (section 5.0); added Test Procedure steps for AIX1000 2.0.3.0 or later (section 8.3); corrected product names to match labels (section 4.0); corrected address format (section 13.0).
L	02/03/2023	Removed Authorized Representative