

SERODIA®-TP•PA

REAGENTS FOR THE DETECTION OF ANTIBODIES TO *Treponema pallidum*

Part No. 1626 (100 test)

Part No. 1633 (220 test)

INTENDED USE

Serodia®-TP•PA is a qualitative gelatin particle agglutination assay intended to be used for the detection of *Treponema pallidum* antibodies in human serum or plasma as an aid in the diagnosis of syphilis. This product is not cleared or approved by the U.S. Food and Drug Administration (FDA) for use in screening blood or plasma donors.

SUMMARY AND EXPLANATION

The identification of *Treponema pallidum* antibodies aids in the diagnosis of syphilis caused by the microorganisms belonging to the genus *Treponema* and provides epidemiological information on syphilis.

Serological tests for syphilis were first used in 1906 with the development of the nontreponemal (reagin) test by Wasserman.⁽¹⁾ Soon, many tests were developed each with its own modification of a lipoidal antigen, a crude alcohol extract of liver, heart, or other animal organs. The earliest tests were based on the complement fixation procedure of Bordet and Gengou.⁽²⁾ All nontreponemal tests measure both immunoglobulin G (IgG) and M (IgM) anti-lipid antibodies formed by the host in response both to lipoidal material released from damaged host cells early in infection and to lipid from the cell surfaces of the treponeme itself.⁽³⁾ The nontreponemal tests for syphilis in use today are flocculation tests using the basic antigen formula of the Venereal Disease Research Laboratory (VDRL) test, which contains cardiolipin, cholesterol, and lecithin.⁽⁴⁾ The rapid plasma reagin (RPR) test uses a stabilized suspension of VDRL antigen to which charcoal particles have been added.⁽⁴⁾ The antigen is not attached to these particles, but the charcoal is trapped in the lattice formed by the antigen-antibody complex when reactive serum is added. Because of either the lipidic nature of the antigen or some unusual property of the antibodies, the antigen-antibody reaction remains suspended, and flocculation occurs, rather than agglutination or precipitation as in most other serologic tests.⁽⁴⁾

Two treponemal tests have attained standard status for confirmatory syphilis serological testing. The FTA-ABS test is an indirect immunofluorescent antibody test. The FTA-ABS test is sensitive and must be well controlled. The 1+ reading of the standard control, correct conjugate dilution and proper adjustment of the fluorescent microscope are critical to the reliability and reproducibility of the test results. Hemagglutination (MHA-TP) tests using treponemal antigen for *T. pallidum* have gained acceptance since their emergence in mid 1960's⁽⁵⁻⁷⁾ as a confirmatory procedure following a reactive nontreponemal assay or as a screening procedure. This Serodia®-TP•PA test uses the same treponemal antigen as the MHA-TP, but offers the advantage of gelatin particles, which eliminate non-specific reactions with plasma samples.

PRINCIPLE OF THE TEST

The Serodia®-TP•PA test is based on the agglutination of colored gelatin particle carriers sensitized with *T. pallidum* (Nichols Strain) antigen. Serum or plasma samples are serially diluted in Sample Diluent in microplate wells. Sensitized Gelatin Particles are added to respective wells and the contents of the plate mixed by hand or on a tray mixer. The mixture is incubated stationary for 2 hours at room temperature. Serum or plasma containing specific antibodies will react with the antigen-sensitized colored gelatin particles to form a smooth mat of agglutinated particles in the microtitration tray. A compact button formed by the settling of the non-agglutinated particles characterizes negative reactions. The test is designed to be used exclusively with microtitration techniques. The agglutination patterns and interpretation of the test are clear cut and easy to read visually or with the aid of a tray viewer.

MATERIALS SUPPLIED

The kit contains sufficient reagents to perform 100 or 220 qualitative tests. Each kit contains the following reagents and accessories:

Table 1

MAXIMUM ASSAYS	REAGENTS				
	Reconstituting Solution (liq) (A)	Sample Diluent (liq) (B)	Sensitized Particles (lyo) (C)	Unsensitized Particles (lyo) (D)	Positive (Reactive) Control (liq) (E)
100 (20X5)	1 vial X 8 mL	1 vial X 29 mL	5 vials X 0.6mL*	5 vials X 0.6mL*	1 vial X 0.5mL
220 (55X4)	1 vial X 18mL	1 bottle X 60mL	4 vials X 1.5mL*	4 vials X 1.5mL*	1 vial X 0.5mL

* Reconstitution Volume per vial

- A. **Reconstituting Solution:**
Aqueous solution of 0.05M Phosphate Buffer containing 0.2M NaCl, 0.6% normal rabbit serum, and 0.06% sodium azide, at pH 7.00-7.60. The solution is used for reconstituting the Sensitized and Unsensitized Particles.
- B. **Sample Diluent:**
The solution is used for diluting human specimens in the assay. Aqueous solution of 0.05M Phosphate Buffer containing 0.9M NaCl, 2% normal rabbit serum, 0.1% rabbit testicular extract, and 0.1% sodium azide at pH 6.70-7.30.
- C. **Sensitized Particles:**
Lyophilized preparation of colored gelatin particles sensitized with *Treponema pallidum* antigen. At the time of use, add the Reconstituting Solution to the volume indicated on the Particles vial label and in Table 1 above. The rehydrated reagent contains a 1% suspension of sensitized gelatin particles and 0.8% (w/v) sodium azide as preservative.
- D. **Unsensitized Particles:**
Lyophilized preparation of colored gelatin particles. At the time of use, add the Reconstituting Solution to the volume indicated on the Particles vial label and in Table 1 above. The rehydrated solution contains a 1% suspension of unsensitized gelatin particles and 0.8% (w/v) sodium azide as preservative.
- E. **Positive (Reactive) Control Serum:**
This liquid serum containing rabbit antibodies to *T. pallidum* should demonstrate a titer of 1:320 final dilution ± one doubling dilution, when tested according to the procedure described below. Control contains 0.099% sodium azide as preservative.
- F. **Dropper:** 2 pcs. in 100 and 220 Test Kits - To dispense approximately 25 µL per well. One dropper to be used exclusively for dispensing reconstituted Sensitized Particles and the other dropper for dispensing the Unsensitized Particles.
- G. **Non-Reactive Control Serum:** Vial and directions for use provided in separate packaging with this kit.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Polystyrene microplate with "U" shaped wells. Plates should be free from dust, lint, and scratches.
2. Micro-pipette with tips - to dispense 25 µL and 100 µL - for dispensing and diluting serum samples.
3. Pipettes - 1.0 mL serological for reconstitution.
4. Tray mixer (automatic vibratory shaker) - optional
5. Tray viewer - optional

PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. All reagents should be brought to room temperature before use.
3. Proper plate mixing, after the addition of all reagents, is important. Use an automatic vibratory plate mixer or tap the plate sharply with your finger to assure proper mixing. The use of a rotator, such as those used for RPR card test, will not provide adequate mixing.
4. During incubation, cover the microplate with an empty plate or a microplate cover and keep free from vibration.
5. Do not intermix reagents from different kit lots.
6. Ideally, the lyophilized reagents in this kit should be used on the same day as reconstituted. However, when stored at 2-10°C they have a reconstituted stability of 7 days.
7. Some reagents contain small amounts of sodium azide as preservative. Sodium azide may react with lead or copper plumbing, which may result in the formation of highly explosive metal azides. If these reagents are to be disposed of in a laboratory sink, flush with generous amounts of water to avoid azide build-up.
8. Do not pipette patient specimens by mouth (use precision pipettes). All solutions should be handled as if capable of transmitting HIV, Hepatitis or other potentially infectious agents, and disposed of as potential biohazards at "Biosafety Level 2" as recommended in the "Biosafety in Microbiology and Biomedical Laboratories", 1999 or latest edition.⁽⁸⁾
9. Use separate pipette tips for each sample, control and reagent to avoid cross contamination.

STORAGE

Store all reagents at 2-10°C both before and after opening or reconstitution. DO NOT FREEZE. Reconstituted Sensitized and Unsensitized Particles should be used within 7 days. Liquid reagents are stable through the labeled expiration date. Do not use reagents after the expiration date marked on the kit.

SPECIMEN COLLECTION AND PREPARATION

Human serum is the specimen of choice for this kit ⁽⁴⁾, however EDTA, Sodium Citrate or Heparin plasma may be used when serum cannot be obtained. Specimens containing erythrocytes or other visible matter should be centrifuged prior to testing to prevent interference with test results. Store patient serum samples in a refrigerator at 2-8°C if testing is done within 5 days. Sera may be frozen and thawed only once. Heat-inactivation is not necessary for the patient sera. However, previously heat-treated (56°C for 30 minutes) sera may be used. Do not inactivate or freeze plasma. Test plasma samples within 48 hours.

PREPARATION OF REAGENTS

1. Reconstituting Solution, Sample Diluent and Positive (Reactive) Control are liquids ready for use and require no reconstitution.
2. Sensitized Particles and Unsensitized Particles must be reconstituted with the Reconstituting Solution using the volume listed on the vials (See Table 1). Once opened, dispense the appropriate amount of Reconstituting Solution. Mix the reconstituted reagents thoroughly and allow them to stand at room temperature for at least 30 minutes prior to use. Mix particles again prior to dispensing. When the particles are reconstituted, write the date reconstituted plus 7 days out-date on the label. The particles should not be used past this 7 day reconstituted date.

ASSAY PROCEDURE (See Table 2)

Four wells are required for each patient sample and Control(s) run in this assay. Wells # 1 & 2 are for dilution of sample, Well #3 for Unsensitized Particles and Well # 4 for Sensitized Particles. The Positive (Reactive) and Non-Reactive Controls should be included in each assay run.

1. Place 4 drops (100µL) of Sample Diluent in Well #1, and 1 drop (25 µL) in Wells #2 through #4 using a calibrated pipette dropper.
2. Using a micropipette, add 25 µL of patient specimen or Positive or Non-Reactive Control Sera into Wells #1.
3. Mix the contents of Well #1 by filling and discharging the micropipette 5 or 6 times. Then, using the micropipette, transfer 25 µL of the diluted solution from Well #1 into Well #2. Mix the contents of Well #2 in the same manner as stated above and transfer 25 µL into Well #3. Following the same procedure, mix the contents of Well #3 and transfer 25µL into Well #4, mix and discard the 25µL of solution remaining in the pipette after mixing Well #4.
4. Place 1 drop (25µL) of Unsensitized Particles in Well #3, and 1 drop (25µL) of Sensitized Particles in Well #4 using the droppers supplied in the kit.
5. Mix the contents of the wells thoroughly (for approximately 30 seconds) using a plate mixer (automatic vibratory shaker). DO NOT USE A ROTATOR. Then cover the plate with an empty plate or microplate cover, and let stand at room temperature (15-30°C) for 2 hours before reading. The incubation can be extended to overnight without any perceptible difference in patterns.
6. Place the plate onto a flat surface, preferably with a white background, and visually observe the pattern of agglutination in each well. A plate viewer may be used to aid in enhancing the visual interpretation. Carefully place the microplate on a plate viewer with indirect lighting. Observe the agglutination pattern for each patient and Control(s) wells. Ensure each of the Unsensitized Particle wells is non-reactive and interpret the agglutination pattern of the Sensitized Particles using the criteria shown in Table 4:

Table 2

WELL #	1	2	3	4
Sample Diluent (µL) Specimen (µL)	100 25) ↗	25 25) ↗	25 25) ↗	25 25) →
Specimen Dilution	1:5	1:10	1:20	1:40
Unsensitized Particles (µL) (D)			25	
Sensitized Particles (µL) (C)				25
Final Dilution			1:40	1:80
Mix, cover plate, and incubate for 2 hours				
Interpretation				

QUALITY CONTROL

1. The Positive (Reactive) Control should be processed at least once on the day of testing or when a batch of specimens is run and should yield a positive reaction. The Positive (Reactive) Control Serum may be titrated to confirm the 1:320 endpoint, ± one doubling dilution, as additional quality control for the assay. To titrate the Control, follow the Titration Procedure described below.
2. A Non-Reactive Control should be run at least once on the day of testing or when a batch of specimens is run and should yield a negative reaction. A separate Non-Reactive Control is provided with the kit or an in-house specimen can be used. If the Non-Reactive Control is not with the kit or more is needed, the control can be obtained from the Technical Service Dept.
3. Confirm that the reaction with Unsensitized Particles (1:40 final dilution) is Negative (-) for each patient sample.
4. When titrating the Positive (Reactive) Control Serum, confirm that the titer of the Positive (Reactive) Control Serum is 1:320 ± one doubling dilution, (final dilution) for the Sensitized Particles for each assay run. (See Table 3)
5. If an assay does not meet Quality Control parameters listed above, the patient results from that assay should not be reported.

Titration Procedure for Positive (Reactive) Control (See Table 3)

1. Use a "U" shaped microplate sideways. One row (12 Wells) is necessary to test one Positive (Reactive) Control dilution series.
2. Place 4 drops (100 µL) of Sample Diluent into Well #1 and 1 drop (25 µL) into Wells # 2 through # 12.
3. Using a micropipette, add 25 µL of Positive (Reactive) Control Serum into Well #1. Mix by filling and discharging the micropipette 5 or 6 times with the solution in Well #1. Then transfer 25 µL of the diluted solution in Well #1 with a micropipette and transfer it into Well #2. Mix well again and transfer 25 µL into Well #3. Repeat mixing and transfer respectively through Well #12 to obtain serial doubling dilutions. Discard the 25 µL of the solution remaining in the pipette after mixing Well #12.
4. Place 1 drop (25 µL) Unsensitized Particles into Well #3, and 1 drop (25 µL) of Sensitized Particles into Wells # 4 through # 12 using the droppers supplied in the kit.
5. Mix the contents of the wells thoroughly (for approximately 30 seconds) using a plate mixer (automatic vibratory shaker). DO NOT USE A ROTATOR. Then cover the plate with an empty plate or microplate cover, and place it on a level surface. Allow it to stand at room temperature (15-30°C) for 2 hours before reading. The incubation can be extended to overnight without any perceptible difference in patterns.

Table 3

Well #	1	2	3	4	5	6	7 - 11	12
Sample Diluent (µL) (B)	100	25	25	25	25	25	Continue serial dilutions in Wells 7 through 12	25
Reactive Control (µL) (E)	25)	25)	25)	25)	25)	25)		25) →
Dilution	1:5	1:10	1:20	1:40	1:80	1:160		1:10,240
Unsensitized Particles (µL) (D)			25					
Sensitized Particles (µL) (C)				25	25	25		25
Final Dilution			1:40	1:80	1:160	1:320		1:20,480
Mix, cover plate, and incubate for 2 hours								
Interpretation								

INTERPRETATION OF RESULTS

Place the plate onto a flat surface, preferably with a white background, and visually observe the pattern of agglutination in each well as described below. Note: A plate viewer may be used to aid in enhancing the visual interpretation.

Carefully place the microplate on a plate viewer (with indirect lighting), and read the agglutination patterns of the Unsensitized and Sensitized Particles for each specimen and Controls. Readings are scored using the criteria described in Table 4 below:

Table 4

Settling Patterns of Particles	Reading	Interpretation
Particles are concentrated in the shape of a button at the center of the well with a smooth round outer margin.	(-)	Non-Reactive
Particles are concentrated in the shape of a compact ring with a very small "hole" in the center and a smooth round outer margin.	(-)	Non-Reactive
Particles are concentrated in the shape of a compact ring with a "hole" in the center and a smooth round outer margin.	(±)	Inconclusive
Defined large ring with a rough multiform outer margin and peripheral agglutination.	(+)	Reactive
Agglutinated particles spread out covering the bottom of the Well uniformly, edges sometimes folded.	(++)	

REACTIVE: A specimen showing Non-Reactive with Unsensitized Particles (final dilution 1:40) but demonstrating a reaction of + or ++ at any dilution 1:80 or over with Sensitized Particles is interpreted as Reactive in this test. The endpoint titer is determined as the highest serum dilution showing a + or ++ reactive pattern. If a serum sample demonstrates a positive reaction with both the Sensitized and Unsensitized Particles, retest using the Absorption Procedure described below. A reactive treponemal test indicates past or present infection and usually remains reactive for life.

INCONCLUSIVE/INDETERMINATE: A specimen showing Non-Reactive with Unsensitized Particles (final dilution 1:40) but demonstrating a plus/minus reaction with Sensitized Particles at a 1:80 final dilution, is regarded as inconclusive or indeterminate. In such cases, it is recommended that the assay be re-run, or another sample drawn and assayed in two weeks. The result of the repeated test should be reported if found to be Reactive or Non-Reactive. Repeated inconclusive results should be reported as Inconclusive for further follow-up and/or confirmed by other methods such as FTA-Abs.

NON-REACTIVE: Regardless of the reaction pattern with Unsensitized Particles, a specimen showing Non-Reactive with Sensitized Particles at a 1:80 final dilution is regarded as Non-Reactive in this test. A Non-Reactive result indicates no past or present infection, but during incubating-stage syphilis, a Non-Reactive result may also occur.

ABSORPTION PROCEDURE

In most cases, test samples do not show agglutination with Unsensitized Particles. However, if a test sample produces agglutination with both Unsensitized and Sensitized Particles, it should be re-tested after using the following absorption procedure:

- Place 0.95 mL of reconstituted Unsensitized Particles in a small test tube.
- Add 50 µL of test specimen and mix thoroughly using tube mixer and incubate at room temperature for 20 - 30 minutes (mix manually 1 or 2 times).
- Centrifuge for 5 minutes at 2,000 rpm. Place 50 µL of supernatant (absorbed at a 1:20 ratio of test specimen to Unsensitized Particles) to Well #3 of the microplate.
- Add 1 drop (25 µL) of Sample Diluent into Well #4 of the microplate using a micropipette, transfer 25 µL of Well #3 (absorbed 1:20 diluted sample) into Well #4. Mix completely by filling and discharging the micropipette 3 or 4 times with fluid in Well #4, in order to make a doubling dilution, and then discard the 25µl of solution remaining in the pipette after mixing.
- Place 1 drop (25 µL) of Unsensitized Particles in Well #3 and 1 drop (25 µL) of Sensitized Particles in Well #4.
- Follow the original procedure and read the patterns.

LIMITATIONS

- The Serodia[®]-TP•PA test is specific for detecting *Treponema pallidum* antibodies in serum or plasma samples. It does not detect *T. pallidum* directly.
- As with all serological tests for syphilis, interpretation of results obtained with the Serodia[®]-TP•PA syphilis Antibody test must be used in conjunction with the patient's clinical symptoms, medical history and other clinical and/or laboratory findings to produce an overall clinical diagnosis.
- Specimens giving inconclusive results in the assay should be re-tested. A repeated inconclusive specimen should be reported as Inconclusive for follow-up and another specimen drawn in two weeks for testing and/or confirmed by other methods, such as FTA-Abs.
- The Serodia[®]-TP•PA is less sensitive than the fluorescent treponemal antibody absorption (FTA-Abs) test in untreated primary syphilis but compares favorably in all other stages of syphilis.
- All treponemal tests tend to remain reactive following treponemal infection; therefore, they should not be used to evaluate response to therapy.

Because of the persistence of reactivity, probably for the life of the patient, the treponemal tests are of no value to the clinician in determining relapse or re-infection in a patient who has had a reactive Serodia[®]-TP•PA result.

- The Serodia[®]-TP•PA may be reactive in a small percentage (less than 1%) of normal or healthy persons; these false-positive results are often transient, their cause unknown. False positive Serodia[®]-TP•PA results may occur in association with other underlying illnesses.
- The Serodia[®]-TP•PA may be reactive in persons from areas where yaws or pinta was, or is, endemic.
- The Serodia[®]-TP•PA, as do all laboratory tests, performs best in populations at risk for the disease for which the test has been developed.
- Samples from patients with HIV, Leprosy, Toxoplasmosis, H. pylori, and drug addiction may react, on occasion, with either the sensitized or the unsensitized particles, causing false-positive or inconclusive results.

EXPECTED RESULTS

Studies performed on 175 normal male and female and 171 normal pregnant female populations indicated a reactive rate of 2.6% (9/346) from people located in the Southeast United States. Six of these donors were also reactive on RPR testing. The population ranged in age from 14 to 40 years of age. The rate of reactivity will vary according to geographic location and social demographics of the population.

One hundred percent (100%) of samples tested from both treated and untreated patients in both the primary and secondary stages of the disease were detected by the Serodia[®]-TP•PA test. Samples from individuals with autoimmune disease, Toxoplasmosis, H. pylori, IV drug users, and HIV demonstrated an 11.4% (18/158) reactive rate. These same samples were also reactive by the MHA-TP Hemagglutination assay.

PERFORMANCE CHARACTERISTICS

Studies were conducted which compared the Serodia[®]-TP•PA to SERA-TEK MHA-TP, using a total of 422 sera specimens from patients referred by physicians for testing as suspected positive for syphilis based on symptoms and history. A total of 425 specimens from healthy donors considered as normal population, randomly collected including both males and females, were also evaluated. One site used 253 pregnant women as their normal population; and the other site collected and tested 57 suspected positive specimens from pregnant women. These specimens were tested by the sites using a routine RPR test and SERA-TEK MHA-TP test in order to assess the performance of the Serodia[®]-TP•PA kit.

The two clinical study sites selected to perform the testing are located in the Southeastern United States. One facility is a state department of health clinic whose routine population consists of prenatal testing of pregnant females and patients for transmitted disease testing and treatment. The second facility is a university hospital microbiology and immunology laboratory whose general population of all ages consists of blood donors, routine general testing, and infectious disease referrals. All specimens used in this study were serum samples since potential non-specific reactions may occur using plasma with animal cell-based particles used in the reference tests.

The following Tables demonstrate the performance of the Normal and Suspected Positive groups pulled from each site's general population. In each population, the RPR reactive and negative samples are shown using the Serodia[®]-TP•PA test compared to the SERA-TEK MHA-TP test. Relative sensitivity and specificity determinations were made with 95% Confidence Intervals () calculated by the exact method.

Indeterminate interpretations (+/-) in the MHA-TP (3 samples) and Serodia[®]-TP•PA (1 sample) tests were treated as a positive result for the calculations of sensitivity and specificity below.

NORMAL POPULATION

		SERODIA +	SERODIA -
RPR REACTIVE	MHA-TP +	6	0
	MHA-TP -	0	4
RPR NONREACTIVE	MHA-TP +	3	1
	MHA-TP -	0	411

Relative Sensitivity = 6 / 6 plus 3 / 4 = 90% (C.I. = 55.5 - 99.7%)
 Relative Specificity = 4 / 4 plus 411 / 411 = 100% (C.I. = 99.1 - 100%)

SUSPECTED POSITIVE SYPHILIS PATIENTS

		SERODIA +	SERODIA -
RPR REACTIVE	MHA-TP +	387	0
	MHA-TP -	6*	5
RPR NONREACTIVE	MHA-TP +	23	0
	MHA-TP -	0	1

* FTA-ABS results were also positive on these samples

Relative Sensitivity = 387 / 387 plus 23 / 23 = 100% (C.I. = 99.1 - 100%)
Relative Specificity = 5 / 11 plus 1 / 1 = 50% (C.I. = 21.1 - 78.9%)

Six (6) samples from the testing groups resulted in "false positive" results as compared to the reference MHA-TP test. Further evaluation confirmed these samples reactive by FTA-ABS IFA.

Documented Positive Primary and Secondary Samples:

An additional panel of 100 documented VDRL and RPR reactive syphilis sera, obtained from patients medically diagnosed with primary and secondary stage infection, was sent blinded for evaluation to assess the inter-laboratory performance of the Serodia test. Half of the patients were on penicillin therapy at the time the blood sample was drawn. This panel consisted of specimens from 27 treated-primary infection, 23 untreated-primary infection, 24 treated-secondary stage, and 26 untreated-secondary stage patients. Both laboratories identified all samples reactive and demonstrated equivalent reactive interpretations of the Serodia results to the reference MHA-TP titers. Incubating-stage syphilis samples were not specifically identified and tested in these studies.

Potential Cross Reactors:

To evaluate potential interference, 174 samples confirmed positive for each respective disease condition, were assayed in the Serodia®-TP•PA and MHA-TP tests. The bulk of the samples were obtained from each laboratory's routine testing population based on diagnostic condition. Thirty-five specimens were purchased as medically diagnosed for Systemic Lupus Erythematosus (SLE), HIV, Toxoplasmosis, H. pylori, Arthritis, and drug addiction. The total number of disease condition categories and reactive results obtained is listed in the following table. The Serodia®-TP•PA test showed no difference in results as compared to MHA-TP, in the samples evaluated. Serodia®-TP•PA reactive patients were also reactive with the reference MHA-TP assay, but further FTA-ABS evaluation was not performed.

Category	Number Tested	Serodia TP-PA Number Reactive
Drug Users	10	1
Toxo (IgM & IgG)	21	2
SLE	23	0
HIV	81	19
H. pylori	10	1
Arthritis	19	1
Lyme Disease	10	0

REPRODUCIBILITY:

Studies were performed in both laboratories to demonstrate the Inter-day, Inter-Lot, and Intra-Assay reproducibility of the Serodia® TP-PA Test kit. Three lots of kits were used in each laboratory for the Inter-Day testing with a panel of 3 reactive samples at different titers, three non-reactive, and the Reactive Control Serum from the kit. The panel samples were serially diluted into tubes each day and run in duplicate for 5 days on the 3 lots of kits. The Intra-Assay testing was run on one lot with the 6 panel sera samples run 10 times each in each laboratory. The same thirty-five potential cross-reacting samples (above) were sent to each site for evaluation. These purchased samples were diagnosed with other diseases, such as: SLE, HIV, Toxoplasmosis, H. pylori, Arthritis, and drug addiction. Both laboratories identified the same 33 samples as non-reactive and two as reactive in the Serodia test. In all cases, the Intra-Assay, Inter-Lot, Inter-Lab, and Inter-Day reproducibility was 0% C.V. for the Negatives and no greater than +/- one titer for reactive sera and the Reactive Control.

BIBLIOGRAPHY

1. Wassermann, A., Neisser, A., Bruck, C., *Eine serodiagnostische reaktion bei Syphilis*, Dtsch Med Wochenschr. 32:745-6, 1906.
2. Bordet, J., Gengou, O., *Sur l'existence de substances sensibilisatrice dans la plupart des serums antimicrobiens*, Ann Inst Pasteur, 15:289, 1901.
3. Matthews, H.M., Yang, T.K., Jenkin, H.M., *Unique lipid composition of Treponema pallidum (Nichols virulent strain)*, Infect Immun. 24:713-19, 1979.
4. Larsen, S.A., Hunter, E.F., Kraus, S.J., Manual of Tests for Syphilis, Public Health Service Publication no. 411, Washington, D.C. Government Printing Office, 1990.
5. Rathlev, T., *Hemagglutination tests utilizing antigens from pathogenic and apathogenic Treponema pallidum*, WHO Document, WHO/VDI/RES. L77:65, 1965.
6. Tomizawa, T. and Kasamatsu, S., *Hemagglutination tests for diagnosis for Syphilis*, A preliminary report, Jap J Med Sci Bio. 19:305-308, 1966.
7. Rathlev, T., *Hemagglutination test utilizing pathogenic Treponema pallidum for the Serodiagnosis of Syphilis*, Brit. J. Ven. Dis. 43:181-185, 1967
8. US Department of Health and Human Services, Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, Washington, D.C., U.S. Government Printing Office, May 1999. (May be obtained from: Government Printing Office at (202)512-2356 or Superintendent of Documents, U.S. GPO, Washington, D.C. 20402)



Distributed By:
Fujirebio Diagnostics, Inc.
Malvern, PA, USA
(610) 240-3800
(888) 499-9998

Part No.: 000-976 06/2006
Printed in U.S.A.