Read this insert carefully before performing the assay and keep for future reference. The reliability of assay procedure other than those described in this package insert cannot be guaranteed.

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SERODIA®-TP•PA

(For In Vitro Diagnostic Use)

Passive Particle Agglutination Test for Detection of antibodies to Treponema Pallidum

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1. PRINCIPLE OF THE PROCEDURE

SERODIA-TP･PA kit is manufactured using gelatin particle carriers sensitized with purified pathogenic Treponema Pallidum (Nichols Strain). The test is based on the principle that sensitized particles are agglutinated by the presence of antibodies to Treponema Pallidum in human serum/plasma.

2. INTENDED USE

SERODIA-TPPA is a qualitative assay, and the antibody titer of positive samples can be determined in a "semi-quantitative" serial dilution assay. SERODIA-TPPA is intended to serve as an aid in the diagnosis of infection by Treponema Pallidum in serum or plasma specimens. Patient population is patients suspected syphilis.

3. FEATURES

SERODIA-TP･PA offers following features:

(1) Simple test procedure: The test does not require special equipment and the procedure utilizes standard "U" shaped microtray, it is suitable for the testing of small number of specimens as well as mass screening.

(2) Short reaction time: The procedure allows for visual interpretations to be made after a two-hour incubation.

(3) High specificity: The kit uses artificial particles, specifically developed by Fujirebio Inc. as carriers. These particles can minimize the nonspecific agglutination usually observed with use of other carriers.

4. KIT COMPONENTS

The kit contains sufficient reagents to perform 100, 220, 550, and 600 qualitative tests. Each kit contains the following reagents and droppers:

Reagents / Maximum Assays	Reconstituting Solution	Sample Diluent	Sensitized Particles	Unsensitized Particles	Positive Control
100 (5×20)	1 vial × 8 mL	1 vial × 29 mL	5 vials × → 0.6 mL	5 vials × → 0.6 mL	1 vial × 0.5 mL
220 (4×55)	1 vial × 18 mL	1 bottle × 60 mL	4 vials × → 1.5 mL	4 vials × → 1.5 mL	1 vial × 0.5 mL
550 (5×110)	2 vials × 18 mL	2 bottles × 60 mL	5 vials × → 3.0 mL	5 vials × → 3.0 mL	1 vial × 0.5 mL
600 (2×300)	2 vials × 18 mL	2 bottles × 60 mL	2 vials × → 8.0 mL	2 vials × → 8.0 mL	1 vial × 0.5 mL

→ After Reconstitution

A. RS Reconstituting Solution (Liquid)
Use for reconstitution of Sensitized Particles and Unsensitized Particles. This reagent contains 0.06% (w/v) of sodium azide per vial as a preservative.

B. DIL Sample Diluent (Liquid)
Use for dilution of Specimens. This reagent contains 0.10% (w/v) of sodium azide per vial as a preservative.

C. SP Sensitized Particles (Lyophilized)
Lyophilized preparation of gelatin particles coated with Treponema Pallidum. Reconstituted by adding prescribed quantity of Reconstituting Solution. The reconstituted solution contains 0.08% (w/v) of sodium azide per vial as a preservative.

D. USP Unsensitized Particles (Lyophilized)
Lyophilized preparation of tanned gelatin particles. Reconstituted by adding prescribed quantity of Reconstituting Solution. The reconstituted solution contains 0.08% (w/v) of sodium azide per vial as a preservative.

E. PC Positive Control (Liquid)
This control is prepared from rabbit serum containing antibodies to T. Pallidum. The control should show a titer of 1:320 ±1 dilution (final dilution) when tested according to the procedure described in Table 2. This reagent contains 0.10% (w/v) of sodium azide as a preservative.

Droppers (25 μL): 2 droppers (100, 220, 550)
4 droppers (600)

The droppers are designed for the sole purpose of dispensing the reconstituted Sensitized and Unsensitized particles.

5. MATERIALS REQUIRED BUT NOT PROVIDED

Prepare the following laboratory equipment before testing:

(1) "U" shaped microplate ····· FASTEC MICROPLATE U
(2) Diluters ····· 25 μL (0.025 mL)
(3) Calibrated pipette droppers ··· 25 μL (0.025 mL)
(4) Pipettes ····· Micropipette and Volumetric pipettes
(5) Plate Mixer (automatic vibratory shaker)*
(6) Plate Viewer
(7) Tips

*Do not use rotator

6. PREPARATION

(1) Preparation of specimens:
Erythrocytes of other visible components present in the serum or plasma samples should be removed by centrifugation prior to testing in order to prevent interference with test results. Serum inactivation has no affect on test result. Do not inactivate plasma.

Store specimens in a refrigerator at 2-8°C. Do not perform freeze/thaw cycle 2 or more times.

(2) Reconstitution of lyophilized particles:
Reconstitute Sensitized and Unsensitized Particles with

prescribed quantity of Reconstituting Solution, respectively, at room temperature (15-30℃) 30 minutes prior to use. In order to obtain suitable test results, make sure to mix the Sensitized and Unsensitized Particles thoroughly before testing.

(3) After adding Sensitized and Unsensitized Particles into the microplate wells, mix the contents of the wells THOROUGHLY.

(4) During the incubation, cover the microplate and keep free from vibration.

(5) The positive control should be processed at least once on the day of testing or when a batch of specimen are run.

7. TEST PROCEDURE

The test procedure is as follows:

(1) QUALITATIVE TEST (see Table 1 for further details)

1) Place 4 drops (100 μL) of Sample Diluent in well #1, and 1 drop (25 μL) in wells #2 through #4 using calibrated pipette dropper.

2) Add 25 μL of specimen to well #1 using micropipette.

3) Fill the micropipette or a diluter with 25 μL of the diluted solution well #1 and mix well. Transfer 25 μL of the mixture of specimen and Sample Diluent into well #2. Then mix well and repeat this procedure again with wells #2, #3, and #4 to obtain serial doubling dilutions.

4) Place 1 drop (25 μL) of Unsensitized Particles in well #3, 1 drop (25 μL) of Sensitized Particles in well #4 using 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 ROTATOR. Then cover the plate and let it stand at room temperature (15-30℃) for 2 hours before reading. The incubation can be extended to overnight without any perceptible difference in patterns.

Table 1. QUALITATIVE TEST PROCEDURES (SUMMARY)

WELL NO.	1	2	3	4	
Sample Diluent (μL) Test Specimen (μL)	100 25	25 25	25 25	25 25	(discard) 25 μL
Test Specimen Dilution	1:5	1:10	1:20	1:40	
Unsensitized Particles (μL)			25		
Sensitized Particles (μL)				25	
Final Dilution			1:40	1:80	
Mix the content using a plate mixer, cover the plate and incubate for 2 hours					
Interpretation					

It is recommended that specimens showing positive reactions and/or indeterminate in the Qualitative Assay be confirmed in the Semi-Quantitative Assay for accurate interpretation.

(2) SEMI-QUANTITATIVE TEST (see Table 2 for further details)

1) Place 4 drops (100 μL) of Sample Diluent in well #1 and drop 1 drop (25 μL) in wells #2 through #12.

2) Add 25 μL of specimen to well #1, using micropipette.

3) Fill the micropipette or a diluter with 25 μL of the diluted solution well #1 and mix well. Transfer 25 μL of the mixture of specimen and Sample Diluent into well #2. Then mix well and repeat this procedure again to well #12 to obtain serial doubting dilutions.

4) Place 1 drop (25 μL) of Unsensitized Particles in well #3, 1 drop (25 μL) of Sensitized Particles in wells #4 through #12 using droppers supplied in the kit.

5) Mix the contents of the wells thoroughly (for approximately

30 seconds) using plate mixer (automatic vibratory shaker). DO NOT USE ROTATOR. Then cover the plate and let it stand at room temperature (15-30℃) for 2 hours before reading. The incubation can be extended to overnight without any perceptible difference in patterns.

(3) CONTROL TESTS

1) Confirm that each specimen reacts negatively (at the final dilution titer of 1:40) with Unsensitized Particles.

2) Confirm that the Sample Diluent reacts negatively with both Sensitized and Unsensitized Particles for each test run (reagents control).

☆3) Confirm that the titer of the Positive Control is 1:320 ±1 dilution (at the final dilution) for the Sensitized Particles for each test kit (see Table 2).

Table 2. SEMI-QUANTITATIVE AND POSITIVE CONTROL TEST PROCEDURES (SUMMARY)

Well No.	1	2	3	4	5	6		12	
Sample Diluent (μL) Test Specimen or Positive Control (μL)	100 25	25 25	25 25	25 25	25 25	25 25		25 25	(discard) 25 μL
Test Specimen Dilution	1:5	1:10	1:20	1:40	1:80	1:160		1:10240	
Unsensitized Particles (μL)			25						
Sensitized Particles (μL)				25	25	25		25	
Final Dilution			1:40	1:80	1:160	1:320		1:20480	
Mix the content using a plate mixer, cover the plate and incubate for 2 hours									
Interpretation									

8. INTERPRETATION OF RESULTS

Place the microplate gently on a plate viewer, compare the agglutination patterns with those of the reagents control and interpret according to the criteria shown in Table 3.

Table 3. INTERPRETATION OF RESULTS

Settling Patterns of Particles	Reading
Particles concentrated in the shape of a button in the center of the well with a smooth round outer margin	(−)
Particles concentrated in the shape of a compact ring with a smooth round outer margin	(±)
Definite large ring with a rough multiform outer margin and peripheral agglutination	(+)
Agglutinated particles spread out covering the bottom of the well uniformly	(++)

*Specimens which show an indeterminate result (±) should be retested following the Table 1 Test Procedure and test results shall be interpreted according to the criteria in Table 3. A repeated ± should be confirmed by other methods for accurate interpretation.

9. CRITERIA FOR INTERPRETATION

Positive

A specimen showing (−) with Unsensitized Particles (1:40 final dilution) but demonstrating (+) with Sensitized Particles (1:80 final dilution or more) is interpreted as POSITIVE. In semi-quantitative tests, the antibody titer is determined as the final dilution giving a (+) pattern.

Negative

Regardless of the reading of the reaction pattern with Unsensitized Particles, a specimen showing (−) with Sensitized Particles (1:80 final dilution) is interpreted as NEGATIVE.

Indeterminate

A specimen which showing (−) with Unsensitized Particles (1:40 final dilution) and demonstrating (±) with Sensitized Particles (1:80 final dilution) is interpreted as INDETERMINATE.

*For specimens showing positive or indeterminate results with SERODIA-TP･PA test, the results should be confirmed by testing with other methods and retesting on another day using a specimen freshly collected. A comprehensive assessment of the patient's condition should comprise the careful analysis of the patient's clinical symptoms and interpretation of results of available tests for the disease.

10. ABSORPTION PROCEDURE

If a specimen causes agglutination with both Unsensitized and Sensitized Particles or shows indeterminate, it should be retested after the following absorption procedure.

1) Place 0.95 mL of reconstituted Unsensitized Particles in a small test tube.
2) Add 50 μL of specimen into the tube and mix thoroughly. Then, incubate at room temperature (15-30℃) for 20 minutes or more (mix one or twice during incubation).
3) Centrifuge for 5 minutes at 2000 rpm. Take the supernatant (absorbed 1:20 diluted specimen) carefully, then place 50 μL in well #3 of the microplate.
4) Place 1 drop (25 μL) of Sample Diluent in wells #4 through #12. Using a diluter or a micropipette, prepare serial doubling dilutions from wells #3 through #12.
5) Place 1 drop (25 μL) of Unsensitized Particles in well #3, 1 drop (25 μL) of Sensitized Particles in wells #4 through #12 using droppers supplied in the kit.
6) Mix the contents of the wells thoroughly (for approximately 30

seconds) using a plate mixer (automatic vibratory Shaker). DO NOT USE ROTATOR. Then cover the plate and let it stand at room temperature (15-30℃) for 2 hours before reading. The incubation can be extended to overnight without any perceptible difference in patterns.

11. PERFORMANCE CHARACTERISTICS

1) Specificity
When 496 SERODIA-TP non-reactive samples were tested by SERODIA-TP·PA, all samples were negative. The specificity was 100% (95% confidence limits: 98.04-100%).

2) Sensitivity
When 391 SERODIA-TP reactive samples were tested by SERODIA-TP·PA, all samples were positive. The sensitivity was 100% sensitivity (95% confidence limits: 98.04-100%).

3) Reproducibility
When 3 in-house reference samples (final dilution 1:160 or more) were tested 5 consecutive times respectively according to the test procedure, all results were found to be within ±1 doubling dilution.

4) Positive Primary and Secondary Samples
100 Venereal Disease Research Laboratory (VDRL) and Rapid Plasma Reagin (RPR) reactive Syphilis sera, obtained from patients medically diagnosed with primary and secondary stage disease, were used for the evaluation of the SERODIA-TP·PA. 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. All specimens were positive by SERODIA-TP·PA. Incubating-stage syphilis samples were not specifically identified and tested in these studies.

5) Influence by Biological substances

The following biological substances were investigated in respect to their influence on the test result. They were added to make below-mentioned concentrations in the in-house reference samples, which consist of 3 Positive specimens and 3 Negative specimens.

As all results are found to be within ± 1 doubling dilution, specimens are acceptable for testing if they do not exceed any of the concentration written in Table 4.

Table 4. INFLUENCE BY BIOLOGICAL SUBSTANCES

Biological substances & concentrations		No.1	No.2	No.3	No.4	No.5	No.6
Bilirubin (mg/dL)	Not added	1:160	1:640	1:2560	<1:80	<1:80	<1:80
	6.45	1:160	1:640	1:2560	<1:80	<1:80	<1:80
	12.9	1:160	1:640	1:2560	<1:80	<1:80	<1:80
	21.5	1:160	1:640	1:2560	<1:80	<1:80	<1:80
Free Bilirubin (mg/dL)	Not added	1:160	1:640	1:5120	<1:80	<1:80	<1:80
	5.25	1:160	1:640	1:2560	<1:80	<1:80	<1:80
	10.5	1:160	1:640	1:2560	<1:80	<1:80	<1:80
	17.5	1:160	1:640	1:5120	<1:80	<1:80	<1:80
Hemolytic Hemoglobin (mg/dL)	Not added	1:160	1:640	1:2560	<1:80	<1:80	<1:80
	168	1:160	1:640	1:2560	<1:80	<1:80	<1:80
	336	1:160	1:640	1:2560	<1:80	<1:80	<1:80
	560	1:160	1:640	1:2560	<1:80	<1:80	<1:80
Chylomicron (Turbidity)	Not added	1:160	1:640	1:2560	<1:80	<1:80	<1:80
	700	1:160	1:640	1:2560	<1:80	<1:80	<1:80
	1400	1:160	1:640	1:2560	<1:80	<1:80	<1:80
	2300	1:160	1:640	1:2560	<1:80	<1:80	<1:80

6) Potential Cross Reactors

To evaluate the potential cross reactors, 174 samples confirmed

positive for each respective disease condition, were assayed by the SERODIA-TP·PA and SeraTek MHA-TP Assay, which is a hemagglutination test kit. The total number of disease categories and reactive result is listed in Table 5.
SERODIA-TP·PA showed no difference in results as compared to SeraTek MHA-TP Assay, in the samples evaluated. SERODIA-TP·PA reactive patients were also reactive with the SeraTek MHA-TP Assay, but further evaluation by the Fluorescent Treponema Pallidum absorption (FTA-ABS) was not performed.

Table 5. POTENTIAL CROSS REACTORS

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

7) Traceability
Positive Control of SERODIA-TP·PA E. PC was established with in-house standard, because international reference materials for TP antibody detection kit does not exist.

12. CORRELATION RESULTS

The results of the correlation test performed SERODIA-TP using 391 samples are shown in Table 6. The measured values were within ±1 doubling dilution of the control value in the specification, with 100% consistency. As a result of the linear regression analysis, an excellent correlation (correlation coefficient r = 0.964, regression formula y = 1.009x + 0.296) was observed.

Table 6. CORRELATION RESULTS

SERODIA-TP·PA (Final dilution rate)	SERODIA-TP (Final dilution rate)										
	<1:80	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240	1:20480	1:40960<
1:20480<									2	5	3
1:10240								3	2		
1:5120							4	4			
1:2560						8	7				
1:1280					13	27	1				
1:640				36	37	1					
1:320			39	62	1						
1:160		20	71								
1:80		44	1								
<1:80											

13. PRECAUTIONS

1) When a specimen shows reactive or indeterminate in the Qualitative Assay, the specimen should be retested in the Semi-Quantitative Assay. A repeated reactive or indeterminate specimen should be confirmed by other methods (FTA-ABS).

2) This kit is designed for the sole purpose of detecting Treponema Pallidum antibodies in serum/plasma specimens. It does not, however, detect TP directly. The test results should not be used in isolation but used in conjunction with the patient's clinical symptoms, clinical history, and any other available data to produce an overall clinical diagnosis.

3) At the early stage of infection, in case of extremely low concentration of the antibodies, it is recognized that presently available methods (including this kit) for detection of antibodies to TP are not sensitive enough to detect existing antibodies. Therefore, in case infection is suspected, even if test results are negative, specimens should be retested and interpreted in conjunction with the results of other test methods and also

with patient's clinical symptoms, clinical history and any other available data to produce an overall clinical diagnosis.

4) Note that some specimens with very high antibody titer may exhibit the prozoning phenomenon at lower dilutions.
5) When patients specimen injected blood derivatives/preparations including immunoglobulin is interpreted, positive reaction might be observed.
6) Quality assurance is given for each production lot. Do not use the reagents in combination with the kit of other production lots.
7) The kit is designed for use with the "U" shaped FASTEC microplate.
8) When using any equipments or device with SERODIA-TP·PA, follow the instructions given with the equipment/device.
9) Ideally, lyophilized reagents contained in the kit should be used within the same day of reconstitution. However, under proper storage conditions at 2-10℃, they will remain stable for 7 days after reconstitution. In such a case, perform a Control Test to confirm their quality before use. Reconstituted Sensitized and Unsensitized particles should be sealed with sealing film to prevent contamination from any foreign bodies during storage.

14. HEALTH AND SAFETY INSTRUCTION

1) All the kit reagents are intended to "*in vitro*" diagnostic use.
2) Wear disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
3) Do not pipette by mouth.
4) Because no known test method can offer complete assurance that the HIV, Hepatitis B or C virus or other infectious agents are absent, consider patient samples, as potentially infectious and handle them carefully.
5) Any equipment directly in contact with samples should be considered as contaminated products and treated accordingly.

6) Avoid spilling samples or solutions containing samples.
7) Contaminated surfaces should be cleaned 10 % diluted bleach. If the contaminating fluid is an acid, the contaminated surfaces should be first neutralized with sodium bicarbonate, then cleaned with bleach, and dried with absorbent paper. The material used for cleaning should be discarded into a biohazardous waste container.
8) Samples, as well as contaminated material and products should be discarded after decontamination:
   -either by soaking into bleach at a final concentration of 5 % sodium hypochlorite (1 volume of bleach per 10 volumes of contaminated fluid or water) for 30 minutes.
   -or by autoclaving at 121℃ for 2 hours minimum.
   Autoclaving is the best method to inactivate HIV and HBV.
   CAUTION : DO NOT PLACE SOLUTIONS CONTAINING SODIUM HYPOCHLORITE IN THE AUTOCLAVE
9) Do not forget to neutralize and/or autoclave the wash waste solutions or any fluid containing biological samples before discarding them into the sink.
10) The Material Safety Data Sheet is available upon request.
11) Handle any medical waste produced by this assay in compliance with waste related regulations in each country or region.
12) All reagents contain sodium azide as a preservative. Sodium azide may form copper or lead azides in laboratory plumbing. Such azides are explosive. To prevent azide built-up, flush the pipes with a large amount of water if solutions containing azide are discarded into the sink after inactivation.
    Sodium azide: NaN_3 0.10% (w/v); Sample Diluent, Positive Control
    EUH032: Contact with acids liberates very toxic gas.

15. STORAGE

Always store reagents at 2-10℃ when not in use. Do not freeze.

16. SHELF LIFE

Shelf life is indicated by the expiration date printed on the package and on the reagent labels.

17. REFERENCES

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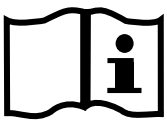
☆ Revised in January, 2018 (Ver. 10)
☆ : Note changes

■GLOSSARY OF SYMBOLS

CE	CE Marking (European directive 98/79/EC on *in vitro* diagnostic medical devices)		
EC REP	Authorised Representative in the European Community		
IVD	*In Vitro* Diagnostic Medical Device	LOT	Batch Code
	Consult Instructions for Use	REF	Catalogue Number
	Fragile, handle with care		Use by
	Manufacturer		Temperature Limitation
	Date of manufacture		Contains Sufficient for <n> Tests
CONTENTS	Contents of Kit	A. RS	Reconstituting Solution
B. DIL	Sample Diluent	C. SP	Sensitized Particles
D. USP	Unsensitized Particles	E. PC	Positive Control
→	After Reconstitution		