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.

REF 207727

SERODIA®-MYCO II

(For In Vitro Diagnostic Use)

PARTICLE AGGLUTINATION TEST KIT FOR DETECTION OF ANTI-MYCOPLASMA PNEUMONIAE ANTIBODIES

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1. ASSAY PRINCIPLE AND ADVANTAGES

SERODIA-MYCO II is an in vitro diagnostic test for the detection of antibodies to *Mycoplasma pneumaniae*, which is manufactured using artificial gelatin particles, sensitized with cell membrane components of *Mycoplasma pneumoniae* (Mac strain). SERODIA-MYCO II is based on the principle that sensitized particles are agglutinated by the presence of antibodies to *Mycoplasma pneumoniae* in human serum.

1. The test procedure of SERODIA-MYCO II is remarkably simple than that of conventional complement fixation test.
2. In order to eliminate as much nonspecificity derived from erythrocyte carrier as possible, the originally developed artificial carrier is used in SERODIA-MYCO II.
3. SERODIA-MYCO II, by using the colored artificial carrier, produces more clear-cut and easy-to-read agglutination patterns compared with hemagglutination patterns.
4. SERODIA-MYCO II requires 3 hours to obtain test results. Readings after overnight incubation is also feasible without a notable change in the patterns.

2. KIT COMPONENTS

The complete SERODIA-MYCO II kit contains the following reagents and droppers.

Reagents / Packaging	B. DIL Sample Diluent (Liquid)	C. SP Sensitized Particles (Lyophilized)	D. USP Unsensitized Particles (Lyophilized)	E. PC Positive Control (Liquid)
25 semi-quantitative assays	1 vial × 30 mL	5 vials × ↓ 1.5 mL	3 vials × ↓ 0.5 mL	1 vial × 0.5 mL

↓ After Reconstitution

B. DIL Sample Diluent (Liquid)
For use in diluting specimens and reconstituting Sensitized Particles and Unsensitized Particles.

C. SP Sensitized Particles (Lyophilized)
Lyophilized preparation of gelatin particles sensitized with *Mycoplasma pneumoniae* (Mac Strain) antigen. At the

time of use, add the prescribed amount of Sample Diluent. The reconstituted reagent includes 0.9% Sensitized Particles.

D. USP Unsensitized Particles (Lyophilized)
Lyophilized preparation of tanned gelatin particles. At the time of use, add the prescribed amount of Sample Diluent.

E. PC Positive Control (Liquid)
1:10 diluted preparation of *Mycoplasma pneumoneae* (Mac strain) antibody positive rabbit serum with Sample Diluent. The Control gives a 1:320 end point titer at final dilution when tested according to the Semi-quantitative Assay Procedure (See Table 3).

Traceability
Positive Control of SERODIA-MYCO II E. PC was established with in-house standard.

Droppers 25μL 2 pcs.
The droppers are designed for the sole purpose of dispensing the reconstituted Sensitized and Unsensitized Particles.

3. INTENDED USE

SERODIA-MYCO II is an in vitro diagnostic test for the detection of anti-*Mycoplasma pneumoniae* antibodies in human serum.
SERODIA-MYCOII is a qualitative assay, and the antibody titer of positive samples can be determined in a "semi-quantitative" serial dilution assay. SERODIA-MYCOII is intended for use in assisting in the diagnosis of mycoplasma pneumonia. Patient population is patients suspected mycoplasma pneumonia.

4. MATERIALS REQUIRED BUT NOT PROVIDED

Prepare the following laboratory equipments for use with this kit:

1. Equipments required for microtiteration technique
   1) "U" shaped microplate
      .....FASTEC MICROPLATE U
   2) Diluter for 25μL (0.025 mL)
      .....For diluting specimens
   3) Dropper* calibrated for 25μL (0.025 mL)

*Droppers supplied in the kit are used exclusively for dropping Sensitized and Unsensitized Particles. Prepare another calibrated dropper for use with Sample Diluent.

   4) Plate mixer — optional
      .....Automatic vibratory shaker (not a rotating mixer) to mix contents thoroughly
   5) Plate viewer — optional
      .....For reading
2. Pipettes
   25μL and 50μL micropipettes with tips
      .....For dispensing and diluting specimens
   0.2 mL, 2.0 mL and 5.0 mL volumetric pipettes
      .....For absorption procedures and reconstitution of lyophilized reagents
3. Test tubes

5. PROCEDURAL PRECAUTIONS

1. Erythrocytes or other visible components present in the serum specimens should be removed by centrifugation prior to testing to prevent interference with the test results. Serum inactivation has no affect on test result.
2. Mix reconstituted Sensitized and Unsensitized Particles thoroughly before use.
3. After dropping Sensitized and Unsensitized Particles, mix thoroughly the contents of the microplate well.
4. During incubation, cover the microplate and avoid vibrations.
5. Store specimens in a refrigerator at 2-10°C. Do not perform freeze/thaw cycle 2 or more times. Heat-inactivation is not necessary for the patient sera. However, previously heat-treated sera may be used.
6. Specimens were mixed with various concentrations of potential interference substances to confirm their effects. Even with the use of concentrations of up to 19.1 mg/dL of bilirubin F, 20.0 mg/dL of bilirubin C, 489 mg/dL of Hemolytic hemoglobin, and 1440 FTU of chyle, no influence in reactivity with SERODIA-MYCOII was observed in any of the specimens.

Influences of interfering substances

	Positive specimen			Negative specimen		
	P-1	P-2	P-3	N-1	N-2	N-3
Bilirubin·F Blank	1:80	1:160	1:160	Negative	Negative	Negative
Bilirubin·F 19.1 mg/dL	1:80	1:160	1:160	Negative	Negative	Negative
Bilirubin·C Blank	1:80	1:160	1:160	Negative	Negative	Negative
Bilirubin·C 20.0 mg/dL	1:80	1:160	1:160	Negative	Negative	Negative
Hemolytic hemoglobin Blank	1:80	1:160	1:160	Negative	Negative	Negative
Hemolytic hemoglobin 489 mg/dL	1:80	1:160	1:160	Negative	Negative	Negative
Chyle Blank	1:80	1:160	1:160	Negative	Negative	Negative
Chyle 1440 FTU	1:80	1:160	1:160	Negative	Negative	Negative

6. TEST PROCEDURES

1. Preparation of Reagents

Reconstitute Sensitized Particles, Unsensitized Particles with the prescribed amount of Sample Diluent 30 minutes before testing.

2. Qualitative Assay (See Table 1)

1) Using a calibrated dropper, place 100 µL (4 drops of 25µL) of Sample Diluent in well #1, and 25µL (1 drop of 25µL) in wells #2 and #3.
2) Using a micropipette*, add 25µL of specimen into well #1.
3) Using a diluter or micropipette*, prepare a two-fold dilution from wells #1 through #3 (or more).

***Procedure without micropipette**

Place precisely 25µL of the specimen into well #1 using a diluter and perform dilution from well #1 through #3. Otherwise, dispense 25µL of Sample Diluent into well #2 and #3, and then place 25µL of 1:5 diluted specimen prepared separately (e.g. mixture of 0.2 mL of the Sample Diluent and 50µL of the serum specimen pipetted into a small test tube) into well #2 using a diluter and perform dilution at well #2 and #3.

4) Using one of the droppers supplied in the kit, add 25µL (1 drop of 25µL) of Unsensitized Particles to well #2. Using the other dropper supplied in the kit, 25µL (1 drop of 25µL) of Sensitized Particles to well #3.

5) Mix the content of the wells thoroughly for about 30 seconds using a plate mixer (Be careful not to splatter the contents of the wells). Then cover the plate and allow it stand at room temperature (15-30ºC) for 3 hours before reading agglutination patterns on the plate viewer. The incubation may be extended overnight without any perceptible difference in patterns.

Table 1. Qualitative Assay Procedure

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

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

3. Semi-quantitative Assay (See Table 2)

1) Using a calibrated dropper, place 100µL (4 drops of 25µL) of Sample Diluent in well #1, and 25µL (1 drop of 25µL) in wells #2 through #8 (or more).
2) Using a micropipette*, add 25µL of specimen into well #1.

3) Using a diluter or micropipette*, prepare a two-fold dilution from wells #1 through #8 (or more).

***Procedure without micropipette**

Place precisely 25µL of the specimen into well #1 using a diluter and perform dilution from wells #1 through #8 (or more). Otherwise, dispense 25µL of Sample Diluent in wells #2 through #8 (or more) and then place 25µL of 1:5 diluted specimen prepared separately (e.g. mixture of 0.2 mL of the Sample Diluent and 50µL of the serum specimen pipetted into a small test tube) into well #2 using a diluter and perform dilution from well #2 through #8 (or more).

4) Using one of the droppers supplied in the kit, add 25µL (1 drop of 25µL) of Unsensitized Particles to well #2. Using the other dropper supplied in the kit, 25µL (1 drop of 25µL) of Sensitized Particles to wells #3 through #8 (or more).
5) Mix the content of the wells thoroughly for about 30 seconds using a plate mixer (Be careful not to splatter the contents of the wells). Then cover the plate and allow it stand at room temperature (15-30ºC) for 3 hours before reading agglutination patterns on the plate viewer. The incubation may be extended overnight without any perceptible difference in patterns.

Table 2. Semi-quantitative Assay Procedure

WELL NO.	1	2	3	4	5	6	12
Sample Diluent (µL)	100 or 25	25	25	25	25	25	25
Specimen (µL)	25 (1:5)	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	25
Final Dilution		1:20	1:40	1:80	1:160	1:320	1:20480
Interpretation	Mix using a plate mixer, cover the plate and incubate for 3 hours						

4. Control Test

1) Confirm that the reaction of each specimen and Unsensitized Particles (1:20 final dilution) is negative (-).
2) Confirm that the mixture of Sample Diluent both with Sensitized Particles and Unsensitized Particles show negative (-) for each run of tests (Reagent Control).
3) Confirm that the titer of Positive Control is 1:320 at final dilution according to the test procedure outlined in Table 3 for each test kit.

The Positive Control is pre-diluted to 1:10. Place 25µL (1 drop of 25µL) of Sample Diluent into wells #3 through #12. Then add 50µL of the Positive Control to well #2 and perform the test following the Semi-quantitative assay procedure.

Table 3. Positive Control Test Procedure

WELL NO.	1	2	3	4	5	6		12
Sample Diluent (µL)		25	25	25	25	25		25
Positive Control (µL)		50 →	25	25	25	25		25 (discard) 25 µL
Test Specimen Dilution		1:10	1:20	1:40	1:80	1:160		1:10240
Unsensitized Particles (µL)		25						
Sensitized Particles (µL)			25	25	25	25		25
Final Dilution		1:20	1:40	1:80	1:160	1:320		1:20480
Mix using a plate mixer, cover the plate and incubate for 3 hours								
Interpretation								

7. INTERPRETATION

1. Reading of reaction patterns

Place the microplate gently on a plate viewer and compare the agglutination patterns with those of the Reagent Control and interpret according to the criteria shown in Table 4.

Table 4. Interpretation

Settling Patterns	Reading
Definite compact button in center of well with a smooth round outer margin.	(-)
Particles settle to form a small ring with a smooth round outer margin	(±)
Definite large ring with firmly agglutinated particles spread within the ring	(+)
Agglutinated particles spread out to cover the bottom of the well entirely under the uniform agglutination	(++)

2. Criteria for interpretation

Positive

A specimen showing (-) with Unsensitized Particles (1:20 final dilution) but demonstrating (+) or more with Sensitized Particles (1:40 final dilution) is interpreted as POSITIVE. The end 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 (1:40 final dilution) is interpreted as NEGATIVE.

Indeterminate

A specimen showing (-) with Unsensitized Particles (1:20 final dilution) and demonstrating (±) with Sensitized Particles (1:40 final dilution) is interpreted as INDETERMINATE.

For specimens showing positive or indeterminate results with SERODIA-MYCO II, 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.

8. ABSORPTION PROCEDURE

If a specimen shows more than (±) agglutination patterns both with Unsensitized Particles and Sensitized Particles, the specimen should be retested after performing the following Absorption Procedure:

1) Dispense 450µL of Unsensitized Particles, reconstituted with the prescribed amount of Sample Diluent, into a small test tube.
2) Add 50µL of the specimen, mix thoroughly and incubate at room temperature (15-30ºC) for 30 minutes (Mix once or twice during incubation).
3) Centrifuge at 2000 r.p.m. for 5 minutes. Place 50µL of the supernatant (absorbed 1:10 diluted specimen) into well #2. Dispense 25µL (1 drop of 25µL) of Sample Diluent into wells #3 through #12. Using a diluter or micropipette, prepare a 2^n dilution from wells #2 through #12.
4) Using one of the droppers supplied in the kit, add 25µL (1 drop of 25µL) of Unsensitized Particles to well #2. Using the other dropper supplied in the kit, 25µL (1 drop of 25µL) of Sensitized Particles to wells #3 through #12.
5) Mix the content of the wells thoroughly for about 30 seconds using a plate mixer (Be careful not to splatter the contents of the wells). Then cover the plate and allow it stand at room temperature (15-30ºC)

for 3 hours before reading agglutination patterns on the plate viewer. The incubation may be extended overnight without any perceptible difference in patterns.

9. PERFORMANCE CHARACTERISTICS

1. Specificity

When in-house reference samples are tested according to the prescribed procedures, the 5 negative reference samples show NEGATIVE results and the 5 positive reference samples show POSITIVE results.

2. Sensitivity

When the Positive Control supplied in the kit is tested according to the prescribed procedure, indicated titer is 1:320 at the final dilution. (±1 dilution)

3. Reproducibility

When in-house reference samples are tested 5 consecutive times respectively according to the test procedure, all results are found to be within one doubling dilution.

10. CORRELATION

86 positive specimens were tested by both SERODIA-MYCO II and FUJIREBIO's In-house PHA test and the following results were obtained.

Specimens tested	N=86
Range of titers	1:40 ~ 1:10240
Correlation (±1 dilution)	
	96.5% (83/86)

11. PRECAUTIONS

1. SERODIA-MYCO II is designed for the sole purpose of detecting anti-*Mycoplasma pneumoniae* antibody. It does not detect *Mycoplasma pneumoniae* directly. Therefore, positive results does not indicate a conclusive *Mycoplasma pneumoniae* infection diagnosis. 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.
2. There is a possibility that an extremely low concentration of the antibody cannot be detected by this test. In some patients infected with *Mycoplasma pneumoniae*, antibodies are not produced or a very small amount of antibodies are produced. Specimens of those patients may show negative results with SERODIA-MYCO II. When infection is suspected, even if the specimen shows negative results with SERODIA-MYCO II, the patient specimen should be retested at different time intervals and 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.
3. Note that some specimens with very high antibody titer may exhibit the prozoning phenomenon at lower dilutions.
4. Quality assurance is given for each production lot. Do not use the reagents in combination with a kit of a different production lot.
5. Quality standards of SERODIA-MYCO

II are set using FASTEC "U" shaped microplate available separately by FUJIREBIO INC.

6. When using any equipments or device with SERODIA-MYCO II, follow the instructions given with the equipment/device.
7. The lyophilized reagents must be used on the day of reconstitution. However, if they are stored at 2-10ºC, they can be used up to 5 days later. 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.
8. Avoid freezing the reagents contained in the kit.

12. 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 121ºC 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 0.15% (w/v)
EUH032: Contact with acids liberates very toxic gas.

13. STORAGE
Store the reagents of the SERODIA-MYCO II kit at 2-10ºC.

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

15. PACKAGE
25 semi-quantitative assays

16. REFERENCES

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5) Taylor, P.: Evalution of an indirect Hemagglutination kit for the rapid serogical diagnosis of *Mycoplasma pneumoniae* infections. J. Clin. Pathol., 32:280, 1979
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☆ Revised in April, 2015 (Ver. 8)
☆ : 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
	Contains Sufficient for <n> Tests	CONTENTS	Contents of Kit
B. DIL	Sample Diluent	C. SP	Sensitized Particles
D. USP	Unsensitized Particles	E. PC	Positive Control
→	After Reconstitution		