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SERODIA[®]-AMC

(For In Vitro Diagnostic Use)

**A REAGENT FOR DETECTION AND TITRATION OF
THYROID MICROSOMAL ANTIBODIES**

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1. INTENDED USE

SERODIA-AMC is a semi-quantitative microtiter particle agglutination test for the in vitro diagnostic detection and titration of microsomal antibodies in human serum.

2. SUMMARY AND EXPLANATION

Autoimmune diseases can comprise conditions in which structural or functional damage, or both, is produced by humoral and cell-mediated immune reactions with normal components of the body. These may be classified as tissue specific or generalized.(1) An organ specific disease such as chronic thyroiditis (Hashimoto's disease) may commonly produce antibodies to thyroglobulin or microsomal antigen of the thyroid. Autoimmune thyroid disease is common, affecting approximately 1% of the population while subclinical, focal thyroiditis and/or circulating thyroid antibodies can be found in about 15% of otherwise healthy subjects who are euthyroid.(1,2) In addition to being found in cases of thyroiditis, these antibodies may be found with other thyroid disorders, such as primary myxedema, hyperthyroidism, goiter and thyroid tumors.(1)

Microsomal antibodies can be demonstrated by several procedures, such as passive agglutination. SERODIA-AMC is prepared using gelatin particles sensitized with purified microsomal antigen. Since thyroid autoimmune disease may demonstrate an immunological response to antigens other than microsomal antigen, SERODIA-AMC should always be used in conjunction with clinical findings and other immunological thyroid tests. A number of references pertaining to antibody agglutination tests for microsomal antibody in thyroid diseases have been published.(3-13)

3. PRINCIPLE OF THE TEST

SERODIA-AMC is based on the agglutination of gelatin particle carriers sensitized with microsomal antigen, extracted and purified from human thyroid tissue. Serum containing specific antibodies will

react with the microsomal antigen-sensitized colored gelatin particles to form a smooth mat of agglutinated particles in the microtitration plate. Negative reactions are characterized by a compact button formed by the settling of the nonagglutinated particles. The test is designed to be used exclusively with microtitration techniques. The agglutination patterns and interpretation are clear cut and easy to read.

4. MATERIALS SUPPLIED

A : Reconstituting Solution: 1 x 11 mL for 25 Test Kit and 1 x 36 mL for 100 Test Kit - Aqueous solution containing Phosphate Buffer, sodium azide (0.06% w/v), and stabilizers at pH 7.0-7.5. The solution is to be used for reconstituting the Sensitized and Unsensitized Particles.

B : Sample Diluent: 1 x 30 mL for 25 Test Kit and 2 x 64 mL for 100 Test Kit - Aqueous solution containing Phosphate Buffer, sodium azide (0.10% w/v), and stabilizers at pH 7.0-7.5. The solution is used for diluting human serum in the assay.

C : Sensitized Particles: 5 x 1.5 mL for 25 Test Kit and 5 x 6.0 mL for 100 Test Kit - Lyophilized preparation of tanned gelatin particles sensitized with microsomal antigen containing sodium azide as preservative. At the time of use, add the Reconstituting Solution as prescribed on the vial label. The rehydrated reagent contains a 1% suspension of sensitized gelatin particles. (sodium azide 0.08% w/v after reconstitution)

D : Unsensitized Particles: 3 x 0.5 mL for 25 Test Kit and 3 x 1.2 mL for 100 Test Kit - Lyophilized preparation of tanned gelatin particles containing sodium azide as preservative. At the time of use, add the Reconstituting Solution as prescribed on the vial label. The rehydrated solution contains a 1% suspension of unsensitized gelatin particles. (sodium azide 0.11% w/v after reconstitution)

E : **Positive Control (goat):** 1 x 0.2 mL for 25 Test Kit and 2 x 0.2 mL for 100 Test Kit - This liquid serum containing goat antibodies to microsomal antigen should demonstrate a titer of 1:1,600 final dilution when tested according to the procedure described below. Control contains sodium azide (0.10% w/v) as preservative.

F : **Dropper:** 2 pcs. in 25 Test Kit and 4 pcs. in 100 Test Kit - To dispense approximately 25 μ L per well. One dropper to be used exclusively for dispensing reconstituted Sensitized Particles and the other dropper for dispensing the Unsensitized Particles.

5 MATERIALS REQUIRED BUT NOT SUPPLIED

1. "U" shaped microplate
2. Calibrated pipette droppers - to dispense approx. 25 μ L.
3. Micro-pipettor with tips - to dispense 25 μ L- for dispensing and diluting serum samples.
4. Pipettes - 1.0 mL and 5.0 mL for reconstitution and 10 μ L for dispensing.
5. Plate mixer (automatic vibratory shaker)
6. Plate viewer

6. PRECAUTIONS

1. For in vitro diagnostic use only.
2. All reagents should be brought to room temperature before use.
3. Proper plate mixing, after addition of all reagents, is important. Use an automatic vibratory plate mixer or tap the plate sharply with your finger or against a hard surface such as the side of a workbench to assure proper mixing. The use of a rotator, such as those used for RPR card test will not give adequate mixing.
4. During incubation, cover the microplate and keep free from vibration.

5. Reuse of microplates is not recommended. However, if microplates are to be reused, it is critical that special care be taken when cleansing the microplate before reusing it, otherwise the reaction may be adversely affected. Be sure not to leave any disinfectant or detergent residues on the plate. Only high quality, rigid, “U” shaped microplates, such as Fujirebio FASTEC plates, should be used for the assay. Plates with oil residue or other surface contaminants will interfere with the test results.
6. Do not intermix reagents from different kit lots.
7. 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
8. 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.
9. Do not pipette patient specimens by mouth (use precision pipettors). 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 CDC/NIH Manual “Biosafety in Microbiology and Biomedical Laboratories”, 1984 or latest edition.
10. Use separate pipette tips for each sample, control and reagent to avoid cross contamination.
11. Avoid freezing reagents contained in the kit.

7. 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.

8. SPECIMEN COLLECTION AND PREPARATION

The specimen for this kit is serum free of particulate matter. Specimens containing erythrocytes or other visible matter should be centrifuged to testing to prevent interference with test results. Store sera in a refrigerator at 2-8°C. Sera may be frozen and thawed once. Heat-inactivation is not necessary for the patient sera, however, previously heat-treated sera may be used.

9. PREPARATION OF REAGENTS

1. Reconstituting Solution, Sample Diluent and Positive Control are liquid 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. Once opened, dispense the appropriate amount of Reconstituting Solution. Mix reconstituted reagents thoroughly and allow to stand for at least 30 minutes prior to use. Mix again prior to dispensing.

Reconstitute the reagents following the Table 1 below:

Table 1

Reagent	Volume of Reconstituting Solution	
	100 Test Kit	25 Test Kit
Sensitized Particles (Vial C)	6.0 mL	1.5 mL
Unsensitized Particles (Vial D)	1.2 mL	0.5 mL

10. ASSAY PROCEDURE

1. Uses “U” shaped microplate sideways. One row (12 wells) is

necessary to test one patient sample.

2. Using a pipette calibrated to deliver 25 μL , place 2 drops (50 μL) of Sample Diluent into well #1 and #2 and 3 drops (75 μL) into wells #3 - #12.
3. Using a micropipette, add 10 μL of serum specimen or Positive Control into well #1. Mix well by filling and discharging the micropipette 3 or 4 times with fluid in well #1. Then draw up 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 through well #12 to obtain a four-fold dilution.
4. Place 1 drop (25 μL) Unsensitized Particles into well #2 and 1 drop (25 μL) of Sensitized Particles into wells #3 through #12 using the droppers supplied in the kit.
5. Repeat the above steps for each patient specimen and Positive Control.

Table 2

Well No.	1	2	3	4	5	6	...		12
Sample Diluent (μL) Serum Specimen or Positive Control (μL)	50 10	50 25	75 25	75 25	75 25	75 25	...		75 25
Test Specimen Dilution	1:6	1:18	1:72	1:288	1:1,152	1:4,608	...		1:18,874,368
Unsensitized Particles (μL)		25					...		
Sensitized Particles (μL)			25	25	25	25	...		25
Final Dilution		1:27	1:100* (10 ²)	1:400 (20 ²)	1:1,600 (40 ²)	1:6,400 (80 ²)	...		1:26,214,400 (5,120 ²)
Mix the content using a plate mixer, cover the plate and incubate for 3 hours									
Interpretation									

(discard)
25 μL

6. Mix the contents of the wells thoroughly using a plate mixer (automatic vibratory shaker) or tapping plate with finger. Then cover the plate and place it on a level surface. Allow it to stand at room temperature (15-30°C) for 3 hours (or overnight, if desired) and read the patterns.

Mix the plate using a plate mixer (automatic vibratory shaker), cover the plate and incubate for 3 hours, or overnight if desired. Protect from vibration during incubation

11. INTERPRETATION OF RESULTS

Results are obtained by reading the settling patterns of the colored gelatin particles using a plate viewer. Carefully place the microplate on a plate viewer (with indirect lighting), and compare the Unsensitized Particle Control agglutination patterns with those of the Positive Control. Readings are scored using the criteria shown in Table 3:

Table 3

Settling Patterns of Particles	Reading	Interpretation
Particles are concentrated in the shape of a button at the center of the well. There is a smooth round outer margin.	(-)	Non-Reactive
Particles are concentrated to form a compact-ring shape with a smooth outer margin.	(±)	Indeterminate
Particles form a large ring with a rough multiform outer margin. Peripheral agglutination occurs.	(+)	Reactive
Firmly agglutinated particles spread out covering the bottom of the well uniformly.	(++)	Reactive

12. CRITERIA FOR INTERPRETATION

1. Reactive

A specimen showing Non-Reactive with Unsensitized Particles (final dilution 1:27), but demonstrating a reaction of (+) or more at any dilution 1:100 or greater with Sensitized Particles is

interpreted as REACTIVE.

2. **Non-Reactive**

Regardless of the reading of reaction pattern with Unsensitized Particles, a specimen showing (-) with sensitized Particles (final dilution 1:100) is interpreted as NON-REACTIVE.

3. **Indeterminate**

A specimen showing (-) with Unsensitized Particles (final dilution 1:27) and demonstrating (\pm) with Sensitized Particles (final dilution 1:100) is interpreted as INDETERMINATE.

13. ABSORPTION PROCEDURE

In most Cases, test specimens do not show agglutination with Unsensitized Particles. However, if a test specimen produces (\pm) or more with Unsensitized Particles and Sensitized Particles, retest after the following absorption procedure:

1. Add 250 μ L of reconstituted Unsensitized Particles into a small test tube.
2. Add 50 μ L of test specimen, mix thoroughly using tube mixer and incubate at room temperature for 30 minutes (mix manually 1 or 2 times).
3. Centrifuge for 5 minutes at 2,000 r.p.m. Place 50 μ L of supernatant (absorbed 1:6 diluted specimen) to well #1 of the plate. Add 50 μ L (2 drops) of Sample Diluent into well #2 and 75 μ L (3 drops) into well #3 through #12. Using a micropipette, transfer 25 μ L of well #1 (absorbed 1:6 diluted specimen) into well #2. Mix completely by filling and discharging the micropipette 3 or 4 times with fluid in well #2. In order to make a four-fold dilution, repeat the same procedure for the rest of the wells, from well #3 to well #12, as shown in Table 2.
4. Place 1 drop (25 μ L) of Unsensitized Particles in well #2 and 1 drop (25 μ L) of sensitized Particles is well #3 through #12.

5. Then follow the original procedure and read the patterns.

14. QUALITY CONTROL

1. The Positive Control should be processed at least once on the day of testing or when a batch of specimens are run.
2. Confirm that the reaction of each specimen and Unsensitized Particles (1:27 final dilution) is non-reactive (-).
3. The mixture of Sample Diluent either with reconstituted Sensitized Particles or Unsensitized Particles should be non-reactive on any run of tests. (Reagent Control)
4. Confirm that the titer of the Positive Control is 1:1,600(± 1 dilution) at final dilution, according to the test procedure outlined in Table 2.

15. LIMITATIONS

1. SERODIA-AMC test is specific for detecting microsomal antibodies.
2. Since thyroid autoimmune disease may demonstrate an immunological response to antigens other than microsomal, this test should always be run in conjunction with a thyroglobulin antibody test, In thyroid autoimmune disease, the frequency of positive results with the microsomal antibody test has been shown to be higher than with thyroglobulin antibody test. (6) However, in some cases, a positive thyroglobulin antibody test can be obtained while the microsomal antibody test results are negative.
3. In other autoimmune disorders, such as Sjogren Syndrome, Systemic Lupus Erythematosus (SLE), rheumatoid arthritis (RA), and autoimmune hemolytic anemia, there is a serologic overlap in which positive reactions may occur with the thyroglobulin and microsomal antibody tests. Antithyroid antibodies, particularly microsomal antibodies, are found in other thyroid disorders and in other autoimmune diseases such as pernicious anemia,

- myasthenia gravis, SLE, and rheumatoid arthritis. (10-12)
4. It has been reported that thyroid autoantibodies have been detected in the sera of patients with other organ-specific autoimmune manifestations. This overlap with other autoimmune disorders suggests that other immunologic tests may be indicated in some patients. (3-13)
 5. Some sera samples with high antibody titer may exhibit the prozoning phenomenon at lower dilutions. When prozoning occurs, the agglutination patterns at low dilutions will exhibit a non-reactive appearance. Upon further dilution, the agglutination pattern will appear as reactive. It is important to dilute all samples through the 12th wells to obtain correct results.

16. EXPECTED RESULTS

Thyroid antibodies are seldom found in serum of normal patients. However, 2-17% of the normal population may exhibit low titers of thyroid antibodies with no symptoms of disease. (12,13) The incidence is higher in women and increases with age. The presence of thyroid antibody may also be indicative of previous autoimmune disorders. Patients with low thyroid antibody titer should be tested periodically, as the presence of the antibody may be an early sign of autoimmune disease.

In active cases of thyroid autoimmune disease and in some cases of thyrotoxicosis, moderate (1:1,600) to very high (1:25,600) antibody titers may be observed. The detection of very high (1:25,600) antibody titers in an individual with a firm, hard, fast-growing, symmetrical goiter strongly suggests Hashimoto's goiter. (6,9-13) Normal or only slightly elevated titers of thyroid antibodies constitute some evidence against the diagnosis of immune thyroiditis. (12,13)

Serum demonstrating a reactive result at any dilution should be interpreted in accordance with clinical findings. Diagnosis of thyroid autoimmune disease should not be made on the basis of the microsomal antibody test alone, but in conjunction with other

immunological tests, thyroid function tests, physical examination, familial studies, and, if necessary, biopsy.

17. PERFORMANCE CHARACTERISTICS

Studies were performed in Fujirebio laboratories and in two clinical sites comparing the performance of the SERODIA Microsomal Antibody Test with gelatin particles (y) to the SERODIA test with sheep erythrocytes (x). The in-house study was performed using the micro technique (micro hemagglutination) for the SERODIA test with erythrocytes; both clinical studies used the macro technique (macro hemagglutination).

The study performed in-house showed the following statistics:

$$n = 100; Y = 1.025x + 0.034; r = 0.955.$$

In all but one case, there was no more than one well difference in titer (one specimen showed a two well difference).(5)

The first clinical study tested sera from 65 patients; the correlation coefficient was $r = 0.953$. (3) The second clinical study tested 276 specimens, 98 of which gave a positive titer by one or both tests. This study showed a positive coincidental rate of 99%.

A slightly lower titer was occasionally obtained with the SERODIA test with gelatin particles when compared to the hemagglutination test. This is attributed to a lower frequency of nonspecific agglutination when using the gelatin particles. (3-4)

18. REFERENCES

1. Bigazzi, P.E., and Rose, N.R., Tests for antibodies to tissue-specific antigens, In: Manual of Clinical Immunology, 2nd ed. (N.R. Rose and H. Friedman, eds.) ASM, Washington, DC., 1980, pp. 874-885.
2. Weetman, A.P., Autoimmune thyroiditis: predisposition and pathogenesis, Clinical Endocrinology, 36, 1992, pp. 307-323.

3. Tsuchiya, H., et al., Study of a reagent for the measurement of microsomal antibody titer, SERODIA-AMC, Yugawara Welfare Annuity Hospital, Central Testing Laboratory, Japan.
4. Abiko, H., et al., Experience of evaluating SERODIA-ATG and AMC, Central testing Dept., Affiliate Hospital to Tohoku University, School of Medicine, Japan.
5. SERODIA-ATG and SERODIA-AMC Tests, Fujirebio, Inc., Tokyo, Japan.
6. Ravel, R., Thyroid function tests, In: Clinical Laboratory Medicine, 4th Ed., Chicago, 1984, p.366.
7. Beeson, P.B. and McDermott, W., (eds). Diseases of the Endocrine System,. In: Textbook of Medicine., 14th Ed., Philadelphia, W.B. Saunders Co., 1975, p.1716.
8. Fischback, F.T., Thyroid hemagglutination tests, In: A Manual of Laboratory Diagnostic Tests, Philadelphia, J.B. Lippincott Co., 1980, pp 417-418.
9. Gornall, A.G., Luxton, A., and Shavnani, B.R., Endocrine Disorders, In: Applied Biochemistry of Clinical Disorders, 2nd Ed., (Gornall, A.G., ed.), Philadelphia. W.B. Saunders Co., 1979, p. 1278.
10. Zweiman, B., and Lisak, R. P., Autoimmunity and Autoimmune Disease, In: Clinical Diagnosis and Management by Laboratory Methods, (Henry. J.B., ed.), 16th Ed., Philadelphia. W.B. Saunders Co., 1979, p. 1278.
11. Ingbar, S.H., and Woeber, K.A., Diseases of the Thyroid, In: Harrison's Principles of Internal Medicine, 9th Ed., (Isselbacher, K.J., et al. Eds), New York, McGraw Hill Book Co., 1980, p.1711.
12. Crolla, L.J., and Maso, C.J., Immunologic Studies of the Endocrine System, In: Clinical Immunochemistry, (Natalson, S., Pesce, A.J., and Dietz, A.A., eds). Washington, DC, AACC, 1978, pp. 282-284.

13. Williams, D.L. and Goodburn, H., The Thyroid Gland, In: Biochem. In Clinical Practice, (Williams, D.L. and Marks, V., eds) New York, Elsevier Pub. Co., 1985, pp. 567-575.

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