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SERODIA[®]-ATG

(For In Vitro Diagnostic Use)

**A REAGENT FOR DETECTION AND TITRATION OF
ANTIBODIES TO THYROGLOBULIN**

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1. INTENDED USE

SERODIA-ATG is a semi-quantitative microtiter particle agglutination test for the in vitro diagnostic detection and titration of thyroglobulin 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, focalthyroiditis and/or circulating thyroid antibodies can be found in about 15% of otherwise healthy euthyroid subjects. (1,2) In addition to being found in cases of thyropiditis, these antibodies may be found with other thyroid disorders, such as primary myxedema, hyperthyroidism, goiter and thyroid tumors.(1)

Thyroglobulin antibodies can be demonstrated by several procedures, such as passive agglutination. SERODIA-ATG is prepared using gelatin particles sensitized with purified thyroglobulin. Since thyroid autoimmune disease may demonstrate an immunological response to antigens other than thyroglobulin, SERODIA-ATG should always be used in conjunction with clinical findings and other immunological thyroid tests. A number of references pertaining to antibody agglutination tests for thyroglobulin in thyroid diseases have been published.(3-8)

3. PRINCIPLE OF THE TEST

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

react with the thyroglobulin-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 thyroglobulin 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 thyroglobulin 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 the 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 provide 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 microplates, 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 human serum free of particulate matter. Specimens containing erythrocytes or other visible matter should be centrifuged prior to testing to prevent interference with test results. Store sera in a refrigerator at 2-10°C. Sera may be frozen and thawed only 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 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. Once opened, dispense the appropriate amount of Reconstituting Solution. Mix the reconstituted reagents thoroughly and allow them to stand for at least 30 minutes prior to use. Mix them 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. Use a “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 wells #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.
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.

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

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 wells #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 in well #3 through #12.
5. 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-ATG is specific for detecting thyroglobulin antibodies.
2. Since thyroid autoimmune disease may demonstrate an immunological response to antigens other than thyroglobulin, this test should always be run in conjunction with a microsomal 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. 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. (10-11)

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. (9-21)
5. Some sera samples with very 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 well 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.(26, 27) 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 antibody titers in an individual with a firm, hard, fast-growing, symmetrical goiter strongly suggests Hashimoto's goiter.(22-27)

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 thyroglobulin 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 conducted comparing the performance of the SERODIA ATG Test to the SERA-TEK Thyroglobulin Antibody Test. Both tests use microtitration with thyroglobulin-sensitized gelatin particles. The first study used 400 specimens from patients with thyroid diseases (including thyroid auto-immune diseases, thyroid cancer, and thyroiditis) and 200 specimens from healthy donors, randomly collected from both sexes. In addition, 121 specimens from patients hospitalized with Rheumatoid Arthritis, and 96 specimens from women who were 3 - 6 months pregnant, were collected. These specimens were tested with the two kits in order to assess the performance of the Serodia ATG test using potentially difficult and cross-reactive samples. The results from the studies demonstrated a Regression formula of: $Y = 1.07x + 2.2$ with a Correlation Coefficient: $r = 0.98$

The results of these studies indicated that the Serodia ATG kit is more sensitive (displaying higher endpoint titers) than the reference test. Twenty-seven (27) samples from the four testing groups resulted in false positive results when compared to the reference test. No false negative results were found.

The 27 discrepant results were investigated further to determine the true status of the patients. Purified thyroglobulin was added to the Sample Diluent to determine if inhibition was observed in these 27 patients and to identify the possibility of non-specific reactions or other interfering proteins causing the false positive results. The patient samples were diluted with the spiked diluent and re-run in the assay. Samples which became non-reactive were assumed to have previously contained thyroglobulin antibody and, therefore, true

positive in the Serodia ATG test. The results of the absorbed and re-tested patient samples from the four groups showed that all of the samples were non-reactive in the Serodia test. This demonstrated that the samples were true positive.

A study was performed, using a highly positive patient sample, to evaluate the effect of dilution on the reaction the assay. A phenomenon in which antibody excess causes a false positive reaction, prozone, is known in some immunologic tests. This reaction can cause a mis-interpretation unless the sample is diluted to reduce the excess antibody. In this study, a high titered positive sample was serially diluted and run in both the SERA-TEK Thyroglobulin Antibody Test and Serodia ATG kit. Two lots of each kit were run to demonstrate the prozone effect. The results of the study showed that the Serodia ATG test did not exhibit a prozone effect (positive reaction) at the lower dilutions.

Studies were performed to demonstrate the Inter-day and Intra-run reproducibility of the Serodia ATG Test kit. Three lots of kits were used to perform the testing. These kits were used for the Inter-Day testing using 5 positive samples at different titers, 1 negative sample, and the Positive Control. These samples were serially diluted into six tubes each day and run in triplicate for 5 days on the 2 lots of kits. Intra-run testing was performed using the same 7 sera samples run 20 times each.

The reproducibility performance of the Serodia ATG kits showed the Coefficient for Variation for the Intra-run and Inter-day reproducibility was 0%.

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