1988.2 Draft
1990.6 Update

In Vitro Diagnostic Reagents
Ref. (63E) No. 1811

TST-RPLA "SEIKEN"

A Reagent Kit for detecting Staphylococcus TSST-1 (Toxic Shock Syndrome Toxin) by Reversed Passive Latex Agglutination 20 tests

Toxic-Shock Syndrome (TSS) by Staphylococcus aureus infection produces various symptoms such as fever, hypotension, systemic erythema, conjunctive congestion, multiple organopathy etc.

Staphylococcus aureus isolated from the patients of this syndrome has been reported to produce the same enterotoxins (Toxic-shock toxin, TST).[1)2)]

The sensitized latex in this test kit is prepared as follows. Rabbits are immunized with the specifically purified TSST-1,[3)] the antisera taken from the rabbits are purified by affinity-chromatography and polystyrene latex particles are sensitized with the purified antisera.

CHARACTERISTICS

1. This test does not require animals or cell cultures and shows good sensitivity and specificity.
2. Since it uses the microtiter technique this procedure is easy.

KIT COMPONENTS

1. **Sensitized latex:** 5 ml 1 vial
   A suspension of latex particles sensitized with specific antibodies (rabbit IgG) to Staphylococcal TSST-1. The reagent contains sodium azide as a preservative at a concentration of 0.1 w/v%.
2. **Control latex:** 5 ml 1 vial
   A suspension of latex particles sensitized with normal rabbit IgG. The reagent contains sodium azide as a preservative at a concentration of 0.1 w/v%.
3. **Control TST (lyophilized):** 0.5 ml equivalent 1 vial
   This reagent is lyophilized TSST-1, and the agglutination titer is shown on the vial label.
4. **Diluent:** 50 ml 1 vial
   Phosphate-buffered saline (PBS) containing 0.5 w/v% bovine serum albumin and the reagent contains sodium azide as a preservative at a concentration of 0.1 w/v%.

INTENDED USE

This reagent kit is intended for use in detecting Toxic-Shock Syndrome Toxin-1 (TSST-1) produced by Staphylococcus aureus grown in the uropoietic organs or genital organs

PRINCIPLE

Latex particles sensitized with antibodies to Staphylococcus aureus TSST-1 react specifically with the TSST-1 produced by the organism in the sample and form an agglutination. This reagent kit employs Reversed Passive Latex Agglutination, based upon this principle.

PROCEDURES

1. Materials necessary for the test

(1) Sample Culture fluid of Staphylococcus aureus

(2) Equipment
- Microtiter plate (V-type)
- Dropper (25 μl)
- Diluter (25 μl)
- Mixer for microtiter plate
- Others (black paper, plate cover, marking tape, moisture box)

(3) Reagent TST-RPLA "SEIKEN"

2. Preparation of reagent and sample

(1) Reagent preparation

Use sensitized latex, control latex and diluent as supplied. Control TST should be used after reconstituting with 0.5 ml diluent.

(2) Sample preparation

Culture the test bacteria in BHI (Brain Heart Infusion) medium, etc. at 37 ℃ for 18－20 hours by shake culture. Centrifuge the culture fluid at 3000 rpm for 20 minutes, and use the supernatant as test specimen.

3. Reversed Passive Latex Agglutination

(1) Use two rows of microtiter wells for each specimen. Using a dropper add 25 μl of diluent to all wells except the first well in each row.

(2) Drop 25 μl of specimen into the first well in each row.

(3) Using two diluters, take 25 µl of sample and carry out two-fold serial dilutions from the second wells up to the second to last wells.

(4) Add 25 μl sensitized latex suspension to each well in one row, and add 25 μl control latex to all the wells in the other row.

(5) As a positive control, prepare one well as follows. Add 25 μl reconstituted control TST and sensitized latex to one well in the microtiter plate.

(6) Shake the microtiter plate well with a microtiter plate mixer.

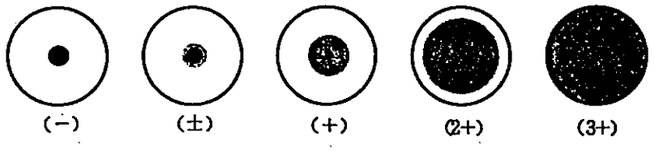
(7) To avoid evaporation of the solutions in the wells, cover the microtiter plate or place it in a moisture box, then leave it for 18－20 hours and observe the results.

(8) Observation of the microtiter plate should be carried out over black paper placed in a well-lit place looking from above.

INTERPRETATION

Refer to the following figures in the observation of results.

Agglutination patterns



Regard agglutination stronger than (+) as positive.

If the control latex produces agglutination stronger than (+), then this should be regarded as non-specific agglutination.

If agglutination stronger than (+) is observed in the last well where only latex reagents and diluent were added, agglutination has been spontaneous and the kit should not be used.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and detection range

Specimen	Dilution							
	× 2	× 4	× 8	× 16	× 32	× 64	× 128	× 256
TST (100 ng/ml)	3+	3+	3+	3+	3+	2+	+	－
	3+	3+	3+	3+	3+	2+	+	－
	3+	3+	3+	3+	3+	2+	±	－
TST 2-fold dilution	3+	3+	3+	2+	+	±	－	
	3+	3+	3+	3+	2+	+	－	
	3+	3+	3+	3+	2+	+	－	
TST 10-fold dilution	3+	2+	+	－				
	3+	2+	+	－				
	3+	2+	+	－				

Marks represent the degree of agglutination. (in-house data)

When samples of purified TSST-1 (TST, 100 ng/ml) prepared by Denka Seiken were tested using this kit according to the instructions, the undiluted toxin solution, a 2-fold dilution, and a 10-fold dilution produced agglutination up to a 64－128× dilution, 32－64× dilution, and 8× dilution, respectively. All control latex produced negative results, and it was concluded that the sensitized latex had a sensitivity of 1－2 ng/ml.

2. Specificity and within-run reproducibility

Sample	Test									
	1	2	3	4	5	6	7	8	9	10
Positive (1)	×512	×1024	×1024	×1024	×1024	×512	×1024	×1024	×1024	×512
Positive (2)	×128	×128	×128	×64	×128	×128	×128	×128	×128	×64
Positive (3)	×512	×256	×512	×512	×512	×512	×512	×256	×512	×512
Negative (1)	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2
Negative (2)	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2
Negative (3)	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2	< ×2

(in-house data)

Figures in the table represent the maximum specimen dilution factor which produced agglutination.

Culture media of the Staphylococcus which produce TSST-1 (positive samples 1, 2 and 3) and a culture media of the Staphylococcus which produce enterotoxins A, B, and C (negative samples 1, 2 and 3) were tested with this kit 10 times. All positive samples produced positive results, and all negative samples produced negative results.

The maximum dilution factor of the positive reaction did not vary by more than one well in 10.

Control latex produced negative results.

NOTE

1. Use this test kit only for in vitro diagnostic purposes.
2. Do not freeze the kit. Ensure the kit has reached room temperature at least 30 minutes before use.
3. Use a microtiter plate which is free from scratches and stains.

4. Thoroughly agitate the latex reagent vial to resuspend the latex particles and form a homogeneous solution.
5. Store the reconstituted control enterotoxin at 2–10°C . In this manner, the reconstituted reagent can be used for up to 3 months.
6. Do not mix reagents of different lot numbers.
7. Materials and equipment used in this test should be sterilized by soaking in 0.1% sodium hypochlorite solution (chloride content about 1000 ppm) for more than 1 hour or by autoclaving them at 121 °C for more than 20 minutes.
8. If necessary, carry out Agglutination Inhibition test. Inhibition Antibodies are separately available from us.
9. Sodium azide contained in the reagents may react with lead and copper piping to form highly explosive metal azides. Upon disposal, flush this reagent away with a large volume of water to prevent accumulation of azides.

STORAGE AND EXPIRATION

Store the reagent at 2–10°C, up to expiration date on the label.

PACKAGE

TST-RPLA "SEIKEN" 20 tests per box

☆ The following product is available from us.

Antibody for Agglutination Inhibition test (for TST-RPLA "SEIKEN") Lyophilized 5 ml equivalent 1 vial

REFERENCES

1. Todd, J., et al.: TOXIC-SHOCK SYNDROME ASSOCIATED WITH PHAGH-GROUP-I STAPHYLOCOCCI, Lancet, ii, 1116 (1978).
2. Cohen, M. L., et al.: Protein Antigens from Staphylococcus aureus Strains Associated with Toxic-Shock Syndrome, Science, **211**, 842 (1981).
3. Igarashi, H., et al: Purification and Characterization of *Staphylococcus aureus* FRI 1169 and 587 Toxin Shock Syndrome Exotoxins, Infect. Immune. **44**, 175 (1984).