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EXT-RPLA "SEIKEN"

A Reversed Passive Latex Agglutination
Test for the Detection of Staphylococcal
Exfoliative Toxins A and B

For 20 tests

INTRODUCTION

Up to 10% of clinical isolates of *Staphylococcus aureus* secrete either or both of two related, but immunologically distinct proteins known as exfoliative toxins, designated ETA and ETB. Both are strongly associated with staphylococcal scalded skin syndrome (SSSS), an acute erythematous disease which results in the peeling and loss of large areas of the skin. The affliction is seen primarily in infants, especially in outbreaks at nurseries, hospitals, etc and may lead to serious complications, including death, in the absence of prompt treatment.

As and aid both in diagnosis and in epidemiological studies aimed at controlling outbreaks of the disease, this kit employs RPLA methodology for the rapid detection and identification of ETA and ETB.

CHARACTERISTICS

1. Excellent sensitivity (1 - 2 ng/mL) and specificity.
2. Simple operation by microplate method.
3. No special equipment needed for judgement.

CONTENTS

1. Sensitized latex EXT-A 5 mL x 1 vial

A suspension of latex particles sensitized with staphylococcal exfoliative toxin A specific antibodies (rabbit , polyclonal) containing sodium azide as a preservative (0.1 w/v %).

2. Sensitized latex EXT-B 5 mL x 1 vial

A suspension of latex particles sensitized with staphylococcal exfoliative toxin B specific antibodies (rabbit , polyclonal) containing sodium azide as a preservative (0.1 w/v %).

3. Control latex 5 mL x 1 vial

A suspension of latex particles sensitized with non-immune rabbit IgG antibody containing sodium azide as a preservative (0.1 w/v %).

4. Control toxins

Exfoliative toxin A (lyophilized) 0.5 mL equiv. x 1 vial
Exfoliative toxin B (lyophilized) 0.5 mL equiv. x 1 vial

Lyophilized toxins contain lactose and bovine serum albumin as stabilizers.

5. Diluent 50 mL x 1 vial

Phosphate-buffered saline (PBS) containing 0.5 w/v % bovine serum albumin and sodium azide as a preservative (0.1 w/v %).

INTENDED USE

To detect and identify staphylococcal exfoliative toxins A and B in isolates of *S. aureus*.

PRINCIPLE OF THE TEST

Latex particles sensitized with antibodies against either ETA or ETB will react in the presence of the respective toxin to agglutinate and settle diffusely over the base of a microtiter plate well; in the absence of toxin, latex particles settle to form a tight button, a pattern easily distinguishable from positives.

PROCEDURE

1. Materials and reagents necessary for the test

1) Sample: Culture supernatant of *Staphylococcus aureus*
2) Equipment: Microtiter plate (V-type)
Dropper (25 μL)
Diluter (25 μL)
Mixer for microtiter plate
Centrifuge capable at 900 g : 3,000 rpm or more in bench-top type
Other (black paper, plate cover, marking tape, moisture box)
3) Media: Brain heart infusion agar containing cadmium nitrate (30 μg/mL)
Brain heart infusion agar (BHI agar medium)
Brain heart infusion broth (BHI liquid medium)

2. Reagent preparation

Use Sensitized latex EXT-A and EXT-B, Control latex reagent and Diluent as supplied. To reconstitute the Control exfoliative toxins, add 0.5 mL of Diluent to the vial and shake gently. After reconstitution, store at 2 - 10 °C and use within 3 months. For longer storage, aliquot and place at - 40 °C or below.

3. Sample preparation

Selection on Cd-BHI agar

As both the gene for exfoliative toxin B and cadmium-resitance are encoded on a plasmid known to be easily lost under non-selective conditions, it is important to maintain selection on cadmium-containing agar media during passage, preparation of stock cultures, etc. Purified isolates should be streaked on both BHI and Cd-BHI. If growth is observed on the latter, the colony for testing should be taken from this plate; otherwise a colony from the BHI plate should be chosen. If the isolate to be tested is not pure, it should be streaked on and then from both plates since some ETA producers are not Cd resistant. For tested clinical specimens, ie, isolates from SSSS patients, several representative colonies should be tested.

Preparation of sample

Inoculate the isolate(s) to be tested into BHI liquid media and culture with shaking (120 rpm) at 37 °C for 18-24 hrs under aerobic conditions. Centrifuge the resultant culture at 900 rpm for 20 minutes and use the supernatant as sample.

(Note: addition of cadmium to this media is not necessary)

4. Procedures for RPLA

Refer to Table 1 and Figure 1 below when performing the test.

Table 1. Method of Dilution

Well No.	1	2	3	4	5	6	7		8
Dilution factor	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128		Diluent control
Diluent (μL)	25	25	25	25	25	25	25	Discard	25
Sample (μL)	25	25	25	25	25	25	25	25	-
Sensitized Latex or Control Latex (μL)	25	25	25	25	25	25	25		25

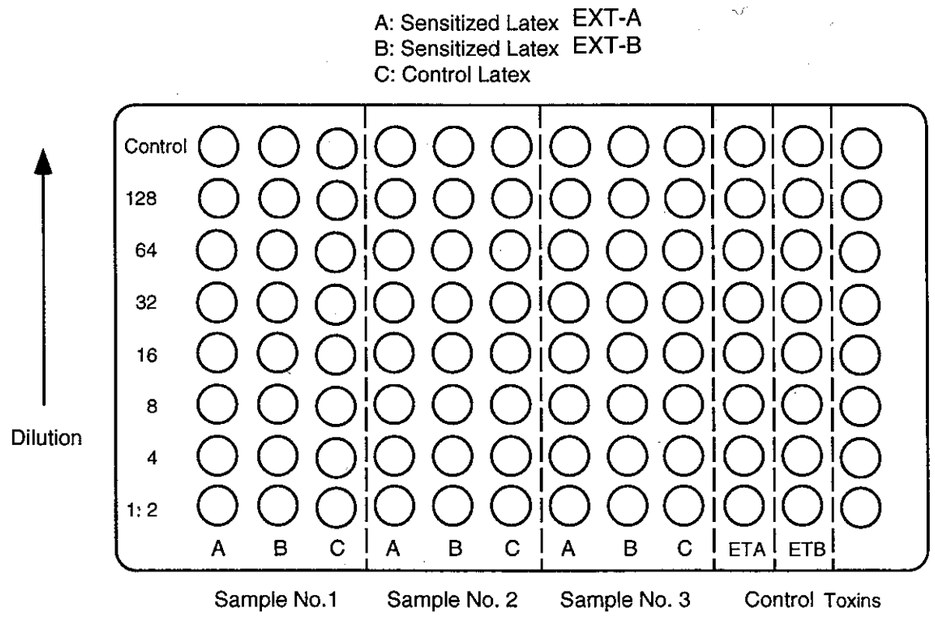


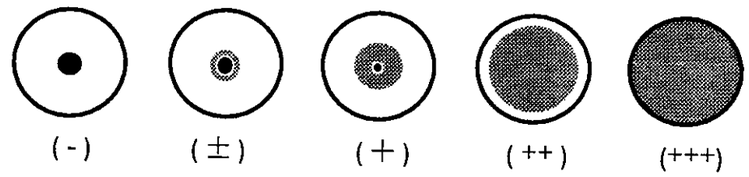
Figure 1. Microtiter plate configuration

1) Arrange and label the plate so that each sample is allotted 3 rows, each consisting of 8 wells. Also, reserve two rows for each toxin control (See Fig. 1).
2) Using a pipette or dropper, add 25 μL of diluent into each well of the three rows.
3) Add 25 μL of the test sample into the first well of the three rows.
4) Using the pipette or dilutor make 2-fold serial dilutions in each row starting from well 1 and continuing through well 7, stopping at the 8th well (the diluent control).
5) Add 25 μL of sensitized latex EXT-A into each well of the first row, 25 μL of sensitized latex EXT-B into each well of the second row and 25 μL of the control latex into each well of the third.

6) Assay the toxin controls in a similar manner to verify the integrity of the reagents.
7) Mix the well contents by rotating the plate on a micromixer or by agitating carefully by hand. Avoid spillage.
8) After covering the plate with a lid or placing in a moisture box to avoid evaporation, incubate in a vibration-free area at room temperature for at least 18 - 20 hours.
   Note: placing the plate over a black sheet of paper for this step will facilitate reading the results later.
9) Read the resulting agglutination patterns (See INTERPRETATION on next page).

INTERPRETATION

Refer to positive patterns obtained using the control toxins and to the following figures when interpreting the results.



(+++), (++), and (+) are considered positive while (±) and (-), negative. To consider the test valid, however, the last well in all rows should be negative. If non-specific agglutination is seen in the wells of the third row, this does not necessarily negate the validity of the test since culture filtrates reacting with sensitized latex at a dilution four times higher (ie, a two-well difference) than that seen with the control latex are regarded as positive.

Prozone effects. In samples from high level toxin-producing strains, negative patterns of agglutination in the lower dilution wells may be observed due to antigen excess; however, since true positive patterns can be observed in the higher dilution wells, it can be distinguishable from negative samples.

PERFORMANCE

1. Sensitivity

Detection limit is 1 - 2 ng/mL.

2. Specificity

No cross-reactivity between staphylococcal exfoliative toxin A and B is seen, nor with staphylococcal enterotoxins A, B, C, D or toxic shock syndrome toxin-1.

PRECAUTIONS

1. Allow the reagents to come to room temperature before use. Do not freeze reagents.
2. Shake latex reagents by gently inverting several times to ensure homogenous suspensions before use.
3. Use microtiter plates which are clean, free of nicks and scratches, etc.

4. Sterilize all samples, equipment, etc. used in this test by one of the following methods:
   1) Soaking in 2 w/v % glutaraldehyde for 1 hour or longer.
   2) Soaking in 0.5 w/v % sodium hypochlorite for 1 hour or longer.
   3) Autoclaving at 121 °C for at least 20 minutes.
5. These reagents contain sodium azide. As sodium azide may react with lead and copper piping to form highly explosive metal azides, dispose of by flushing with copious amounts of water.
6. Do not mix or interchange reagents with different lot numbers.

STORAGE AND SHELF LIFE

Storage : 2 - 10 °C protected from light.
Shelf life : Up to the expiry date on the label.

PACKAGE

EXT-RPLA "SEIKEN" 20 tests

REFERENCES

Susumu Sakurai; Staphylococcal exfoliative toxins produced by *Staphylococcus aureus*, Medical Bacteriology, published by Saikon Shuppan, 2, 329 (1987)