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In Vitro Diagnostic Reagents
Ref. (01E) No. 0109

CRET-RPLA "SEIKEN"

The Detection of Bacillus Cereus Enterotoxin by Reversed Passive Latex Agglutination 20 tests

Such as in soil, water and air Bacillus cereus is widely distributed in the environment. It persists in foods because it forms spores resistant to heat and chemicals. It is known to cause food poisoning due to the enterotoxin it produces.

The sensitized latex in this test kit is prepared by purifying the enterotoxin produced by B. cereus, and hyperimmunizing healthy rabbits with it. The antibody to this toxin is then purified by affinity chromatography and is adsorbed onto latex particles. This kit is used to detect B. cereus enterotoxin by Reversed Passive Latex Agglutination.

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 CONTENTS

1. **Sensitized latex:** 5 ml 1 vial
   A suspension of latex particles sensitized with specific antibodies (rabbit) to B. cereus enterotoxin. The reagent contains sodium azide as a stabilizer 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 stabilizer at a concentration of 0.1 w/v%.
3. **Control enterotoxin:** 0.5 ml 1 vial
   This reagent is lyophilized Bacillus cereus enterotoxin, 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 stabilizer at a concentration of 0.1 w/v%.

INTENDED USE

This reagent kit is intended to be used to detect Bacillus cereus enterotoxin.

PRINCIPLE

Latex particles sensitized with antibodies to Bacillus cereus react specifically with the enterotoxin produced by the organism and form an agglutination. This reagent kit employs Reversed Passive Latex Agglutination based upon this principle.

PROCEDURES

1. **Materials necessary for the test**
   1) **Equipment**
      Microtiter plate (V-type)
      Dropper (25 μl)

      Diluter (25 μl)
      Mixer for microtiter plate
      Others (black paper, plate cover, marking tape, moisture box)
   2) **Reagent**
      BHI (Brain-Heart infusion)
2. **Preparation of reagent and specimen**
   1) **Reagent preparation**
      Use sensitized latex, control latex and diluent as supplied. Control enterotoxin should be used after reconstituting with 0.5 ml diluent.
   2) **Preparation of specimens**
      The test strain is cultured on BHI culture and is shake-cultured at 32 ℃ (110 times or more /minute) for 6－20 hours. After shake-culturing, the culture medium is centrifuged at 3000 rpm for about 20 minutes and the supernatant is used.
3. **Procedures**
   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, transfer 25 μl of sample from the first well of each row to the second wells and carry out two-fold serial dilutions 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 enterotoxin and sensitized latex to one well in the microtiter plate.
   6) A negative result should be seen for the reaction between the control latex and the control enterotoxin.
   7) Shake the microtiter plate well with a microtiter plate mixer.
   8) To avoid evaporation of the solutions in the wells, cover the microtiter plate or plate it in a moisture box and leave it for 18－20 hours then observe the results.

NOTE

Culture time for the test organism should be 6－20 hours. After a longer culture, enterotoxin may not be found.

INTERPRETATION

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

Refer to the following figures in the observation of results.

1) Regard stronger agglutination than (+) as positive.
2) If the control latex produces agglutination stronger than (+), then this should be regarded as non-specific agglutination.
3) 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
CRET (100 ng/ml)	3+	3+	3+	3+	3+	2+	±	−
	3+	3+	3+	3+	3+	2+	±	−
	3+	3+	3+	3+	3+	2+	+	−
CRET 2-fold dilution (50 ng/ml)	3+	3+	3+	2+	+	±	−	
	3+	3+	3+	2+	+	±	−	
	3+	3+	3+	2+	2+	+	−	
CRET 10-fold dilution (10 ng/ml)	3+	2+	+	−				
	3+	2+	+	−				
	3+	2+	+	−				

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

When samples of purified B. cereus enterotoxin (CRET 100 ng/ml) prepared by Denka Seiken were tested using this kit according to the instructions, the undiluted enterotoxin 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)	×256	×256	×256	×256	×256	×256	×256	×256	×256	×256
Positive (2)	×128	×128	×128	×64	×128	×128	×128	×128	×128	×128
Positive (3)	×64	×64	×64	×64	×64	×64	×64	×64	×32	×64
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 B. cereus strains producing enterotoxin (positive specimens 1, 2, and 3) and strains not producing enterotoxin (negative specimens 1, 2 and 3) were tested with this kit 10 times. All positive specimens produced positive results, and all negative specimens 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.

The results of tests using various bacteria which produce toxins (Staphylococcus, Vibrio cholerae, Vibrio parahaemolyticus, Clostridium perfringens) were negative.

CORRELATION

The correlation between RPLA and mouse intestinal ligation test was examined using 138 strains of B. cereus and a complete agreement between the two test was observed.

	Loop test (positive)	Loop test (negative)	Total
RPLA positive	56	0	56
RPLA negative	0	82	82
Total	56	82	138

Figures represent number of specimens. (in-house data)

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. Thoroughly agitate the latex reagent vial to resuspend the latex particles and form a homogeneous solution.
4. Store the reconstituted control enterotoxin at 2−10℃. In this manner, the reconstituted reagent can be used for up to 3 months.
5. Use a microtiter plate which is free from scratches and strains.
6. 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℃ for more than 20 minutes.
7. Do not mix reagents of different lot numbers.
8. This reagent contains 0.1 w/v% sodium azide as a stabilizer. Sodium azide 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 SHELF LIFE

Storage: 2 − 10℃

Shelf life: Up to the expiry date on the label.

PACKAGE

CRET-RPLA "SEIKEN" 20 tests per box

REFERENCES

1. Thompson, N. E., et al.: Isolation and Some Properties of an Enterotoxin Produced by *Bacillus cereus*. Infect. Immun., **43**, 887 (1984).
2. Shinagawa, K., et al.: The Relation Between the Diarrheal and Other Biological Activities of *Bacillus cereus* Involved in Food Poisoning Outbreaks. Jpn. J. Vet. Sci., **47**, 557 (1985).

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