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VET-RPLA「生研」
In Vitro Diagnostic Reagents
Ref. (62E) No. 1436

VET-RPLA "SEIKEN"

A Reagent kit for detecting Vibrio cholerae entrotoxin-Escherichia coli heat-labile enterotoxin by Reversed Passive Latex Agglutination 20 tests

It is well known that enterotoxigenic Escherichia coli produces two kind of enterotoxins, that is heat-labile enterotoxin (LT), and heat-stable enterotoxin (ST),[1][2] and also that the former has parts in its antigenic structure common with Vibrio cholerae enterotoxin (CT).[3-7]

The reagent kit is prepared, based on this knowledge, for detecting CT and LT by the Reversed Passive Latex Agglutination method, utilizing latex particles sensitized with purified antibodies against CT.

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 CT. 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 (lyophilized):** 0.5 ml equivalent 1 vial
   This reagent is lyophilized CT and the agglutination titer is shown on the vial label.
4. **Diluent:** 50ml 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 enterotoxins produced by Vibrio cholerae and Escherichia coli.

PRINCIPLE

Latex particles sensitized with antibodies to CT will react specifically with the CT or LT 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 which are necessary for the test but not provided

(1) Samples — Culture fluid of Vibrio cholerae or Escherichia coli.

(2) Equipment — Microtiter plate (V-type)
Dropper (25 μl)
Diluter (25 μl)

Mixer for microtiter plate
Others (black paper, plate cover, marketing tape, moisture box)

(3) Reagent — VET-RPLA"SEIKEN"
Polymyxine B, Physiological saline

2. Preparation of reagent and sample

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) Sample preparation

(1) Culture fluid of V. cholerae of E. coli
Inoculate test bacterial strain to the culture media[*1,*2] prepared for producing toxin, then cultivate it by shake culture (more than 110 times/min) at 30℃ for V. cholerae and at 37℃ for E. coli for 18－20 hours.
After that, centrifuge the culture fluid at 3000 rpm for 20 minutes, and use the supernatant as the test specimen.

(2) E. coli solution treated with Polymyxin B
As for E. coli having poor LT producibility, the quantity of toxin in the supernatant may sometimes be too small to be detected. In such cases, or if the shaking machine for the shake culture is not available, extract from test bacteria treated with Polymyxin B as described below is used as the test sample.
① Inoculate the test bacterial strain on 10 ml of the slant agar medium for producing toxin (BHI with Lincomycin added at a concentration of 90 ug/ml,[8] etc.) and culture it at 37℃ for 18－20 hours.
② Suspend the bacterial cells in 1 ml of physiological saline mixed with 5000 units/ml Polymyxin B.
③ Shake the solution at 37℃ for 30 minutes, then centrifuge it at 3000 rpm for 20 minutes.
④ Decant the supernatant gently and use it as test specimen.

*Note 1: Culture medium for producing V. cholerae enterotoxin:
Syncase culture medium devised by Finkelstein et al. is generally used.

Casamino acid	10 g
Sucrose	5 g
Na_2HPO_4	5 g
K_2HPO_4	5 g
NH_4Cl	1.18 g
Na_2SO_4	0.089 g
$MgCl_2 .6H_2O$	0.042 g
$MnCl_2.4H_2O$	0.004 g
$FeCl_3.6H_2O$	0.005 g
Distilled water	1000 ml

pH 7.2

*Note 2: Culture medium for producing E. coli enterotoxin:
CAYE culture medium devised by Evans et al. is generally used.

Casamino acid	20 g
Yeast extracts	6 g
NaCl	2.5 g
K_2HPO_4	8.71 g
Salts mixture solution[*3]	1 ml
Distilled water	1000ml

pH 8.5

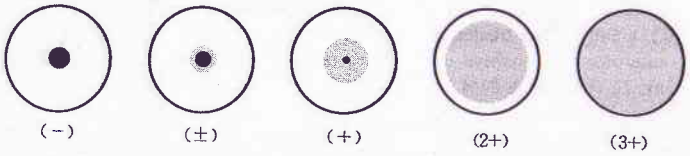
*Note 3: 0.001N H_2SO_4 solution including $MgSO_4$ at 5%, $MnCl_2$ at 0.5% and $FeCl_3$ at 0.5% concentration.

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, 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) Negative figures will be given as references in the reaction of control latex and 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 place it in a moisture box, then leave it for 18－20 hours and observe the results.
9) 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.



Regards agglutination stronger than (+) as positive.

If the control latex produces agglutination stronger than (+), then this should be regarded as non-specific agglutination. If necessary, carry out Agglutination Inhibition test.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and detection range

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

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

When samples of purified V. cholerae enterotoxin (V-ET, 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)	×128	×128	×128	×64	×128	×128	×64	×128	×128	×128
Positive (2)	×256	×512	×512	×512	×512	×512	×512	×256	×512	×512
Positive (3)	×64	×64	×64	×32	×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 Vibrio cholerae strain which produces enterotoxins (positive samples 1, 2), a Polymyxin-treated solution of enterotoxigenic E. coli which produces heat-labile enterotoxins (positive sample 3), and a Polymyxin-treated solution of enterotoxigenic E. coli which does not produce this phenomenon (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℃. 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℃ for more than 20 minutes.
8. 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 SHELF LIFE

Storage: 2－10℃
Shelf life: Up to expiry date on the label.

PACKAGE

VET-RPLA "SEIKEN" 20 tests per box

*The following product is available from us.

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

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