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腸炎ビブリオ型別用免疫血清「生研」
In Vitro Diagnostic Reagents
Ref. (62E) No. 1433

Vibrio parahaemolyticus Antisera "SEIKEN"

Vibrio parahaemolyticus is a non spore forming gram negative bacillus with monopolar flagella.

These antisera are K typing sera and O grouping sera used for the serological test of *V. parahaemolyticus* and each sera contains agglutinins specific to the K type and O group.

K typing and O grouping are carried out by slide agglutination or test tube agglutination methods. The product is prepared by hyperimmunizing healthy rabbits with formalin treated bacterial cells possessing K antigens (in the case of K sera) or O group antigens (in the case of O sera). After bleeding, the immune sera are inactivated by heating at 56 ℃ for 30 minutes, and cross agglutinins are removed by absorption. The sera are then sterilized by filtration and sodium azide is added at 0.1w/v% as a preservative.

PRODUCTS

[O grouping sera] Set of 11 vials
O sera (O1, O2, O3, O4, O5, O6, O7, O8, O9, O10 and O11)

[K typing sera] Set of 74 vials
Polyvalent 9 vials (Ⅰ , Ⅱ , Ⅲ , Ⅳ , Ⅴ , Ⅵ , Ⅶ , Ⅷ and Ⅸ)
Monovalent 65 vials (K1 - K7 except K2, K14, K16, K27, K35 and K62)

Polyvalent K antiserum	K agglutinins present						
Ⅰ	1	3	4	5	6	7	8
Ⅱ	9	10	11	12	13	15	17
Ⅲ	18	19	20	21	22	23	24
Ⅳ	25	26	28	29	30	31	32
Ⅴ	33	34	36	37	38	39	40
Ⅵ	41	42	43	44	45	46	47
Ⅶ	48	49	50	51	52	53	54
Ⅷ	55	56	57	58	59	60	61
Ⅸ	63	64	65	66	67	68	69
	70	71					

INTENDED USE

Determination of *V. parahaemolyticus* serotype

PRINCIPLE OF MEASUREMENT

This product is mixed with *V. parahaemolyticus* to cause an antigen-antibody reaction and forms an aggregate of bacterial cells which is observable macroscopically. The serotype is determined using this principle.

PROCEDURES

After isolating organisms using conventional procedures, carry out K typing and O grouping of organism whose biochemical properties are identical as *V. parahaemolyticus*.

1. Determination of K type

1) Slide agglutination test using polyvalent sera

(1) Using a glass pencil, divide a glass slide into several parts, and place one drop of each polyvalent serum and physiological saline (control) onto each section of the slide.

(2) Using a bacteriological loop, place one drop of the test bacteria which has been densely suspended* in physiological saline in the vicinity of the drop of solution previously put on the slide. Using the bacteriological loop, mix the antigen and serum drop well and also mix the antigen solution and physiological saline in each section.

(3) Tilt the glass slide back and forth and observe the slide for agglutination. Only strong agglutination occurring within 1 minute should be positive, whereas agglutination occurring later than 1 minute or weak agglutination should be negative. Spontaneous agglutination of antigen should be checked using physiological saline as a control.

2) Agglutination test for typing

When a positive reaction is observed with one of the K polyvalent sera, carry out a slide agglutination test in the same way as described above, using monovalent sera comprising the polyvalent serum which agglutinated.

(1) The antigen for typing is prepared as follows. Test bacterium is inoculated onto the agar plate supplemented with 3w/v% sodium chloride (containing 0.1w/v% Teepol), then a turbid S type colony is selected and inoculated on slant agar of the plate and this is incubated overnight. It is then densely suspended in a small quantity of 3w/v% sodium chloride solution.

(2) Since this antiserum is OK type antiserum, when test bacteria lacking K antigens or with few K antigens us used, the organism may produce O agglutination to yield incorrect test results. Appropriate care should be taken in selection of the colony.

(3) When the test produced negative results for all the polyvalent and monovalent K sera, it is recommended to add the same volume of 0.2mol/L hydrogen chloride solution to the test bacteria suspension and, after mixing well, the solution is left intact at the room temperature for 30 minutes. The suspension is then centrifuged, cells are collected and the supernatant is discarded. The sediment is then resuspended in an appropriate volume of physiological saline for washing cells, the suspension is centrifuged, the supernatant is discarded, and cells in the sediment are used for K typing using the above method. If the result is again negative, the bacteria are presumed to possess a new type of K antigen which has not yet been included in the existing list of types.

2. Determination of O grouping

The test bacteria are suspended in 3w/v% sodium chloride solution to make the concentration of the cells about 10 mg/mL, the suspension is heated at 121 ℃ for 1 hour, it is centrifuged, the supernatant is discarded and the sediment is used as antigen for the test. The K antigen of the test bacteria is occasionally not inactivated after heating at 121 ℃ for 1 hour and O agglutination may be inhibited by K antigen. However, *V. parahaemolyticus* tends to produce spontaneous agglutination by heating. It is recommended to avoid such problems by using 3w/v% sodium chloride solution supplemented with 5w/v% glycerin to suspend the cells. When glycerin supplemented saline is used, the suspension must be centrifuged before use and the sediment should be used in the test.

The slide agglutination method is convenient for O groupings as well although tube agglutination us recommended when required. In this case, use of serum of a volume of 1/10 that of antigen suspension is appropriate.

1) Slide agglutination test

(1) When K type is known:

The O group is guessed according to the table of below, and one drop each of the O group antiserum corresponding to the K antigen is placed on a clean glass slide, the antisera are mixed well with one loopful of the heated test bacteria and is observed for agglutination. If the result is negative, all other groups of O antisera are tried in the same manner as described above, and the result is observed.

The O antiserum group, which produces agglutination of cells, is the O group of test bacteria. Agglutination should be observed within 1 minute after the addition of bacterial suspension.

(2) When K typing of the test bacterium cannot be carried out or when this has not been done:

One drop each of the O antisera is placed onto a glass slide. These are mixed with one loopful of test bacteria and agglutination of the cells is observed with one group of antisera. The O group of antisera which produced agglutination of cells is the same as the O group of the test bacteria.

2) Test tube agglutination

Heated test bacteria are suspended in 3w/v% sodium chloride solution at a concentration of about 1 mg/mL, and 0.5 mL aliquot of these are put into 12 small test tubes (1 test tube is used for the control). Then, 3 drops each of the O grouping sera are added to these small test tubes which are incubated at 37 ℃ for 2 hours, and then left intact in a refrigerator overnight to observe if agglutinations occur. The O group of the antisera which produced positive results is the same as the O group of the test bacteria.

Note: If the heated test bacteria do not react with any of the O group antisera, the antigen should be resuspended in 3w/v% saline supplemented with 5w/v% glycerin, and should be retested after heating the suspension at 121 ℃ for 1 hour.

Antigenic Schema of *Vibrio parahaemolyticus*

O group	K type
1	1, 25, 26, 32, 38, 41, 56, 58, 64, 69
2	3, 28
3	4, 5, 6, 7, 29, 30, 31, 33, 37, 43, 45, 48, 54, 57, 58, 59, 65
4	4, 8, 9, 10, 11, 12, 13, 34, 42, 49, 53, 55, 63, 67, 68
5	15, 17, 30, 47, 60, 61, 68
6	18, 46
7	19, 52
8	20, 21, 22, 39, 70
9	23, 44
10	19, 24, 52, 66, 71
11	36, 40, 50, 51, 61

PRECAUTIONS

1. If the serum freezes, it may produce a sediment when thawed. Take care not to allow antisera to freeze.
2. All living cells, and instruments such as glass slides and test tubes used in this test should be sterilized by either soaking in 0.1w/v% sodium hypochlorite (available chlorine approximately 1,000 ppm) for more than 1 hour or by autoclaving at 121 ℃ for more than 20 minutes.
3. This reagent contains 0.1w/v% sodium azide as a preservative. Sodium azide may react with lead and copper piping to form highly explosive heavy metal azide. Upon disposal, flush this reagent away with a large volume of water to prevent accumulation of azides.
4. On storage, some sera become slightly turbid. This does not necessarily indicate deterioration and the sera may be clarified by centrifugation or membrane filtration (0.45 μm) before use. Gross turbidity indicates contamination and such sera should be discarded.

STORAGE AND SHELF LIFE

Storage: 2 - 10 ℃

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

PACKAGES

[O grouping sera] each group in a 2 mL vial with a pipette
Set 11 vials 1 box

[K typing sera] each type in a 2 mL vial with a pipette
Set 74 vials 1 box

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