

コレラ菌AD「生研」

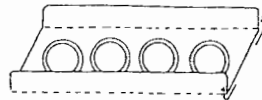
In Vitro Diagnostic Reagents
Ref. (61E) No. 1356* 1996.9 Updated
1990.7 Updated**Vibrio Cholerae O1 AD "SEIKEN"**A kit for the detection and serotyping of
Vibrio cholerae O1 by slide latex agglutination
50 tests

Cholera, which has been designated as an international quarantinable contagious disease, is caused by a bacterium, *Vibrio cholerae* O1. It can be differentiated from other *V. cholerae* (non-O1 *V. cholerae*) by serotyping.

The antigenic structure of *V. cholerae* O1 has been reported by Sakazaki et al.¹⁾ According to their report, the *Vibrio* antigen consists of three types of antigenic factors a, b and c and the mode of combination of these factors determines the type of *Vibrio*, i.e. Ogawa, Inaba and Hikojima types. In this test, a slide latex agglutination test is employed. Monoclonal antibodies are produced against the antigenic factors a, b and c of *V. cholerae* O1 and polystyrene latex particles are sensitized with monoclonal antibodies. These sensitized latex particles will agglutinate in the presence of corresponding *V. cholerae* O1 antigen factors. This test kit is designed to perform serotyping of a culture isolate and also to detect *V. cholerae* O1 antigen directly from stool specimens taken from patients in a screening test. The test is very specific because of the use of monoclonal antibodies and is also very rapid, easy and sensitive.

CONTENTS

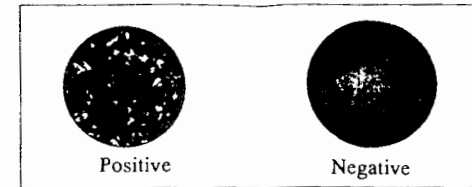
- Sensitized latex a:** 2 ml 1 vial
Latex suspension sensitized with monoclonal antibodies which recognize factor a of *V. cholerae* O1 antigen, containing 0.1 w/v% sodium azide as a preservative.
- Sensitized latex b:** 2 ml 1 vial
Latex suspension sensitized with monoclonal antibodies which recognize factor b of *V. cholerae* O1 antigen, containing 0.1 w/v% sodium azide as a preservative.
- Sensitized latex c:** 2 ml 1 vial
Latex suspension sensitized with monoclonal antibodies which recognize factor c of *V. cholerae* O1 antigen, containing 0.1 w/v% sodium azide as a preservative.
- Control latex:** 2 ml 1 vial
Latex suspension sensitized with normal mouse immunoglobulins, containing 0.1 w/v% sodium azide as a preservative. This reagent is used as a control for sensitized latex.
- Slides for agglutination:** 55 sheets
Turn up two edges of the plate before use as indicated below.

**PROCEDURE****1. Serotyping of culture isolate**

After confirming that the culture isolate is *V. cholerae* O1 by biochemical examinations, the typing of *Vibrio* is carried out as described below.

- Distribute one drop of sensitized latexes a, b, c and the control latex to the four circles on a slide.
- Add a small volume of bacterial culture using a platinum wire to each circle and mix the sample and latex suspension.
- Tilt the slide back and forth for approximately 10 seconds.

- Read the results with the naked eye in a light place. Clear agglutination is regarded as positive while reactions in which the control agglutinates are interpreted as non-specific.*



- Determine serotype as indicated below.**
Positive reaction with sensitized latex a and bserovar Ogawa
Positive reaction with sensitized latex a and cserovar Inaba
Positive reaction with sensitized latex a, b, and cserovar Hikojima
- Direct detection of *Vibrio* from stool samples**
 - One drop of watery stools taken from a patient suspected of having a cholera infection are placed on two circles on a slide.***
 - Add one drop of sensitized latex a and the control latex to the specimen on the slide and mix.
 - Tilt the slide back and forth for approximately 1 minute.
 - Read the results with the naked eye in light place. Clear agglutination is regarded as positive while reactions in which the control agglutinates are interpreted as non-specific.*

- * If misleading results are observed or a non-specific reaction occurs with the control latex the specimen must be heated at 100 °C for 10 minutes and retested using the same procedures as described above.
- ** Please note that a prolonged contact with Ogawa type *V. cholerae* may also lead to agglutination of sensitized latex c.
- *** Specimens containing solid substances should be centrifuged at 3000 rpm for 5 minutes and the supernatant used in the test as a specimen.

Performance of the kit and substances which may interfere with reactions.**1. Sensitivity in detecting the antigen**

Sensitivity of sensitized latex a in detecting Ogawa and Inaba type: 5×10^6 cells/ml
Sensitivity of sensitized latex b in detecting Ogawa type: 1×10^7 cells/ml
Sensitivity of sensitized latex c in detecting Inaba type: 4×10^7 cells/ml

2. Specificity

In our test to check the specificity of the test, sensitized latexes a, b and c reacted solely with *V. cholerae* O1 bearing corresponding antigenic factors and they did not react with non-O1 *V. cholerae*, *E. coli*, *Shigella*, *Salmonella*, *Vibrio parahaemolyticus* or *Yersinia enterocolitica*.

3. Interfering substances

Solid or viscous substances may interfere with reactions. The influence by these can be eliminated by centrifugation or heating.

NOTE

- The reagents are for in vitro diagnostic use only.
- Take care not to freeze the reagents. Bring the reagents to room temperature before use by leaving the reagent vials at room temperature for more than 30 minutes.
- Shake the reagent vials well before use to ensure homogeneous suspension.
- Take care not to mix up the lids of the reagents. *

5. After use slides must be sterilized with disinfectant solution or by autoclaving before disposal.
6. The direct detection of *V. cholerae* O1 using this test kit is regarded as a screening test and for confirmation of antigen existence, cultivation of bacteria is necessary.

STORAGE AND SHELF LIFE

Storage: 2–10 °C

Shelf life: Up to expiry date on the label.

PACKAGE

V. cholerae O1 AD "SEIKEN" 50 tests per box

REFERENCE

1. Sakazaki, R. et al.: Japanese Journal of Medical Scientific Biology **24**, 93 (1971).
2. Shimada, T. et al.: A serogroup of non-O1 *Vibrio cholerae* possessing the Inaba antigen of *Vibrio cholerae* O1, *J. Appl. Bacteriol.*, **64**, 141 (1988).
3. Shimada, T. et al.: *Vibrio fluvialis*: A new serogroup (19) possessing the Inaba factor antigen of *Vibrio cholerae* O1, *Japan. J. Med. Sci. Biol.*, **40**, 153 (1987).
4. Shimada, T. et al.: A bioserogroup of marine vibrios possessing somatic antigen factors in common with *Vibrio cholerae* O1, *J. Appl. Bacteriol.*, **62**, 453 (1987).