Cholera DFA
89-114100
Revision 2-09212011

A Direct
Fluorescent-Monoclonal Antibody
Staining Kit
for Detection and Enumeration of *Vibrio cholerae* O1

For Laboratory Use Only.
Not for Human Use.
INTENDED USE

The DFA Cholerae Test Kit, by New Horizons Diagnostics, is intended for the direct detection of *Vibrio cholerae* O1 in clinical, food, and water samples.

INTRODUCTION

*Vibrio cholerae* O1 is the causative agent of epidemic cholera. The vibrios are aquatic bacteria found in a wide variety of environmental water sources. Cholera is spread primarily by ingestion of contaminated water or raw, poorly cooked, or recontaminated seafood. While the majority of people infected with *V. cholerae* O1 have no or mild symptoms, a certain proportion of the population have severe diarrhea with massive fluid loss that can lead to death. Thus, the spread of cholera through carrier can be extensive. A rapid and reliable test for *V. cholerae* O1 in water is of great value to public health officials in controlling spread of the bacteria and the associated disease 1,2.

A number of studies have shown that the organism can exist in a viable, but non-culturable state. Animal studies have demonstrated that such cells remain pathogenic. The organisms can also regain culturability upon changes in environmental conditions including water temperature and salinity. Detection of organisms by fluorescent antibody staining has been shown to be highly sensitive in detecting both culturable and non-culturable organisms3.

The Cholera DFA Test consists of a monoclonal antibody, specific for the A antigen of O1 lipopolysaccharide in the outer membrane of *V. cholerae* O1 that is directly labeled with fluorescein isothio cyanate (FITC) for the rapid, simple detection and enumeration of *V. cholerae* O1 in water, food, and stool samples 4.

PRINCIPLE

The test kit is comprised of the Cholera DFA reagent and two control reagents. Water samples are concentrated and a sample is fixed onto a microscope slide. The test sample and control samples are then incubated with the DFA reagent. If the sample contains *V. cholerae* O1, the FITC-labeled monoclonal antibody will bind *V. cholerae* O1. After washing, the slide is examined under the fluorescent microscope.
## MATERIALS PROVIDED

For 100 determinations

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>QUANTITY</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cholera DFA Reagent</td>
<td>1 amber bottle</td>
<td>Mouse monoclonal antibody to <em>V. cholerae</em> O1 - labeled with fluorescein.</td>
</tr>
<tr>
<td></td>
<td>(lyophilized)</td>
<td>Contains 0.01% sodium azide, rhodamine counterstain, and protein in phosphate buffer.</td>
</tr>
<tr>
<td>2. Cholera Positive Control</td>
<td>1 bottle</td>
<td>Formalin fixed inactivated <em>V. cholerae</em> O1.</td>
</tr>
<tr>
<td></td>
<td>(1 mL)</td>
<td>Contains 0.05% sodium azide as a preservative.</td>
</tr>
<tr>
<td>3. Cholera Negative Control</td>
<td>1 bottle</td>
<td>Formalin fixed inactivated <em>V. cholerae</em> non-O1.</td>
</tr>
<tr>
<td></td>
<td>(1 mL)</td>
<td>Contains 0.05% sodium azide as a preservative.</td>
</tr>
<tr>
<td>4. Glass Slide</td>
<td>14 slides</td>
<td>Reusable glass slide with 8 wells for samples.</td>
</tr>
<tr>
<td>5. Fluorescent Mounting Medium</td>
<td>1 bottle</td>
<td>Vialled at working dilution. Contains an inhibitor that retards photobleaching of fluorescein.</td>
</tr>
</tbody>
</table>
MATERIALS REQUIRED BUT NOT SUPPLIED

1. Pipettes and micropettes and safety pipetting devices.
2. Coverslips, 22x50mm, No. 1 thickness.
3. Filters, 10.0 µm pore size and 0.45 µm pore sizes (available at NHD).
4. Ethanol or methanol.
5. Filtering device with hand pump (available at NHD).
6. Distilled or deionized water.
7. Moist chamber - could be a petri dish with wet paper towel in bottom.
8. Incubator (35°C).
9. Epifluorescent Microscope. Refer to manufacturer instructions manual for the filter system which gives optimum results for FITC. (Maximum excitation wavelength = 490 nm and maximum emission wavelength = 520 nm.)

PRECAUTIONS

1. FOR USE WITH WATER SAMPLES ONLY. NOT FOR USE IN DIAGNOSIS OF HUMANS OR ANIMAL DISEASE.
2. Positive control and negative control materials have been fixed with formalin (2%). Good laboratory procedures dictate that these materials, as well as the samples, be handled and disposed of as potentially hazardous material.
3. Some of the reagents contain sodium azide. Sodium azide may react with lead and copper plumbing to form a highly explosive metal azide. On disposal, flush liberally with water.

REAGENT PREPARATION

1. Cholera DFA Reagent. Reconstitute in 1.0 mL of distilled or deionized water. Keep at 4-8°C following reconstitution. Store reagent away from bright light.
SAMPLE PREPARATION

1. Water samples of 100 to 500 mL should be collected in a clean container. If the water sample is turbid, it should be initially filtered through a 10.0 \( \mu \text{m} \) filter.

2. Using negative pressure from pump or other vacuum source, concentrate the 100 to 500 mL water sample. Place the filter on a clean petri dish and add 1 mL of sterile PBS onto it in order to obtain a thick suspension of organisms.

3. Stool samples need to be filtered through specimen filtering devices (available upon request from NHD).

SUGGESTED PROCEDURE

1. Prepare Cholera DFA Reagent and samples. All materials should be at room temperature.

2. Make a thin smear of resuspended sample by adding 5 \( \mu \text{l} \) on a well, then spreading the contents to cover the well.

3. Controls should be run at least once a day. Make a thin smear of the positive control by adding a small drop of the control on a well, then spreading the drop to cover the well. Make a similar thin smear of the negative control. Make a thin smear of the sample by adding approximately 5 \( \mu \text{l} \) of the sample to the slide and spreading.

4. Air dry.

5. Add one drop of absolute methanol or ethanol to each control or sample well to fix the smear, then air dry.

6. Add 10 \( \mu \text{l} \) of reconstituted Cholera DFA Reagent to each well.

7. Place the slides in a covered, moist chamber, and incubate at 35\( ^\circ \text{C} \) for 30 \( \pm \) 5 minutes. Protect from light.

8. Rinse the slides thoroughly with PBS. Protect from light.

9. Absorb off excess moisture using a blotter paper.

10. Add a drop of Fluorescent Mounting Medium on the slide and cover with a 22x50mm, No. 1 coverslip.
11. For best results, the slides should be read immediately at a magnification of 1000 X with oil immersion. Equivalent readings may be obtained if the slides are read within 24 hours. The slides must, however, be kept cool, in the dark, and sealed, or kept humid to prevent drying.

**INTERPRETATION**

1. Quality Control: For the test samples to be valid, the Positive Control should exhibit bright, apple-green, fluorescence and there should be no fluorescence on the Negative Control well.

2. The sample is considered Positive if apple-green fluorescence is observed that appears in a ring shaped pattern.

**LIMITATIONS**

The DFA procedure is capable of detecting as little as 10,000 organisms/mL. Given a concentration of the sample of at least 100-fold, the test should be capable of detecting the presence of any significant amount of *V. cholerae* O1 contamination in a sample. The sensitivity of the procedure will be affected by a number of factors, including the number of *V. cholerae* O1 in a sample, the quality of the sample, the sample age, etc. The presence of a heavy load of other organisms may mask a positive reaction or contribute to non-specific binding. Questionable samples should be repeated and additional samples from the same source should be obtained. Detection can also be enhanced by enriching the sample in alkaline peptone water (APW, pH 8.4 ± 0.2) for 6-8 hours. Call the Technical Service Department at New Horizons Diagnostics (NHD) at 1-443-543-5746 if you require assistance.
REFERENCES


