

# **Bengal DFA**

89-116111

Revision 1-080501

**A Direct  
Fluorescent-Monoclonal Antibody  
Staining Kit**  
for Detection and Enumeration of  
*Vibrio cholerae* O139

**For Laboratory Use Only.  
Not for Human Use.**

## **New Horizons Diagnostics Corporation**

9110 Red Branch Road  
Columbia, Maryland USA 21045  
410 / 992-9357 (fax) 410.992.0328  
e-mail: [NHDiag@aol.com](mailto:NHDiag@aol.com)  
[www.NHDiag.com](http://www.NHDiag.com)

## INTENDED USE

The Bengal DFA Cholerae Test Kit, by New Horizons Diagnostics, is intended for the direct detection of *Vibrio cholerae* O139 in clinical, food, and water samples. Not for use in diagnosis or treatment of human or animal disease.

## INTRODUCTION

Until recently, only toxigenic *V. cholerae* serogroup O1 was believed to be capable of causing epidemic cholera. The other 137 serogroups, collectively called the "non-O1" serogroups (O2-O138) were not thought to have pandemic potential, and to include only organisms that cause sporadic diarrheal illness or occasional limited outbreaks. However, in the late part of 1992, large epidemics of cholera broke out in southern and eastern India and southern Bangladesh, and has since spread to the entire Indian subcontinent and several neighboring countries. The epidemic strain is a new serogroup of *V. cholerae* non-O1 and assigned to serogroup O139 with the synonym Bengal, to refer to its first isolation from areas surrounding the Bay of Bengal. By January, 1994, the organism had officially been reported from Bangladesh, India, Malaysia, Nepal, Pakistan, China, Thailand, the United Kingdom, and the United States<sup>1</sup>.

New epidemics of cholera due to O139 strains are affecting persons of all ages in an area where most of the population except for young children has some level of acquired immunity to *V. cholerae* O1. This suggests that prior to immunity to O1 does not protect against O139 infection. It also suggests that existing and experimental O1 cholera vaccines will not induce immunity to this strain. Widespread transmission of *V. cholerae* O139 with outbreaks similar to those caused by the O1 can, therefore, be expected to occur in Latin America once O139 is introduced into the region<sup>1</sup>.

The vibrios are aquatic bacteria found in a wide variety of environmental water sources<sup>2</sup>. Cholera is spread primarily by ingestion of contaminated water or raw, poorly cooked, or recontaminated seafood. A rapid and reliable test for *V. cholerae* O139 is of great value to public health officials in monitoring and in controlling spread of the bacterium<sup>3</sup>.

The Bengal DFA Test consists of a monoclonal antibody, specific for the surface antigen of O139 lipopolysaccharide in the outer membrane of *V. cholerae* O139<sup>4</sup> that is directly labeled with fluorescein isothiocyanate (FITC) for the rapid, simple detection and enumeration of *V. cholerae* O139 in clinical food and water samples<sup>5</sup>. New Horizons also manufactures a similar kit, Cholera DFA, for direct detection and enumeration of *V. cholerae* serogroup O1<sup>5</sup>.

## PRINCIPLE

The test kit is comprised of the Bengal 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* O139, the FITC-labeled monoclonal antibody will bind *V. cholerae* O139. After washing, the slide is examined under the fluorescent microscope.

## MATERIALS PROVIDED: For 100 determinations

<u>MATERIAL</u>	<u>QUANTITY</u>	<u>DESCRIPTION</u>
1. Bengal DFA Reagent	1 amber bottle (lyophilized, 1mL)	Mouse monoclonal anti- body to <i>V. cholerae</i> O139 – labeled with fluorescein. Contains protein in phosphate buffered saline.
2. Bengal Positive Control	1 bottle (2 mL)	Formalin inactivated <i>V. cholerae</i> O139. Contains 0.05% sodium azide as a preservative.
3. Cholera Negative Control	1 bottle (2 mL)	Formalin inactivated <i>V. cholerae</i> non-O1. Contains 0.05% sodium azide as a preservative.
4. Glass Slide	10 slides	Reusable glass slide with 10 wells for samples.
5. Fluorescent Mounting Medium	1 bottle (2 mL)	Vialed at working dilution. Contains an inhibitor that retards photobleaching of fluorescein as well as a counterstain.

## **MATERIALS REQUIRED BUT NOT SUPPLIED**

1. Pipettes and micropettes and safety pipetting devices.
2. Coverslips, 22x50mm, No. 1 thickness.
3. Filters, 10.0  $\mu\text{m}$  pore size and 0.45  $\mu\text{m}$  pore sizes (available at NHD).
4. Absolute ethanol or methanol.
5. Filtering device with hand pump (available at NHD).
6. Distilled or deionized water.
7. Phosphate buffered saline (PBS).
8. Moist chamber - could be a petri dish with wet paper towel in bottom.
9. Incubator (35°C).
10. 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.)

## **REAGENT PREPARATION**

1. Bengal 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 Bengal 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 or incubate at 37°C until dry.
5. Add 5  $\mu\text{l}$  of absolute ethanol or methanol to each control or sample well to fix the smear, then air dry.
6. Add 10  $\mu\text{l}$  of reconstituted Bengal DFA Reagent to each well.
7. Place the slides in a covered, moist chamber, and incubate at 35°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 22 x50mm, 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 minimal or no green fluorescence on the Negative Control well. The counterstain appears red and should block out most, if not all background.
2. The sample is considered Positive if apple-green fluorescence is observed around the perimeter of the cell, creating an outline. The bright green color of a positive sample will clearly contrast the red counterstain background.

## **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* O139 contamination in a sample. The sensitivity of the procedure will be affected by a number of factors, including the number of *V. cholerae* O139 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 1-800-888-5015 or 410/992-9357 if you require assistance.

## **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.

## **REFERENCES**

1. Cholera situation in the Americas. 1994. Pan American Health Organization/World Health Organization.
2. Colwell, R.R., M.L. Tamplin, P.R. Brayton, A.L. Gauzens, B.D. Tall, D. Herrington, M.M. Levine, S. Hall, A. Huq, and D. Sack. 1990. Environmental aspects of *Vibrio cholerae* O1 in transmission of cholera, p. 327-343. In R.B. Sack, and Y. Zinnaka (ed.), *Advances in Research on Cholera and related diarrheas*. KTK Scientific Publishers, Tokyo.
3. Hasan, J.A.K., L. Loomis, D. Bernstein, A. Huq, R.B. Sack, G.B. Nair, and R.R. Colwell. 1993. Development of rapid immunodiagnostic kits to detect *Vibrio cholerae* O139 synonym Bengal, abstr. P. 222. Abstr. 29<sup>th</sup> Joint Conference on cholera and related Diarrheal Diseases. The United States-Japan Cooperative Medical Science Program. National Institute of Health.
4. Garg S., T. Ramamurthy, A.K. Mukhopadhyay, B.C. Deb, G.B. Nair, T. Shimada, T. Takeda, A. Huq, R.R. Colwell, and Y. Takeda. 1994. Production and cross-reactivity patterns of a panel of high affinity monoclonal antibodies to *Vibrio cholerae* O139 Bengal. *FEMS Immunol. Med. Microbiol.* 8:293-298.
5. Hasan, J.A.K., A. Huq, G. B. Nair, S. Garg, A.K. Mukhopadhyay, L. Loomis, D. Bernstein, and R.R. Colwell. 1995. Development and testing of monoclonal antibody-based rapid immunodiagnostic test kits for direct detection of *Vibrio cholerae* O139 synonym Bengal. *J. Clin Microbiol.* 33:2935-2939.
6. Hasan, J.A.K., D. Bernstein, A. Huq, L. Loomis, M.L. Tamplin, and R.R. Colwell. 1994. Cholera DFA: an improved direct fluorescent monoclonal antibody staining kit for rapid detection and enumeration of *Vibrio cholerae* O1. *FEMS Microbiol. Lett.* 120:143-148.

**Reorder No. : 89-116111**

## **New Horizons Diagnostics Corporation**

9110 Red Branch Road

Columbia, Maryland USA 21045

410 / 992-9357 (fax) 410.992.0328

e-mail: [NHDIag@aol.com](mailto:NHDIag@aol.com)

[www.NHDIag.com](http://www.NHDIag.com)

P.N.: 88-116001