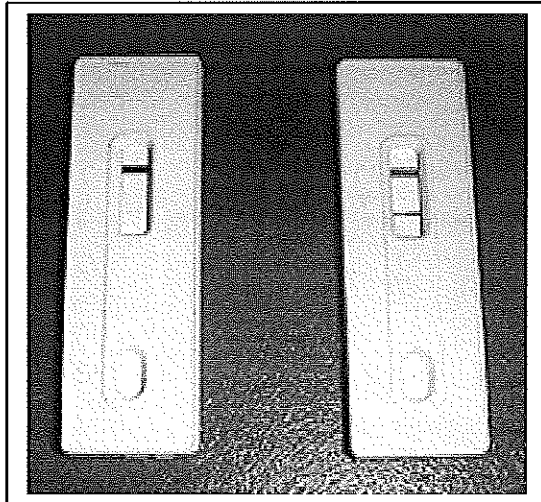


# CHOLERA O139 SMART™ II

25 Determinations

Reorder No. 89-115225R



A Colorimetric Immunoassay for the  
Direct Detection of *Vibrio cholerae* O139  
Synonym Bengal strain

For Research Use Only

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## INTENDED USE

Cholera O139 SMART™ II (Sensitive Membrane Antigen Rapid Test) is a rapid, lateral flow, colorimetric immunoassay designed for the direct presumptive detection of *Vibrio cholerae* O139 in clinical samples as an adjunct to classical culture methods.

## 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 (O2-138) were not thought to have pandemic potential and included 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; then spreading 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 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.

## PRINCIPLE OF THE TEST

The Cholera O139 SMART™ II assay is a rapid, qualitative test in the lateral flow format. Cholera O139 specific monoclonal antibody-coated colloidal gold particles (red-colored) are applied to a membrane surface and dried. A sample is placed in a specimen filtering device and if necessary, treated with extraction buffer. When 4 drops of an appropriately treated specimen, from the specimen filtering device, are squeezed into the (S) sample well, the gold conjugate reacts with cholera O139 antigen in the sample as it migrates across the length of the membrane to where it encounters two zones of capture antibody (T) Test and (C) Control. Those antibody-gold conjugates, which have been bound to the antigen in the sample, are then bound in the *V. cholerae* O139 capture antibody zone (T),

presenting a visually detectable line of color and indicating a positive test result at (T). If no *V. cholerae* O139 is present, no line will form at (T) and the sample will continue to migrate to the Positive Control Line (which is not specific for the cholera antigen) and will bind with any excess gold-conjugated antibody yielding a red line. The (C) Line must be visible to ensure the device is working properly. Appearance of one line at (C) is indicative of a sample negative for *V. cholerae* O139. Appearance of two lines, one at (T) and one at (C) is indicative of a positive *V. cholerae* O139 sample. The total time to perform the test is less than 20 minutes.

## MATERIALS PROVIDED

Each kit contains the following in quantities sufficient to perform 25 determinations.

- FOIL POUCH:** Each foil pouch contains one SMART™ II device.  
**CHASE BUFFER:** Each bottle of Chase buffer contains processed water, detergents, and 0.05% sodium azide (preservative).  
**POSITIVE CONTROL REAGENT:** The bottle of positive control reagent contains formalin-treated *V. cholerae* O139 organisms in buffer with 0.05% sodium azide (preservative).  
**EXTRACTION BUFFER:** The bottle of extraction buffer contains tris buffered saline with EDTA and 0.05% sodium azide (preservative).

## SPECIMEN FILTERING:

- Specimen Filtering Device:** Soft plastic tube and a snap-on filter.  
**Glass dropper:** The glass dropper is marked at 0.3/0.5 ml.  
**Plastic droppers:** Disposable paddle-end plastic droppers.

## STORAGE AND STABILITY: CAUTION: DO NOT FREEZE!

The expiration date of the kit is indicated on the outer box label and is based on proper storage of the components. Reagents can be stored either refrigerated or at room temperature (2 °C to 30°C or 34 °F to 86°F).

## PRECAUTIONS

1. Safety precautions should be observed in handling and disposing of processed test materials as with any other microbiological/clinical materials.
2. All reagents contain 0.05% sodium azide. Sodium azide may react with lead and copper plumbing to form a highly explosive metal azide. On disposal, flush liberally with water.
3. The reagents have been tested as a unit. Do not substitute reagents from other kit lots.

4. Do not use reagents beyond the indicated expiration date.
5. Do not dilute any of the reagents. This will have an impact both on test sensitivity and stability.

## SPECIMEN COLLECTION AND HANDLING

Samples should be stool specimens. Samples that will not be tested directly should be frozen or can be placed in Alkaline Peptone Water (APW) at a 1:10 (volume of sample): (volume of APW) ratio and incubated for a maximum of 24 hours prior to testing.

Use of rectal swabs is not recommended. If a rectal swab is to be used, the swabs should be placed directly in 1 ml of APW and incubated at 23°C to 40°C for a maximum of 24 hours prior to testing. Transporting a rectal swab in Cary-Blair transport medium may reduce sensitivity. However, if Cary-Blair transport medium is used to transport the rectal swab, place it directly into a specimen filtering device containing 1 ml of extraction buffer and mix thoroughly.

## SPECIMEN PREPARATION

1. If stool specimen is liquid, use a disposable plastic dropper to place the specimen into the soft plastic tube of the specimen filtering device. Fill up approximately half of the tube. Angle the snap-on filter (cap) to the top of the soft plastic tube. Snap on cap and mix. Large particles in the specimen should be allowed to settle before placing the specimen into the filtering device.
2. If stool specimen is semi-soft or formed, use a stick to place the specimen into the soft plastic tube of the specimen filtering device. Fill up approximately one-fourth of the tube. Using a glass dropper, add 0.5 ml of extraction buffer to the tube. Angle the snap-on filter (cap) to the top of the soft plastic tube. Snap on cap and shake vigorously. Vigorous shaking should produce a sufficient volume of extracted sample that can be processed. If not, add another 0.5 ml of extraction buffer and shake well. If the specimen is still difficult to pass through the specimen filtering device, large particles in the sample should be allowed to settle and the specimen should be placed into a second device. **It is important that the specimen not be diluted anymore than suggested. Over dilution may reduce the sensitivity of the test.**

## TEST PROCEDURE

1. Collect samples and follow dilution guidelines to ensure sample is in a liquid form.
2. Open Cholera SMART™ II pouch. Remove contents. Label device with Sample Identification using permanent marker.

- Invert and squeeze the specimen filtering device to place 3 drops into the sample well of a lateral flow device.
- Wait approximately three (3) minutes or for the sample to be absorbed into the sample well. Then place three (3) free falling drops of Chase buffer from the dropper bottle into the sample well.
- Read results after 15 minutes (no longer than 30 minutes) of sample addition. Observe the development of color on the Control (C) and Test Line (T) and record result. See RESULTS table to interpret test.

### QUALITY CONTROL

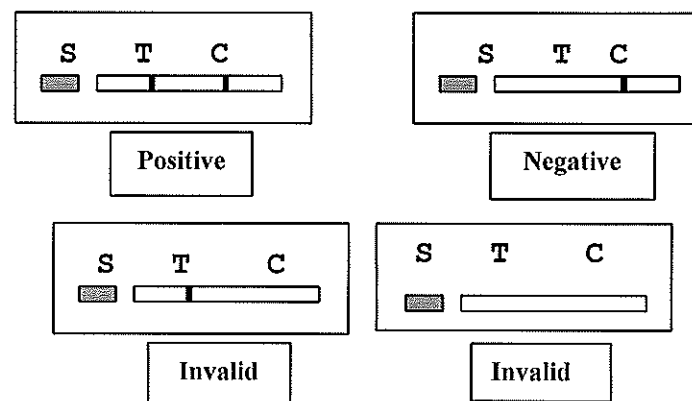
Perform quality control on a SMART™ II device using the Positive Control reagent each day the kit is used to ensure proper kit performance.

- Open Cholera SMART™ II lateral flow device pouch. Remove contents. Label device as Positive Control sample using permanent marker.
- Add 3 drops of cholera Positive Control reagent into the sample well of the lateral flow device.
- Follow steps 4-5 in the Test Procedure.
- Two distinct red lines should appear at the Control and Test Line indicating a positive sample. If no red line appears at the Test Line or at the Test Line and Control Line, review the instructions and repeat the test. If the quality control result is still unsatisfactory, do not report out results of test performed that day. Please contact New Horizons Diagnostics for technical assistance or replacement at 1-800-888-5015 or (410) 992-9357.
- The Chase Buffer could be used as a Negative Control reagent and the procedure outlined in the previous steps for positive control followed.
- The appearance of a distinct red line only at the Control Line would indicate a negative sample. A separate Negative Control comprising a *V. cholerae* non-O139 organism may also be included in the daily check as an additional control.

### RESULTS:

POSITIVE TEST	Appearance of a <u>distinct</u> red line on both <b>CONTROL</b> and <b>TEST</b> Lines.
NEGATIVE TEST	Appearance of a red line only at the <b>CONTROL</b> Line and absence of a red line on the <b>TEST</b> Line.
INVALID	Appearance of red line at the <b>TEST</b> Line and absence of a red line on the <b>CONTROL</b> Line.
INVALID	No lines appeared. Sample did not flow.

### ILLUSTRATION:



## LIMITATIONS OF THE PROCEDURE

1. Results obtained from this test should be used as an adjunct to other information available including symptoms and culture results as appropriate. Cholera SMART II is not intended for use as the sole diagnosis of the *V. cholerae* O139 disease.
2. Cholera O139 SMART™ II does not detect *V. cholerae* O1 or non-O1/O139 strains. The non-O1/O139 strains may cause diarrhea and other symptoms similar to those caused by *V. cholerae* O1.
3. Cholera O139 SMART™ II has been devised for use with fecal specimens. Use of swabs and specimens such as urine, saliva, or wound exudates has not been confirmed.
4. Cholera O139 SMART™ II recognizes an antigen in the LPS of *V. cholerae* O139. The test may detect both viable and non-viable bacteria and may be positive following successful treatment.

## EXPECTED VALUES

Cholera occurs in epidemic outbreaks and is endemic in certain areas of the world. Outside of these areas, the occurrence of cholera is very rare. Sporadic cases of gastroenteritis caused by *V. cholerae* O1 or O139 have been identified in non-endemic areas usually associated with consumption of raw seafood, travelling from epidemic areas, accidental trauma infected with contaminated food or water or other risk behaviors.

## PERFORMANCE CHARACTERISTICS

Cholera O139 SMART™ II (lateral flow format) has been shown to be equivalent to Cholera SMART™ (flow-through format) in laboratory tests.

### Analytical Sensitivity

The analytical sensitivity of Cholera SMART™ II was tested using suspensions of *V. cholerae* O139 from pure culture. Dilutions were made from a starting suspension and bacterial numbers were assessed by optical density at 650nm. Cholera SMART™ II consistently detected suspensions that contained at least  $2 \times 10^7$  colony forming units/ml based on optical density.

Cholera O139 SMART™ II was tested with four strains of *V. cholerae* O139 and was positive on all strains tested.

### Cross-reactivity

The cross-reactivity of Cholera O139 SMART™ II for other organisms was assessed using suspensions of pure cultures of organisms containing  $>10^8$  CFU/ml. None of the other organisms tested showed any cross-reactivity in the test. Organisms tested for cross-reactivity were (number of strains is indicated in parentheses):

*Aeromonas hydrophila* (2), *Escherichia coli* (3), *Pseudomonas aeruginosa* (1), *Salmonella typhi* (1), *Serratia marcescens* (1), *Shigella dysenteriae* type 1 (1), *Vibrio cholerae* O1 and non-O1/non-O139 (3), *Vibrio cincinnatiensis* (1), *Vibrio damsela* (1), *Vibrio harveyi* (1), *Vibrio hollisae* (1), *Vibrio ordalii* (1) and *Vibrio vulnificus* (2).



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