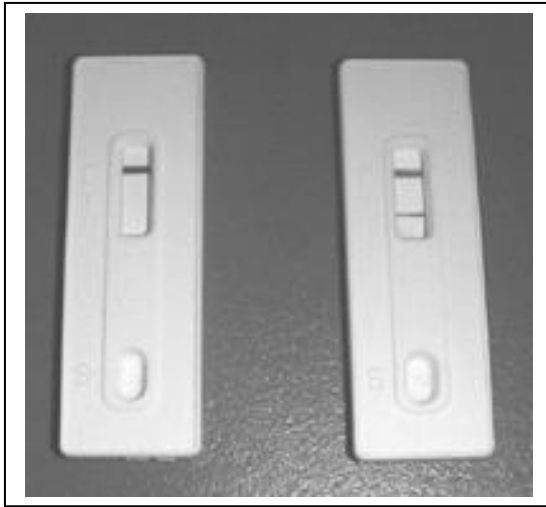


CHOLERA SMART™ II

25 Determinations

Reorder No. 89-113225R



A Colorimetric Immunoassay for the

Direct Detection of *Vibrio cholerae* O1

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INTENDED USE

Cholera SMART™ II (Sensitive Membrane Antigen Rapid Test) is a rapid, lateral flow, colorimetric immunoassay designed for the direct presumptive detection of *Vibrio cholerae* O1 in clinical samples as an adjunct to classical culture methods.

INTRODUCTION

Cholera epidemics, caused by *V. cholerae* serotype O1, continue to be a devastating disease of immense global significance in many developing countries. Clinically, cholera may range from asymptomatic colonization to severe diarrhea with massive fluid loss, leading to dehydration, electrolyte disturbances, and death. *V. cholerae* O1 causes this secretory diarrhea by colonization of the small intestine and production of a potent cholera toxin.

Because of the clinical and epidemiological importance of cholera, it is critical to determine as quickly as possible whether or not the organism from a patient with watery diarrhea is positive for *V. cholerae* O1. A fast, simple and reliable method for detecting *V. cholerae* O1 is a great value for clinicians in managing the disease and for public health officials in instituting control measures. NHD's monoclonal antibody provides specificity for the *cholerae* O1 antigen thereby circumventing the many inherent problems encountered when polyclonal anti-O1 antibody is used to identify *V. cholerae* O1 from samples. The Cholera SMART™ II utilizes a monoclonal antibody based lateral flow format. The monoclonal antibodies are specific for the A antigen of O1 lipopolysaccharide (LPS) of *V. cholerae* O1. The Cholera SMART™ II test is simple, and can be performed in approximately 15 minutes. The lateral flow Cholera SMART™ II assay replaces the flow through Cholera SMART™ assay, which utilized a monoclonal antibody – polyclonal antibody sandwich. In-House testing of Cholera SMART™ II has shown it to be equivalent to Cholera SMART™.

PRINCIPLE OF THE TEST

The Cholera SMART™ II assay is a rapid, qualitative test in the lateral flow format. Anti-A antigen specific monoclonal antibody-coated colloidal gold particles (red-colored) are applied to a membrane surface and dried. A sample is placed in a specimen tube and if necessary, treated with extraction buffer. 3 to 4 drops of an appropriately treated specimen are added with the disposable plastic dispenser into the (S) sample well. The dried gold conjugate reacts with any anti-A antigen that is present 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* O1 capture antibody zone (T), presenting a

visually detectable line of color and indicating a positive test result at (T). If no *V. cholerae* O1 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 A 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* O1. Appearance of two lines, one at (T) and one at (C) is indicative of a positive *V. cholerae* O1 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 heat-inactivated *V. cholerae* O1 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). The plastic dropper is marked 0.3 and 0.5 mls

SPECIMEN COLLECTION TUBE: Soft plastic tube and a snap-on top.

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. Alternatively, samples 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 collection tube. Fill up approximately half of the tube. Add 2 drops of the extraction fluid. Snap on cap and mix. Large particles in the specimen should be allowed to settle before placing the specimen into the lateral flow device. Add 4 drops of test tube contents into the lateral flow device with the disposable plastic pipette.
2. If stool specimen is semi-soft or formed, use a stick to place the specimen into the soft plastic tube. Fill up approximately one-fourth of the tube. Using a plastic dropper, add 0.5 ml of extraction buffer to the 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. Large particles in the sample should be allowed to settle before adding to the lateral flow test 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 pouch of Cholera SMART™ II lateral flow device. Remove contents. Label device with Sample Identification using permanent marker.
3. Place liquid sample into plastic sample tube. Add 1 to 2 drops of extraction fluid. Close tube and shake. Add 3 to 4 drops of test sample to the lateral flow device with the disposable plastic pipette.
4. Wait approximately three (3) minutes or for the sample to be absorbed into the sample well. Then place two (2) free falling drops of Chase buffer from the dropper bottle into the sample well.
5. 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 table to interpret test. **High positive reaction can produce result in less than 10 minutes.

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.

1. Open Cholera SMART™ II lateral flow device pouch. Remove contents. Label device as Positive Control sample using permanent marker.
2. Add 4 drops of cholera Positive Control reagent into the sample well of the lateral flow device.
3. Follow steps 4-5 in the Test Procedure.
4. 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

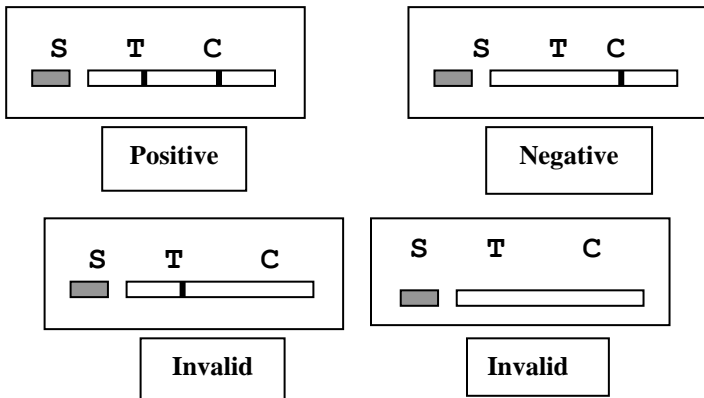
the test. If the quality control result is still unsatisfactory, do not report out results of test performed that day. Please contact New Horizons Diagnostics for technical assistance or replacement at (443)-543-5746.

5. The Chase Buffer could be used as a Negative Control reagent and the procedure outlined in the previous steps for positive control followed.
6. 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-O1 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 CONTROL and TEST Lines.
NEGATIVE TEST	Appearance of a red line only at the CONTROL Line and absence of a red line on the TEST Line.
INVALID	Appearance of red line at the TEST Line and absence of a red line on the CONTROL 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* O1 disease.
2. Cholera SMART™ II recognizes an antigen in the LPS of *V. cholerae* O1. The test may detect both viable and non-viable bacteria and may be positive following successful treatment.
3. Cholera SMART™ II can differentiate *V. cholerae* serotype O1 from serotype non O1 but it does not support further serotyping of O1 into Inaba or Ogawa and also does not support susceptibility testing.

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 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 SMART™ II has been shown to be equivalent to Cholera SMART™ in laboratory tests.

Analytical Sensitivity

The analytical sensitivity of Cholera SMART™ II was tested using suspensions of *V. cholerae* O1 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 2×10^7 colony forming units/ml of either Inaba or Ogawa serotypes of *V. cholerae* O1 based on optical density.

Cholera SMART™ II was tested with eight strains of *V. cholerae* O1, including both Inaba and Ogawa strains and was positive on all strains tested.

Cross-reactivity

The cross-reactivity of Cholera 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 are

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 non-O1* (3), *Vibrio cincinnatiensis* (1), *Vibrio damsela* (1), *Vibrio harveyi* (1), *Vibrio hollisae* (1), *Vibrio ordalii* (1) and *Vibrio vulnificus* (2).



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