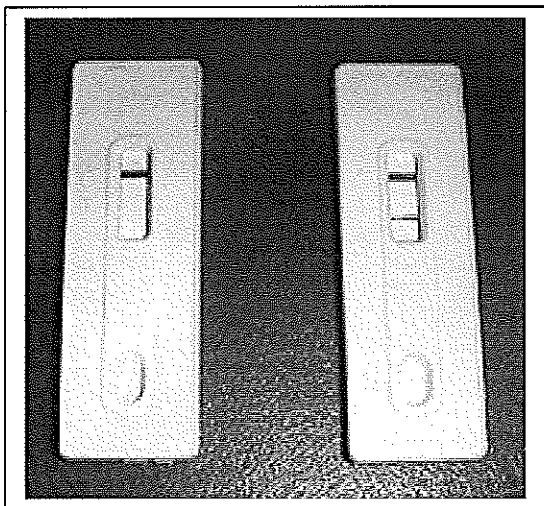


SMART™ II CHOLERA O1 Water Test

10 Determinations

Reorder No. 89-113210



A Colorimetric Immunoassay for
Direct Detection of *Vibrio cholerae* O1
in Water

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I. INTENDED USE

Cholera SMART™ II (Sensitive Membrane Antigen Rapid Test) Water Test is a rapid, lateral flow, colorimetric immunoassay designed, when combined with auxiliary reagents for preparation of water samples, for the direct presumptive and qualitative detection of *Vibrio cholerae* O1 water samples. The test device can also be used to test Alkaline Peptone Water (APW) medium from Moore swabs, as a confirmation of culture results, or as a monitor of presence of *V. cholerae* O1 from food specimens. Not for Human use.

II. 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, Cholera SMART II is useful in rapidly tracking the spread of the bacteria in the environment, in predicting the appearance of cholera in an area, and in monitoring the effectiveness of public health measures. The test can also be used to confirm culture results. Cholera SMART™ II uses a lateral flow format and monoclonal antibodies directed against the 'A' antigen of *V. cholerae* O1 LPS; 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 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™.

III. PRINCIPLE OF THE TEST

The Cholera SMART™ II assay is a rapid, qualitative test in the lateral flow format. Basically, water samples (500 mL) are collected into a Filtration Device (not provided) and passed through the filters provided in the kit. Filter A removes large particulates, debris, and other materials with a high flow rate, but very low binding for *V. cholerae* O1. Filter B retains essentially 99% of the *V. cholerae* O1 with a good flow rate. Filter B is then placed into tubes containing APW and incubated for 6-24 hours. Using provided droppers, 3 drops of the sample from the incubated APW tube are transferred to the (S) Sample Well of the SMART II Cholera Water device, followed by Chase Buffer to ensure the flow of antigen from (S) Sample Well to the (C) Control

Line and (T) Test Line.

Anti-A antigen specific monoclonal antibody-coated colloidal gold particles (red-colored) are applied to a membrane surface and dried. When 3 drops of an appropriately treated specimen are squeezed into the (S) sample well, this 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 (C) 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.

IV. MATERIALS PROVIDED

Each kit contains the following in quantities sufficient to adequately test 10 water samples as specified. Additional devices or accessory reagents can be obtained separately.

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).

SPECIMEN FILTERING:

Filter A: Filter with high flow-rate and low bacterial binding capacity

Filter B: Filter with high bacterial binding capacity and high bacterial recovery.

APW TUBE: Conical plastic tube containing 10mL of alkaline peptone water.

DECONTAMINATION REAGENT: Reagent is a 10X solution used to decontaminate the filter chambers between samples.

NEUTRALIZATION REAGENT: Reagent is a 10X solution used to neutralize the filter vessels after decontamination.

PLASTIC DROPPERS: Disposable plastic droppers.

V. MATERIALS REQUIRED BUT NOT PROVIDED WITH KIT:

The following items are available as an accessory kit from New Horizons Diagnostics Corporation ...

- 1) Filtering Device, reusable vessel that uses 47 mm diameter filters and 500 mL of sample
- 2) Forceps
- 3) Hand Pump

V.1 ASSEMBLY OF THE FILTERING DEVICE (not provided):

The reusable filter apparatus supplied with New Horizons Diagnostics Corporation's accessory kit for water testing consists of an upper and lower chamber, a filter holder which fits onto the lower chamber, and a collar that holds the filter holder and upper chamber together. The lower chamber has two side arms, one of which is plugged with a cap that comes with the apparatus. A graduated tubing adaptor that fits on the second side arm is also supplied with the filter apparatus.

1. To assemble the apparatus, push the filter holder snugly onto the lower chamber. Place the appropriate filter (A or B) onto the top of the filter holder. Be sure the filter sits flat and centered on the filter holder and covers the entire surface area of the filter holder with no buckling or overlap.
2. Place the collar onto the filter holder and screw the upper chamber to the filter holder by tightening the collar.
3. One sidearm should be securely fitted with the rubber sidearm cap, the second sidearm should be fitted with the graduated tubing adaptor. Tubing should fit securely onto the adaptor.
4. Once a sample has been added to the upper chamber the other end of the tubing should be connected with either the hand pump (supplied as a part of NHD's accessory kit) or an external vacuum source.

V.2 CLEANING THE FILTER APPARATUS (not provided)

The filter apparatus should be cleaned with the decontamination and neutralization solutions between samples.

1. Prepare a working solution of Decontamination Reagent by diluting 5.0 ml of 10X Decontamination Reagent to 50 ml of water. Rinse the filter apparatus including all internal parts thoroughly.
2. Prepare a working solution of Neutralization Reagent by diluting 5.0 ml of 10 X Neutralization Reagent to 45 ml of water. The water used for the preparation of working Neutralization Reagent should be known to be free of *V. cholerae* 01. Rinse the filter apparatus including all internal parts thoroughly.
3. Shake excess water from the apparatus. It is now ready to be used for the next sample.

VI. 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).

VII. 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.

VIII. SPECIMEN PREPARATION

The kit and test device can be used to test a variety of samples including clear, potable water, contaminated or sludge water, or APW from Moore swabs. The sample preparation and processing is slightly different for each of these three sample types. Both types of water samples require filtration. The procedure for preparing samples for each of these cases is discussed below.

A) Clear, potable water with minimal debris or contamination.

- A-1. Collect water samples (500 ml) into a clean container.
- A-2. Assemble the filter apparatus with Filter B onto the filter holder as discussed above. Pour or collect the sample into the upper chamber of the filter apparatus and connect the hand pump or vacuum source.
- A-3. Apply pressure from either the pump or an external source until the sample has entirely passed through to the lower chamber.
- A-4. Once the sample has passed through to the lower chamber, remove the upper chamber to expose the filter.
- A-5. Using forceps, place the filter into one of the tubes containing APW medium supplied with the kit. Secure the cap tightly and lay the tube on its side so that the filter is covered with the APW medium. Carefully label APW Tube.

Maximal sensitivity is obtained if the tube is gently rocked side to side at $36^{\circ} \pm 1^{\circ}\text{C}$. Growth will also occur, although slower, in a stationary tube kept at room temperature ($18\text{-}30^{\circ}\text{C}$). Incubation at temperatures higher than 37°C or when the filter is not covered with medium will severely impact the growth of *V. cholerae* 01.

- A-6. Test the APW medium at 6 hours following the steps outlined below in the Test Procedure.
- A-7. If the test result is negative, retest the APW medium containing the filter after an additional 16-20 hours of incubation. In contrast to culture, the performance of the Cholera SMART™ II test is not adversely affected by growth of other bacteria in the media.

B) Surface water or water samples that are contaminated or that contain particulates.

If the sample contains significant numbers of large particles, Filter B may clog. To prevent this, the sample should first be passed through Filter A.

- B-1. Assemble the filter apparatus as discussed above, except that filter A should be placed onto the filter holder.

- B-2. Pour or collect the water sample (500 ml) into the upper chamber of the filter apparatus and connect the hand pump or vacuum source.

- B-3. Pressure from either the pump or external source should be applied until the sample has entirely passed through to the lower chamber.

- B-4. Once the entire sample is collected in the lower chamber, carefully remove the lower chamber containing the sample and disassemble the filter apparatus.

- B-5. Place Filter B onto the filter holder and reassemble the filter apparatus with the second lower chamber attached.

- B-6. Pour the sample from the original lower chamber into the upper chamber of the filter apparatus and apply the vacuum until the entire sample is in the second lower chamber.

- B-7. Once the sample has passed through to the lower chamber, remove the upper chamber to expose the filter.

- B-8. Using forceps, place the filter into one of the tubes containing APW medium supplied with the kit. Secure the cap tightly and lay the tube on its side so that the filter is covered with the APW medium. Carefully label APW Tube.

Maximal sensitivity is obtained if the tube is gently rocked side to side at $36 \pm 1^{\circ}\text{C}$. Growth will also occur, although slower, in a stationary tube kept at room temperature ($18\text{-}30^{\circ}\text{C}$). Incubation at temperatures higher than 37°C or when the filter is not covered with medium will severely impact the growth of *V. cholerae* 01.

- B-9. Test APW medium at 6 hours following the steps outlined below in the Test Procedure.

- B-10. If the test result is negative, retest the APW medium containing the filter after an additional 16-20 hours of incubation. In contrast to culture, the performance of the Cholera SMART™ II test is not adversely affected by growth of other bacteria in the media.

C) Moore Swabs

Sewage waters are best tested using a modification of the procedure for Moore swabs.

- C-1. Place and collect the Moore swab according to the site's current protocol and introduce the swab into APW medium. Carefully label APW Tube.
- C-2. Incubate the APW medium at 36° ±1°C for 6 hours and test according to the Test Procedure outlined below. If the medium is negative, incubate the APW for an additional 16-20 hours and retest.

IX. TEST PROCEDURE

1. Collect water sample and filter through Filter Apparatus using Filter A or B, as described in sections A, B or C above. Select carefully labeled APW tube.
2. Open pouch of Cholera SMART™ II lateral flow device. Remove contents. Label device with Sample Identification using permanent marker to match labeling on APW Tube.
3. Using one of the plastic droppers provided, draw up 3 drops of sample from the APW tube (containing Filter B). Place the 3 drops into the sample well of a SMART™ II lateral flow device.
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.

X. 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.

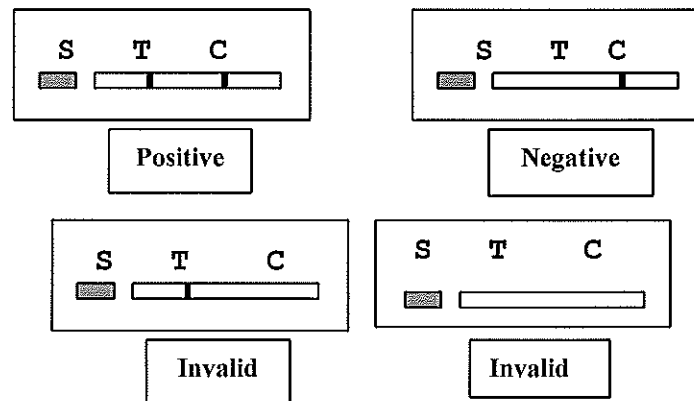
1. Open Cholera SMART™ II lateral flow device pouch. Remove contents. Label device as Positive Control sample using permanent marker.
2. Add 3 drops of cholera Positive Control reagent into the sample well of the lateral flow device.

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 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.
5. 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.

XI. RESULTS:

POSITIVE TEST	Appearance of two <u>distinct</u> red lines: one on the CONTROL and one on the TEST Line.
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.

XII. ILLUSTRATION:



XIII. 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 Water Test is not intended for the diagnosis of *V. cholerae* O1 disease.
2. Cholera SMART™ II Water Test does not detect *V. cholerae* non-O1, including *V. cholerae* O139, a new epidemic strain causing cholera in southern Asia. The non-O1 strains may cause diarrhea and other symptoms similar to those caused by *V. cholerae* O1.
3. Cholera SMART™ II Water Test 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.
4. Cholera SMART™ II Water Test 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.

XIV. 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.

XV. 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

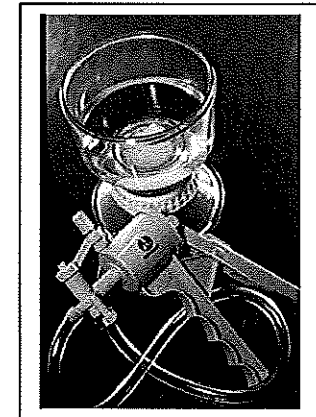
The cross-reactivity of Cholera SMART™ II for other organisms was assessed using suspensions of pure cultures of organisms containing $>10^8$ CFU/ml. None of the other organisms tested showed any cross-reactivity in the test. Organisms tested for cross-reactivity were (number of strains are indicated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

Reorder SMART II Cholera Water Kit 89-113210

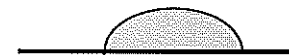
Cholera SMART II Water Accessory Kit / Order #... 89-113011

contains:

- 1 ea Filtration Unit, 500 mL
(Upper & Lower Chambers)
- 1 ea Tubing
- 1 ea Hand Operated Vacuum Pump
- 1 ea Forceps



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