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* 01 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

III. 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).

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

PLASTIC DROPPERS: Disposable plastic droppers.

IV. PRINCIPLE OF THE TEST

A. For high levels of cholera > 10^5 /cc

The Cholera SMART™ II assay is a rapid, qualitative test in the lateral flow format. Using provided droppers, 3 drops of the sample are transferred to the (S) Sample Well of the SMART II Cholera Water device, followed by Chase Buffer (3 drops) which is added after the test sample has completely entered into the device

When 3 drops of a specimen are placed 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 (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.

B. For low levels of cholera

This will require an incubation step with the APW alkaline Peptone water provided in the kit.

The intrinsic sensitivity of the assay is 10^5 organisms per ml. If your test sample is below this level it will have to be preincubated for at least 9 hours at 37 ^oC by placing 1 ml of the sample into 10 mls of APW that is provided in the kit. If the sample is incubated at room temperature, the incubation time should be at least 20 hours. The charts below describe the growth of cholera in APW incubated at 37C where the doubling time of the organism is every 30 minutes.
If the incubation temperature is at room temperature (22 ^oC), the doubling time of the cholera would be increased to 1 hour.

CALCULATIONS FOR NUMBER OF ORGANISMS DOUBLING EVERY HALF HOUR

Level required for detection by SMART TEST II

HOUR	ORGANISMS	HOUR	ORGANISMS
0.5	2	6.5	8,192 (10^4)
1.0	4	7.0	16,384
1.5	8 (10^1)	7.7	32,768
2.0	16	8.0	65,536
2.5	32	**8.5**	**131,072 (10^5)**
3.0	64	9.0	262,144
3.5	128 (10^2)	9.5	524,288
4.0	256	10.0	1,048,576 (10^6)
4.5	512	10.5	2,097,152
5.0	1024 (10^3)	11.0	4,194,304
5.5	2048	11.5	8,388,608 (10^7)
6.0	4096	12.0	16,777,216

CALCULATIONS FOR NUMBER OF ORGANISMS DOUBLING EVERY HOUR

Level required for detection by SMART TEST II

HOUR	ORGANISMS	HOUR	ORGANISMS
1	2	13	8,192 (10^4)
2	4	14	16,384
3	8 (10^1)	15	32,768
4	16	16	65,536
5	32	**17**	**131,072 (10^5)**
6	64	18	262,144
7	128 (10^2)	19	524,288
8	256	20	1,048,576 (10^6)
9	512	21	2,097,152
10	1024 (10^3)	22	4,194,304
11	2048	23	8,388,608 (10^7)
12	4096	24	16,777,216

V. 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).

VI. 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.

VII. 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**. Test as is, but should be above ph5

B) **Sludge water**. It can be filtered by placing it in a funnel with a coarse filter or by letting particles settle.

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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° $\pm$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.

VIII. TEST PROCEDURE

1. If sample is potable H_2O and above ph5, sample is ready to use.

2. For maximum sensitivity, place 1 ml of test sample and place into 10 ml tube of APW, and incubate overnight at 37 ^{o}C.

3. Open pouch of Cholera SMART^{TM} II lateral flow device. Remove contents. Label device with Sample Identification using permanent marker to match labeling on APW Tube.

4. Using one of the plastic droppers provided, draw up 3 drops of sample from the APW tube. Place the 3 drops into the sample well of a SMART^{TM} II lateral flow device.

5. 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.

6. 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.

IX. QUALITY CONTROL

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

1. Open Cholera SMART^{TM} 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 (443) 543-5746.

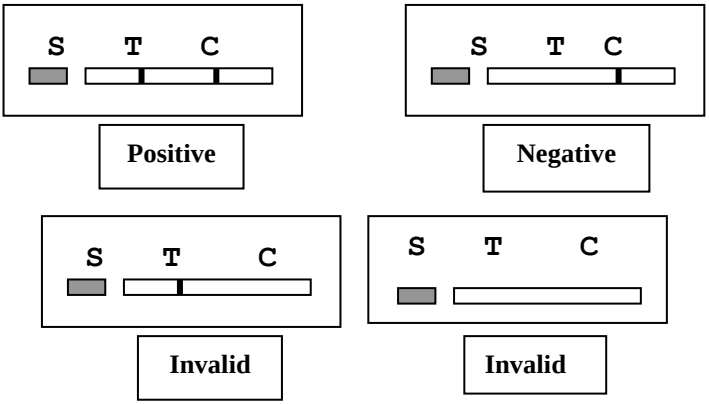
5. The Chase Buffer could be used as a Negative Control Reagent and

the procedure outlined in the previous steps for positive control followed. The appearance of a distinct red line only at the Control Line would indicate a negative sample.

X. RESULTS:

POSITIVE TEST	Appearance of two distinct 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.

XI. ILLUSTRATION:



XII. 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.

XIII. 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.

XIV. 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 2×10^6 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 dysenterae 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).*

Note:

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