2. 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, non-O139 (3), Vibrio cincinnatiensis (1), Vibrio damsela (1), Vibrio harveyi (1), Vibrio hollisae (1), Vibrio ordalii (1) and Vibrio vulnificus (2).*

VIII. 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).

IX. PRECAUTIONS

1. Safety precautions should be observed in handling and disposing of processed test materials as with any other microbiological/clinical materials.
2. The reagents have been tested as a unit. Do not substitute reagents from other kit lots.
3. Do not use reagents beyond the indicated expiration date.
   Do not dilute any of the reagents. This will have an impact both on test sensitivity and stability.

New Horizons DIAGNOSTICS CORPORATION

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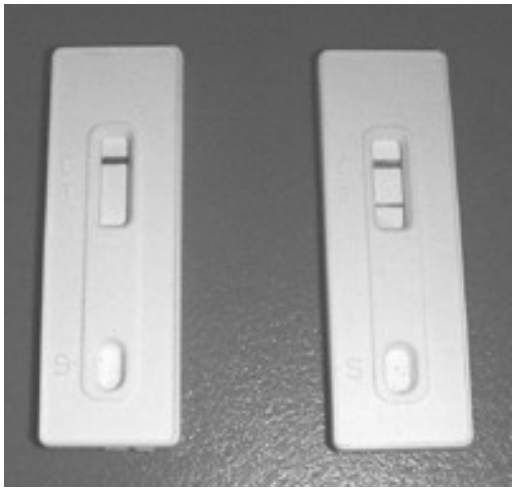
SMART™ II
CHOLERA O1/O139
Ballast Water Test

5 Determinations each of O1/O139

Reorder No. 89-150001

A Colorimetric Immunoassay for Direct Detection of *Vibrio cholerae* O1and O139 in Ballast Water



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I. INTENDED USE

Cholera SMART™ II (Sensitive Membrane Antigen Rapid Test) Ballast Water Test is a rapid, qualitative colorimetric immunoassay in the lateral flow format, designed, when combined with auxiliary reagents for the direct presumptive and qualitative detection of *Vibrio cholerae* O1 and O139 in ballast water samples.

II. INTRODUCTION

Cholera has been located globally in all bodies of water. It is important for all water going vessels to determine whether there is any presence of cholera in the ballast water before exposing it to the environment. The Ballast Water test utilizes overnight growth of a sample of ballast water to test for the presence of cholera organisms at the detection rate of one viable cholera organism in 100ml of ballast water. The test utilizes a lateral flow format with very specific monoclonal antibodies. The Cholera SMART™ II Ballast Water test is highly reliable, simple to use and can be performed on site aboard ship.

III. MATERIALS PROVIDED

Each kit contains the following in quantities sufficient to adequately test 10 ballast water samples as specified 5 Cholera O1 tests and 5 Cholera O139 tests. Additional devices or accessory reagents, such as a **Positive Control Reagent**, can be obtained separately. Each bottle of growth media is used to test both O1 and O139.

5 FOIL POUCHES: Each contains one SMART™ II Cholera O1 Device.
5 FOIL POUCHES: Each contains one SMART™ II Cholera O139 Devices
1 CHASE BUFFER
5 APW BOTTLES: Each contains 200 ml of APW (2.5X)
10 PLASTIC DROPPERS

IV. PRINCIPLE OF THE TEST

In order to be able to detect viable cholera at the low level of one viable organism per 100ml of ballast water, it is necessary to transfer the ballast water in our specially prepared APW, so as to achieve the most probable number equivalency (MPN)

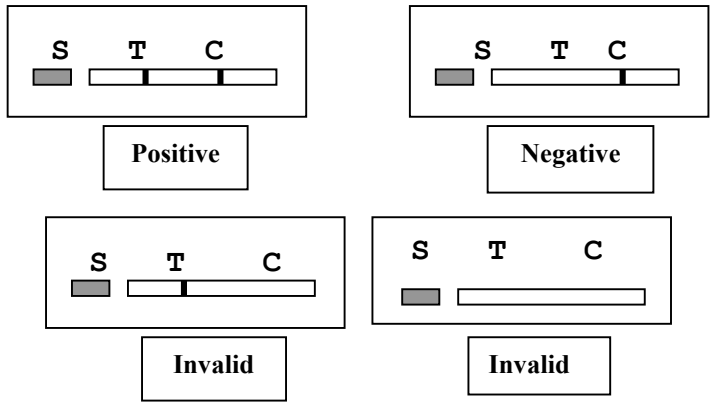
1. Preheat the APW media at 37^0C.
2. Add 300ml ballast water sample to the preheated APW bottle and gently mix.
3. Incubate the inoculated sample for 18- 24 hours at 37^0C.
4. Place 3 drops or 100 µl ($1/10^{th}$ ml) of the inoculated sample into the sample well (**S**) of each of the Cholera LFA O1 and O139 devices.
5. Wait 2-3 minutes for the inoculated sample to be absorbed into the sample well of the device.

6. Add 2 free falling drops of the Chase Buffer to the sample well.
7. Read results after 15 minutes, but no longer than 30 minutes.

V. TEST 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.

VI. ILLUSTRATION:



S: Sample well T: Test line C: Control line

VII. PERFORMANCE CHARACTERISTICS:

1. <u>Analytical Sensitivity</u>

The analytical sensitivity of Cholera SMART™ II was tested using suspensions of *V. cholerae* O1and 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 x 10^6 colony forming units/ml of either Inaba or Ogawa serotypes of *V. cholerae* O1 or O139 based on optical density.