INTENDED USE
Crystal VC is an in vitro qualitative screening test for presence of LPS antigen of V.cholerae O139 & O1 in human stool.

INTRODUCTION
Cholera, an ancient disease, remains a major public health problem in many countries of the World. This disease is caused by V. cholerae. Two serogroups, viz. O139 and O1 of V. cholerae are responsible for most of the outbreaks. The characteristic symptom of the disease is copious, painless, watery diarrhea induced by an enterotoxin commonly referred to as Cholera Toxin. The toxin causes water and electrolyte loss due to profuse diarrhea, resulting in malaria to severe dehydration which may even result in death. Infection is transmitted by fecal-oral route, by ingestion of food or water contaminated with toxigenic serotypes of V. cholerae. The incubation period ranges from few hours to 5 days. Despite all the major advances in medical sciences, Cholera still remains a challenge to the modern medicine. Successful intervention and containment depends largely on early detection of Cholera outbreaks. Culture still remains the gold standard for diagnosis of Cholera but requires a functional microbiology laboratory and is time consuming. Non-availability of such a set up in most endemic areas hinders efficient management of Cholera outbreaks. Prompt and accurate diagnosis of Cholera is, therefore, of great importance in order to implement proper epidemiological measures.

Crystal VC is a Lateral Flow Immunocromatographic test for the qualitative detection of Lipopolysaccharide (LPS) antigen of both V.cholerae O139 and O1 in the stool specimen, using monoclonal antibodies specific to V.cholerae O139 and O1 LPS. Many Rapid Diagnostic tests for Cholera targets only V.cholerae O1 whereas as Crystal VC is designed to detect both V.cholerae O1 and O139. Crystal VC is well suited for the rapid diagnosis of Cholera in endemic areas.

PRINCIPLE
1. Monoclonal antibodies specific to LPS antigen of V.cholerae O139 & O1 are immobilised at Test regions ‘T1’ and ‘T2’ respectively.
2. The conjugate releasing pad of the Dipstick is impregnated with colloidal gold coupled with monoclonal antibodies to LPS antigen of V.cholerae O139 & O1.
3. As the sample passes through the conjugate releasing pad, if LPS antigen of V.cholerae O139 & O1 are present, they bind with corresponding monoclonal antibodies to LPS of V.cholerae O139 & O1 coupled with colloidal gold.
4. This colloidal gold antibody-antigen complex moves through the nitrocellulose membrane and bind to the immobilised antibodies to V.cholerae and form pinkish red colour bands at ‘T1’ and ‘T2’ regions which indicate reactive (positive) results.
5. An inbuilt immobilised control at ‘C’ ensures the performance validation of the test.

KIT CONTENTS AND DESCRIPTION

REAGENTS

<table>
<thead>
<tr>
<th>Reagent no.</th>
<th>Reagent name</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>Dipstick</td>
<td>Dipstick having nitrocellulose membrane impregnated with monoclonal antibodies to LPS of V.cholerae O139 &amp; O1 at ‘T1’ &amp; ‘T2’ regions respectively, Control reagent impregnated at ‘C’ region and conjugate releasing pad impregnated with colloidal gold coupled with monoclonal antibody to V.cholerae O139 &amp; O1 LPS antibodies.</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>Reaction Buffer</td>
<td>Sample processing vial filled with Reaction Buffer</td>
</tr>
</tbody>
</table>

ACCESSORIES

Disposable plastic droppers
Test tubes
Test tube stand

REAGENT STORAGE AND STABILITY
All kit components are stable until the expiry date printed on the label, when stored between 4-30 °C. Store the kit in cool and dry place and protect it from direct sunlight.

MATERIALS REQUIRED BUT NOT PROVIDED IN THE KIT
1. Timer
2. Disposable waste bag

BIOSAFETY
1. Handle all samples with care, as they can be potentially infectious.
2. Wear disposable gloves throughout the test procedure and dispose them off as biohazard waste.
3. Wear protective laboratory clothing while performing the test.
4. Do not smoke, eat or drink in area where samples are being handled.
5. Technicians with wound, cut or skin abrasions on the hand must refrain from performing the test without proper precautions.
6. Avoid spilling of samples or solutions containing samples. In case of spillage, immediately clean it with 1:10 dilution of 5% freshly prepared Sodium hypochlorite solution and dispose off the cleaning material by a suitable method.
7. Remnants of samples and used Dipstick should be collected in a waste container. Discard them as biohazard waste in a suitable container. The container should be finally incinerated or autoclaved at 121 °C for 1 hour.
8. Wash hands thoroughly with disinfectant after completion of the test.

SPECIMEN COLLECTION AND HANDLING

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Storage at</th>
<th>Stable for</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool (semi solid/viscous/liquid)</td>
<td>2 - 4°C</td>
<td>24 hours</td>
<td>Stool sample should be collected in a clean and dry container and preferably tested immediately after collection. The frozen sample should be totally thawed and brought to room temperature before testing.</td>
</tr>
<tr>
<td>Stool (semi solid/viscous/liquid)</td>
<td>-20°C</td>
<td>long term</td>
<td></td>
</tr>
</tbody>
</table>

ASSAY PRECAUTIONS
1. Bring the reagents and sample to room temperature before starting the procedure.
2. For every new kit, check the inactness of the reagent bottle. Do not use the reagent if the bottle/vial is already open or leaked.
3. Do not use reagents after expiry date.
4. Do not combine the reagents 1 & 2 from different lots of kits, in one test.
5. Do not use the reagent 2, if it appears turbid or discoloured.
6. At the time of opening the pouch, if the desiccant color has turned from blue to white, don't use that test strip.
7. Open the sealed pouch for performing the test only after it attains the room temperature.
8. Gently mix the reagent 2 and sample prior to application onto the dipstick.

PROCEDURE
A. Sample Processing (for initial screening test)
Take the sample processing vial and unscrew the cap with the sampling stick attached to it (keep the sample processing vial in vertical position to avoid loss of solution).

1. For solid, semisolid or viscous stool samples:
   With the help of sampling stick collect a small portion of stool sample from two or more areas in the sample (Figure-1A).
   Note: Take only the stool content that got stuck to the sampling stick, avoid taking separate, large pieces of stool sample.
2. Reciprocate the sample processing vial lightly and shake it to mix the content properly (Avoid any spillage while doing this) (Figure-2).
3. Break the outer end of the sampling stick (Figure-3).

(Figure-1A)
B. Sample Processing (For Confirmation)

1. Use Alkaline Peptide Water (APW) vial instead of Reagent 2 (sample processing vial containing Reaction Buffer) & perform step nos. 1 & 2 of the sample processing described above [APW vials are available separately (Code no. 96970-10-1)].

2. Incubate this APW vial at ambient temperature between 20-40 °C for 4 hours.

3. Perform as per step no. 3 of the sample processing described above.

Note: Please use freshly collected stool samples for sample processing (for confirmation).

ASSAY PROCEDURE

1. Remove the Dipstick from the pouch and label it with patient identification code.

2. Take a test tube and dispense 4 drops of processed sample from sample processing vial by breaking the lip of its cap as shown in Figure-3.

3. Place the Dipstick vertically into the test tube in such a way that the arrows point toward the bottom.

4. At the end of 15-20 minutes remove the Dipstick from the test tube and read the results.

Note: Do not read/interpret the results after 30 minutes.

INTERPRETATION OF RESULTS

NEGATIVE

Presence of only one pinkish red band at Control 'C' region and no band at Test regions 'T1' and 'T2' indicates that the sample is negative for V. cholerae.

POSITIVE

1. Presence of two pinkish red bands, one at 'C' and one at 'T1' region indicate that the sample is positive for LPS antigen of V. cholerae O1

2. Presence of two pinkish red bands, one at 'C' and one at 'T2' region indicate that the sample is positive for LPS antigen of V. cholerae O139.

3. Presence of three pinkish red bands at 'C', at 'T1', and at 'T2' regions indicate that the sample is positive for LPS antigens of V. cholerae O139 & O1.

Note: Compare the used Dipstick with Template Dipstick as shown below to have exact idea about location of different bands.

INVALID

Absence of pinkish red band at Control 'C' region with or without presence of band at 'T1' and/or 'T2' regions indicate invalid result. This can be due to deterioration of sample/reagent or presence of particulate matter in the sample. In such case repeat the test using new Dipstick and fresh sample.

QUALITY CONTROL

It is vital to deliver analytical services in useful, convenient and meaningful manner as it is first and foremost step on the way to correct treatment. To ensure the reliability of the final report it is recommended:

1. to follow kit's instructions meticulously.
2. to run known controls as a part of internal and external quality control programme.

PERFORMANCE CHARACTERISTICS

The over all sensitivity of the Crystal VC rapid test as compared to culture technique for the detection of V. cholerae O1 ranges from 88 % to 100 % and that of V. cholerae O139 was 99 %. While the specificity was found to be 61 % to 87.3 % for V. cholerae O1 and 96 % for V. cholerae O139, without performance of confirmatory step as per procedure B described above.

ACKNOWLEDGMENT

Crystal VC has been developed under technical collaboration with the laboratory of Production of Monoclonal Antibodies and Recombinant Proteins and with the National Reference Center for Vibrio & Cholera, Institute Pasteur, Paris, France.

PRESENTATION

<table>
<thead>
<tr>
<th>Reagent No.</th>
<th>Reagent name</th>
<th>18C101-05</th>
<th>18C101-10</th>
<th>18C101-50</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dipstick</td>
<td>6 Nos.</td>
<td>10 Nos.</td>
<td>50 Nos.</td>
</tr>
<tr>
<td>2</td>
<td>Reaction Buffer</td>
<td>5 Nos.</td>
<td>10 Nos.</td>
<td>50 Nos.</td>
</tr>
</tbody>
</table>

ACCESSORIES

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>5 Nos.</th>
<th>10 Nos.</th>
<th>50 Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disposable plastic dropper</td>
<td>Test tubes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test tube stand</td>
<td>Test tubes</td>
<td>1 Nos.</td>
<td>2 Nos.</td>
<td>5 Nos.</td>
</tr>
</tbody>
</table>

REFERENCES


SYMBOL LEGENDS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Explanation of symbol</th>
<th>Symbol</th>
<th>Explanation of symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC</td>
<td>In vitro diagnostic device</td>
<td>REP</td>
<td>Contains sufficient for &lt; n tests</td>
</tr>
<tr>
<td>mL</td>
<td>Do not use if package is damaged</td>
<td>LIT</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>C</td>
<td>Store at 4-30°C</td>
<td>MAN</td>
<td>Batch code No.</td>
</tr>
<tr>
<td>KEEP</td>
<td>Keep away from sunlight</td>
<td>MAN</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>..</td>
<td>Keep dry</td>
<td>MAN</td>
<td>Date of manufacture</td>
</tr>
<tr>
<td>..</td>
<td>Do not reuse</td>
<td>MAN</td>
<td>Use by (date or month of expiry)</td>
</tr>
</tbody>
</table>

LIMITED EXPRESSED WARRANTY DISCLAIMER

SPAN DIAGNOSTICS LTD, GPPA tests are not to be used in the testing of the test kit for any consequential losses that may arise.

For Technical Support & Queries Contact: Customer Service Cell (CSC),
Span Diagnostics Ltd., Phone No.: +91 981 2340712-15,
Span Diagnostics Ltd., Plot No. 336, MS vsk, Road No. 8, Bhubaneshwar, Odisha, India,
Fax: +91 781 2525714,
E-mail: info@spandiagnostics.com

Span Diagnostics Ltd.