C. difficile GDH Rapid Test
Immunochromatographic rapid test card for qualitative detection of C. difficile GDH antigen in feces

INTRODUCTION
C. difficile Ag (GDH) is a rapid chromatographic immunoassay for the qualitative detection of Clostridium difficile glutamate dehydrogenase antigen in human feces specimens to aid in the diagnosis of Clostridium difficile.

Clostridium difficile is an anaerobic gram-positive spore-forming bacillus. The key feature in enabling it to persist in patients and the physical environment for long periods and thereby facilitating its transmission is the ability of C. difficile to form spores. C. difficile is transmitted through the fecal-oral route. Clostridium difficile is the principal pathogen related to antibiotic-associated diarrhea and/or pseudomembranous colitis in hospitalized patients. Mature colonic bacterial flora in a healthy adult is generally resistant to C. difficile colonization. However, if the normal colonic flora is altered, resistance to colonization is lost. Thus, any factor associated with alteration of the normal enteric flora increases the risk of C. difficile colonization after exposure to antibiotics, especially those with broadspectrum activity such as penicillins, cephalosporins and clindamycin. C. difficile can release two high-molecular-weight toxins, toxin A and toxin B, which are responsible for the clinical manifestations, which range from mild, self-limited watery diarrhea to fulminating pseudomembranous colitis, toxic megacolon, and death. Clostridium difficile Glutamate Dehydrogenase (GDH) is an enzyme produced in large quantities by all toxigenic and non-toxigenic strains, making it an excellent marker for the organism.

PRINCIPLE OF THE TEST
The GDH Test Device employs red gold-conjugated monoclonal antibodies against antigen GDH of C. difficile, and solid-phase specific another GDH antibodies. In this test the specimen is first treated with an extraction solution to extract GDH antigens from the fecal. Following extraction, the only step required is to add the extract to the reaction device. As the sample flows through the test membrane, the colored particles migrate. In the case of a positive result the specific antibodies present on the membrane will capture the colored particles. Red color lines will be visible, depending upon the virus content of the sample. These lines, after 5-15 minutes of incubation at room temperature, are used to interpret the result.

REAGENTS AND MATERIALS
GDH Device 25pcs
Extraction Vials 25pcs
Instruction for use 1

PRECAUTION FOR USERS
1. For in-vitro diagnostic use only.
2. Handle all specimens as if they contain infectious agents. When the assay procedure is completed, dispose of specimens carefully after autoclaving for at least one hour. Alternatively, treat with a 0.5 or 1% solution of sodium hypochlorite for one hour before disposal.
3. Wear protective clothing (laboratory coats and disposable gloves) when assaying samples.
4. Do not eat, drink or smoke in areas where specimens and kit reagents are handled.
5. Avoid contact between hands and eyes or nose during specimen collection and testing.

SPECIMEN COLLECTION
Stool samples must be taken as soon as the symptoms appear. Viral particles decrease in number after one week, making the diagnosis more difficult. The samples can be stored in the refrigerator for 1 to 2 days. For longer storage they must be kept frozen at -20°C. In this case, the sample should be totally thawed, and brought to room temperature and homogenised before testing.

STORAGE OF TEST KIT
The GDH Test Device can be stored at any temperature between 4-30°C. Do not freeze. The stability of the kit under these storage conditions is 24 months. Use up the reagents as soon as possible after the kit is unpacked within 3 months.

ASSAY PROCEDURES
Preparations:
Allow all reagents and samples to equilibrate to room temperature before proceeding with the test.
Write the specimen number on the cassette and sample preparation device.

Procedure:
1. Unscrew the cap of the Sample Extraction Vial. Collect sample by immersing the applicator stick into the faeces ensuring that the sample has impregnated the stick surface properly (about 50mg). For liquid or semi-solid stools add 100 microliter of stool using an appropriate pipette into the vial.
2. Reinsert the applicator stick into the vial and screw the cap tightly. Shake the vial to release the virus into diluent.
3. Break the tip off. Add 3 drops to the sample well of the test device.
4. Incubate the test at room temperature and read the test after 5-15 minutes.

INTERPRETATION OF RESULTS

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>T</th>
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<tbody>
<tr>
<td>Negative</td>
<td></td>
<td></td>
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<tr>
<td>Positive</td>
<td></td>
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<tr>
<td>Invalid</td>
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Negative: One pink colored control line, showing the test has been carried out correctly. There will be no line in test region.
Positive: In addition to a pink colored control line, a distinct pink colored band will also appear in the test region.
Invalid: A total absence of color in both regions is an indication of procedure error and/or that the test reagent has deteriorated. The test should be repeated using a new strip.

LIMITATIONS OF THE ASSAY
1. The test should be used only for the detection of GDH antigen in faecal samples, not the toxins.
2. The test is qualitative and no quantitative interpretation should be made with respect to the intensity of the positive line, when reporting the result.
3. More than 400 samples were evaluated to assure the correct performance of the test. The correlation of the results with other techniques (ELISA) was excellent. However, differences in the performance of the tests should not be excluded.

PERFORMANCE

<table>
<thead>
<tr>
<th>Biocare GDH Device</th>
<th>Positive</th>
<th>Negative</th>
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<tbody>
<tr>
<td>Commercial GDH Test</td>
<td>103</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>303</td>
</tr>
</tbody>
</table>

Specificity: 303/(303+2)= 99.3 %
Sensitivity: 103(103+1)=99 %

Litterature:

Manufacturer:
Biocare Diagnostics Ltd.
6F, Building B, 108 Xinghua Road
Xiangzhou, Zhuhai, China 519000
Tel:+86-756-2529237
E-mail: info@ivdbiocare.com
Website: www.ivdbiocare.com

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