QuickStripe™ Rotavirus
One-Step Test for Determination of Rotavirus in Human Feces

Instruction Manual

Test kit for 25 determinations (Catalog No.41205)

For In Vitro Diagnostic Use
Store at 4-30°C. Do Not Freeze

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Intended Use
QuickStripe™Rotavirus is a rapid immunochromatographic test for use in the qualitative screening of human fecal samples for detection of the presence of rotavirus antigen.

Summary and Explanation
Rotaviruses are one of the major causes of pediatric gastroenteritis and diarrhea. Untreated, rotavirus infection may result in severe illness with dehydration and disturbances of the body's normal electrolyte balance, especially in babies and preschool children [1]. Rotavirus is the cause of up to 50% of the hospitalized cases of diarrheal illness in infants and young children [2]. Rotavirus induced dehydration is a major cause of infant morbidity in both developed and underdeveloped countries, and a major cause of infant mortality in the latter regions (up to 4% per year) [3].

The highest prevalence of the disease is experienced in temperate climates during the cooler months of the year [4]. In tropical climates rotavirus infection can occur year round [2]. The age groups most susceptible to the disease are that of infants and children [4] and geriatric patients [9,12].

Diagnosis of rotavirus gastroenteritis is based on the identification of rotavirus particles in the feces. These particles, shed in large numbers during infection, may be observed by electron microscopy (EM) or detected by immunological methods, such as the immunochromatographic method used in the Rotavirus assay.

Principle of the Procedure
The Rotavirus test strip contains a unique monoclonal antibody that is conjugated to colloidal gold particles. The second antibody is a polyclonal of rabbit origin and is immobilized in the test area of the strip. If the stool sample extract contains rotavirus antigen, these will form an antigen-antibody complex with the gold particles. As this complex migrates along the test strip to the immobilized capture antibody, a pink/purple line is formed indicating a positive test. The remaining conjugate migrates to a second antibody on the control area of the test strip forming a pink/purple band. This indicates proper performance of the test.

Kit Contents (25 determinations)
25 rotavirus Rotavirus dipstick tests
25 dilution tubes, each containing 1 ml dilution buffer
1 plastic test holder with lid
1 instruction sheet

Warnings and Precautions
1. Do not use kit or components beyond expiration date.
2. All components in the kit are for in-vitro diagnostic use only, not for internal or external use in humans or animals.
3. Infectious agents may be present in test specimens. Therefore all specimens should be regarded and handled as potential biohazards. Never pipette by mouth and avoid contact with open wounds.
4. Dilution buffer contains sodium azide, which can react with lead and copper plumbing to form explosive metal azides. Azide build-up may be avoided by flushing with large volumes of water following disposal of reagents.
5. Do not mix reagents from kits of different lots.
6. Incubation times or temperatures other than those specified may give erroneous results.
7. After use the product should be discarded into a suitable biological waste container. Sterilize used test strips, strip holder, and test tubes before releasing into the environment.

Storage of reagents
Kits should be stored at 4-30°C. Avoid extreme heat or cold. Do not freeze. When stored correctly the product can be kept until the expiry date stamped on the box label.

Specimen collection and handling
Fecal samples should be collected in clean, dry containers, free of calf or bovine serum (which may contain antibodies to rotavirus) or detergents. Approximately 0.05g (0.05ml) is sufficient to perform the test. Swab samples are acceptable provided that this amount of feces can be collected. For best results samples should be collected 3-5 days after appearance of symptoms of rotavirus infection. Samples collected eight days or more after symptoms are first noted may not contain sufficient antigen or virus particles to be detected [13,16].

Refrigerate all specimens until ready for testing. If specimens are not going to be tested with 48 hours, they should be stored at -20°C or below. Avoid repeated freezing and thawing. Storage in a self-defrosting freezer is not recommended. Samples diluted in Sample Diluting Solution should be discarded after use.

Procedure
1. Open a sample tube.
2. Add a small portion of feces (about 0.05g) to the sample tube. Cap the tube.
3. Shake tube vigorously until sample dissolves into test fluid.
4. Wait until the large particles settle to the bottom of the tube.

Test Procedure
1. Remove the dipstick test from its pouch.
2. Label the test with a patient name or ID number. Insert the dipstick test vertically (with the blue arrows pointing downwards) into the sample tube with test fluid.
3. Remove dipstick from sample tube when fluid reaches the middle of the test area of the dipstick.
4. Place the dipstick test horizontally on a flat surface.

Interpretation of Results
Read results 5 minutes after removal from the test fluid. Low positive results may take up to 15 minutes. Do not consider results after 15 minutes.

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Interpretation of Results
Read results 5 minutes after removal from the test fluid. Low positive results may take up to 15 minutes. Do not consider results after 15 minutes.
Negative: only one pink/purple band appears in the Control window. No band is visible in the Test window.

Positive: in addition to the Control band a clearly distinguishable pink/purple band also appears in the Test window.

Inconclusive: if no control band is visible (with or without a visible band in the test window) the test is inconclusive. The test should be repeated using a new device.

Quality control

Each test strip contains a built in procedural control. Correct device performance is confirmed when a colored line appears in the control area of the strip. Good laboratory practice requires running a known positive control sample when a new lot of strips is used. If a positive result is not obtained, test results are not valid and the kit should not be used.

Limitations of the Procedure

- Rotavirus has been identified as a factor in the cause of gastroenteritis. The specificity of this test does not preclude the possibility of bacterial gastroenteritis. It is advisable, therefore, to test the fecal sample for bacterial diarrheal pathogens as well.
- Do not use samples containing preservatives or detergents.
- Infrequently a diluted stool sample may fail to diffuse up the stick. In such a case transfer 0.3 ml (about half the volume) of the liquid from the diluted sample to another of the provided dilution tubes, mix vigorously, and test again with a new Rotavirus.

A negative result does not exclude the possibility of rotavirus infection. The quantity of virus or antigen may be too small, or the sampling may be inadequate or improper.

Expected Results

Age of patient, geographical location, season of the year, weather [14] and general health environment are all factors influencing the prevalence of rotavirus infection. In temperate climates, the disease is more prevalent during the winter months [5,11,13,14] and infection is less common in the summer [5,13].

A. Infants: this group has the highest rate of incidence of rotavirus illness. It is reasonable to expect that about 50% of samples from patients with acute diarrhea from this age group will test positive [1,5,11]. The strongest reaction will be seen in samples taken from 1 to 4 days after onset of symptoms [16].

B. Adults: attacks are rare in this group and are usually asymptomatic or very mild [8].

C. Geriatric patients: institutionalized patients in this age group are especially susceptible, and it may be expected that 20-80% of samples will yield a positive result for rotavirus. Most frequent symptoms are diarrhea, nausea and occasionally elevated temperature [9,12].

Specific Performance Characteristics

A. Clinical Comparison

In studies conducted in Israel the Rotavirus test was compared to two different ELISA assays, and the following results were obtained (Table I).

Table I. Comparison of clinical results (n=221)

<table>
<thead>
<tr>
<th>Result against</th>
<th>Savyon ELISA</th>
<th>DAKO ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positive</td>
<td>73</td>
<td>55</td>
</tr>
<tr>
<td>True negative</td>
<td>37</td>
<td>51</td>
</tr>
<tr>
<td>False positive</td>
<td>1</td>
<td>4*</td>
</tr>
<tr>
<td>False negative</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>97.3% (72/74)</td>
<td>92.7% (51/55)</td>
</tr>
<tr>
<td>Specificity</td>
<td>97.4% (37/38)</td>
<td>100% (51/51)</td>
</tr>
<tr>
<td>Predictive value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>98.7% (74/75)</td>
<td>93.2% (55/59)</td>
</tr>
<tr>
<td>Negative</td>
<td>94.9% (37/39)</td>
<td>100% (51/51)</td>
</tr>
<tr>
<td>Overall agreement</td>
<td>97.3% (214/220)</td>
<td></td>
</tr>
</tbody>
</table>

* At a higher dilution two of the four samples were positive by ELISA

B. Cross-reactivity

The anti-rotavirus antibodies employed in this kit were found not to recognize the following common intestinal pathogens and viruses found in feces:

1) Adenovirus I, II 11) Polio virus I,II
2) Entamoeba histolytica 12) Salmonella B, C
3) Ascaris lumbricoides 13) Salmonella infantis
4) Campylobacter jejuni 14) Salmonella typhi
5) Vibrio cholerae 15) Shigella sonnei
6) Clostridium difficile 16) Shigella flexneri
7) Eche virus 3, 7 17) Shigella dysenteriae
8) Escherichia coli 18) Vibrio parahemolytica
9) Giarda lamblia 19) Trichurus trichiura
10) Pirovirus 20) Corona-like virus

C. Reproducibility

To negative fecal samples and 10 samples of low to high positive responses were analyzed for the presence of rotavirus antigen on 10 consecutive days. No change in intensity of result color was detected upon repeated testing of the same sample.

D. Prozone Effect

Concentrated rotavirus antigen was prepared from a cell culture displaying 100% cytopathic effect. Dilutions of this concentrated preparation were tested in order to determine whether antigen concentration influences the assay. The following results were recorded, according to the intensity of the colored band:

<table>
<thead>
<tr>
<th>Antigen dilution</th>
<th>1:10</th>
<th>1:20</th>
<th>1:40</th>
<th>1:80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result</td>
<td>++++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

The results clearly show the absence of prozone effect.

E. Level of detection

A positive sample with known concentration of antigen particles was diluted with buffer and assayed for presence of rotavirus. The lowest virus concentration giving a positive result was about 1 x 10^7 particles/ml. This concentration of virus is far below those found during the active phase of the disease (10^8 - 10^11 particles/ml).
Bibliography


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