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# *Rota-Stat* Latex Agglutination Test for Determination of Rotavirus in Human Feces

Catalog no. R-5113 for 30 Determinations

Directions for Use

## Intended Use:

*Rota-Stat* is a rapid latex agglutination slide 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. Among the latter methods, the latex agglutination technique enables the rapid and convenient screening of fecal samples for the presence of rotavirus particles. Although this method is less sensitive than EM or ELISA methods, it has the advantage of being simple to perform without any special equipment, and results are available in less than 5 min. If an optimal sample is collected during a period of peak viral shedding, the lower sensitivity of the latex test may not diminish its effectiveness.

## Principle of the procedure

This is a rapid slide test in which latex particles coated with specific antibody react with the rotavirus antigen present in the fecal sample, resulting in agglutination.

The fecal specimen is first suspended in the Sample Diluting Solution (Reagent A) and centrifuged. One drop of the clear supernatant fluid is mixed with a drop of Test Latex reagent and a second drop of supernatant fluid is mixed with Control Latex reagent on the plastic slide provided. A positive result is indicated when agglutination (clumping) occurs in the Test Latex reagent, while the Control Latex reagent remains smooth and free of agglutination.

## KIT CONTENTS (30 determinations)

**Reagent A -** Sample-diluting solution, 1 bottle glycine buffered saline with detergent containing 0.1 % sodium azide **75 ml**

**Reagent B -**Test Latex, 1 vial with dropper. Latex particles coated with immunoglobulin fraction of rabbit antiserum to simian rotavirus SA-11 in buffer solution with 0.1% sodium azide. **1.5 ml**

**Reagent C -** Control latex, 1 vial with dropper. Latex particles coated with immunoglobulin fraction of normal rabbit serum buffer solution with 0.1% sodium azide **1.5 ml**

**Reagent D -** Positive control antigen, 1 vial with dropper. Inactivated simian rotavirus SA-11 in minimum essential medium (MEM), phosphate buffered saline (PBS) 0.01 M, pH 7.35 with 2% bovine serum albumin (BSA) and 0.1% sodium azide. **0.5 ml**

- Disposable slides **30**
- Disposable mixing sticks **60**
- Directions for use **1**

**Equipment required but not provided:**

* Laboratory centrifuge capable of centrifuging at 1000 x g.
* Stoppered or screw-capped centrifuge tubes
* Timer, to time minutes ± 30 sec. * 50 µl micropipette

**WARNINGS AND PRECAUTIONS**

1) Do not use kit or components beyond expiration date.
2) All the reagents in the kit are for in-vitro diagnostic use only, not for internal or external use in humans or animals. 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.
3) Do not allow latex suspensions to cool below 2°C.
4) Reagents contain 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) Do not use kit if indications of instability are evident.
7) Although test reagents do not contain infectious particles of rotavirus, they are prepared from biological materials and, therefore, should be handled as potentially hazardous.
8) Incubation times or temperatures other than those specified may give erroneous results.

## Indications of Instability

The Test Latex and Control Latex should both contain a uniform milky suspension of latex particles. Sample Diluting Solution and Positive Control Antigen should be clear solutions, free of gross particulate matter. The kit should not be used if:

(a) Sample Diluting Solution or Positive Control show marked turbidity.
(b) Clumping in Test Latex or Control Latex is evident and cannot be removed by gentle mixing.
(c) Positive Control Antigen does not react with Test Latex or does react with Control Latex.

## STORAGE OF REAGENTS

Refrigerate all reagents. DO NOT FREEZE!

Latex suspensions begin to clump irreversibly below 2°C.

## Specimen collection and handling

1. Fecal samples should be collected in clean, dry containers, free of calf or bovine serum (which may contain antibodies to rotavirus) or detergents. Approximately 0.2 g (0.2 ml) 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]. Samples of infant stools should contain as much possible solids, avoiding urine-diluted feces. Excessive amounts of urine in the sample may affect the test results.
2. 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**

I. Preparation of Sample

1. Take up at least 0.2 ml of the fecal sample using a pipette or a swab. If the sample is too viscous, cut off the tip of the pipette to enlarge the opening. Dilute the sample with 2 ml of Sample Diluting Solution in a 10 ml centrifuge tube and cap securely.
2. Shake the mixture vigorously for about 10-15 seconds, ensuring that the sample is still capped, in order to prevent the escape of pathogenic microorganisms.
3. Centrifuge the suspension at about 1000 x g for 10 min at ambient temp. Use the clarified supernatant fluid for the test.

II. Test Procedure

Bring kit components and samples to room temperature 15-30°C).

**Note:** mix Test and Control latexes with **vortex mixer** before use.

The test uses a special disposable slide comprising two wells - one for the Test Latex and one for the Control Latex. Be sure the slides are clean and free of lint before use.

1. **Dispense** one drop (about 50 µl) of the clarified sample supernatant fluid onto each well.

2. **Add** one drop of the resuspended Test Latex onto the left-handed well of the slide and one drop of the resuspended Control Latex onto the right-hand well of the slide. Thoroughly mix sample and latex suspension using a separate mixing stick for each well.
3. **Rock** the slide gently for 2 minutes. Avoid mixing the contents of the two wells.

## INTERPRETATION OF RESULTS

**Positive**

A test is positive if agglutination occurs in the test well (left) within 2 minutes, and a milky suspension remains in the negative control well (right).

**Negative**

A test is negative if a milky suspension remains in both the Test and Control wells. *Any agglutination of the control latex is an indication that the test was performed incorrectly or that the reagents are defective*. In such a case results are invalid.

**Result Summary Table**

| Test Latex (left well) | Control Latex (right well) | Results |
|---|---|---|
| Agglutination | No agglutination | Positive |
| No agglutination | No agglutination | Negative |
| Agglutination | Agglutination | Not valid |
| No agglutination | Agglutination | Not valid |

## QUALITY CONTROL

Ready-for-use Positive Control antigen is supplied with the kit. Good laboratory practice requires running the Positive Control each time the kit 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.
- Samples containing 50% v/v or greater amounts of urine may yield erroneous results.

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 VALUES

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 1-4 days after onset of symptoms [16].

B. *Adults*: attacks are rare in this group and are usually asymptotic 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 the United States and Israel, this kit was compared to immunoelectronmicroscopy [17] and to Novamed's Rotavirus ELISA assay. Ninety-five samples were evaluated and the following results were obtained (Table I).

**Table I.** Comparison of results from this kit, ELISA and IEM (n=95)

| Result | IEM | ELISA | This Kit |
|---|---|---|---|
| True positive | 36 | 36 | 30 |
| True negative | 59 | 59 | 59 |
| False positive | 0 | 0 | 0 |
| False negative | 0 | 6 | 3 |
| Sensitivity | 100% (36/36) | 100% (36/36) | 90.9% (30/33) |
| Specificity | 100% (59/59) | 100% (59/59) | 100% (59/59) |
| Efficiency | 100% (95/95) | 100% (95/95) | 96.7% (89/92) |
| Predictive value | | | |
| Positive | 100% (36/36) | 100% (36/36) | 100% (30/30) |
| Negative | 100% (59/59) | 100% (59/59) | 100% (59/59) |
| No clinical answer | 0 | 0 | 3* |

**Not included in efficiency calculation*

In clinical studies conducted in Israel and in two US laboratories, A total of 235 fecal samples were analyzed for the presence of rotavirus antigen using this kit, NOVAMED's Rota ELISA and another latex agglutination kit commercially available in the US (Table II).

**Table II.** Comparison of results from this kit, NOVAMED's Rota ELISA and another latex agglutination kit commercially available in the US (n = 235)

| Result | ELISA | This Kit | Competitor |
|---|---|---|---|
| True positive | 91 | 82 | 80 |
| True negative | 144 | 143 | 130 |
| False positive | 0 | 0 | 12 |
| False negative | 0 | 6 | 7 |
| Sensitivity | 100% (91/91) | 93 % (82/88) | 92 % (80/87) |
| Specificity | 100% (144/144) | 100 % (143/143) | 92 % (130/142) |
| Efficiency | 100% (235/235) | 97 % (225/231) | 92 % (210/229) |
| Predictive value | | | |
| Positive | 100% (91/91) | 100% (82/82) | 92 % (80/87) |
| Negative | 100% (144/144) | 96% (143/149) | 95 % (130/137) |
| No clin. answer | 0 | 4* | 6* |

**Not included in efficiency calculation*

B. Cross-reactivity

The kit was tested against the following common intestinal pathogens and viruses found in feces:

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

The results were negative, demonstrating no cross-reactivity with these pathogens.

C. Reproducibility

Ten negative fecal samples and 10 samples of low to high positive responses were analyzed with for the presence of rotavirus antigen on 10 consecutive days. No change in intensity of agglutination 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 agglutination. The following results were recorded, according to the intensity of the agglutination:

| **Antigen dilution** | 1:1 | 1:2 | 1:4 | 1:8 |
|---|---|---|---|---|
| **Agglutination** | ++++ | +++ | ++ | + |

The results clearly show the absence of prozone effect.

E. Level of detection

Positive samples (different rotavirus serotypes) with known concentrations of antigen particles were diluted with buffer and assayed for presence of rotavirus. The lowest virus concentration giving a positive result is shown in the following table:

| Strain | Detection Limit | Strain | Detection Limit |
|---|---|---|---|
| WA | $7 \times 10^3$ | SA-11 | $1 \times 10^4$ |
| DS Ir | $1 \times 10^4$ | HOCHI | $1.2 \times 10^4$ |

These concentrations of virus are far below those found during the active phase of the disease ($10^7$ - $10^{11}$ particles/ml).

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