Detection of rotavirus by Latex Agglutination Test (Rotalex) ; Comparison with Electron Microscopy and Complement Fixation Test

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Summary

A commercially available kit for latex agglutination test, Rotalex (Orion Diagnostics, Finland), for detection of rotavirus was evaluated and compared with 2 other tests, namely, elecron microscopy and complement fixation test. Although Rotalex was the least complex method, it showed good specificity and sensitivity when carried out according to the manufacturer's instructions. The procedure gave results which were comparable with those obtained by electron microscopy and complement fixation tests.

Introduction

Rotaviruses are now widely recognized as the major etiological agents of gastroenteritis of infants and young children in most areas of the world(1,2,3,4,5,6,7,8). The discovery in 1973 of the 70 nm human rotavirus in association with gastroenteritis of infants and young children represents a major advance in elucidating the cause of acute infectious nonbacterial gastroenteritis(9). These viruses are readily detectable in stools by a variety of methods(10,11,12,13,14,15,16,17,18,19,20,21,22). Recently a latex agglutination test, Rotalex (Orion Diagnostics, Helsinki, Finland), has been developed for the detction of rotaviruses. The test depends on the agglutination of latex particles, coated with specific antibody, by rotavirus present in stool extracts.

The advantages claimed for this test are, mainly, that it can be carried

out on a slide, read with the naked eye or a hand lens, and is suitable for use in paediatric hospitals. The availability of Rotalex prompted us to compare it with other tests, routinely used in our laboratories, namely , electron microscopy and complement fixation tests and the results are reported in this paper.

Materials and methods

Specimens:All stool specimens used in this study had been stored at- 70°C for different periods of time before use . They came from children and infants admitted to hospitals in different parts of Tehran. They had been at 4°C for a maximum of 48 h before freezing.

Electron microscopy: Specimens for electron microscopy were extracted in 5 ml of PBS, shaked well by hand, and clarified in an International portable Refrigerated centrifuge model PR-2 at 3000 rpm for 10 min at 4°C . The supernatent was then centrifuged at 40,000 rpm for l h at 4°C in a Spinco L50 Serial # 1225-J centrifuge to sediment any available virus. The pellet was resuspended in 5 drops of DDW and examined by negative contrast in a Philips EM 300 or 400 electron microscopy using 2-3% phosphotungstic acid adjusted to pH 7 with 0-1 M potassium hydroxide.

Rotalex: Rotalex kits were obtained from Orion Diagnostics (Helsinki, Finland) and the test was initially performed exactly according to the instruction in the enclosed manual.

Briefly, this entailed preparing a 10% stool suspension in Rotalex buffer (provided by the manufacturer, composition not available) and mixed well with a vortex-type mixer, centrifuged at 3000 rpm for 10 min and 2 drops of the supernatent transferred onto a test slide. One drop was mixed with test latex (Coated with antibody to rotavirus), left for 2 min, and examined for the development of agglutination. Positive and negative controls were included in each run and treated the same as the tests samples.

Micro CF test: This was carried out according to the KOLMER 17 with a minor modification for detection of rotavirus in faeces of suspicious patients.

Antigen: In infected patients, the faeces is the best source of complement fixing antigen. For extraction of the antigen, a ten percent suspension of faeces prepared in PBS (pH 7.2), shaked vigorously in vortex, clarifed by

centrifugation at 2500 rpm for 15 min at 4°C. Aquous phase separated and heated at 56°C for 30 min, then used as CF antigen throughout the test. In rare cases of anticomplementary, 10% chlorophorm was added to each samples and kept at 4°C for lh with occasional shaking, then centrifuged and the aquous phase used as antigen.

Complement: Guinea pig complement used throughout the tests. two full units of complement titrated in the presence of each antigen sample were used for the test.Rota positive sera; convalescent sera collected from already infected children and stored at-20°C untill use . Sera were inactivated at 56°C for 30 min right before were the test.

Test procedure;

a) Serial 2-fold dilutions of sera prepared in U shape microplate with a volume of 25 microliter per well.

b) 0.025ml (1 drop) antigen was added to each well.

c) One drop complement (2 full units) added to each well . Fixation period; plates kept at +4°C overnight and warmed at +37°C for 30 min on the following day before adding haemolytic system .

d) Adding hemolytic system (2% sensitized sheep red blood cell) 50 microliter per well.

e) shaked well and incubate at +37°C incubator.

f) Test should be read when the controls of antigen, serum and complement were lysed.

Results

A series of experiments was designed to evaluate the suitability of Rotalex for detecting rotavirus in clinical specimens in a comparative study with two other tests, namely, electron microscopy and complement fixation test routinely used in our laboratories for detecting rotavirus. Table 1 shows the results obtained with the tests. From 97 samples 19 were positive with electron microscopy (19.5%) and 21 samples were positive with Rotalex latex reagent (21.5%), and 5 samples were positive with negative control latex reagent (5%) false positive. Nineteen samples were positive in CF test (19.5%). Results from electron microscopy and complement fixation tests, when applied to detect rotaviruses in the faeces, correlated with each other.

Discussion

This study was undertaken to investigate the suitability of latex agglutination test,(Rotalex) for detection of rotavirus and the comparison of its sensitivity with other two more routinely used methods, electron microscopy and complement fixation test . Rotalex is quicker and easier to run , as it can be carried out every where and it is based on agglutination of latex particles, which can be read with the naked eye or a hand lens, within a short period of time. Rotalex can give results which somehow correlate with two other techniques, electron microscopy and complement fixation test. The results of latter techniques, when applied to detected rotavirus, correlated with each other .

The experience we gained suggests that Rotalex can be considered as a possible standard method for detecting rotavirus if false positive samples are retested by complement fixation method. It may find a useful application in areas where high technology like electron microscopy is not available.

Table 1. Comparisons of 3 different methods for detection of rotaviruses

No	*Test*	*Pos.*	*%Pos.*	*Neg.*	*%Neg.*
1	*electron microscopy*	*19*	*19.5*	*78*	*80.5*
2	*Rotalex Latex reagent*	*21*	*21.5*	*89*	*78.5*
	Rotalex control reagent	*5*	*5*	*93*	*95*
3	*Complement Fixation test*	*19*	*19.5*	*78*	*80.5*

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