Study on Small Round Shaped Virus (SRSV) Infection and Research on gene based Detection

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INTRODUCTION

Nowadays, immunoassays have advances greatly and it presents us an interesting history in rapid diagnosis of infectious disease. The first immunoassay, radioimmunoassay (RIA), was discovered in 1959 as a method of measuring human insulin for the diagnosis of diabetes (Yalow el al., 1959). Then, rather than using the biohazardous hot compound of ^{125}I, a non-RIA called enzyme immunoassay (EIA) was developed. The non-RIA has become a great tool for immunoassay in laboratoriesn (Nakane et al., 1966) (Engvall et al., 1971). However, EIA takes several hours to obtain results and is not the best way of rapidly testing for infectious disease, because many steps are needed in the procedure. Therefore, a simple and rapid assay has been required to indicate positive or negative results from clinical samples (Singer et al., 1965) (Kohno., 1985).

To obtain a quick result, we attempted to develop a rapid and simple testing method by using synthetic polystyrene latex particles, which had been sensitized with high-affinity purified specific antibodies. The development of a long term stable latex particle reagent has enabled us to diagnose a variety of infectious diseases, such as rotavirus and adenovirus infections. More recently, an immunochromatography method has been introduced as a simple and rapid immunologic test. A comparison of immunoassay methods with others and their usefulness are shown. Here, we describe a simple, rapid and cost-effective manual test for rotavirus infection.

METHODS

Cell culture of human rotavirus (Odelia strain)

MA-104 cells were incubated in Eagle's minimal essential medium (MEM) with 10% fetal calf serum (FCS) in a 5% CO_2 incubator at 37°C for 5 days. The incubated cells formed a monolayer sheet on the surface of the culture bottle. For maintenance of the rotavirus-infected cells, we used Eagle's MEN with 10% tryptone phosphate broth, 0.05% yeast extract, 0.5% sodium glutamate, 0.1% glucose and 1 μg trypsin. The cells were incubated at pH 7.4.Rotavirus was incubated with infected MA-104 cells in Eagle's MEN in addition to 10μg/mL trypsin.

Purification of rotavirus antigen

The harvested virus was purified as follows. The infected cells and the supernatants were frozen and thawed three times. Then, the fluid was centrifuged at 6000 *g* for 30 min. The supernatant was collected, put on the 30% sucrose cushion and centrifuged at 90,000 *g* for 3 h. The precipitate was dissolved and applied to the density gradient of CsCl and was ultracentrifuged at 150,000 *g* for 18 h. After ultracentrifugation, the viral band was collected directly from the centrifugation tube by syringe and then dialyzed .

Preparation of rotavirus antibody

The virus inactivated in 0.1% formaldehyde solution at 4°C for 7 days and then dialyzed with PBS at pH 7.4. The protein concentration was adjusted to 2 mg/mL. After that, 0.35 mL of the antigen solution and 0.5 mL Freund's adjuvant were mixed to make an emulsion. The antigen (1 mg) was injected subcutaneously into the rabbits. Four weeks after the first injection, another 0.5 mg antigen was immunized as booster. The serum was collected within 1 week after the second injection .

Preparation of antirotavirus latex reagent

The antiserum was extracted with 33% ammonium sulfate. The purified immunoglobulin was coated on the surface of the latex beads. Briefly, 3 mg immunoglobulin

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was added to 1% polystyrene latex bead emulsion under vigorously stirred conditions. The latex suspension was treated with 1% bovine serum albumin for 20 min. After the supernatant was discarded, 2mL 0.2 mmol/L glycine-NaOH buffer at pH 8.2 was added.

Latex agglutination test of paper slide

A stool suspension of approximately 10% was prepared. The supernatant was collected after centrifugation. The supernatant (100μL) and 50 μL reagent (one drop) were mixed and stirred on a paper slide for 3 min at room temperature. Then, agglutination of the latex was checked visually.

Measurement of latex agglutination by the LPIA system

An aliquot (40μL) of 0.25% latex solution and 250 μL stabilizing buffer were added to 10μL of extracted sample and mixed. The agglutination process was monitored and calculated by computer and the results were available within 10 min. All of the process was carried out automatically. On the basis of a calibration curve, the antigen concentration was calculated. Precise information about the LPIA system has been reported previously (Kohno., 1992).

Enzyme immunoassay and electron microscopy

The EIA was carried out using commercially available reagents (Rotazyme II, Abbott, Chicago, IL, USA). The procedure was done according to manufacture's instructions. For electron microscopic study, the virus was extracted with phosphate-buffered saline (PBS(-)) after ultracentrifugation. The diluted sample was put on the grid (H-400; Veco, Eerbeek, Holland). It was vaporized on the collodion membrane. The sample was negatively stained with 3% uranium oxide. The extracted viral sample was diluted 10 fold. The number of viruses was counted in five meshes.

Comparative study of commercially available latex kits

We used two commercially available latex reagent kits, Slidex (Bio Merieux, Macy-1'Etolle, France) and Rotalex (Orion, Espoo, Finland) for our comparative study. All rests were performed according to the instructions of the manufactures.

RNA polyacrylamide gel electrophoresis

Briefly, the 10% stool suspension in PBS was homogenized and centrifuged at 6000 *g* for 30min. Supernatant (2 mL) was collected and was put on the cushion of 30% sucrose. The tube was centrifuged at 100,000g for 3h. The precipitate was collected and suspended in 0.5 mL 0.4 mol/L NaCl. After the addition of an equal volume of 10% SDS, the extraction of RNA was carried out with phenolchloroform. The precipitate was dissolved in 1 mL PBS(-) and the same amount of chloroform containing 4% isoamylalcohol. After centrifugation, the upper layer was collected and electrophoresed in a 10% polyacrylamide gel. The gel was stained with silver solution.

RESULTS AND DISCUSSION

In our findings (Honna el al., 1990), 50% of infants under 2 years of age suffer from severe diarrhea caused by rotavirus infection. We isolated human rotavirus from a patient's stool and named it the 'Odelia strain'. The virus was used as an antigen to prepare antibody for the development of rapid detection latex kit for rotavirus. One hundred and nine patients were investigated in a comparative study between electron microscopy and our rapid diagnostic latex reagent. Furthermore, we compared our latex reagent (Rota-check, Tokyo, Japan) with the other commercially available latex test and an EIA test kit (Rotazyme-II) for sensitivity and specificity (Table 1). The comparison of the latex agglutination test and EM showed that results from both are in agreement. The sensitivity of the latex agglutination test was equal to that of the EIA test kit.

A number of 307 stool samples were examined by electron microscopy and Rota-check. The cases of (±), treated with delipidation reagent, were found to be positive. The final results showed good correlation between the alternative methods. The EM method showed positive results with 113 cases and negative 194 cases, whereas the latex test showed 112 positive cases.

Table 1 comparison of the latex agglutination test, electron microscopy and ELISA

Method	Results		
	Positive	Negative	Equivocal
Elecron Microscopy	57 (52.3%)	52 (47.7%)	
Rota-check (our latex test)	60 (55.6%)	47 (43.1%)	2 (1.8%)
Slidex	62 (56.9%)	44 (40.3%)	3 (2.8%)
Rotalex	57 (52.3%)	49 (45.0%)	3 (2.8%)
Rotazyme-II	55 (32.1%)	54 (49.5%)	

Sensitivity and specificity was 98.2% and 94.8%, respectively (Table 2).

Table 2 comparison of the latex agglutination test and electron microscopy.

Rota-check	Electron microscopy		
	+	−	Total
+	95	4	99
±	17	6	23
-	1	184	185
Total	113	194	307

In order to measure the rotavirus concentration in stool samples, we used the LPIA system (Model L-100, Mitsubishi Chemical, Tokyo, Japan). The calibration curve for standard materials was prepared using a known concentration of virus solution, which was counted by EM. Based on the standard material, we found that the range of virus measurable by the LPIA system was between 3×10^7and10^9 virions/mL (Fig. 1).

The results of three methods, EM, RNA-PAGE and LPIA, were compared. This comparative study revealed that 10^8 virions/mL was the cut-off value for positivity and both by microscopy and RNA-PAGE. We found that 10^8 virions/mL was the clinically significant value of rotavirus infection to be detected by the LPIA system. In all of 94 samples, the results were correlated between the two alternative methods. This LPIA measurement is rather quick; with results within 10 min, and it is effective in an emergency test to know the concentration of virus (Fig. 2).

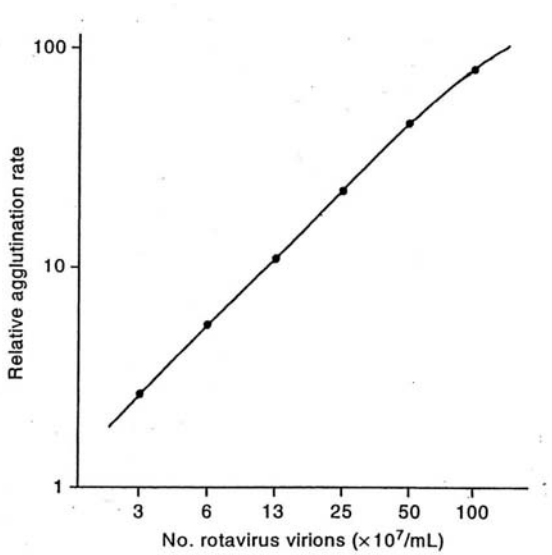

Fig. 1 Calibration curve for the relative agglutination rat and the number of rotavirus virions

CONCLUSION

We developed a latex agglutination test based on the immunoassay to detect the clinically isolated Odelia strain (human rotavirus, serotype IV, subgroup II). From the development of the latex agglutination test and clinical investigation, we understood the fact as follows.

1. Our latex test was evaluated as an alternative method to EM and RNA-PAGE.
2. The latex test is available for rapid detection of rotavirus.
3. To measure rotavirus in stool samples, the LPIA system was applicable for automatic measurement.

4. In the process of development of a rapid and simple test for rotavirus infection, we feel strongly that we need more rapid testing for effective therapeutic treatment, especially in infantile infectious diseases.

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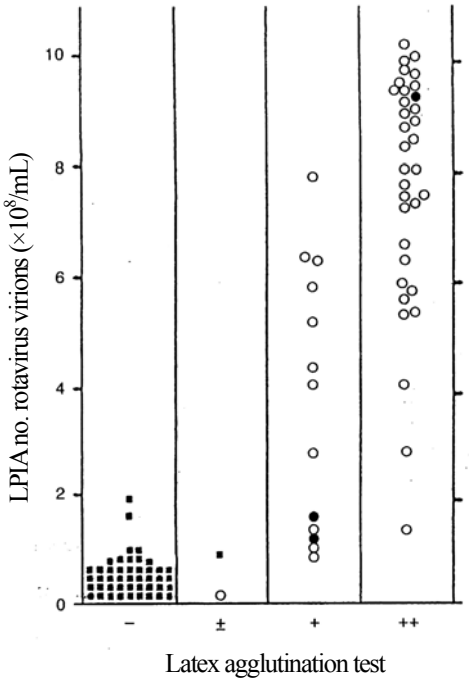

Fig. 2 Comparison between the latex photometric immunoassay system and the latex agglutination test. (○), Positive by electron microscopy (EM) and polyacrylamide gel electrophoresis (PAGE); (●), positive by EM but negative by PAGE; (■), negative by both EM and PAGE. (-), Negative; (±), weakly positive; (+), positive; (++), strongly positive.

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