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A New Screening Test for Rotavirus Infection

Summary: A simple and rapid staphylococcal coagglutination test, using rabbit antisera prepared against Nebraska calf diarrhea virus (NCDV), is described for the detection of rotavirus in neonatal fecal specimens. When the samples were examined directly using the coagglutination test, more than 60% of the specimens agglutinated the control reagent. These non-specific reactions were markedly reduced by preincubation of the specimens with non-immune rabbit serum and further heating at 80° C for 45 min. Such treatment did not reduce the specific activity in the coagglutination test when rotavirus-containing stools were tested. The coagglutination test was compared with ELISA in 290 stools positive or negative for rotavirus. The sensitivity of the coagglutination test was 92%, the specificity 91% and the predictive value 31%. These results indicate that coagglutination is a suitable test for rapid screening of rotavirus infection in clinical practice.

Zusammenfassung: *Neuer Screening-Test für Rotavirus-Infektion.* Für den Nachweis von Rotavirus in Stuhlproben Neugeborener wird ein einfacher und schneller Staphylokokken-Koagglutinationstest mit Anwendung von Kaninchen-Antiserum gegen das Nebraska-Kälberdiarrhöe-Virus (NCDV) vorgestellt. Bei direkter Prüfung im Koagglutinationstest agglutinierten mehr als 68% der Proben die Kontrollsubstanz. Diese unspezifischen Reaktionen wurden durch Vorinkubation der Proben mit dem Serum nicht immunisierter Kaninchen und 45minütiges Erhitzen auf 80° C erheblich vermindert. Bei Testung Rotavirushaltiger Stühle wurde durch diese Vorbehandlung keine Verminderung der spezifischen Aktivität im Koagglutinationstest hervorgerufen. Beim Vergleich der Ergebnisse von Koagglutinationstest und ELISA in 290 Rotavirus-positiven oder -negativen Stühlen ergab sich für den Koagglutinationstest eine Sensitivität von 92%, Spezifität von 91% und ein prädiktiver Wert von 31%. Diese Ergebnisse zeigen, daß sich der Koagglutinationstest für das Schnellscreening auf Rotavirus-Infektionen in der klinischen Praxis eignet.

Introduction

Rotaviruses are the most important cause of acute non-bacterial gastroenteritis in infancy and childhood (1) and a common cause of severe diarrheal disease in newborn calves (2) and piglets (3). The diagnosis of rotavirus infection has traditionally been made by electron microscopy (EM) (4). In fact, although cultivation of rotavirus has now been achieved (5), the relatively low isolation rate and the frequent need for initial "blind" passages preclude cultivation as a viable diagnostic method at the present time. Recently, many tests have been described for the detection of rotavirus (6–9). The main reason for the development of these many tests was a desire to have a reliable and sensitive technique which could be used to screen a large number of specimens.

In this paper we are presenting a simple staphylococcal agglutination assay for the detection of rotavirus in human stools.

Materials and Methods

Fecal samples were obtained from November 1984 to May 1985 from 290 five-day-old full-term neonates born in the Obstetric Clinic of the University of Padova, Italy. Specimens were suspended and diluted to approximately 10% in PBS and centrifuged at 3000 rpm for 30 min. Supernatants were stored at −70° C until used. Rotavirus diagnosis was based on the detection of rotavirus antigen in stool by enzyme-linked immunosorbent assay (ELISA), using microplates pre-coated with anti-SA 11 rabbit antiserum (Behring Institute) to perform the test as described by *Yolken* et al. (7). A reading on the spectrophotometer (Behring) > 0.2 absorbance, measured at a wavelength of 405 nm, was considered positive. The specificity of positive specimens was established by a blocking test, as previously described (7). All the specimens were also tested by a coagglutination test (CoA).

Staphylococcal reagent: Staphylococcus aureus "Cowan" was grown in brain-heart infusion overnight at 37° C. The bacteria were fixed with 0.5% formalin in PBS and heat-treated at 80° C for 1 h.

Antibody coupling: 1 ml of 10% staphylococcal suspension in PBS was mixed with 0.1 ml rabbit hyperimmune serum and incubated for 3 h at 22–25° C. The antibody-coated staphylococci were washed and resuspended in PBS as a 10% solution. Staphylococci coated with rabbit non-immune serum were used as a control reagent.

Coagglutination test: Before use, all the reagents and fecal specimens were equilibrated at room temperature and the CoA reagents were shaken to obtain a uniformly distributed bacterial suspension. Two separate drops of the fecal extract were mixed on a glass slide with the staphylococci coated with antibodies against rotavirus and with the control reagent. Specimens developing agglutination with the suspension of staphylococci coated with hyperimmune serum and not in the control test were taken to be rotavirus-positive. The agglutination pattern was visible microscopically after a period of 2 to 3 min of continuous circular motion.

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Treatment of fecal specimens: Initiallys, the CoA test was performed directly on fecal samples. Most of the specimens reacted with the control reagent. To eliminate this non-specific agglutination, a pool of ELISA-negative fecal specimens were pretreated with the following different regimens: heating and filtration, preincubation with N-acetylcysteine, preincubation with uncoated staphylococci, preincubation with non-immune rabbit serum, preincubation with non-immune rabbit serum and heating.

Therefore, all the specimens were tested after being pretreated with the last regimen, which had shown the best specificity in relation to ELISA. 1 ml of fecal specimen was incubated with an equal volume of non-immune rabbit serum at 37° C for 2 h. Then the suspension was heated at 80° C for 45 min.

Results

22 of 290 fecal specimens examined were positive by ELISA. 12 of 22 were also confirmed by a blocking test. On the basis of ELISA results, 52 negative fecal specimens were examined by CoA test. When the samples were examined directly, 32 specimens agglutinated with the control reagent. To reduce these non-specific agglutination reactions, 35 ELISA-negative fecal suspensions were pretreated with different regimens. The effects of preincubation of these specimens are presented in Table 1.

All 290 fecal specimens were tested by CoA after preincubation with non-immune rabbit serum and heating at 80° C for 45 min. The results, compared to ELISA, are shown in Table 2. The specificity was 91%, the sensitivity 92% and the predictive value 31%.

Table 1: Effect of pretreatment on CoA specificity.

Treatment	Specificity
No treatment	33%
Preincubation	
10% uncoated staphylococci	53%
20% N-acetylcysteine	0%
Non-immune rabbit serum	73%
Pretreatment	
Heating and filtration	80%
Heating	75%
Preincubation with non-immune rabbit serum and heating	90%

Table 2: Comparison between ELISA and CoA.

	ELISA		
	Positive	Negative	Total
CoA			
Positive	11	25	36
Negative	1	253	254
Total	12	278	290
Sensitivity	92%		
Specificity	91%		
Predictive value	31%		

Discussion

Staphylococcal protein A reacts with the Fc fragment of IgG from many mammalian species (10). The Fab fragment of IgG, so bound to protein A, is free to combine with homologous antigen. In this study, staphylococci coated with antibodies against rotavirus were used to detect rotavirus in human fecal specimens.

Rotavirus has been associated with gastrointestinal infections in a variety of animals (11). The antigenic similarity of animal and human rotavirus allows the use of animal virus for preparations of immuno-reagents (12). This is important since human rotavirus cannot be cultivated in generally available tissue culture systems. For coating staphylococci, we used a rabbit hyperimmune serum against Nebraska calf diarrhea virus (NCDV) proteins (kindly supplied by Dr. *Cancellotti,* Institute "Zooprofilattico", Padova) with high reactivity with protein A (92–94%).

Non-specific reactions, present when the fecal specimens were examined directly by the CoA test, were probably due to immunoglobulins against IgG present in the stools (13). Preincubation with N-acetylcysteine, as proposed by *Yolken* et al. (13), failed to improve the specificity of CoA due to the presence of a non-specific reaction between N-acetylcysteine and uncoated staphylococci. In our study preincubation with uncoated staphylococci, as described (14), did not influence the reaction. This could suggest that IgM is largely responsible for non-specific activity of fecal specimens due to the presence of a weak interaction between human IgM and protein A (10).

From a practical standpoint it would be desirable to eliminates, or at least reduce the non-specific responses without reducing the sensitivity of the test and its most valuable characteristic, rapidity. The goal was achieved by preincubation with non-immune rabbit serum. Serum IgG bound to IgM anti-IgG present in the stool, thus eliminating part of the non-specificity. A further heating of fecal suspensions, so treated, markedly improved the reaction. Such pretreatment did not result in any reduction of specific activity when rotavirus-containing stools were tested. We had a high rate of presumably false positive reactions (25 of 278 ELISA-negative specimens). A possible explanation is that for coating staphylococci we used a rabbit non-purified immune serum against NCDV, which probably contained antibodies against antigens other than Rotavirus which were present in stools. The use of monoclonal antibodies or a highly purified antiserum would eliminate false positive results.

In conclusion, because of its rapidity of performance, simplicity and low cost, CoA is a suitable test to screen a large number of fecal specimens with minimum effort, even in the smallest laboratories. However, to eliminate non-specific reactions, it is advisable to incubate fecal specimens with non-immune serum and to heat the resulting suspension at 80° C for 45 min. Positive results should, whenever possible, be confirmed by ELISA or EM.

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