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CONCISE ARTICLE

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New Immunochromatographic Method for Rapid Detection of Rotaviruses in Stool Samples Compared with Standard Enzyme Immunoassay and Latex Agglutination Techniques

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Abstract Three different commercial immunologic tests for rapid detection of group A rotavirus (an immunochromatographic method, latex agglutination, and enzyme immunoassay) were used to evaluate 228 faecal specimens obtained from Spanish children with acute gastroenteritis. After resolution of 30 (13.2%) discordant results by reverse transcription-polymerase chain reaction for rotavirus, the statistical values of the enzyme immunoassay, latex agglutination, and immunochromatographic method were respectively 96%, 68%, and 99% for sensitivity; 99%, 99%, and 96% for specificity; 98%, 96%, and 92% for positive predictive value; and 98%, 88%, and 99% for negative predictive value. The immunochromatographic technique showed high sensitivity and specificity and was rapid and easy to perform in the routine clinical laboratory.

Introduction

According to World Health Organisation estimates, group A rotaviruses cause 130 million cases of childhood gastroenteritis yearly and almost one million deaths throughout the world [1]. Rapid diagnosis of these infections allows us to determine the underlying aetiology and prognosis, define preventive measures in order to avoid nosocomial or community transmission, and reduce unnecessary use of antibiotic therapies.

At present, detection of rotavirus antigens in stool samples is carried out by immune analytical methods; in this context, latex agglutination (LAT) or enzyme immunoassay (EIA) techniques are well established [2–5]. EIA technology is recommended by the World Health Organisation because of its sensitivity, specificity, simplicity, and low cost [6–8] in contrast to other more time-consuming methods such as electron microscopy, viral RNA electrophoresis in polyacrylamide gel electrophoresis, or dot-blot nucleic acid hybridisation procedures.

Reverse transcription-polymerase chain reaction (RT-PCR) has been used for detecting rotaviruses in faecal samples and for characterising G and P serotypes. Given appropriate purification of faecal extracts, RT-PCR is 100–1,000 times more sensitive than immunoassay techniques, but its application for routine clinical diagnosis is currently limited [9].

This study evaluates a novel immunochromatographic (ICG) method for rapid detection of rotavirus in stool specimens and compares it with LAT and EIA, two commercial techniques that are widely used in microbiology laboratories [7, 10, 11].

Materials and Methods

A total of 228 faecal samples collected from children under 4 years of age (mean age, 1.03 years; range, 0.1–5.1 years) with acute gastroenteritis, were studied in the Hospital Severo Ochoa during 1999. This centre is the reference hospital of Health Care Area IX in Madrid, Spain, which has a total population of 350,000 inhabitants. Stool specimens were obtained by direct deposition in a sterile container, and aliquots were stored undiluted at –70°C until processing.

The following commercial techniques were used to evaluate the samples for the presence of rotavirus antigen. In all cases, the stools were initially diluted to 5–10% with extraction buffer supplied and subsequently analysed according to the manufacturer's instructions.

We employed IDEIA Rotavirus (Dako Diagnostics, UK), a commercial EIA method. It uses a microplate-based solid phase sandwich-type immunoassay with a polyclonal antibody to detect

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specific group A rotavirus proteins, particularly the internal capsid protein (VP6). The minimum performance time of the technique is 90 min. Reading of the assay is carried out by spectrophotometry. According to the manufacturer, this assay can detect rotavirus concentrations as low as 7.8×10^5 viral particles/ml and shows good correlation, sensitivity and specificity when compared to electron microscopy (99.5%, 100%, and 99.2%, respectively).

For LAT, we used the MUREX Rotavirus kit (Abbott, UK). It is a slide-based assay employing latex particles coated with rabbit antibodies specific for rotavirus detected in Nebraska calves with diarrhoea. The duration of the test is 20 min. Its sensitivity and specificity with respect to electron microscopy are 90% and 99%, respectively.

For ICG, we used ROTA-strip (CorisBioConcept, Belgium), a commercial method. It uses immunogold-based technology in a vertical-flow membrane to detect group A rotavirus. The diluted faecal specimen is contacted to the bottom port of the device. The sample mixes with gold particles coated with antirotavirus monoclonal antibody and migrates along the nitrocellulose membrane through the capture antibody area and the control (goat anti-mouse antibody) area over a 10 min period at room temperature. The control line serves to ensure that the sample has migrated the appropriate distance along the membrane. The test line contains antirotavirus polyclonal antibody (capture system). If rotavirus antigen is present in the sample, a complex is formed between the capture antibody and the monoclonal antibody-gold conjugate, which can be seen as a red-purple line in the test area.

Accuracy of ICG results for faecal swabs was determined by a parallel analysis carried out in 20 specimens collected in duplicate (in sterile container and faecal swab) from children with gastroenteritis. To confirm the reliability of ICG for detecting all rotavirus types commonly circulating in Spain, a total of 27 stool samples containing serotypes G1 (n = 12), G3 (n = 6), and G4 (n = 9), previously detected by EIA and RT-PCR [10], were selected and tested using ICG.

Specimens with discordant results in the immunologic assays were evaluated by RT-PCR. Viral dsRNA was extracted from stool suspensions using the RNAID kit (Bio 101, USA) following the instructions of the manufacturer with some modifications [9]. RT-PCR amplification was performed in a one-step process according to the method previously described [10]. The primers employed are specific for the gene coding for VP7 [12].

To determine the sensitivity, specificity, predictive values, and the Youden index [13] of the EIA, LAT, and ICG, each faecal specimen with identical results using all three techniques was considered as true positive or negative. Discordant results were classified as positive or negative based on the RT-PCR results, which were considered the gold standard.

Results and Discussion

Seventy-four (32.5%) of 228 cases were positive by any method employed, whereas 184 (80.7%) samples were negative in at least one test. Positive rates varied according to the technique employed: 31.6% for ICG, 28.1% for EIA and 20.6% for LAT. When ICG and LAT were compared with EIA, the ICG method had only one false-negative result, but the LAT technique showed a larger number of false-negative results (n=20).

No discrepancies were observed in 198 samples tested using the three immunologic assays. Positive results were obtained in 44 (19.3%) cases, and 154 (67.5%) were negative; thus, the concordance level for the three methods was 86.8%. Discordant results were detected in a low percentage of stool specimens (13.2%, n=30). RT-PCR analysis of these cases are shown in Table 1. All samples with EIA-positive, LAT-negative, and ICG-positive results were RT-PCR-positive (n=18).

Table 1 Concordance between enzyme immunoassay (EIA), latex agglutination (LAT) and immunochromatographic (ICG) methods plus results of RT-PCR performed on specimens with discordant results in rotavirus assays

Test method			No. (%) of samples	No. (%) of RT-PCR-positive samples
EIA	LAT	ICG		
+	+	–	0 (0.0)	0 (0.0)
+	–	+	18 (7.9)	18 (100.0)
+	–	–	2 (0.9)	1 (50.0)
–	+	+	2 (0.9)	1 (50.0)
–	+	–	1 (0.4)	0 (0.0)
–	–	+	7 (3.1)	2 (28.7)

RT-PCR, reverse transcription polymerase chain reaction; +, positive; –, negative

Table 2 Statistical results of immunologic assays after resolution of discordant results by RT-PCR

Parameter	Test method		
	EIA	LAT	ICG
True positives	63	45	66
True negatives	157	157	152
False positives	1	2	6
False negatives	3	21	1
Sensitivity	95.5	68.2	98.5
Specificity	99.4	98.7	96.2
Positive predictive value	98.4	95.7	91.7
Negative predictive value	98.1	88.2	99.3
Younden index	94.8	66.9	94.7

RT-PCR, reverse transcription polymerase chain reaction; EIA, enzyme immunoassay; LAT, latex agglutination; ICG, immunochromatographic method

Table 2 shows statistical results of the immunologic assays after resolution of discordant results by RT-PCR. The ICG method was considered more sensitive (98.5%) than EIA (the technique recommended by World Health Organisation), whereas LAT was more specific (98.7%), even though ICG specificity (96.2%) was also high. ICG and EIA concordance was greater than data between LAT and EIA. Likewise, the Youden index indicated that ICG is a more precise technique than LAT (94.7% vs. 66.9%).

ICG tests performed on stool samples simultaneously collected by swab and sterile container from 20 patients proved concordant in all cases (14 positive and 6 negative). However, as the intensity of positive reactions was slightly lower for swab-collected samples, we recommended collection of whole faecal specimens. The ICG technique detected all group A rotaviruses commonly circulating in Spain; every sample with G1, G3, and G4 genotypes was positive using the ICG method.

The usefulness of a rapid diagnostic technique must be evaluated according to criteria such as simplicity, ease of performance and interpretation, and reliability of results. Each laboratory must also consider its needs on the basis of its patient population, cost and the need for tech-

nical help to determine what is best for its specific situation. In our experience, ICG showed high sensitivity (98.5%) and good consistency with EIA for early diagnosis of group A rotavirus infection; these results are in agreement with those of other authors who used similar EIA methods from other commercial sources [14].

A current limitation of the ICG technique is that it only detects rotavirus belonging to group A. This problem occurs also with EIA and LAT, but does not occur with other more laborious methods such as electron microscopy, polyacrylamide gel electrophoresis, or RT-PCR. The development of monoclonal antibodies capable of recognising non-A rotaviruses and their inclusion in immune analytical techniques would be desirable.

On the basis of the results shown in Table 2, as well as its simplicity, low cost, and rapid technique, we consider ICG particularly suitable for routine detection of rotavirus antigen in stool samples, especially in areas with a low incidence of childhood rotavirus gastroenteritis. In addition, ICG strips can be kept at room temperature, which is important for field laboratories where the availability and maintenance of the equipment for EIAs is scarce.

The EIA method facilitates many simultaneous determinations and it is considered the reference procedure because of its high sensitivity and specificity [15]. Nevertheless, this procedure is more time-consuming than other methods and requires specific equipment and longer performance times. The latex technique requires simple processing and each sample must be worked individually, but sensitivity and specificity performances fall short of EIA.

In our study, ICG technology offered the advantages of both assays, including simplicity of performance and statistical results similar to EIA. Another advantage is that commercial ICG strips are already available to detect both rotaviruses and adenoviruses using the same strip, resulting in a decrease in time and cost.

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References

1. Glass RI, Kilgore PE, Holman RC: The epidemiology of rotavirus diarrhea in the United States: surveillance and estimates of disease burden. Journal of Infectious Diseases (1996) 174:5–11
2. Steele AD, Williams MM, Bos P, Peenze I: Comparison of two rapid enzyme immunoassays with standard enzyme immunoassay and latex agglutination for the detection of human rotavirus in stools. Journal of Diarrhoeal Disease Research (1994) 12:117–120
3. Gilchrist MJR, Bretl TS, Moultney K, Knowlton DR, Ward RL: Comparison of seven kits for detection of rotavirus in fecal specimens with a sensitive, specific enzyme immunoassay. Diagnostic Microbiology and Infectious Disease (1987) 8:221–228
4. Thomas EE, Puterman ML, Kawano E, Curran M: Evaluation of seven immunoassays for detection of rotavirus in pediatric stool samples. Journal of Clinical Microbiology (1988) 26:1189–1193
5. De Vries WP, Houben AW, Stobberingh EE: Comparison of four commercial assays for detection of rotavirus in childhood gastroenteritis. European Journal of Clinical Microbiology & Infectious Diseases (1986) 5:542–544
6. Ibrahim OS, Sunderland D, Hart CA: Comparison of four methods for detection of rotavirus in faeces. Tropical Doctor (1990) 20:30–32
7. Flewett TH, Arias CF, Avendano LF, Ghafoor A, Mathan MM, Mendis L, Moe K, Bishop RF: Comparative evaluation of the WHO and DAKOPATTS enzyme-linked immunoassay kits for rotavirus detection. Bulletin of the World Health Organization (1989) 67:369–374
8. Dennehy PH, Gauntlett DR, Spangenberger SE: Choice of reference assay for the detection of rotavirus in fecal specimens: electron microscopy versus enzyme immunoassay. Journal of Clinical Microbiology (1990) 28:1280–1283
9. Buesa J, Colomina J, Raga J, Villanueva A, Prat J: Evaluation of reverse transcription and polymerase chain reaction (RT/PCR) for detection of rotaviruses: applications of the assay. Research in Virology (1996) 147:353–361
10. Sanchez-Fauquier A: The molecular epidemiology of the rotavirus in Spanish children. Enfermedades Infecciosas y Microbiología Clínica (1999) 17:509–514
11. De Beer M, Peenze I, Da Costa VM, Steele AD: Comparison of electron microscopy, enzyme-linked immunosorbent assay and latex agglutination for the detection of bovine rotavirus in faeces. Journal of the South African Veterinary Association (1997) 68:93–96
12. Gouvea V, Glass RI, Woods P, Taniguchi K, Clark HF, Forester B, Fang ZY: Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. Journal of Clinical Microbiology (1990) 28:276–282
13. Youden WJ: Index for rating diagnostic tests. Cancer (1950) 3:32–35
14. Dennehy PH, Hartin M, Nelson SM, Reising SF: Evaluation of the ImmunoCardSTAT! Rotavirus assay for detection of group A rotavirus in fecal specimens. Journal of Clinical Microbiology (1999) 37:1977–1979
15. Kapikian AZ, Chanock RM: Rotaviruses. In: Fields BN, Knipe DM (eds) Field's virology. Lippincott-Raven, Philadelphia (1996) pp 1657–1708