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# Rapid Screening Test for the Diagnosis of Rotavirus Infection

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**The early diagnosis of human rotavirus infection is essential for effective patient management and infection control. We report here a rapid, easy-to-perform, and inexpensive test for rotavirus detection. The viral RNA is extracted directly from the stools and electrophoresed on 1% agarose gels. Currently available immunoassays for routine diagnostic purposes are directed at the common group A-specific antigen. As reports become available on human gastroenteritis caused by the atypical or novel rotaviruses, this technique presents an added advantage in that it can detect both group A and non-group A rotaviruses.**

Rotaviruses are among the most important etiological agents of acute gastroenteritis in infants, young children, and the elderly. The rotavirus infection peak usually occurs during the winter months, particularly in temperate climates. Virus shedding begins on days 2, 3, and 4 following infection and lasts for approximately 6 days (7). The duration of virus excretion in infants has been found to vary from 1 to 9 days (2, 4). Electropherotyping (distribution of the segmented RNA genome of the rotavirus on polyacrylamide gels) has become an important laboratory and epidemiological procedure for the characterization of rotaviral strains (8, 10, 11). As workers in a public health laboratory, we are often confronted with institutional outbreaks which involve children or the elderly and in which isolation and/or identification coupled with a typing scheme become essential features in the reporting of results.

This paper describes a rapid, inexpensive, and easy-to-perform technique for the screening of stools for rotavirus when it is suspected as the causative agent for diarrheal episodes.

Recently, Pyndiah et al. (14) described a rapid procedure for the extraction of rotaviral RNA from diarrheal stools. We used this method with a minor modification of the extraction buffer, i.e., the addition of EDTA to prevent the aggregation of nucleic acids mediated by $Mg^{2+}$ (1). Stools were suspended in 500 μl of extraction buffer (0.02 M Tris hydrochloride [pH 7.4], 0.3 M NaCl, 0.01 M $MgCl_2$, 0.1% sodium dodecyl sulfate, 0.005 M EDTA, 4% sucrose, 0.04% bromophenol blue) to a final concentration of 20%. Each preparation was mixed with an equal volume of phenol-chloroform (1:1) and vortexed for 30 to 60 s to yield a homogeneous suspension which was then spun in a microcentrifuge at 10,000 × *g* for 10 min. The upper layer (dark blue solution) containing the double-stranded segments of RNA was collected and stored at room temperature for electrophoretic analysis by the standard 5% polyacrylamide gel electrophoresis (PAGE) method and the rapid 1% agarose gel method. The procedures for PAGE and silver staining were those of Kasempimolporn et al. (8), and 1% agarose gels were prepared as described by Perbal (13). Briefly, 1 g of agarose (ultrapure DNA grade; Bio-Rad Laboratories) was suspended in 100 ml of TBE buffer (0.09 M Tris-borate, 0.002 M EDTA) and melted in a microwave oven for 4 min. The agarose was poured onto a plastic plate (16 by 16 cm), and a comb was inserted to form wells for loading the specimens. Each gel was 5 mm thick and contained 15 sampling wells. Once solidified, the gel was submerged horizontally in TBE buffer, loaded with 50 μl of the sample (specimen RNA extract), and electrophoresed at room temperature and 100 V for 2.0 h. The gel was then immersed in ethidium bromide solution (1 μg/ml) for 5 min and visualized under a 300-nm UV transilluminator constructed in the laboratory at a cost of $40.00 (Canadian dollars).

Over 97 rotavirus stools positive by electron microscopy were analyzed by the standard PAGE technique for epidemiological studies (manuscript in preparation); the same 97 specimens were examined on agarose gels, and the migration

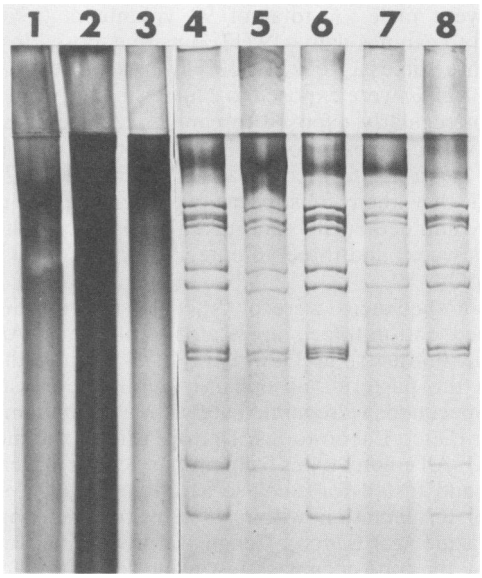


FIG. 1. Electropherogram of rotaviruses on a 5% polyacrylamide gel stained with silver stain. Lanes: 1 to 3, rotavirus-negative stools; 4 to 8, rotavirus-positive stools (single cases). Lanes 4 to 7 were run in parallel on a 1% agarose gel (see Fig. 2).

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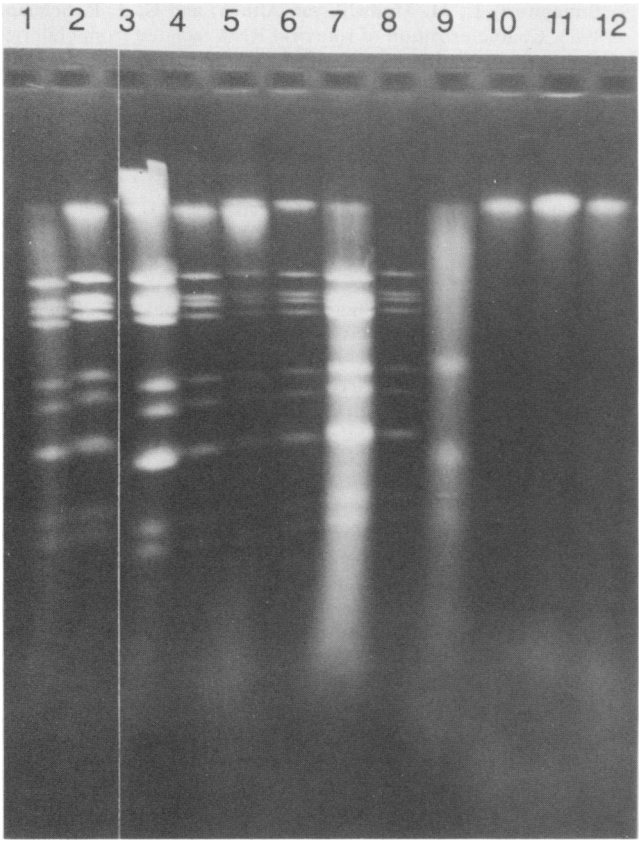


FIG. 2. Electropherogram of rotaviral RNA on a 1% agarose gel stained with ethidium bromide. Lanes: 1 to 8, rotavirus-positive stools (single cases); 9 to 12, rotavirus-negative stools. Lanes 1 to 4 were run in parallel on a 5% polyacrylamide gel (Fig. 1, lanes 4 to 7, respectively).

of their RNAs was compared. All specimens positive by PAGE were positive on agarose gels; a typical rotaviral pattern was noted with one consistent observation: triplet segments 7, 8, and 9 migrated as a single band on the agarose gels (Fig. 1 and 2).

Figure 3 compares the steps involved in both of the PAGE methods and the agarose gel method, with final results made available in 3 days, 9 h, and 2.5 h, respectively. Electropherotyping is now an accepted method not only for epidemiological studies but also as a diagnostic tool (7, 10). Our results with the rapid extraction procedure of Pyndiah et al. (14) and agarose gel electrophoresis (Fig. 4) indicate that this rapid and simple assay could be applied on a wide scale for the screening of stools suspected for rotaviral outbreaks in day-care centers, nurseries, hospitals, homes for the elderly, etc., as well as in veterinary institutions. The assay does not need sophisticated equipment, and the cost per test is minimal (±$1.00) as compared with that of enzyme-linked immunosorbent assays or latex agglutination tests currently available on the market (6, 11, 15). Ethidium bromide staining is quick (5 min) and specific, as the stain binds well to nucleic acids and poorly to protein contaminants, so it has little effect on the electrophoresis results. It has been reported (1, 12) that PAGE and agarose gels are equally sensitive in that both can detect up to ~5 ng of double-stranded DNA or RNA. In our experiments, all stools positive by PAGE were also detected on the agarose gels. Another distinct advantage of this method is its ability also to detect non-group A rotaviruses (10), which are antigenically dissimilar and therefore are not detected by available serological diagnostic reagents (9).

An inexpensive, sensitive, and specific test which provides results within 2.5 h following receipt of the specimens could be of value in the health-care field, in which stools from large numbers of individuals are examined.

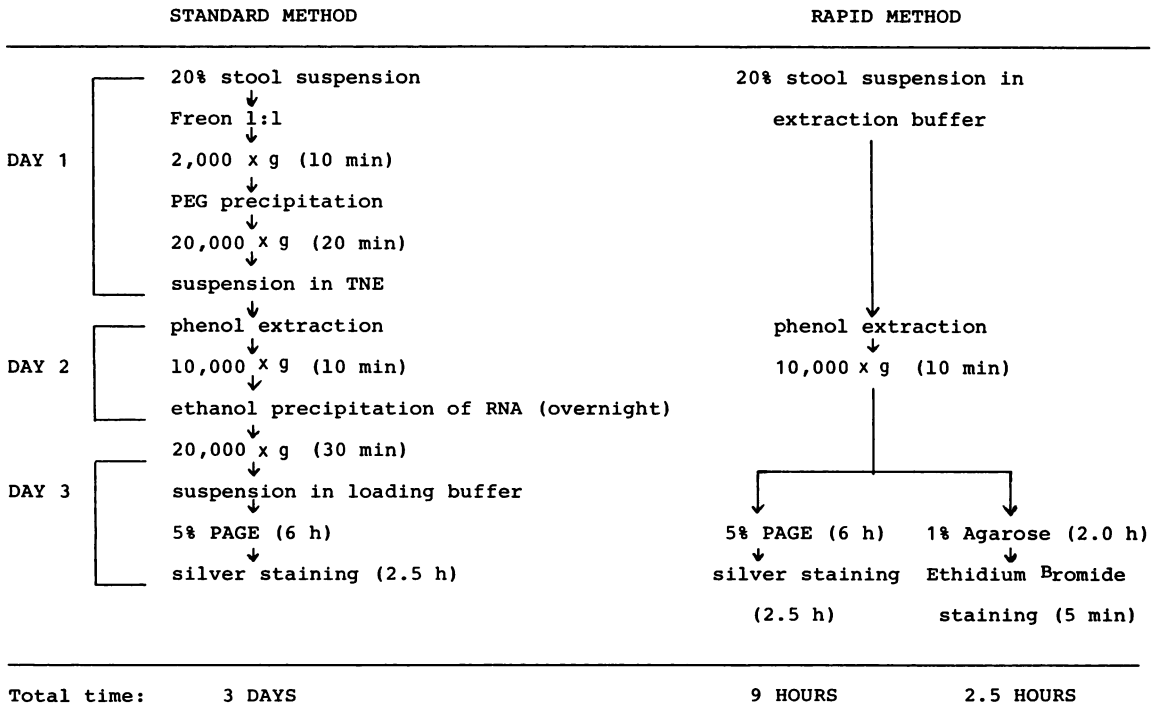


FIG. 3. Comparison of standard (3) and rapid methods for the diagnosis of rotavirus. PEG, PEG 6000 (polyethylene glycol); TNE, 10 mM Tris hydrochloride (pH 7.4)–100 mM NaCl–10 mM EDTA.

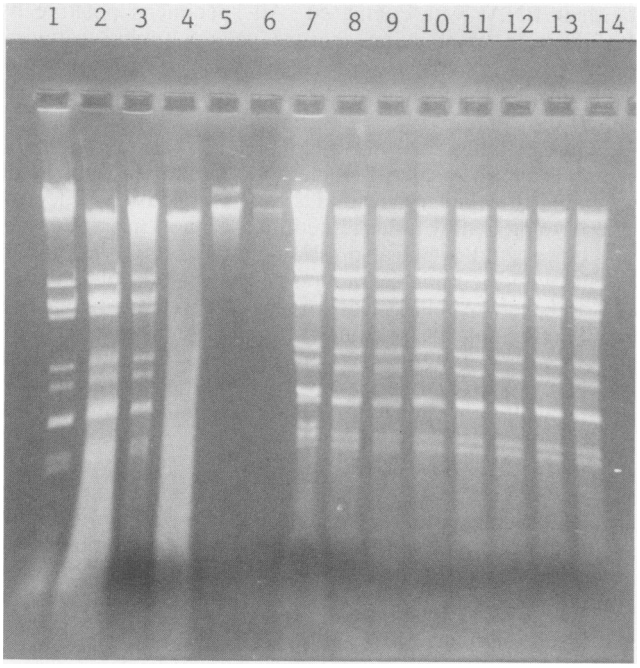


FIG. 4. Electropherogram of rotavirus RNA extracts. Stools from an outbreak were screened on a 1% agarose gel stained with ethidium bromide. Lanes: 1 to 3 and 8 to 14, rotavirus-positive stools; 4 to 6, rotavirus-negative stools; 7, internal laboratory standard.

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