# RESEARCH NOTE

# COMPARISON BETWEEN RT-PCR AND RAPID AGGLUTINATION TEST FOR DIAGNOSIS OF HUMAN ROTAVIRUS INFECTION

Suwanna Noppornpanth[1] and Yong Poovorawan[2]

[1]Department of Microbiology and [2]Viral Hepatitis Research Unit, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University and Hospital, Bangkok 10330, Thailand

**Abstract.** Rotavirus represents the major cause of dehydrating diarrhea among infants and young children on worldwide scale and has recently become the target of research aimed at developing a vaccine. To that end, screening tests of clinical specimens ought to provide high sensitivity and specificity. Hence, in order to achieve that aim we compared a commercially available latex agglutination (LA) kit with reverse transcription polymerase chain reaction (RT-PCR) using primers amplifying the gene for the major neutralization antigen in 71 stool samples of children with acute gastroenteritis during November 1998-Apirl 1999. Based on accuracy (76.05%), specificity (86.8%) and sensitivity (63.6%) determined for LA with RT-PCR serving as the gold standard, we recommend LA for field studies where speed and simplicity are crucial. Yet, for the purpose of further studies as to epidemiology and vaccine trials RT-PCR with its higher specificity and sensitivity will be required.

## INTRODUCTION

Rotavirus constitutes the major etiologic agent of severe dehydrating diarrhea in infants and young children on a worldwide scale. The mortality rate in the developing world is estimated at more than 800,000 deaths per year . Rotavirus infection also represents a health problem in developed countries where a wide spectrum of clinical symptoms can be encountered including high fever, vomiting, mild to severe diarrhea, occasionally culminating in fatal dehydrating illness (Kapikian and Chanock, 1996). Recently, rotavirus has become an important target of intense research aimed at eventually developing an effective vaccine. Although screening of clinical specimens for the presence of rotavirus has become a routine diagnostic procedure, the test is required to exhibit high sensitivity and specificity not only for diagnostic purposes, but also to provide the information necessary for vaccine studies and epidemiological surveillance, especially in developing countries.

The techniques most commonly applied for screening of rotavirus focus on the detection of virus particles or viral antigen. Based on previous studies, electron microscopy (EM) had been established as exhibiting high specificity for virus detection. Subsequently, numerous techniques have been developed to detect rotavirus antigen (Ag) directly from fecal specimens, such as enzyme-linked immunosorbent assay (ELISA), latex agglutination (LA), radioimmunoassay, counter-immunoelectro-osmophoresis and polyacrylamide gel electrophoresis (PAGE). In addition, detection of rotavirus can also be accomplished by molecular techniques, such as RNA hybridization with specific probes and more recently, by reverse transcriptase polymerase chain reaction (RT-PCR) aimed at detecting rotavirus RNA (Hoshino and Kapikian, 1994).

The purpose of the present study has been to compare between two screening methods for human rotavirus as to their respective sensitivity and specificity, one employing a commercial latex agglutination kit (Rotalex, Orion Diagnostica, Espoo, Finland) and the other RT-PCR using primers directed at amplifying the gene coding for the major neutralization antigen, the outer capsid VP7 protein.

Between November 1998 and April 1999, altogether 71 stool specimens had been collected from children, below 3 years of age in most cases, treated for acute gastroenteritis at the Department of Pediatrics, Chulalongkorn University and Hospital, Bangkok. Individual samples were prepared by

Correspondence: Prof Yong Poovorawan, Vital Hepatitis Research Unit, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University and Hospital, Bangkok 10330, Thailand.
Tel: +662-256-4909; Fax: +662-256-4929; E-mail: Yong.P@chula.ac.th

resuspension in phosphate buffered saline (PBS) at a 10-20% concentration, followed by through mixing and centrifugation at 1,500 rpm for 10 minutes. The supernatant was subjected to LA according to the manufacturer's specifications. Viral RNA was extracted from the supernatant as described by Chomczynski and Sacchi (1987). Briefly, 50 µl of the supernatant were mixed with 500 µl of 6 M guanidinium thiocyanate, 0.5% sodium-N-lauroyl-sarcosinate, 0.1 M 2-mercaptoethanol and 50 µl 2 M sodium acetate followed by phenol/chloroform/isoamylalcohol extraction and subsequent precipitation in isopropanol. The RNA pellet was resuspended in 10 µl Dep-C (diethylpyrocarbonate) treated sterile water and directly used as a template for RT-PCR. To that end, the RNA was heated to 65°C for 5 minutes, cooled on ice, and incubated in a reaction mixture containing 1 x RT buffer, 0.3 µM primer each, 0.5 mM dNTP, 20 U RNasin® ribonuclease inhibitor and 100 U M-MLV reverse transcriptase (Promega, Madison, Wis.) at 37°C for 1 hour. In order to amplify the resultant cDNA, 5 µl of it were incubated in the PCR reaction mixture (10 mM Tris-HCl pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCl, 0.3 µM primer each, 0.5 mM dNTP) and 1 U of Taq polymerase (Pharmacia Biotech) for 30 cycles comprising an initial step at 94°C for 3 minutes, denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 1 minute, concluded by a final extension step at 72°C for 4 minutes in an automated thermocycler (Perkin Elmer Cetus). The PCR products were analyzed by electrophoresis in a 1.5% agarose gel with ethidium bromide added upon preparation and visualized on a UV transilluminator.

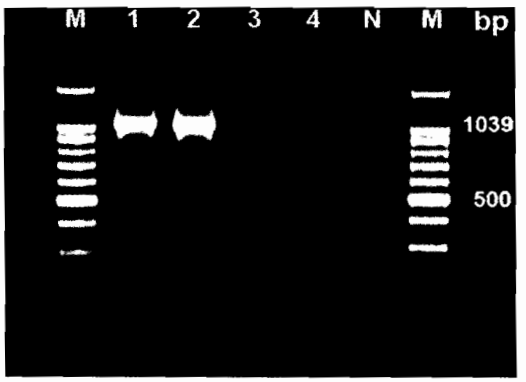


Fig 1–1.5% Agarose gel electrophoresis of the rotavirus VP7 amplified by RT-PCR. From left to right: M=100 base pair DNA ladder (Promega, Madison, Wis); 1 and 2=rotavirus-positive samples showing the product band at 1039 base pairs; 3 and 4=rotavirus negative samples; N=negative control.

Table 1
Comparison between rotavirus screening by LA and RT-PCR.

| | | RT-PCR rotavirus RNA | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| LA | Positive | 21 | 5 | 26 |
| | Negative | 12 | 33 | 45 |
| | Total | 33 | 38 | 71 |

The primers employed for the PCR had been selected from the conserved region of the VP7 gene of human rotavirus type A (Gouvea *et al*, 1990). The primer sequences were for the forward primer 5′-CCG TCT GGC TAA CGG TTA GCT-3′ (nt. 21-41) and for the reverse primer 5′-GGT CAC ATC GAA CAA TTC TAA-3′ (nt 1041-1059). The PCR results exemplified by five discrete specimens are shown in Fig 1. Table 1 depicts a comparison between the results obtained by LA and RT-PCR using accuracy, specificity and sensitivity attained by the latter method as the gold standard. To further specify, the sensitivity of LA amounted to 63.6%, its specificity to 86.8% and its accuracy to 76.05% compared with RT-PCR. The positive predictive value was 80.7%, the negative predictive value 73.3%. Our findings were comparable to those reported in a previous study comparing LA with ELISA, with the sensitivity amounting to 80% and the specificity to 81%, respectively (Hendricks *et al*, 1995). Another study compared LA with enzyme immunoassay (EIA) then confirmed the discordance results with electron microscopy (EM). The sensitivity and specificity of LA were reported as 70-80% and 97-100% respectively. In the case of equivocal results, EIA has been found to be specific at least as sensitive as EM (Sambourg *et al*, 1985). Here in Thailand, a comparative study in the detection of rotavirus yielded a sensitivity and specificity of Mini-LA amounting to 87.5% and 99.6%, respectively, in comparison with enzyme linked immunosorbent assay (ELISA) (Punnarugsa *et al*, 1989).

Based on these data, LA represents a method easily performed and providing the most rapid screening for rotavirus without the need for expensive equipment. Therefore, the tests with high specificity are appropriate for field studies where speed and simplicity are required (Dennehy *et al*, 1988). Also, in children with rotavirus diarrhea the clinical symptoms displayed can be suggestive, whereas other

methods such as stool test for rotavirus antigen or electron microscopy are certainly needed for a definite diagnosis. For arriving at a precise diagnosis in order to enable the most appropriate treatment and prevent the unnecessary consumption of antibiotics LA can certainly be recommended. Yet, a test solely based on agglutination results is sometimes difficult to interpret, especially, agglutination test (Rotalex®) shown the high number of nonspecific results which can occur in the presenting of protein A of *Staphylococcus aureus*, heptoglobin, fibonectin, and fiber in stool supernatant fluids. (Sambourg *et al*, 1985). Additionally, decreased sensitivity of the LA test compared with EM or ELISA was shown to be related to the amount of rotavirus virions in the stage of the disease at which specimens were collected (Brandt *et al*, 1987). Contrasting that, RT-PCR constitutes a highly efficient method for the detection and characterization of different serotypes of rotavirus. Hence, this technique will be required to perform further studies regarding epidemiology and vaccine trials.

## ACKNOWLEDGEMENTS

We would like to thank Ms Apiradee Theamboonlers for advice as to the laboratory techniques. We also would like to express our gratitude to the entire staff of the Viral Hepatitis Research Unit, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University and Hospital, Bangkok, for their support in the sample collection. Our special thanks go to Ms Petra Hirsch for preparing and reviewing the manuscript. This work was supported by the Thailand Research Fund, Senior Research Scholar, in partial fulfilment of the requirement of the Royal Golden Jubilee PhD Program, Thailand Research Fund.

## REFERENCES

Brandt CD, Arndt CW, Evans GL, *et al.* Evaluation of a latex test for rotavirus detection. *J Clin Microbiol* 1987; 25: 1800-2.

Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; 162: 156-9.

Dennehy PH, Gauntlett DR, Tente WE. Comparison of nine commercial immunoassays for the detection of rotavirus in fecal specimens. *J Clin Microbiol* 1988; 26: 1630-4.

Gouvea V, Glass RI, Woods P, *et al.* Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. *J Clin Microbiol* 1990; 28: 276-82.

Hendricks MK, Cuevas LE, Hart CA. Rotavirus diarrhoea in Thai infants and children. *Ann Trop Paediatr* 1995; 15: 147-52.

Hoshino Y, Kapikian AZ. Rotavirus antigens. *Curr Top Microbiol Immunol* 1994; 185: 179-227.

Kapikian AZ, Chanock RM. Rotaviruses. In: Fields BN, Knipe DM, Howley PM, *et al*, eds. Fields Virology, 3rd ed. Philadelphia: Lippincott-Raven Publishers, 1996: 1657-708.

Punnarugsa V, Woodtayakorn J, Mungmee V. Comparative study in the detection of rotavirus in feces, using mini-latex agglutination and enzyme linked immunosorbent assays. *Chula Med J* 1989; 33: 113-7.

Sambourg M, Goudeau A, Courant C, Pinon G, Denis F. Direct appraisal of latex agglutination testing, a convenient alternative to enzyme immunoassay for the detection of rotavirus in childhood gastroenteritis, by comparison of two enzyme immunoassays and two latex tests. *J Clin Microbiol* 1985; 21: 622-5.