Detection of Human Rotavirus Using One-Step Sandwich Enzyme-Linked Immunosorbent Assay

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ABSTRACT

Human rotavirus is a major causative agent of acute viral gastroenteritis in infants and young children. Rapid and accurate diagnosis of rotavirus is essential to identify those infected individuals who are potential sources of infection to others. A total of 320 stool specimens were taken from pediatric patients under five years of age suffering from acute diarrhea at Songklanagarind Hospital, Songkhla, Thailand, between March 2001 and April 2002. Diagnostic tests were performed using the conventional two-step sandwich enzyme-linked immunosorbent assay (ELISA), which is the standard method and the one-step sandwich ELISA. Forty-six (14.4%) specimens gave positive result and 274 (85.6%) specimens gave negative result by both procedures. The sensitivity, specificity, false-positive rate, false-negative rate and percent agreement were 100 percent, 100 percent, 0 percent, 0 percent and 100 percent, respectively. In addition, all stool samples gave the same result determined the color by either the ELISA plate reader or the naked eye. The between-run variation was only 4 percent. This study suggests the one-step sandwich ELISA can be an alternative assay to detect human rotavirus in stool specimens. (J Infect Dis Antimicrob Agents 2005;22:15-20.)

INTRODUCTION

Rotaviruses are members of the Reoviridae family and recognized as a major etiologic agent of acute gastroenteritis in infants and young children.1,2 Worldwide, the incidence has been reported between 18-70 percent of the acute gastroenteritis in young children under 5 years of age. In Thailand, the incidence of rotavirus ranges between 22-24 percent of acute gastroenteritis, and rises as high as 80-90 percent in winter.3,4 Rotavirus causes a serious problem in nursery and day-care centers. The accurate diagnosis of rotavirus infection is important for identification of infected individuals who are potential sources of infection to others. Rapid and accurate detection of rotavirus is important in the hospital environment for newly admitted patients, so that they can be properly isolated to prevent transmission of the virus and ensure adequate treatment. Most routine studies of rotaviruses have been based on the detection of a common group antigen by enzyme-linked immunosorbent assay (ELI-
SA) which has high sensitivity and specificity.\textsuperscript{3,6} But the conventional ELISA is a two-step sandwich method which is relatively time-consuming. We developed a rapid one-step sandwich ELISA for use as a qualitative test for detection of the rotavirus antigen.

**MATERIALS AND METHODS**

**Fecal sample and preparation**

Consecutive 320 stool specimens were collected from 320 pediatric patients under 5 years of age suffering from acute diarrhea who attended at Songklanagarind Hospital, Songkla, Thailand, between March 2001 and April 2002. Each stool specimen was prepared as either a 10 percent (weight/volume) solid suspension or a 20 percent (volume/volume) suspension of liquid feces in 0.01 M phosphate buffer saline, pH 7.2. After incubation at room temperature, the suspension was centrifuged at 1,500Xg for 5 minutes. The supernatant was then tested and stored at -20°C, which had no influence on specimen quality.

**Conventional sandwich ELISA for human rotavirus antigen**

100 µl of an optimal concentration of anti-rotavirus antibody (Cat.No.B0218 Dako, Denmark) and normal rabbit immunoglobulins (Cat. No. X0903 Dako, Denmark) in carbonate buffer pH 9.6 were alternately adsorbed in vertical rows into the wells of a microtiter plate (Maxisorp, Nunc-Immunoplate, Denmark), and then incubated overnight at 4°C in the moist chamber. The microtiter plate was washed 5 times with a phosphate buffer saline, pH 7.2, and then 100 ml of each sample supernatant was added into both the test wells (coated with anti-rotavirus antibody) and the control wells (coated with normal rabbit immunoglobulin). Then, the plate was incubated for 60 minutes at 37°C before washing 5 times as described above. 100 µl of an optimal concentration of peroxidase-conjugated rabbit anti-rotavirus antibody (Cat. No. P219, Dako, Denmark) was added into both wells, and further incubated at 37°C for 60 minutes. After washing 5 times, 100 µl of freshly prepared chromogenic substrate [37 mg ortho-phenylenediamine dihydrochloride (Sigma Chemical, USA) in 10 ml of 0.1 M citric acid phosphate buffer, pH 5.0, with 10 µl of 30 percent H₂O₂] were added into the wells. The plate was incubated at room temperature for 15 minutes. The reaction was then stopped with 100 µl of 4 M sulphuric acid, and the optical density (OD) was measured at the wave-length of 490/600 nm by the ELISA plate reader (BEP III, Dade-Behring ELISA Processor III). Both positive and negative controls were included in each examination. A sample showing a difference in the OD between the test and control wells greater than 0.2 was considered as positive.

**One-step sandwich ELISA for human rotavirus antigen**

This method was modified from the conventional two-step sandwich ELISA. All steps the followed from the standard protocol, except 100 µl of the sample supernatant and 100 µl of an optimal concentration of the peroxidase-conjugated anti-rotavirus antibody were added together into both test and control wells, and then incubated at 37°C for 60 minutes. After measuring an OD by the ELISA plate reader, each well was then visually observed and recorded for the color intensity. Positive reaction was represented by brown color in the test well and colorlessness in the control well, and negative reaction was represented by colorlessness in both the test and control wells.

**Statistic analysis**

The sensitivity, specificity, false-positive rate and false-negative rate were calculated according the method described by Griner.\textsuperscript{7}
RESULTS

Three hundred and twenty stool samples from pediatric patients under 5 years of age suffering from acute diarrhea were tested using the conventional two-step and one-step sandwich ELISAs for detection of rotavirus antigen. The two-step sandwich ELISA served as the standard method. Forty-six (14.4%) specimens were positive and 274 (85.6%) specimens were negative by both methods. The sensitivity, specificity, false-positive rate, false-negative rate and percent agreement were 100 percent, 100 percent, 0 percent, 0 percent and 100 percent, respectively (Table 1). The kappa statistic value for agreement between the two-step and one-step sandwich ELISAs showed perfect agreement of 1.0. The difference in the OD between the test and control wells among all samples by the one-step sandwich ELISA are shown in Figure 1. All colorimetric reactions were clearly detected and gave the same result determined by either the ELISA plate reader or by visual inspection using the naked eye.

Table 1. The statistic parameters of one-step ELISA for detection of rotavirus antigen in stool specimens using colorimetric reader and visual reading.

<table>
<thead>
<tr>
<th>Statistic parameters</th>
<th>Colorimetric reader</th>
<th>Visual reading</th>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>46/46 (100%)</td>
<td>46/46 (100%)</td>
</tr>
<tr>
<td>Specificity</td>
<td>274/274 (100%)</td>
<td>274/274 (100%)</td>
</tr>
<tr>
<td>False-positive rate</td>
<td>0/46 (0%)</td>
<td>0/46 (0%)</td>
</tr>
<tr>
<td>False-negative rate</td>
<td>0/274 (0%)</td>
<td>0/274 (0%)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>320/320 (100%)</td>
<td>320/320 (100%)</td>
</tr>
</tbody>
</table>

Figure 1. The difference in the optical density between the test and control wells of one-step sandwich ELISA were plotted. Negative and positive groups were identified by conventional two-step sandwich ELISA.
The minimum, mean and maximum difference in OD between the test and control wells of positive results were 0.35, 1.69 and 2.8, respectively. The minimum, mean and maximum difference in the OD between the test and control wells of negative results were -0.3, -0.069 and 0.181, respectively. The reproducibility of the test protocol was performed using one single, reactive sample repeatedly tested on 20 consecutive days. The coefficient of variance (%CV) of the between-run assay was 4.0 percent.

**DISCUSSION**

Previous studies showed that the ELISA is a very sensitive and specific test with high reliability and reproducibility for detection of rotavirus in stool.8-10 We have used the conventional two-step sandwich ELISA in our lab for many years, but it requires two steps of incubation and requires three hours to accomplish. The one-step sandwich ELISA requires only one step of incubation, and thus at least one hour can be reduced. This rapid result can help the clinician for a proper management of pediatric patients with acute diarrhea. This may reduce the overuse of antibiotics which is commonly prescribed in the treatment of acute gastroenteritis.

Rotaviruses are classified into seven serogroups (A-G) on the basis of their distinct antigenic and genetic properties.11 All seven groups are associated with acute diarrhea in animals, but only three (A-C) have been associated with diarrhea in humans. Group A rotaviruses are the most important cause of acute gastroenteritis in children. Previous reports show that most of the cases occur in the first three years of life.12-16 Group A rotaviruses are routinely detected by the ELISA, and both platforms of ELISA technique studied here are able to detect group A rotavirus in stool specimens.17-20

In our study, the two qualitative methods for detection of human rotavirus in stool specimens were compared. All samples gave the same results between the conventional two-step and the one-step sandwich ELISA. The colorimetric reaction was very clear. Visual inspection of the color using the naked eye could be done without any change in the results. This indicated that the one-step sandwich ELISA can be used in the field, without requiring expensive equipment like the ELISA plate reader. In addition, the coefficient of variance (%CV) of the one-step sandwich ELISA was only 4 percent. This showed that the test had high reliability. These are all features that improve the ELISA technique for detection of human rotavirus in stool specimens by using one-step sandwich ELISA.

**ACKNOWLEDGEMENT**

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**References**

7. Griner PF, Mayewski RJ, Muslin AI, Greenland P.


