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Comparison of Premier™ Rotaclone®, ProSpecT™, and RIDASCREEN® Rotavirus Enzyme Immunoassay Kits for Detection of Rotavirus Antigen in Stool Specimens

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Abstract

Background—Rotaviruses are the major cause of severe dehydrating diarrhea in children throughout the world. Enzyme immunoassays (EIA) have been the standard method for detection of rotavirus in stool specimens since the 1980s. The World Health Organization (WHO) Rotavirus Surveillance Network has proposed including three EIA kits in the WHO-GSM (Global Management System/Système Mondial de Gestion) catalogue for easy procurement of EIA kits by participating rotavirus surveillance network laboratories.

Objectives—In this study, we conducted a comparative analysis of 3 commercially available enzyme immunoassay kits: Premier™ Rotaclone® (Meridian Bioscience, Inc.), ProSpecT™ (Oxoid, Ltd.) and RIDASCREEN® (R-biopharm AG) for rotavirus diagnostics.

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Not applicable.

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Not applicable.

Study design—Using reverse-transcriptase-PCR (RT-PCR) as the gold standard, the 3 EIA kits were evaluated by testing a stool panel consisting of 56 rotavirus-positive and 54 rotavirus negative samples.

Results—The sensitivities of the Premier™ Rotaclone®, ProSpecT™ and RIDASCREEN® kits were 76.8%, 75% and 82.1% respectively, but did not differ significantly. The specificity of all the 3 kits was 100%. The use of RT-PCR as a gold standard lowered the observed sensitivity of all 3 EIA kits but helps to reduce equivocal results that can be seen when another EIA or other non-molecular methods are used as the reference assay in comparison studies.

Conclusion—Our study found that all three kits are suitable for use by rotavirus surveillance programs.

Keywords

rotavirus; EIA; comparison; RT-PCR; sensitivity; specificity

Background

Group A rotaviruses are the leading cause of acute gastroenteritis (AGE) in infants and young children worldwide[1, 2]. Enzyme immunoassays (EIAs) offer a simple, rapid, and sensitive method for routine laboratory detection of rotavirus antigen in stool specimens[3]. Commercial EIA kits have been available since the 1980s and evaluations of these kits have been performed[3-5], but a comparison of current generation EIA kits has not been done. The Rotavirus Surveillance Laboratory, Centers for Disease Control and Prevention, Atlanta, Georgia, USA is the Global Reference Laboratory for the World Health Organization (WHO) Rotavirus Surveillance Network.

Objectives

To assist the WHO in recommending the best commercially available kits for rotavirus detection to laboratories that participate in the global network, we conducted a comparative analysis study of 3 EIA kits in order to determine if these 3 kits meet performance criteria required for inclusion in the WHO-GSM (Global Management System/Système Mondial de Gestion) catalogue to facilitate reagent procurement by network surveillance laboratories. The EIA kits included in this study were: 1) the Premier™ Rotaclone® (Meridian Bioscience, Inc., Cincinnati OH, USA); 2) the ProSpecT™ Rotavirus Microplate Assay (Oxoid, Ltd., Basingstoke, Hampshire, UK); and the 3) RIDASCREEN® Rotavirus (R-Biopharm AG, Darmstadt, Germany). All 3 kits use a solid-phase sandwich EIA format. The Premier™ Rotaclone® kit is the only multi-well EIA kit approved by the U.S. Food and Drug Administration (FDA) for *in vitro* diagnostic (IVD) use. It uses monoclonal antibodies raised against rotavirus structural protein VP6. The ProSpecT™ Rotavirus Microplate Assay EIA kit is a replacement kit for the widely-used rotavirus IDEIA™ Rotavirus EIA (Dako Diagnostics Ltd., Ely UK), which was discontinued in March 2009. It uses polyclonal capture and detector antibodies raised against rotavirus structural proteins. The RIDASCREEN® Rotavirus EIA kit uses monoclonal antibodies raised against rotavirus structural protein VP6. In 2010, RIDASCREEN® was reformulated to incorporate a

biotinylated detector antibody and streptavidin-conjugated peroxidase. The analytical performance of these kits has not been compared directly.

Study Design

Stool samples from AGE cases were selected from domestic and international surveillance samples received by the CDC for genotyping of rotavirus strains. All the samples selected for this study were tested for the presence of rotavirus VP4 and VP7 and/or VP6 genes using reverse transcription-PCR (RT-PCR)[6-8]. Fifty-six rotavirus-positive samples and 54 rotavirus-negative samples were selected for this study. All 110 samples were tested for rotavirus antigen according to manufacturers' instructions for each kit. Three operators performed all tests, for a total of 3 replicates per sample. EIA plates were read on an MRXe ELISA plate reader (Dynex Technologies, Chantilly, VA USA). A sample was considered to test positive by a kit if the optical density (OD) values for 2 or 3 replicates were above the calculated cut-off value for that kit. The analytical sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for each kit. Statistical analyses were performed by using Prism Version 5.02 Software for Windows (GraphPad Software, Inc., La Jolla, CA). Testing results were analyzed by chi-square test. OD values were compared by Kruskal-Wallis test, and pairwise comparisons mean OD values from each kit were performed using Dunn's Multiple Comparison test.

Results

The results of testing 110 samples in triplicate by each kit are shown (Table 1). For each of the 3 kits, all EIA-positive samples had tested as rotavirus-positive by RT-PCR for VP4 and VP7 or VP6 and all EIA-negative samples had tested negative by RT-PCR. However, some RT-PCR positive samples tested negative by EIA, ranging from 10 for RIDASCREEN® Rotavirus to 14 for ProSpecT™. Using RT-PCR as the gold standard, the performance characteristics of the kits were: Premier™ Rotaclone® EIA, 76.8% sensitivity, 100% specificity, PPV = 100% , NPV = 80.6%; ProSpecT™ EIA, 75% sensitivity, 100% specificity, PPV = 100%, NPV = 79.4%; and, RIDASCREEN® Rotavirus, 82.1% sensitivity, 100% specificity, PPV = 100%, NPV = 84.4%. When the sample testing results of the 3 kits, expressed as positives and negatives, were analyzed by chi-square test (Table 1), the results obtained by each kit were not found to differ significantly. Distribution plots of OD values for the 3 kits (n = 330; Figure 1) showed that the distribution for the Premier™ Rotaclone® and ProSpecT™ Rotavirus kits were similar, with each plot skewed to the right. For both assays, numerous data points lay within 0.05 OD units on either side of the cut-off value (Rotaclone®, n=23; ProSpecT™, n=20). In contrast, for the RIDASCREEN® kit, OD values were bimodally distributed, with one large peak on the left side of the graph containing all the negative values, and a broad peak on the right side containing the majority of the positive OD values. Only 1 data point lay within 0.05 OD units on either side of the cut-off value. The OD values from the 3 kits were found to differ significantly, and this difference was observed when all data points were analyzed ($p = 0.0131$), when positive OD values only were analyzed ($p < 0.001$), and when negative OD values only were analyzed ($p < 0.001$). Pairwise comparisons showed that the OD values from the RIDASCREEN® kit differed significantly ($p < 0.05$) from those of the ProSpecT™

kit but did not differ significantly from those of the Rotaclone® kit. The OD values generated by the Premier™ Rotaclone® and ProSpecT™ kits did not differ significantly. We found the Premier™ Rotaclone® EIA to have 76.8% sensitivity and 100% specificity compared with 100% sensitivity and 92% specificity as reported by its manufacturer, Meridian Bioscience, Inc. and 100% sensitivity and 99-100% specificity as reported in published studies.[3, 5]. The ProSpecT™ Rotavirus EIA kit exhibited 75% sensitivity and 100% specificity in this study as compared with 100% sensitivity and 99.2% specificity as reported by Oxoid, Ltd. RIDASCREEN® EIA showed 82.1% sensitivity and 100% specificity as compared to 98.4% sensitivity and 100% specificity as reported by R-biopharm AG. The differences between the sensitivity and specificity values that we found and those reported by the manufacturers and others results from using different gold standard methods. We used RT-PCR as a gold standard whereas Meridian Bioscience compared Premier™ Rotaclone® EIA results to electron microscopy (EM) and other studies used a reference EIAs[3, 5]. Oxoid compared ProSpecT™ Rotavirus EIA results to EM and a commercial EIA kit, and R-Biopharm compared RIDASCREEN® EIA results to other certified EIA kit results. The use of the more sensitive RT-PCR technique[9] for establishing the gold-standard lowers the observed sensitivity of all 3 EIA kits but helps to reduce equivocal results that can be seen when another EIA or other methods are used as the reference assay in comparison studies [3, 5] _ENREF_3. In May 2010, Oxoid Ltd. changed the method used to calculate the cut-off value of ProSpecT™ Rotavirus kit by specifying that cut-off limit should be calculated by adding 0.2 absorbance units to the negative control value; previously 0.1 absorbance units were added. When the ProSpecT™ Rotavirus results from this study were reinterpreted using a cut-off value calculated using the original method, the sensitivity increased to from 75 to 87.5%, surpassing sensitivity attained by the RIDASCREEN® kit. Oxoid Ltd. changed the method of calculating the cut-off limit for the ProSpecT™ kit so that its performance would be equivalent to that of the IDEIA™ kit, which it replaced. However, this change appears to have reduced the sensitivity of the ProSpecT™ Rotavirus kit.

Conclusions

In this study, we evaluated and compared 3 EIA kits, Premier™ Rotaclone®, ProSpecT™ Rotavirus, and RIDASCREEN® Rotavirus for detection of rotavirus antigen in stool samples. Testing of the kits against a stringent gold standard showed them to have comparable sensitivity and specificity, thus, all 3 kits are suitable for use in rotavirus surveillance programs worldwide.

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Abbreviations

AGE	acute gastroenteritis
EIA	Enzyme immunoassay

ELISA	Enzyme-linked Immunosorbent Assay
FDA	Food and Drug Administration
GSM	Global Management System/Système Mondial de Gestion
IVD	*in vitro* diagnostic
NPV	negative predictive value
OD	optical density
PPV	positive predictive value
WHO	World Health Organization

References

1. Parashar UD, Gibson CJ, Bresse JS, Glass RI. Rotavirus and severe childhood diarrhea. Emerg Infect Dis. 2006; 12:304–6. [PubMed: 16494759]
2. Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD, et al. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect Dis. 2012; 12:136–41. [PubMed: 22030330]
3. 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. [PubMed: 2846645]
4. Christy C, Vosefski D, Madore HP. Comparison of three enzyme immunoassays to tissue culture for the diagnosis of rotavirus gastroenteritis in infants and young children. J Clin Microbiol. 1990; 28:1428–30. [PubMed: 2199504]
5. Lipson SM, Svenssen L, Goodwin L, Porti D, Danzi S, Pergolizzi R. Evaluation of two current generation enzyme immunoassays and an improved isolation-based assay for the rapid detection and isolation of rotavirus from stool. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2001; 21:17–27. [PubMed: 11255094]
6. Iturriza Gomara M, Wong C, Blome S, Desselberger U, Gray J. Molecular characterization of VP6 genes of human rotavirus isolates: correlation of genogroups with subgroups and evidence of independent segregation. J Virol. 2002; 76:6596–601. [PubMed: 12050372]
7. Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, et al. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J Clin Microbiol. 1992; 30:1365–73. [PubMed: 1320625]
8. Das BK, Gentsch JR, Cicirello HG, Woods PA, Gupta A, Ramachandran M, et al. Characterization of rotavirus strains from newborns in New Delhi, India. J Clin Microbiol. 1994; 32:1820–2. [PubMed: 7929782]
9. Wilde J, Yolken R, Willoughby R, Eiden J. Improved detection of rotavirus shedding by polymerase chain reaction. Lancet. 1991; 337:323–6. [PubMed: 1703618]

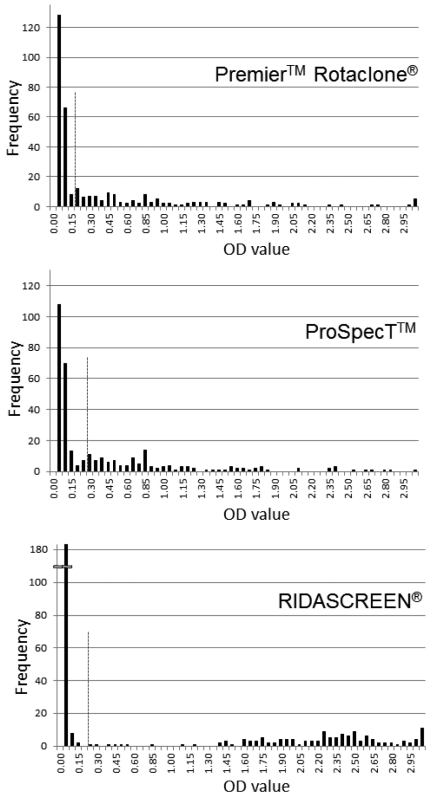

Figure 1.

Frequency distributions of OD values for the Premier™ Rotaclone®, ProSpecT™ Rotavirus, and RIDACREEN® Rotavirus EIA kits. Bins (logical ranges) were set at every 0.05 OD units. The vertical dashed line on each graph indicates the assay cut-off value. The *y*-axis of the bottom graph has been condensed between 100 and 180 to maintain the visibility of the shorter data bars.

Table 1

Comparison of Rotavirus Detection Results by Premiere™ Rotaclone®, ProSpecT ™ Rotavirus and RIDASCREEN® Rotavirus EIA kits using RT-PCR as the Gold Standard.

EIA Kit	RT-PCR		Total
	Positive	Negative	
Rotaclone			
Positive	43	0	43 [a]
Negative	13	54	67 [a]
Total	56	54	110
ProSpecT			
Positive	42	0	42 [a]
Negative	14	54	68 [a]
Total	56	54	110
RIDASCREEN			
Positive	46	0	46 [a]
Negative	10	54	64 [a]
Total	56	54	110

[a] Results obtained by each kit were not found to differ significantly (p = 0.8483, Chi-square = 0.3291, df = 2)