Evaluation of a New Enzyme Immunoassay (TESTPACK ROTAVIRUS®) for Diagnosis of Viral Gastroenteritis

Hitoshi HONMA1,2, Hiroshi USHIJIMA1, Michio TAKAGI3 and Takashi KITAMURA1
1)Division of Special Pathogens, Department of Enteroviruses, National Institute of Health, Tokyo, Japan
2)Department of Bacteriology, The Jikei University School of Medicine, Tokyo, Japan
3)Department of Pediatrics, Maizuru Kyosai Hospital, Kyoto, Japan

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Summary

TESTPACK ROTAVIRUS®, a simple 10 min enzyme immunoassay, was compared with reversed passive hemagglutination assay (RPHA) and polyacrylamide gel electrophoresis of virus RNA (RNA-PAGE) for the detection of rotaviral antigens in feces from 104 diarrheal children. The results of TESTPACK ROTAVIRUS® were identical with those of RNA-PAGE. Discordant results of RPHA were further examined by latex agglutination and electron microscopy, and were finally decided to be negative. Pararotaviruses, adenoviruses and small round viruses were negative in the TESTPACK ROTAVIRUS®.

Introduction

Rotavirus is an important agent causing viral gastroenteritis in infants and children. Rotavirus infection can be diagnosed by electron microscopy (EM), enzyme-linked immunosorbent assay, reversed passive hemagglutination assay (RPHA), RNA-polyacrylamide gel electrophoresis (RNA-PAGE) and latex agglutination (LA) tests.

In recent years, efforts have been made to produce simple and rapid enzyme immunoassays (EIA) using monoclonal antibodies.

Here we report the accuracy of a new, simple, short-incubation, commercially available, solid-phase EIA (TESTPACK ROTAVIRUS®, Abbott Laboratories, North Chicago, Ill., USA) to detect rotaviruses in clinical specimens from patients in Japan which were found to be positive or negative in RPHA and RNA-PAGE.

Materials and Methods

One hundred four diarrheal stool specimens were collected from infants and children who were inpatients and outpatients in the Maizuru Kyosai Hospital, Kyoto, Japan from November 1987 to February 1988. The specimens were frozen and thawed before examinations.

Several other viruses which cause diarrhea in humans and animals were also examined: a mouse rotavirus, a newborn calf diarrheal virus (NCDV; Lincoln strain), group B human rotaviruses, group C human rotaviruses, human adenoviruses type 40 and 41, and two small round viruses (SRVs).

The mouse rotavirus and the calf rotavirus which we used belonged to group A rotaviruses. SRVs had been
Determination of rotavirus using TESTPACK ROTAVIRUS®

They had been negative by RPHA and LA for rotavirus.

RPHA: A commercially available RPHA kit was used with an anti-human rotavirus (Wa strain) rabbit serum as antibody.

RNA-PAGE: About 0.2 g of stool specimens were diluted 5 fold with phosphate buffered saline in microcentrifuge tubes and centrifuged at 10,000 Xg for 10 min. After adding 0.1 ml of 10% SDS, and 0.5 ml of phenol, the supernatant was mixed and centrifuged again. The sample was added with Laemmli solution17) and boiled for 3 min. After electrophoresis in 10% polyacrylamide gel, the gel was stained with silver18).

Enzyme Immunoassay: The Abbott TESRPACK assay was performed as described in the manufacturer’s procedure. Briefly, a 10% suspension of stools was made in a specimen dilution cup, using specimen dilution buffer. The dilution was clarified by placing the filter tube in the specimen dilution cup and pressing slowly and firmly until the filter tube was completely inserted. Latex particles, coated with a guinea pig anti-rotavirus antibody, and alkaline phosphatase conjugate (a mixture of mouse monoclonal and bovine polyclonal anti-rotavirus antibodies) were added to the clarified suspension. Following a 5-min incubation, the entire contents of the tube were poured into the sample focuser on a Reaction Disc. The reaction site was washed with 1 ml of wash buffer and enzyme substrate was added. Color was allowed to develop for 2 min and the stop buffer was added. The results were read visually, with a negative sign indicating that the specimen was negative for the presence of rotavirus. Any color on the vertical bar (+) indicated that the specimen contained rotavirus antigen.

The methods of EM and LA were described previously6).

Table 1 Comparison of TESTPACK ROTAVIRUS®, RPHA and RNA-PAGE for detecting rotavirus in diarrheal stools

<table>
<thead>
<tr>
<th>TESTPACK</th>
<th>RPHA</th>
<th>RNA-PAGE</th>
<th>samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>49</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>4abcde</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>51</td>
</tr>
</tbody>
</table>

Titers of RPHA: a, 16 ×; b, 16 ×; c, 16 ×; d, 32 ×

Table 2 Comparison of TESTPACK and RPHA for detecting rotavirus in stools

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Predictive value</th>
<th>Diagnostic value</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Positive (%)</td>
<td>Negative (%)</td>
</tr>
<tr>
<td>TESTPACK</td>
<td>51/51(100%)</td>
<td>49/49(100%)</td>
<td>51/55(93%)</td>
</tr>
</tbody>
</table>

1) No. of specimens negative by both TESTPACK and RPHA/No. of specimens negative by RPHA
2) No. of specimens positive by both TESTPACK and RPHA/No. of specimens positive by RPHA
3) No. of specimens positive by both TESTPACK and RPHA/No of specimens positive by both TESTPACK and RPHA+No. of specimens positive by TESTPACK and negative by RPHA
4) No. of specimens negative by both TESTPACK and RPHA/No. of Specimens negative by both TESTPACK and RPHA+No. of Specimens negative by TESTPACK and positive by RPHA
5) No. of positive and negative specimens in both TESTPACK and RPHA/No. of specimens tested.

Fig. 1. Comparison of results of TESTPACK and titers of RPHA. Closed circle shows RNA-PAGE positive. Open circle shows RNA-PAGE negative. A big closed or open circle mean 10 samples. A small closed or open circle mean one sample. a, b, c and d mean discordant cases which was shown in Table 1.
Results

Forty-nine of 104 samples (47%) were positive using RPHA, RNA-PAGE and TESTPACK ROTAVIRUS®. The other 51 samples (49%) were negative in all three methods. The other 4 samples (4%) were discordant results (Table 1). Further examinations of the discordant results by EM and LA proved negative.

Within the forty-nine positive samples using all three methods, 32 samples showed more than 256 dilution titer by RPHA. Within the 51 negative samples using all three methods, 35 samples showed less than 4 dilution titer by RPHA. The discordant results originated from 16 and 32 dilution titer by RPHA (Fig. 1).

Using RPHA as the basis of comparison, we found TESTPACK ROTAVIRUS® to have a specificity of 100%, a sensitivity of 92%, a positive predictive value of 100%, a negative predictive value of 93% and a diagnostic value of 96% (Table 2).

Using RNA-PAGE as the basis of comparison, we found TESTPACK ROTAVIRUS® to have a specificity of 100%, a sensitivity of 100%, a positive predictive value of 100%, a negative predictive value of 100% and a diagnostic value of 100%.

Although a mouse rotavirus and a calf rotavirus showed positive using TESTPACK ROTAVIRUS®, other viruses (group B and group C human rotaviruses, human adenovirus type 40 and 41, and small round viruses) showed negative (Table 3).

Discussion

There are several commercial test kits to examine rotavirus and adenovirus gastroenteritis. These kits are usually composed of antigen and antibody reactions by enzyme immunoassay or latex agglutination. TESTPACK was first introduced as a kit for the diagnosis of group A streptococcus infection using enzyme immunoassay on a Reaction Disc. Results are easily read as (+) or (−) which appear on the disc. In the case of TESTPACK ROTAVIRUS®, a filter tube is added to clarify diluted specimens. Chenesky et al. used TESTPACK ROTAVIRUS® to compare with electron microscopy and Pathfinder enzyme immunoassay. Marchlewicz et al. used it to compare with Pathfinder enzyme immunoassay. The sensitivity and specificity were enough for clinical use. We used TESTPACK ROTAVIRUS® comparing RPHA and RNA-PAGE. Our results also indicated the usefulness of TESTPACK. Discordant results by RPHA were negative by two other tests, EM and LA.

Both of our mouse rotavirus and bovine rotavirus belong to group A rotavirus and they showed positive reactions. Other group rotaviruses, adenoviruses and small round viruses, showed negative reactions. The reagent in the kit included anti-mouse and anti-bovine antibodies. Probably those which

<table>
<thead>
<tr>
<th>Viruses by EM and/or PAGE</th>
<th>TESTPACK</th>
<th>No. of test samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDIM</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>NCDV</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>Pararotavirus group B</td>
<td>−</td>
<td>2</td>
</tr>
<tr>
<td>Pararotavirus group C</td>
<td>−</td>
<td>2</td>
</tr>
<tr>
<td>Adenovirus 40</td>
<td>−</td>
<td>1</td>
</tr>
<tr>
<td>Adenovirus 41</td>
<td>−</td>
<td>1</td>
</tr>
<tr>
<td>Small round virus</td>
<td>−</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3 Detection of rotavirus antigen by TESTPACK from various samples

<table>
<thead>
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<th>No. of test samples</th>
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</thead>
<tbody>
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<td>+</td>
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<td>NCDV</td>
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</tr>
<tr>
<td>Pararotavirus group B</td>
<td>−</td>
<td>2</td>
</tr>
<tr>
<td>Pararotavirus group C</td>
<td>−</td>
<td>2</td>
</tr>
<tr>
<td>Adenovirus 40</td>
<td>−</td>
<td>1</td>
</tr>
<tr>
<td>Adenovirus 41</td>
<td>−</td>
<td>1</td>
</tr>
<tr>
<td>Small round virus</td>
<td>−</td>
<td>2</td>
</tr>
</tbody>
</table>
only react against group A rotavirus.

TESTPACK ROTAVIRUS® was proved to be useful, both rapidly and easily, in clinics for the diagnosis of rotavirus gastroenteritis.

Acknowledgement

We thank Dr. Atsushi Mukoyama for his help of electron microscopic examination.

References

ウイルス性胃腸炎の診断のための新しい酵素抗体法
（テストパック・ロタウイルス®）の評価

1) 国立予防衛生研究所腸内ウイルス部
2) 慈恵医科大学細菌学教室
3) 豊橋共済病院小児科

本間 靖1)2) 牛島 廣治1) 高木 道生3) 北村 敬1)

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（平成1年7月4日受理）

胃腸炎患者104例の下痢便について、新しい10分で診断可能なテストパック・ロタウイルス®と従来の逆受身赤血球凝集法、RNAポリアクリルアミドゲル電気泳動法（RNA-PAGE）を用いてロタウイルス抗原の検出を比較した。

テストパック・ロタウイルス®の成績は、RNA-PAGEの成績と一致し、逆受身赤血球凝集法の結果とは一部低い抗原濃度で異なった。逆受身赤血球凝集法でテストパックと結果が異なって陽性を示した例ではラテックス凝集法、電子顕微鏡法では陰性を示した。

バラロタウイルス、アデノウイルス、小球形ウイルスはテストパック・ロタウイルス®で陰性を示した。