



RESEARCH NOTE

# Comparative evaluation of sensitivity and specificity of immunochromatography kit for the rapid detection of norovirus and rotavirus in Bangladesh [version 1; peer review: 2 approved with reservations]

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**Abstract**

We report a comprehensive analysis of sensitivity and specificity of immunochromatography kit (IC Kit) for the rapid detection of norovirus and rotavirus in Bangladesh. The IC kit (IP-Noro/Rota) provides highest sensitivity (100%) to both viruses compared to the reference method reverse transcription- polymerase chain reaction (RT- PCR) for diagnosis. Furthermore, the test provides a high specificity of 98.9% and 96.1% to diagnose norovirus and rotavirus, respectively, as well as good agreement with the reference method. We also found high prevalence of rotavirus infection (74%) among Bangladeshi pediatric population, of which most of the patients were less than five years old, suffering from severe dehydration, abdominal pain and vomiting. This study is the first to report the ease and rapid detection of norovirus and rotavirus by IC kits in Bangladesh. Therefore, IP-Noro/Rota kit is recommended for the rapid detection of these viruses in routine diagnosis as well as during outbreaks.

**Keywords**

Immunochromatography kit, Norovirus, Rotavirus, Rapid detection, Bangladesh.

**Open Peer Review**

**Reviewer Status** ? ?

	Invited Reviewers	
	1	2
<b>version 1</b>		
published 11 Feb 2019	report	report

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- Nicola Page** , National Institute for Communicable Diseases (NICD), Johannesburg, South Africa  
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Any reports and responses or comments on the article can be found at the end of the article.

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**Author roles:** **Shaha M:** Formal Analysis, Writing – Original Draft Preparation, Writing – Review & Editing; **Sifat SF:** Formal Analysis, Methodology, Writing – Review & Editing; **Mamun MA:** Investigation, Methodology, Writing – Review & Editing; **Billah MB:** Methodology, Writing – Review & Editing; **Sharif N:** Formal Analysis, Investigation, Writing – Review & Editing; **Nobel NU:** Formal Analysis, Investigation, Writing – Review & Editing; **Parvez AK:** Investigation, Supervision, Writing – Review & Editing; **Talukder AA:** Investigation, Validation, Writing – Review & Editing; **Nomura A:** Conceptualization, Supervision, Writing – Review & Editing; **Ushijima H:** Supervision, Writing – Review & Editing; **Dey SK:** Conceptualization, Funding Acquisition, Supervision, Writing – Review & Editing

**Competing interests:** No competing interests were disclosed.

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## Introduction

Diarrheal diseases represent a major worldwide public health problem, particularly in developing countries<sup>1</sup>. Acute gastroenteritis is a very common disease in young children<sup>2</sup>. It has been reported that about 3–5 billion cases of acute gastroenteritis occur each year in children less than 5 years old and 1.5 to 2.5 million children of that group die from severe diarrhoea<sup>3–5</sup>. Of that, about half a million deaths are caused by rotavirus infections<sup>6</sup>. On the other hand, norovirus is responsible for almost half of the foodborne gastroenteritis outbreaks and 75–90% of non-bacterial gastroenteritis outbreaks<sup>7</sup>.

When outbreaks of gastroenteritis occur in communities, rapid identification of pathogens is essential to ensure the administration of the appropriate treatment and control. Furthermore, definite diagnosis plays an important role to decrease the unnecessary use of antibiotics. In the case of emergency, there is no rapid detection method available in Bangladesh. In this regard, a rapid diagnosis kit with good sensitivity and specificity is essential. Developing such a kit may raise the reliability for rapid diagnosis in developing countries, where the prevalence of norovirus and rotavirus is increased. Herein, we report a comprehensive analysis of the sensitivity and specificity of an immunochromatography kit (IC Kit) for the rapid detection of norovirus and rotavirus in Bangladesh.

## Methods

### Participants

In this study, we evaluate the newly developed IC test kit for norovirus and rotavirus detection (IP-Noro/Rota; ImmunoProbe Co., Ltd., Saitama, Japan) in 100 stool samples collected from paediatric patients with acute gastroenteritis (severe dehydration, abdominal pain and vomiting) in Bangladesh during January to June 2015. The study was ethically approved by the ethical review committee of Jahangirnagar University, Bangladesh.

### Test methods

Reverse transcription PCR (RT-PCR) was used as the reference test for both norovirus and rotavirus detection<sup>3</sup>. The PCR is a molecular biology technique that allows for nucleic acid fragmentation from a complex pool of DNA. Faecal specimens were

thawed, diluted with distilled water to 10% suspensions, and centrifuged at 10,000xg for 10 min. Viral RNA was extracted from 140µl of the supernatant using a spin-column technique (QIAamp Viral RNA kit; Qiagen, Hilden, Germany) according to the manufacturer's instructions. For reverse transcription, 3µl of extracted RNA was mixed with a reaction mixture consisting of 1µl of oligo dT primer (Promega, Madison, USA) and 1µl of nuclease free water in microcentrifuge tube, then kept at 70°C for 5 mins and then chill for 5 mins. After that 4µl of 5X reaction buffer (Promega, Madison, USA), 2µl of MgCl<sub>2</sub>, 1µl of PCR Nucleotide Mix (Promega, Madison, USA), 0.5µl of Ribonuclease Inhibitor (Promega, Madison, USA), 1µl of Reverse Transcriptase (Promega, Madison, USA), 6.5µl of nuclease free water were mixed with the same microcentrifuge tube. Then the solution was heated at 25°C for 5 mins, 42°C for 60 min and 70°C for 15 mins. Norovirus and rotavirus were detected by PCR analysis of cDNA with specific primers previously published<sup>8–9</sup>. The amplification was carried out in a thermal cycler (2720 Thermal Cycler, Applied Biosystems, USA). The PCR was performed at 94°C for 3 mins followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 60 s and a final extension at 72°C and then held at 4°C. The PCR products were electrophoresed in a 1.5% agarose gel, followed by staining with ethidium bromide (0.5 g/ml) for 20 min and then visualized under ultraviolet (UV) light. The bands were recorded by photography.

To evaluate the sensitivity and specificity of this IP-Noro/Rota test kits, all the 100 samples were tested for norovirus and rotavirus antigens by this kit following manufacturer's instructions (IP-Noro/Rota; ImmunoProbe Co., Ltd., Saitama, Japan). It took only 10–15 min to obtain the result. A positive result for both pathogens is two lines; the left control line (C) and the right test-positive line (T), whereas, a negative result consisted of a single left control line (C) (Figure 1).

### Analysis

The sensitivity and specificity of IP-Rota/Noro test kit were calculated mathematically as described below:

Sensitivity for IC kit =  $\frac{\text{Both IC kit and RT-PCR Positive}}{\text{RT-PCR positive}} \times 100$



**Figure 1.** Detection method of the IP-Rota/Noro kit. The test is positive if two lines appear in the membrane (a). The test is negative when only one line appears in the control area (b).

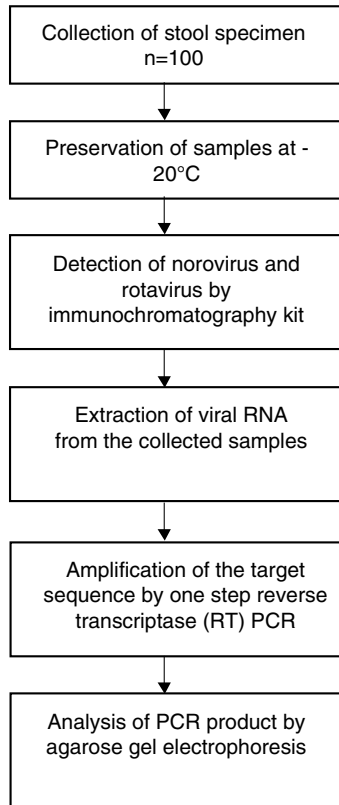
Specificity for IC kit = Both IC kit and RT-PCR negative × 100 / RT-PCR negative

**Results**

The working plan for evaluation of sensitivity and specificity of immunochromatography methods for rapid detection of rotavirus and norovirus associated with paediatric diarrhoea in Bangladesh is described in Figure 2.

By the RT-PCR method, 10 and 74 samples were confirmed as norovirus and rotavirus, respectively. It was found that all the isolated norovirus belongs to the genogroup II (data not shown). On the other hand, G1P<sup>8</sup> rotavirus strain was found the most prevalent among the Bangladeshi pediatric population after characterization of G-types (VP-7) and P-types (VP-4) of rotavirus-positive samples. The youngest patient was 21 days and the oldest 56 months; the average age was 14 months. The most common clinical symptoms of rotavirus and norovirus infected patients were dehydration, vomiting, fever and abdominal pain.

Of the 10 and 74 samples positive for norovirus and rotavirus, respectively, by RT-PCR, IP- Noro/Rota kit recognized all positive samples with 100% sensitivity. However, the kit gave one false positives for norovirus and three false positives for rotavirus detection, resulting in a specificity of 98.9% and 96.1%, respectively (Table 1 and Table 2).



**Figure 2.** Methods for rapid detection of rotavirus and norovirus associated with paediatric diarrhoea in Bangladesh.

**Table 1.** Comparison of norovirus detection in stool samples by IP-Noro/Rota kit and RT-PCR method (n=100).

Test Result	RT- PCR		Total	Sensitivity	Specificity
	+	-			
<b>IP-Noro/Rota kit:</b>					
+	10	1	9	100%	98.9%
-	0	91	91		
<b>Total</b>	10	90	100		

**Table 2.** Comparison of rotavirus detection in stool samples by IP-Rota/Noro kit and RT-PCR method (n=100).

Test Result	RT- PCR		Total	Sensitivity	Specificity
	+	-			
<b>IP-Rota/Noro kit:</b>					
+	74	3	71	100%	96.1%
-	0	29	29		
<b>Total</b>	74	26	100		

**Conclusions**

The clinical symptoms of the patients with acute gastroenteritis are generally not indicative of a specific pathogen. In Bangladesh, the outbreak of norovirus and rotavirus diarrhea occurs mainly in the winter season<sup>3</sup>, when the IC kits could be used for rapid screening, as other existing diagnosis methods are time consuming. The rapid IC kit test is easy to perform at a low cost and it takes only 10–15 min to diagnose with a simple procedure and does not require special equipment or a skilled technician.

Our findings clearly indicate that rotavirus and norovirus are the most important enteropathogen responsible for acute viral gastroenteritis among infants and children in Bangladesh, where 74% of the cases were caused by rotavirus only. The IC kit provides a high specificity and sensitivity as well as good agreement with the reference method, RT-PCR, for the detection of rotavirus and norovirus. Therefore, IC-Noro/Rota kit will be easy and useful assay for the rapid detection of these viruses in routine diagnosis as well as during the outbreaks. This is the first report about the rapid detection of rotavirus by IC kits in Bangladesh. Finally, it is strongly recommended to use the IC kit as an alternative method for rapid diagnosis of norovirus and rotavirus infections, especially in developing countries like Bangladesh.

**Data availability**

**Underlying data**

Figshare: Raw Data- IC kit.csv, <https://doi.org/10.6084/m9.figshare.7616630.v1><sup>10</sup>.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

## Grant information

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## Acknowledgements

We thank the ImmunoProbe Co., Ltd. (Saitama, Japan) for kindly providing the IP-Rota/Noro kit.

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<http://www.doi.org/10.6084/m9.figshare.7616630.v1>

# Open Peer Review

Current Peer Review Status: ? ?

**Version 1**

Reviewer Report 26 March 2019

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? **Nicola Page**

National Institute for Communicable Diseases (NICD), Johannesburg, South Africa

The paper reports the performance of an immunochromatography cartridge for the rapid detection of norovirus and rotavirus against an RT-PCR reference method. The specimens selected (n=100) were collected between January and June 2015 in Bangladesh and had been frozen prior to use. The authors also calculated the sensitivity and specificity of the kit and made recommendations regarding the potential use. However, the paper has a few problems:

1. The calculations in Table 1 and 2 are incorrect. The sum of the rows and columns do not total 100. Consider correcting the tables as indicated below.

Table 1. Comparison of norovirus detection in stool samples by IP-Noro/Rota kit and RT-PCR method (n=100)

Test result	RT-PCR		Total
	Positive	Negative	
IP-Noro/Rota kit			
Positive	10	1	11
Negative	0	89	89
Total	10	90	100

Table 2. Comparison of rotavirus detection in stool samples by IP-Noro/Rota kit and RT-PCR method (n=100)

Test result	RT-PCR		Total
	Positive	Negative	
IP-Noro/Rota kit			
Positive	74	3	77
Negative	0	23	23
Total	74	26	100

The calculations for specificity are, therefore, incorrect. This should be correct in the next version of the paper.

2. The authors briefly mention the broad typing results for norovirus (genogroup II) and rotavirus (G1P[8])

but fail to give any indication of the methods used to obtain these results. It makes it difficult for the reviewers and readers to assess the results presented. In addition, no detailed genotyping results were presented for the viral strains detected. Most current papers evaluating diagnostic assays for enteric viruses include genotyping data for the virus strains detected - see references below.

3. The authors failed to report the manufacturers' sensitivity and specificity for the kit or to compare their results to other studies that evaluated the same or similar kits – see articles below. Alternatively, if this is a new formulation of the kit being evaluated the authors should indicate this fact.

4. The authors utilized stool specimens that were frozen for an unspecified period (but approximately three years if the study was completed in 2018). The prolonged storage or repeated freeze/thawing could have contributed to a degree of degradation of the viruses in the stool material and should have been listed as a potential limitation of the study. However, the screening of the frozen stool specimens is a previously utilized approach for evaluating diagnostic kits.

5. If the kit is introduced according to the author's recommendations, it is more likely that fresh stool specimens will be screened. Therefore, the authors should consider evaluating the kit using fresh stool specimens from routine surveillance or during an outbreak which would mimic real world use and provide better estimates of sensitivity and specificity.

6. Based on the detection rate of rotavirus in the stool specimens selected for the evaluation (74%), these specimens were collected during the rotavirus season in Bangladesh. While the results indicate that the kit would be adequate for use during the rotavirus season (barring a few false positive results), it does not give any indication of the kits performance when the rotavirus prevalence declines, outside of the rotavirus season.

#### Additional comments:

- The term “developing country” is imprecise and inaccurate with no standard definition of what constitutes a developing country. The World Bank has defined standards for classifying countries so a better definition or term is a low-income country. Please correct in paragraphs 1 and 2 of the Introduction and in the last paragraph of the conclusions.
- Please check your nomenclature for rotavirus genotypes – G1P[8] and not G1P<sup>8</sup>.
- The statement “*Our findings clearly indicate that rotavirus and norovirus are the most important enteropathogen responsible for acute viral gastroenteritis among infants and children in Bangladesh, where 74% of the cases were caused by rotavirus only*” is not valid. The authors only screened for rotavirus and norovirus. Other enteric viruses may also be important in gastroenteritis in children in Bangladesh but because the authors failed to screen for these pathogens, these observations would have been missed and, therefore, conclusions regarding viral enteropathogen importance are incorrect.

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**Is the work clearly and accurately presented and does it cite the current literature?**

Partly

**Is the study design appropriate and is the work technically sound?**

Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**

Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**

No

**Are all the source data underlying the results available to ensure full reproducibility?**

Partly

**Are the conclusions drawn adequately supported by the results?**

Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Viruses associated with diarrhoeal diseases, rotavirus vaccines, molecular characterization and phylogenetics of enteric viruses, epidemiology and public health

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Reviewer Report 20 February 2019

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**Mustafizur Rahman**

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In this manuscript, the authors evaluate a newly developed immunochromatography (IC) kit for detecting norovirus and rotavirus by comparing the results with Reverse transcription PCR method as gold standard. They conducted comprehensive analysis of the sensitivity and specificity of the IC kit and concluded that the kit could be performed for rapid detection of norovirus and rotavirus with low cost and recommended the kits for routine use in low-income countries like Bangladesh.

Major comments:



**1. Lack of information about sample quality:** Authors claimed that the samples were collected from January to June 2015 and stored in  $-20^{\circ}\text{C}$ , but did not mention the duration of storage in the freezer before testing, which could affect the evaluation. The reviewer suggests to use freshly collected samples for this type of comprehensive analysis.

**2. Selection of inappropriate gold-standard for comparison:** Authors selected Reverse transcription PCR (RT-PCR) for comparison. This PCR depends on primers which were designed more than 15 years ago (published in 2003); however, according to recent data and considering high diversity of the viruses, these primers may fail to detect the viruses correctly in many samples. Thus, this assay cannot be used as gold standard. Using more reliable and latest detection methods such as real time RT-PCR can be used as a gold standard.

**3. Miscalculation:** Analysis for sensitivity and specificity was incorrectly performed. For example, specificity of the kit for rotavirus detection (in Table 2) should be 89.6%.

**4. Concluding remarks:** The statement “*Our findings clearly indicate that rotavirus and norovirus are the most important enteropathogen responsible for acute viral gastroenteritis among infants and children in Bangladesh, where 74% of the cases were caused by rotavirus only.*” cannot be made from the data presented in this paper. This study was particularly designed to evaluate a kit; not for describing rotavirus and norovirus prevalence.

The authors strongly recommended to use the IC kit as an alternative method for rapid and low-cost diagnosis without the cost analysis by comparing with other available detection kits. Mentioning the unit cost for this IC test could be helpful to support this statement.

One of the major limitations of this study is the lack of information on the capability of the IC kit to detect different genotypes of viruses by this IC kit. All norovirus strains detected in this study belonged to norovirus genogroup II and other norovirus genogroups were ignored. For example, in Bangladesh, about 15-30% of noroviruses belong to genogroups other than genogroup II<sup>1,2</sup>. Similarly, the author did not mention genetic variation of rotaviruses detected in this study except G1P<sup>3</sup>. A well-designed study by including different genotypes with appropriate sample size is recommended for this type of kit evaluation.

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**Is the work clearly and accurately presented and does it cite the current literature?**

Partly

**Is the study design appropriate and is the work technically sound?**

Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**

Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**

Partly

**Are all the source data underlying the results available to ensure full reproducibility?**

Partly

**Are the conclusions drawn adequately supported by the results?**

Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Virology; Infectious diseases; Rotavirus vaccine; norovirus epidemiology; viral hepatitis, HIV

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

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