# Comparison of rotavirus detection from rectal swab and feces in patients with diarrhea symptoms

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**Abstrak**

***Latar belakang:*** *Rotavirus adalah agen etiologi yang paling umum sebagai penyebab diare berat pada bayi dan anak-anak di seluruh dunia. Secara global, setiap tahun terjadi 600.000 kematian pada anak-anak kurang dari 5 tahun terkait dengan infeksi rotavirus. Spesimen yang digunakan untuk mendeteksi Rotavirus biasanya feses, tetapi untuk mendapatkan spesimen feses lebih sulit dibandingkan usap dubur. Untuk itu perlu adanya perbandingan hasil identifikasi rotavirus dari usap dubur dengan feses yang diuji dengan metode RT-PCR.*

***Metode: Melakukan identifikasi rotavirus yang menyebabkan diare dengan menggunakan spesimen usap*** *dubur dan feses yang diambil dari anak balita dan diperiksa dengan metode RT-PCR. Data dianalisis dengan uji sensitifitas dan spesifisitas.*

***Hasil:*** *Hasil RT-PCR rotavirus dari 189 pasangan spesimen didapat 24 adalah negatif pada kedua jenis spesimen dan 112 adalah positif pada kedua jenis spesimen, 42 pasangan positif pada spesimen tinja saja dan 11 pasangan positif pada usap dubur saja. Hasil sensitivitas sampel usap dubur adalah 72,7% dan spesifisitasnya 68,6 %. Nilai penduga positif usap dubur sebesar 91,1% sedangkan nilai penduga negatif sebesar 36,3%.*

***Kesimpulan:*** *Hasil pemeriksaan sensitifitas dan spesifisitas identifikasi rotavirus dari usap dubur masih memadai dibandingkan dengan specimen feses.* ***(Health Science Indones 2012;2:xx-xx)***

***Kata kunci:*** *RT-PCR, rotavirus, rectal swab, feces, diarrhea symptoms*

**Abstract**

**Background:** Rotavirus is the most common etiologic agent causes severe diarrhea in infants and children worldwide. Globally, every year 600,000 deaths in children less than 5 years associated with rotavirus infection. Commonly used to detect rotavirus stool samples, but getting stool samples were more difficult than rectal swab. The purpose of this study was to compare the results of the use of specimen of rectal swabs and feces to detect Rotavirus by RT-PCR method.

M**ethods:** To evaluate rotavirus that cause diarrhea we used rectal swabs and stool samples taken from infants and identified by RT-PCR method. Data were analyzed with sensitivity and specificity analysis tests.

**Results:** A number 189 specimen pairs were included of which 24 were negative in both specimen types and 112 were positive in both specimen types. Forty four 42 pairs was positive in the stool specimen only and 11 pairs was positive in the rectal swab specimen only. Sensitivity of rectal swab specimen was 72.7% and specificity was 68.6%. Rectal swab positive predictive value of 91.1%, while a negative predictive value of 36.3%.

**Conclusion:** The result of the sensitivity and specificity of rectal swab specimen was adequate compared with the feces specimen. ***(Health Science Indones 2012;2:xx-xx)***

**Key words:** RT-PCR, rotavirus, rectal swabs, stool, diarrhea symptoms

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Diarrhea remains a critical global issue with the high morbidity and mortality, especially in developing countries, and as one of the main causes of morbidity and mortality in the world. In general, it is estimated more than 10 million children under five die each year and about 20% died of diarrhea infection.[1]

Based on data from the Integrated Disease Surveillance report in public health centers and hospitals from Directorate General of Communicable Disease and Environmental Health 2006, the overall incidence of diarrhea during the period of five years from 2002 to 2006 tended to increase from 6.7 per 1000 population in 2002 to 9.6 per 1000 population in 2006 (incidence rates varying between 4.5 to 25.7 per 1000 population). It was also reported that diarrhea diseases were the highest cause of death (9.4%) of all infant deaths.[2]

Terms of the etiology, diarrhea can be caused by a variety of causes, namely microbial infection, intoxication, malabsorption, allergic. Acute diarrhea can be caused by infection of viruses, bacteria and parasites. Until 1970, the bacterial infection is considered as the biggest cause of diarrhea in Indonesia. However, several studies have shown that bacteria are not a major cause of diarrhea in children. Even in studies in 2005-2006 at the Hospital of type A in Yogyakarta found that bacteria cause only 5% of diarrhea cases.[3] Viral pathogen is the main cause of gastroenteritis in developing countries, one of which is rotavirus. Rotavirus may cause severe diarrhea.[4]

Rotavirus can not be accurately diagnosed by the clinical symptoms and signs due to rotavirus infection are clinically indistinguishable from other causes. A number of laboratory methods can be used to confirm the diagnosis of rotavirus. Laboratory diagnosis depends on the presence of virus in the feces collected early and the antibody titer rise. Virus found in the stool can be determined by techniques Immune electron microscopy (IEM), latex agglutination tests or enzyme-linked immunosorbent assays (ELISA). Detection of rotavirus nucleic acid from stool specimens by the reaction of polymerase chain reaction (PCR) is the most sensitive method of detection and can also be used to determine the Rotavirus serotypes.[5,6]

The purpose of this study was to compare the results of the use of specimen feces and rectal swabs to detect Rotavirus by RT-PCR method.

## METHODS

The study was conducted at the Virology Laboratory, Center for Biomedical and Basic Technology of Health, National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia, Jakarta. The study was conducted in February-April 2011. The number of specimens feces and rectal swabs were collected in this study amounted to 189. These specimens were obtained from diarrhea patients in Tanjung Karang public health center and Mataram hospital in West Nusa Tenggara province in June 2009 until March 2010.

Stool specimens and rectal swabs were taken from under five years diarrhea patients who come to health facilities (health center/hospital) performed by local medical personnel. Approximately 10 grams of feces was collected in a covered container receptacle screw and waterproof. For rectal swabs, swab inserted into a tube containing PBS (Phosphate Buffer Saline) with antibiotics penicillin-streptomycin 1%. Feces and rectal swab specimens stored in a laboratory. Rectal swab taken by inserting Dacron swab into the rectum of patients and perform rectal swab on the area (± 2-3 cm above the anal canal).

Pathogens cause gastroenteritis can be isolated from the rectal swab. Germs are found from rectal swabs are also present in the digestive tract.[7,8] Specimens were sent to the Virology laboratory Center for Biomedical and Basic Technology of Health, NIHRD, Ministry of Health of Indonesia, Jakarta with cold chain packaging and were sent via services expedition. Once in the laboratory, fecal specimens and rectal swabs were stored in a deep freezer-70$^{o}$C.

Diagnosis for Rotavirus was done by Polymerase Chain Reaction (PCR) technique, which is an enzymatic method to multiply exponentially the specific nucleotide sequence in a way in vitro.[9,10] PCR method is a highly sensitive molecular technique. RNA Isolation used the Qiagen issued by the manufacturer. Into PCR tubes containing a mixture of reagents added 5µL RNA isolation results. Previously made the first program of RT-PCR on the thermal cycler engine, with the following programs: Pre incubation at 50ºC for 30 minutes followed by amplification at a temperature of 94ºC for 1 min followed temperature of 48ºC for 1 min and at a temperature of 68ºC for 3 minutes. The process is repeated 39 times for amplification. Final

extension was at a temperature of 68ºC for 10 min and followed 20ºC forever.

Identification results of RT-PCR performed by electrophoresis using 2% agarose gel electrophoresis. Results from gel electrophoresis viewed and photographed using gel documentation (GEL DOC). The positive results will show ribbon (band) in length 1062 base pair (bp).

To compare the results of the use of specimen of rectal swabs and feces to detect Rotavirus by RT-PCR method, we used sensitivity and specificity analysis tests.[11]

## RESULTS

Table 1 shows that proportion of true positive was high (81.5%), and the sensitivity and specificity may be considered high value (72.73% and 68.57% respectively). Compared with the feces specimen, the sensitivity and specificity of rectal swab specimen were adequate.

Table 1. Comparison of rotavirus detection from rectal swab and feces by RT-PCR

| | | RT-PCR feces | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| RT-CR rectal swabs | Positive | 112 | 11 | 123 |
| | Negative | 42 | 24 | 66 |
| | Total | 154 | 35 | 189 |
| Proportion of true positive | = 154/189 = 81.5% | | | |
| Sensitivity | = 112/154 = 72.7% | | | |
| Specificity | = 24/35 = 68.6% | | | |
| Predictive value of a positive test | = 112/123 = 91.1% | | | |
| Predictive value of a negative test | = 24/66 = 36.4% | | | |

## DISCUSSION

We have been examined for 189 paired fecal and rectal swabs specimens. After examination of RNA isolation RT-PCR, RT-PCR results obtained are qualitative results, in the form of positive or negative outcomes by looking at the presence or absence of bands (band) formed and the size was corresponds to the size of the target DNA base pairs.

The reading of the results of RT-PCR of 189 specimens can be seen in Table 1. Table 1 shows the results of RT-PCR rotavirus positive from rectal swabs and feces was 112 specimens. Rectal swabs negative but feces positive were 42 specimens. Rectal swab positive but feces negative was 11 specimens, this may happen because the process of sending specimens despite use the cold chain is likely to damage the specimen is still there, on the rectal swab specimens using PBS (Phosphate buffered saline) as a transport medium, the possibility can still be reduced, but the stool specimen transport media without using a greater possibility of damage, so the damage to the viral RNA in stool specimens will be greater than rectal swab specimens. The second possibility the amount of virus in the specimen was limited.

To test the validity of rectal swab, we tested the sensitivity and specificity of the rectal swab specimens. As the gold standard was used specimen from feces, as the feces will give more positive results.

Table 1 shows that proportion of true positive was high (81.5%), the sensitivity results obtained rectal swab samples was considered high (72.7%) with the specificity 68.6%. this result means capable of detecting rectal swab positive at 72.7% with feces and detect negative for 68.6% each with feces. Rectal swab positive predictive value was 91.1%, while a negative value estimates was 36.4%.

Based on these results, the value of a positive predictor of rectal swab which is the chance that someone who uses rectal swab specimens classified as positive will actually positive categorized by using fecal specimens. This opportunity is at 91.1%. These results should be compared with the value of the negative estimate of rectal swab specimens are chances that a person with a negative rectal swab specimens actually negative categorized by using fecal specimens, the odds are 36.3%. From the results of the rectal swab specimen positive predictor gives good results, but less good negative predictor. so the use of a rectal swab specimens for diagnosis of rotavirus by RT-PCR method is less accurate, because of the negative results of the rectal swab specimens is not necessarily a negative when used feces specimens.

Gustavsson et al. found in his clinical research that the sensitivity for both types of samples is 97.5%, so the rectal swab as reliable as the sample for PCR-based diagnostic to viral gastroenteritis.[12] Where as our study revealed the sensitivity of rectal swab samples

was 72.7%, the situation was likely to occur due in our field study to the less appropriate of taking rectal swabs, so that was less present in the swab samples.

In conclusion, the rotavirus detection by RT-PCR method from rectal swab may be able to replace feces specimens.

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