Measles virus detection in urine specimens using virus culture and reverse transcriptase polymerase chain reaction (RT-PCR)

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Abstract. Measles is a highly prevalent infectious disease in Indonesia, and these large number of cases need to be confirmed in a laboratory so that precautions can be taken quickly and accurately. This study used urine specimens for laboratory confirmation of measles using viral culture and reverse transcriptase polymerase chain reaction (RT-PCR). Viral culture was performed using vero cells/hSLAM and its cytopathic effect was quantified, and RT-PCR was used to amplify the N gene fragment using measles virus forward primer (MeV216) and measles virus reverse primer (MeV214). We tested 120 urine specimens obtained from nine different provinces in Indonesia in 2016. Virus culture yielded a positivity value of 7 %, whereas RT-PCR positivity was 36 %. These results imply that the RT-PCR method is more sensitive for detecting measles virus compared with viral culture.

Keywords: Cytopathic effect, culture, measles, RT-PCR, vero/hSLAM

1. Introduction

Measles is a contagious infectious disease with a large prevalence, and in Indonesia, data from the Ministry of Health for the period from 2000 to 2015 indicate that the number of measles cases has decreased from 12,867 cases per year to 8,185 cases [1].

The measles viruses belong to the family Paramyxoviridae and the genus Morbillivirus and are pleomorphic negative single-stranded RNA viruses that measure about 100–250 nm [2]. The measles virus envelope is composed of lipid bilayers that serve to protect the genes that code for the major structural proteins of the measles virus. There are six types of genes in the measles virus: H genes, F genes, M genes, N genes, P genes, and L genes [3]. The nucleocapsid (N) is typical and abundantly present [3, 4].

Measles is generally diagnosed on the basis of clinical symptoms alone, but this method is less accurate compared with laboratory detection because measles symptoms can resemble that of other diseases such as rubella and other similar cold-flu like disease. Therefore, molecular techniques are required for early detection by laboratory confirmation.
Selection of the best method depends on the type of specimen used for evaluation and several types of specimens can be used such as serum, those from the genital (urine) and respiratory tracts (nasopharyngeal swab, nasopharyngeal washes, nasopharyngeal aspirate, and oropharyngeal swab), and cerebrospinal fluid [5]. We chose to use urine specimens because they are more practical and sensitive [6]. Thus, the purpose of this study was to compare sample positivity between the reverse transcriptase polymerase chain reaction (RT-PCR)-based method and viral culture to identify a precise and accurate method for the early detection of measles in Indonesia.

2. Experimental method

2.1. Specimens

Urine samples from suspected measles cases in 2016 from different provinces in Indonesia.

2.2. Virus isolation

Vero/hSLAM cells were used for virus isolation, and 200 μL of original specimen was inoculated in the culture flask and then cells were maintained in the incubator at 37 °C. After inoculation, the cells were monitored daily using an inverted microscope to see the cytopathic effect from 24 hours until 21 days.

RT-PCR. Viral RNA was extracted from urine specimens using QIAamp viral RNA mini kit and stored at -80 °C until use.

RT-PCR: A 634 bp fragment at the C-terminal end of the nucleoprotein (N) gene was amplified using RT-PCR; the reaction used 5 μL of extracted RNA and primers MeV214 5'/g397 TAA CAA TGA TGG AGG GTA GG 3' and MeV216 5'/g397 TGG AGC TAT GCC ATG GGA GT 3', along with the Qiagen One-step RT-PCR kit. PCR products were separated by gel electrophoresis on 2 % agarose gels containing GelRed and were visualized under UV light.

3. Results and discussion

A total of 120 urine samples were tested by inoculation and RT-PCR and had been obtained from nine different provinces in Indonesia in 2016, namely, Banten (n = 36), North Borneo (n = 23), Jakarta (n = 17), Bangka Belitung (n = 14), Jambi (n = 11), Central Kalimantan (n = 6), South Sumatra (n = 5), and Lampung (n = 4) and Bengkulu (n = 4) (figure 1).

3.1. Culture

Culture positivity is closely related to duration of the rash. Our results show that highest percent positivity (16.67 %; 3/18) was observed in samples collected on day 3 after the onset of the rash, whereas the lowest percent (5.56 %; 1/18 ) was observed in samples obtained at 8–14 days after the onset of rash (table 1).

Thus, the optimal time for urine sampling appears to be between days 1 and 5 after the appearance of a rash. Samples obtained on or later than 5 days after the onset of a rash may lead to false negativity in measles virus detection [6]. Nonetheless, it is not possible to rule out measles virus detection until day 26 after rash development [7].

3.2. RT-PCR

In contrast, RT-PCR results showed that the measles virus can be detected even beyond 14 days after the onset of the rash. We found highest prevalence (61.11 %; 11/18 cases) in samples obtained with the time span of 8–14 days, whereas no samples were positive for measles when obtained on day 6. Further, RT-PCR could detect the measles virus in 27.27 % (3/11) of the samples that had been collected at day 14 or later after the onset of rash.
Figure 1. Distribution of urine specimens by province in 2016.

Table 1. Comparison between viral culture and RT-PCR positivity at the time of the rash.

| No | Time of rash (day) | Number of samples | Viral culture | RT-PCR |
|----|--------------------|--------------------|--------------|--------|
|    |                    |                    | Number of positive sample | Percentage (%) | Number of positive samples | Percentage (%) |
| 1  | 0                   | 12                 | 0             | 0.00   | 5                     | 41.67          |
| 2  | 1                   | 17                 | 2             | 11.76  | 5                     | 29.41          |
| 3  | 2                   | 22                 | 2             | 9.09   | 7                     | 31.82          |
| 4  | 3                   | 18                 | 3             | 16.67  | 5                     | 27.78          |
| 5  | 4                   | 7                  | 0             | 0.00   | 4                     | 57.14          |
| 6  | 5                   | 7                  | 0             | 0.00   | 2                     | 28.57          |
| 7  | 6                   | 5                  | 0             | 0.00   | 0                     | 0.00           |
| 8  | 7                   | 3                  | 0             | 0.00   | 1                     | 33.33          |
| 9  | 8–14                | 18                 | 1             | 5.56   | 11                    | 61.11          |
| 10 | > 14                | 11                 | 0             | 0.00   | 3                     | 27.27          |
|    | Total               | 120                | 8 positive samples | 43 positive samples |

Positive culture is also closely related to the time of specimen delivery, and our results show that higher percentage positivity values were seen in samples that were sent 1–4 days after specimen collection; samples arriving on or later than day 5 were not of sufficient quality, and positivity could be unambiguously determined. The highest percent positivity value of 45.45 % was seen in 4 day samples, followed by the third day (6.67 %; 1/15), second day (5.26 %; 1/19), and first day (3.85 %; 1/26) delivered samples (table 2). In other side, the RT-PCR has results a bit difference. The highest percentage positivity values were seen in the third day of delivery (73.33 %; 11/15) and still can be detected more than 14 day of delivery even though the results is small (12 %; 3/25). Those results obtained are accordance with the literature which states that the length of time between collection and specimen delivery time affects the positivity value of RT-PCR. Delivery should be done immediately until a maximum of 2 weeks after specimen is taken.
The following precautions need to be followed while collecting samples for laboratory analysis. Urine should be taken as soon as the patient is infected and up to a maximum of 24 h. Samples should not be left at room temperature for too long and should be stored at -81 °C to retain chemical structure and virus viability [8].

The highest positive results of culture viruses occurred in Bengkulu province (37.5 %; 3/8). Followed by the province of Central Kalimantan is (25 %; 2/8), and finally the provinces of Bangka Belitung, Jakarta and North Kalimantan are (12.5 %; 1/8). While the other four provinces did not show positive results. In other side, the RT-PCR has results a bit difference. The highest percentage positivity values were seen in North Kalimantan province (41.86; 18/23), followed by Bangka Belitung province (20.93; 9/14), followed by Jambi, Central Kalimantan, Banten, South Sumatera, and Lampung. Only Jakarta and Bengkulu which did not show the positive results (table 3). The high incidence of measles in certain areas of Indonesia is due to low level of health care facilities and infrastructure in the region and is also related to the duration of rash and specimen delivery.

| Table 2. Comparison between viral culture and RT-PCR positivity in the time of delivery. |
|---------------------------------------------------------------|
| No | Delivery time (day) | Number of samples | Viral culture | RT-PCR |
|----|---------------------|-------------------|--------------|--------|
| 1  | 0                   | 8                 | 0            | 2      |
| 2  | 1                   | 26                | 1            | 7      |
| 3  | 2                   | 19                | 1            | 13     |
| 4  | 3                   | 15                | 1            | 11     |
| 5  | 4                   | 11                | 5            | 4      |
| 6  | 5                   | 4                 | 0            | 2      |
| 7  | 7                   | 2                 | 0            | 1      |
| 8  | 8–14                | 10                | 1            | 0      |
| 9  | > 14                | 25                | 0            | 3      |
|    | Total               | 120               | 8 positive samples | 43 positive samples |

| Table 3. Comparison between viral culture and RT-PCR positivity in provinces in Indonesia. |
|---------------------------------------------------------------|
| No | Provinces | Number of samples | Viral culture | RT-PCR |
|----|-----------|-------------------|--------------|--------|
| 1  | Babel     | 14                | 1            | 9      |
| 2  | Banten    | 36                | 0            | 3      |
| 3  | Bengkulu  | 4                 | 3            | 0      |
| 4  | Jakarta   | 17                | 1            | 0      |
| 5  | Jambi     | 11                | 0            | 5      |
| 6  | Kalteng   | 6                 | 2            | 4      |
| 7  | Kaltara   | 23                | 1            | 18     |
| 8  | Lampung   | 4                 | 0            | 1      |
| 9  | Sumsel    | 5                 | 0            | 3      |
|    | Total     | 120               | 8            | 43     |

| Total | 120 | 8 | 6.67 | 43 | 35.83 |
4. Conclusion

Our results show that, compared with viral culture, RT-PCR-based detection yielded a higher percentage of positive samples, suggesting that the time of sampling should be maintained so that the results are more accurate. Besides, sequencing analysis is needed to determine the measles virus genotype.

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