A point-of-care test for measles diagnosis: detection of measles-specific IgM antibodies and viral nucleic acid

Lenesha Warrener, Rimantas Slibinskas, Kaw Bing Chua, Wondatir Nigatu, Kevin E Brown, Kestutis Sasnauskas, Dhanraj Samuel & David Brown

Objective To evaluate the performance of a newly developed point-of-care test (POCT) for the detection of measles-specific IgM antibodies in serum and oral fluid specimens and to assess if measles virus nucleic acid could be recovered from used POCT strips.

Methods The POCT was used to test 170 serum specimens collected through measles surveillance or vaccination programmes in Ethiopia, Malaysia and the Russian Federation: 69 were positive for measles immunoglobulin M (IgM) antibodies, 74 were positive for rubella IgM antibodies and 7 were positive for both. Also tested were 282 oral fluid specimens from the measles, mumps and rubella (MMR) surveillance programme of the United Kingdom of Great Britain and Northern Ireland. The Microimmune measles IgM capture enzyme immunoassay was the gold standard for comparison. A panel of 24 oral fluids was used to investigate if measles virus haemagglutinin (H) and nucleocapsid (N) genes could be amplified by polymerase chain reaction directly from used POCT strips.

Findings With serum POCT showed a sensitivity and specificity of 90.8% (69/76) and 93.6% (88/94), respectively; with oral fluids, sensitivity and specificity were 90.0% (63/70) and 96.2% (200/208), respectively. Both H and N genes were reliably detected in POCT strips and the N genes could be sequenced for genotyping. Measles virus genes could be recovered from POCT strips after storage for 5 weeks at 20–25°C.

Conclusion The POCT has the sensitivity and specificity required of a field-based test for measles diagnosis. However, its role in global measles control programmes requires further evaluation.

Introduction
Measles is a severe, vaccine-preventable disease that causes extensive morbidity and mortality in large parts of the world. Despite the widespread use of measles vaccine, either as a single antigen vaccine or as a component of the triple vaccine against measles, mumps and rubella (MMR), 278 358 reported cases of measles and an estimated 164 000 deaths from measles occurred worldwide in 2008. Vaccine coverage is highly variable between World Health Organization (WHO) global regions. Measles has been eliminated in the Americas but continues to be endemic in the African and South-East Asia regions, where vaccine coverage is less than 80%. These regions account for approximately 94% of all global measles deaths. Outbreaks continue to occur in other global regions, primarily as a result of measles virus importation into areas where vaccine coverage has fallen to a suboptimal level and a susceptible cohort has accumulated. Most regions have elimination goals and elimination strategies based on the maintenance of high vaccination coverage, for which political commitment is required. A key component of elimination plans is surveillance to monitor impact.

Laboratory confirmation of cases of measles is a vital aspect of surveillance at all stages of control programmes because clinical diagnosis is unreliable. The mainstay of laboratory confirmation is the detection of measles-specific immunoglobulin M (IgM) antibodies in serum samples. More recently, alternative samples such as dried blood spots and oral fluids have been used for diagnosis by antibody detection. Oral fluids can also be used to detect viral ribonucleic acid (RNA) and their use is becoming increasingly common because samples can be obtained safely and non-invasively, without the risks associated with blood collection, and it improves patient compliance with specimen collection, as the procedure is simple and painless.

Point-of-care tests (POCTs) are increasingly used for the rapid diagnosis of infections. They can be performed in a single incubation step at ambient temperature without complex electrical equipment and their results can be read visually. By increasing diagnostic capacity and facilitating rapid diagnosis in resource-poor countries, they have the potential to improve measles surveillance and the response of health authorities to possible outbreaks.

We have developed a POCT capable of detecting measles-specific IgM antibodies in both serum and oral fluid specimens. In this paper we describe the diagnostic performance of this POCT for each specimen type. We also investigated whether viral RNA could be amplified from the used test strips, as that would enhance their use in measles surveillance.

Methods

Test strips
The POCT strips for the detection of measles-specific IgM were constructed essentially as described for mumps-specific IgM POCT strips, except that affinity purified F(\(ab’)\)2 fragment goat anti-human IgM, 1.2mg/ml (Jackson Immunoresearch Labora-

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Abstracts in 中文, Français, Русский and Español at the end of each article.
oral fluid extraction diluent, prepared as assay (EIA) in 20 parts of a measles-IgM-meatls IgM on capture enzyme immuno-
part of a serum that tested positive for prepared. This was done by diluting one antibody test line signal in the POCT was
and purified by caesium chloride ultracen-
Pichia pastoris
Recombinant measles virus nucleoprotein
Serum protocol
America) was used as the test line capture
MB gold conjugate is localized before use; the nitrocellulose membrane
with an immobilized anti-human IgM antibody test
line (T) and an anti-mouse IgG antibody control line
(C) positioned approximately 14 mm and 18 mm
from the conjugate pad, respectively (not visible before testing); and a cotton linter paper wick. The five POCT strips
demonstrate from left to right the results of testing the cut-off control serum (CO), one negative (strip 1, enzyme immunoassay (EIA) T/
CO of 0.34) and three positive specimens of varying intensity (strips 2–4, EIA T/COs of 5.4, 1.7 and 5.5, respectively).
Serum protocol
Recombinant measles virus nucleoprotein
(rNP), expressed in yeast Pichia pastoris
and purified by caesium chlorideultracen-
trifugation,12 was conjugated to 40 nm gold colloid
(BBInternational Ltd, Cardiff, England)
to produce the measles-specific gold con-
jugate (MAB-gold conjugate). Assembled POCT strips are illustrated in Fig. 1.
Oral fluid protocol
The POCT protocol used for oral fluid testing was essentially the same as the one used to test sera, except that oral fluid specimens were tested undiluted, rNP was
used at 35 pg/ml and the test strips were incubated for 20 minutes at room
at a temperature. A cut-off control serum was not
required for interpreting the results since background signals were not observed
when testing oral fluid specimens. If the signal at the anti-human IgM test line ranged from pink to red in colour, the test
was interpreted as positive for measles-specific IgM; if no signal was visible at the test line, the test was interpreted as
negative for measles-specific IgM. For both serum and oral fluid testing, a signal had to be visible at the control line for the
test to be considered valid.
Test performance evaluation
The performance of the POCT for the detection of measles-specific IgM in both serum and oral fluid specimens was evalu-
ated by comparing the results with those obtained with the measles IgM capture
EIA (Microimmune Ltd, Hounslow, England). Two individuals interpreted
the POCT results independently at the specified incubation times. The results
were then classified as positive or negative for measles-specific IgM by consensus. When only one reader observed a weak
signal, the result was interpreted as negative. The EIA was performed and the re-
sults, expressed as a numerical value (quen-
tient) calculated by dividing the optical density obtained for each test specimen
(450/620 nm) by the cut-off value (T/
CO), were interpreted in accordance with
the manufacturer's instructions.
Serum specimens
A total of 170 serum specimens were evaluated; 100 of them had been col-
clected from suspected cases of measles in
Malaysia in 2004 (age range: 6 months to 42 years; mean: 12.4), when outbreaks
were reported. The specimens originated from four federal states within Malaysia:
83 from Selangor, 11 from Melaka, 5 from
Pahang and 1 from Perak.
Sixty-two serum specimens had been collected from children (age range:
6 months to 17 years; mean: 5.8) who
presented with a rash during an outbreak of
these children, 42 had been vaccinated
against measles. Specimens were collected
a mean of 7 days (range: 1–23) after the
onset of the rash.
Eight serum specimens from acute or
suspected cases of rubella in the Russian
Federation were kindly donated by the
World Health Organization's Regional
Office for Europe, in Denmark. All sera
were also tested by rubella IgM capture
EIA (Microimmune Ltd).
Oral fluid specimens
All oral fluids from suspected measles and rubella cases received by the Virus
Reference Department of the Health Pro-
tection Agency of the United Kingdom
of Great Britain and Northern Ireland
over a 10-day period in July 2008 were
used to evaluate the POCT. The speci-
mens had been collected as part of the
national MMR surveillance programme
using Oracol swabs (Malvern Medical
Developments Ltd, Worcester, England)
and extracted as described previously.14
Specimens included 232 oral fluids for
measles investigation, 11 for both measles
and rubella testing and 39 for rubella
investigation only (age range of all oral
fluids: 1 month to 59 years; mean: 5.9).
The 50 oral fluids submitted for rubella
investigation were tested by EIA for the
detection of measles IgM as well as by
Microimmune rubella IgM capture EIA.
Virus nucleic acid amplification
An additional 24 oral fluid specimens
received for measles surveillance testing
in September 2010 were used to evaluate
the extraction and amplification of viral
Reference strain sequences according to NV, Sint-Martens-Latem, Belgium) were sequenced in a Genetic Analyser (Applied Biosystems, Foster City, USA), analysed with Bionumerics software version 6.1 (Applied Maths, USA), and genotyped by comparison with NV sequences. The same strain of measles virus was included as a control in all experiments.

Genome from POCT strips after IgM detection. Following routine surveillance testing, the specimens were stored for up to 6 weeks at 2–8 °C before this evaluation.

To investigate the stability of measles virus nucleic acid on POCT strips, the oral fluids were tested in duplicate by POCT as described earlier, then dried and stored at 20–25 °C. Nucleic acid was extracted and complementary DNA (cDNA) was prepared as described previously, from an aliquot of each oral fluid on the day of point-of-care testing, from one set of dried POCT strips after overnight storage and from a second set of dried strips after 5 weeks of storage. Measles virus cDNA amplicons were generated by real-time polymerase chain reaction (PCR) for the haemagglutinin (H) gene and by nested PCR targeting the nucleocapsid (N) gene. Amplified N-gene products were sequenced in the EIA. They had been collected 1 and 5 days, respectively, after the onset of fever. Of 74 sera that tested positive for rubella-specific IgM by capture EIA and negative for rubella virus nucleic acid on POCT strips, 69 tested positive by POCT. Thus, with this set of samples the POCT had a specificity of 93.2%.

The overall performance of the POCT with all serum samples was as follows: sensitivity: 90.8% (95% CI: 83.4–97.01); negative predictive value: 92.0% (95% CI: 80.5–95.9); specificity: 96.2% (95% CI: 92.6–98.3); positive predictive value: 88.7% (95% CI: 79.0–95.0); negative predictive value: 96.6% (95% CI: 93.2–98.6).

The POCT data were further analysed as a function of T/CO on EIA and of the timing of specimen collection following the onset of symptoms (Table 3). Approximately 20% of the specimens were collected within 7 days of the onset of symptoms; 25% were collected between 7 and 14 days after onset and 31% were collected after the 14th day. The timing of specimen collection in relation to the onset of symptoms was not known for 24% of oral fluids. Seven out of the 70 oral fluids that tested positive by EIA gave discrepant POCT results. All such specimens had T/COs on EIA just above the positive cut-off value of 1.0 (range: 1.05–1.94). Collection dates were known for six of these specimens. Five of them had been collected more than 14 days after the onset of symptoms (range: 19–38). The sixth oral fluid had been collected three days after the onset of symptoms. However, this patient had also received a single dose of measles-virus containing vaccine 32 days before the specimen was collected.

Table 3 also suggests that the detection of IgM by POCT may depend on the quantity of specific IgM antibody present. All oral fluids in this panel with a T/CO of 5 or higher on IgM capture EIA were identified by POCT, regardless of the timing of specimen collection.

### Results

#### Serum

Concordant results were obtained for 157 (92.4%) of the 170 sera tested by measles-specific IgM capture EIA and POCT (Table 1). Of 76 sera that tested positive for measles-specific IgM in EIA, 69 also tested positive by POCT. This included 5 sera that also tested positive for rubella-specific IgM. Of the 94 sera that tested negative for measles-specific IgM by EIA, 88 were also negative by POCT, including 69 that had tested positive for rubella-specific IgM.

Two of the sera that tested positive for measles-specific IgM by capture EIA and negative by POCT had low T/COs (1.5 and 1.9) in the EIA. They had been collected 1 and 5 days, respectively, after the onset of fever. Of 74 sera that tested positive for rubella-specific IgM only, 69 tested negative by the POCT. Thus, with this set of samples the POCT had a specificity of 93.2%.

The overall performance of the POCT with all serum samples was as follows: sensitivity: 90.8% (95% confidence interval, CI: 81.94–96.22); specificity: 93.6% (95% CI: 86.62–97.62); positive predictive value: 92.0% (95% CI: 83.4–97.01); negative predictive value: 92.6% (95% CI: 85.41–96.99).

The performance of the POCT on oral fluids was evaluated using a total of 282 specimens received for measles and rubella investigations (Table 2). Of the 50 oral fluids submitted for rubella investigation, 48 tested negative for both measles- and rubella-specific IgM by EIA. One oral fluid tested positive for rubella-specific IgM and one other tested positive for measles-specific IgM by EIA.

Concordant POCT and EIA results were obtained for 263 of the 278 (94.6%) oral fluids after excluding the four specimens that gave equivocal results on the measles-specific IgM capture EIA. The overall performance of the POCT with all oral fluids was as follows: sensitivity: 90.0% (95% CI: 80.5–95.9); specificity: 96.2% (95% CI: 92.6–98.3); positive predictive value: 88.7% (95% CI: 79.0–95.0); negative predictive value: 96.6% (95% CI: 93.2–98.6).

The POCT data were further analysed as a function of T/CO on EIA and of the timing of specimen collection following the onset of symptoms (Table 3). Approximately 20% of the specimens were collected within 7 days of the onset of symptoms; 25% were collected between 7 and 14 days after onset and 31% were collected after the 14th day. The timing of specimen collection in relation to the onset of symptoms was not known for 24% of oral fluids. Seven out of the 70 oral fluids that tested positive by EIA gave discrepant POCT results. All such specimens had T/COs on EIA just above the positive cut-off value of 1.0 (range: 1.05–1.94). Collection dates were known for six of these specimens. Five of them had been collected more than 14 days after the onset of symptoms (range: 19–38). The sixth oral fluid had been collected three days after the onset of symptoms. However, this patient had also received a single dose of measles-virus containing vaccine 32 days before the specimen was collected.

Table 3 also suggests that the detection of IgM by POCT may depend on the quantity of specific IgM antibody present. All oral fluids in this panel with a T/CO of 5 or higher on IgM capture EIA were identified by POCT, regardless of the timing of specimen collection.

### Microimmune measles-specific IgM capture enzyme immunoassay (EIA)

<table>
<thead>
<tr>
<th>EIA result</th>
<th>POCT result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>Equivocal</td>
<td>Positive</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>200</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>208</td>
</tr>
</tbody>
</table>
Virus nucleic acid amplification

When measles H gene real-time PCR was performed on oral fluid aliquots and their respective POCT strips, concordant results were obtained in 23 of 24 cases (Table 4). A comparison of the threshold cycle (Ct) values obtained in the H-gene real-time PCR for oral fluids with those obtained for their matched POCT strips showed little difference between the two in the recovery of viral nucleic acid.

The H gene was detected in 16 oral fluids and in 15 of the 16 corresponding POCT strips. The measles virus N gene was amplified by nested PCR from 14 of these oral fluids and the corresponding POCT strips. The N gene was also amplified from the oral fluid aliquot and POCT strip of specimen 20, although the H gene had not been detected in either. Nucleic acid sequence analysis of the nested N-gene PCR amplicons identified viral genotype D4 in 14 of these oral fluids and viral genotype D9 in one. The genotypes and individual strains identified from the corresponding POCT strips were identical to those identified from the oral fluids (Table 4).

The remaining nine oral fluid aliquots were negative for the N gene by nested PCR. However, when nested PCR was performed on the corresponding POCT strips, N-gene amplicons were generated on five occasions. These amplicons were not derived from sequences in the specimens, but from the rNP that had been added during IgM detection, which contained trace levels of residual N-gene sequence that amplified sporadically in the absence of specimen-derived N-gene target. The fact that the contaminating N-gene sequence was not detected after one round of N-gene PCR confirms that it was present in minute quantities. The generated amplicons were confirmed to have the same Schwarz vaccine strain sequence that was used to synthesize the rNP antigen.

The measles virus N gene was amplified from oral fluid 23 and identified as a Schwarz vaccine strain during the routine surveillance testing performed before this study, but it was not detected in the oral fluid during this evaluation. Although the same vaccine strain was identified from the corresponding POCT strip, it was not possible to determine whether this originated from the oral fluid specimen or from the added rNP.

POCT results for this small panel of oral fluids were highly concordant with the results of measles-specific IgM EIA, as were the results obtained with the larger panel of oral fluids. Mesles-specific IgM was not detected by POCT in only one EIA-positive oral fluid, specimen 19 (Table 4). In this case, measles was confirmed by PCR.

Stability of measles nucleic acid on POCT strips

The measles virus H gene was amplified from 16 POCT strips stored at 20–25 °C for 5 weeks. This included all 15 specimens in which the H gene was amplified from the first POCT strip as well as specimen 20, in which the H gene was not detected initially. The measles virus N gene was amplified from 12 of these 16 POCT strips after 5 weeks of storage. The nucleotide sequences obtained from 11 of these stored strips were identical to those identified from the original POCT strips. Mesles viral genotype A, consistent with the nucleic acid sequence present in the rNP antigen, was identified from the stored POCT strip of specimen 20; viral genotype D4 had been previously identified in this specimen.

Discussion

Sensitive and specific tests for measles diagnosis that can be used in low-resource settings can greatly enhance surveillance. Field-based tests would facilitate the diagnosis of measles in patients with a rash illness accompanied by fever, as well as the rapid implementation of control measures. With these objectives in mind we developed a rapid POCT for the detection of measles-specific IgM based on the principle of immunochromatographic flow. In this study, the POCT showed good sensitivity, specificity and positive and negative predictive values (> 88%) and is therefore suitable for field use. However, the potential role of POCT in measles surveillance will remain unknown until wider field-based evaluations are conducted in a range of settings, including those where control activities are in the elimination phase.

An added advantage of the POCT, apart from its ability to detect measles-specific IgM, is that the test strip is an excellent matrix for capturing viral nucleic acid from oral fluid samples and preserving it for up to 5 weeks at ambient temperature. This not only makes it possible to diagnose measles cases immediately, but also permits subsequent molecular characterization of the virus at national laboratories to track transmission pathways and confirm the diagnostic accuracy of the POCT in the field.

Table 3. Results obtained for oral fluid specimens with point-of-care test (POCT) for the detection of measles-specific IgM, analysed with respect to the result quotient (T/CO) obtained in Microimmune measles-specific IgM capture enzyme immunoassay (EIA) and timing of specimen collection

<table>
<thead>
<tr>
<th>EIA Result</th>
<th>T/CO&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. positive by POCT out of total tested</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Days between onset of symptoms and specimen collection</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0–7</td>
<td>8–14</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt;0.8</td>
<td>2/42</td>
<td>2/51</td>
</tr>
<tr>
<td>Equivocal</td>
<td>0.8 to &lt; 1.0</td>
<td>0/0</td>
<td>0/1</td>
</tr>
<tr>
<td>Positive</td>
<td>1– 5</td>
<td>6/7</td>
<td>4/4</td>
</tr>
<tr>
<td></td>
<td>5–10</td>
<td>1/1</td>
<td>7/7</td>
</tr>
<tr>
<td></td>
<td>10–15</td>
<td>0</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>15–20</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>&gt;20</td>
<td>4/4</td>
<td>3/3</td>
</tr>
<tr>
<td>No. positive by POCT out of total tested</td>
<td>12/13</td>
<td>17/17</td>
<td>19/24</td>
</tr>
</tbody>
</table>

<sup>a</sup> This represents the quotient calculated by dividing the optical density obtained for each test specimen (450/620 nm) by the cut-off value.
The results obtained with the larger panel of oral fluids suggest that IgM detection by POCT improves as the concentration of IgM antibody increases, since all specimens with an EIA T/CO greater than 5 were identified (Table 3). However, one oral fluid specimen, number 19 in Table 4, was highly reactive in the measles-specific IgM capture EIA but was negative by POCT. That oral fluid was found in the oral fluid aliquots. The presence of trace levels of measles N-gene sequences from the rNP was identified and each was assigned a different letter in the table: a, b, c, d, e. Strains b, c and d were identified from the POCT strips used for oral fluid testing. POCT strips used for oral fluid testing.

N-gene sequences from the rNP is not likely to seriously impair the identification of current wild-type measles strains, as genotype A viruses have not circulated widely in the past 20 years and the few sporadic cases of infection with these viruses have been frequently associated with recent vaccination.20

### Table 4. Results of polymerase chain reaction (PCR) and IgM antibody testing obtained for 24 oral fluid (OF) specimens used to compare viral nucleic acid recovery from OF aliquots and from used point-of-care test (POCT) strips

<table>
<thead>
<tr>
<th>SN</th>
<th>OF aliquot</th>
<th>Real-time H-gene PCR result and viral strain</th>
<th>IgM antibody</th>
<th>SN</th>
<th>Real-time H-gene PCR result and viral strain</th>
<th>IgM antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H (Ct)</td>
<td>Strain</td>
<td>POCT strip</td>
<td>H (Ct)</td>
<td>Strain</td>
<td>Surveillance</td>
</tr>
<tr>
<td>1</td>
<td>36.43</td>
<td>D4-a</td>
<td>35.88</td>
<td>D4-a</td>
<td>34.58</td>
<td>D4-a</td>
</tr>
<tr>
<td>2</td>
<td>30.72</td>
<td>D4-b</td>
<td>29.48</td>
<td>D4-b</td>
<td>30.37</td>
<td>D4-b</td>
</tr>
<tr>
<td>3</td>
<td>36.64</td>
<td>ND</td>
<td>A</td>
<td>ND</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td>ND</td>
<td>A</td>
<td>ND</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>5</td>
<td>25.16</td>
<td>D4-c</td>
<td>27.62</td>
<td>D4-c</td>
<td>21.07</td>
<td>D4-c</td>
</tr>
<tr>
<td>6</td>
<td>30.69</td>
<td>D9</td>
<td>32.24</td>
<td>D9</td>
<td>30.08</td>
<td>D9</td>
</tr>
<tr>
<td>7</td>
<td>34.89</td>
<td>D4-c</td>
<td>35.01</td>
<td>D4-c</td>
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<td>D4-c</td>
</tr>
<tr>
<td>8</td>
<td>34.23</td>
<td>D4-c</td>
<td>35.12</td>
<td>D4-c</td>
<td>30.29</td>
<td>D4-c</td>
</tr>
<tr>
<td>9</td>
<td>ND</td>
<td>ND</td>
<td>A</td>
<td>ND</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>10</td>
<td>33.20</td>
<td>D4-c</td>
<td>33.28</td>
<td>D4-c</td>
<td>30.34</td>
<td>D4-c</td>
</tr>
<tr>
<td>11</td>
<td>32.40</td>
<td>D4-d</td>
<td>33.53</td>
<td>D4-d</td>
<td>30.29</td>
<td>D4-d</td>
</tr>
<tr>
<td>12</td>
<td>26.85</td>
<td>D4-d</td>
<td>27.91</td>
<td>D4-d</td>
<td>23.21</td>
<td>D4-d</td>
</tr>
<tr>
<td>13</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>14</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>15</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
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</tr>
<tr>
<td>16</td>
<td>30.91</td>
<td>D4-c</td>
<td>28.26</td>
<td>D4-c</td>
<td>23.09</td>
<td>D4-c</td>
</tr>
<tr>
<td>17</td>
<td>31.88</td>
<td>D4-c</td>
<td>31.76</td>
<td>D4-c</td>
<td>30.02</td>
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</tr>
<tr>
<td>18</td>
<td>ND</td>
<td>ND</td>
<td>A</td>
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<td>NT</td>
</tr>
<tr>
<td>19</td>
<td>32.51</td>
<td>D4-b</td>
<td>33.32</td>
<td>D4-b</td>
<td>30.82</td>
<td>D4-b</td>
</tr>
<tr>
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<td>ND</td>
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<td>D4-c</td>
<td>37.50</td>
<td>D4-c</td>
</tr>
<tr>
<td>21</td>
<td>31.23</td>
<td>D4-c</td>
<td>31.86</td>
<td>D4-c</td>
<td>32.34</td>
<td>D4-c</td>
</tr>
<tr>
<td>22</td>
<td>34.07</td>
<td>D4-e</td>
<td>33.99</td>
<td>D4-e</td>
<td>33.16</td>
<td>D4-e</td>
</tr>
<tr>
<td>23</td>
<td>37.32</td>
<td>–</td>
<td>34.34</td>
<td>A</td>
<td>37.41</td>
<td>Vaccine</td>
</tr>
<tr>
<td>24</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

Ct: threshold cycle number; Eqv, equivocal; H, haemagglutinin; ND, not detected; Neg, negative; NT, not tested; Pos, positive; SN, specimen number.

* This represents the quotient calculated by dividing the optical density obtained for each test specimen (450/620 nm) by the cut-off value.

Note: The Ct obtained for the OF aliquots submitted for this evaluation, from POCT strips used in IgM antibody detection and from each oral fluid during routine surveillance testing, are listed. During routine surveillance testing, nested N-gene PCR and nucleic acid sequence analysis were performed on oral fluids in which the H gene had been detected by real-time PCR. For this evaluation, real-time H-gene PCR, nested nucleocapsid-gene (N-gene) PCR and sequence analysis were performed on all 24 OF aliquots and their corresponding POCT strips. The measles virus strain designations listed are based on sequence analysis of nested PCR N-gene amplicons. Five different D4 measles virus strains were identified and each was assigned a different letter in the table: a, b, c, d, e.
In its present configuration the POCT relies on some basic laboratory equipment. A robust field-based test would require further development of methods for extracting oral fluids from swabs without centrifugation, the use of disposable, volumetric pipettes or loops to deliver accurate volumes, and a plastic cassette to enclose the test strip and reduce the handling of the POCT’s analytical membrane. The estimated cost of manufacturing one POCT is approximately one United States dollar.

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Rезюме

Тест у постели больного для диагностики кори: выявление специфических IgM антител к вирусу кори и вирусной нукleinовой кислоты

Цель

Проверить эффективность нового теста у постели больного (point-of-care test, POCT) для выявления специфических IgM антител к вирусу кори в образцах сыворотки крови и жидкости полости рта, и оценить возможность восстановления вирусной нукleinовой кислоты вируса кори с использованием тест-полосок POCT.

Методы

Тест POCT был использован для тестирования 170 образцов сыворотки, собранных в ходе эпиднадзора за корью и осуществления программ вакцинации в Малайзии, Российской Федерации и Эфиопии: 69 из них имели положительную реакцию на антитела иммуноглобулина M (IgM) к вирусу кори, 74 – положительную реакцию на антитела IgM к вирусу краснухи и семь – положительную реакцию на антитела к обоим вирусам. Кроме того, были протестированы 282 образца ротовой жидкости, собранные в рамках программы эпиднадзора за корь, свиньей и краснухой (MMR) в Соединенном Королевстве Великобритании и Северной Ирландии. «Золотым стандартом» для сравнения образцов была тест-система Microimmune measles IgM capture enzyme immunoassay.

Результаты

Для сыворотки чувствительность и специфичность теста POCT составили, соответственно, 90,8% (69/76) и 93,6% (88/94), а для жидкости ротовой полости – соответственно, 90,0 (63/70) и 96,2% (200/208). Как H гены, так и N гены были достоверно выявлены на тест-полосках POCT, причем N гены можно было секвенировать для генотипирования. Гены к вирусу кори можно было восстановить с тест-полосок POCT после их хранения в течение пяти недель при температуре 20–25 ºС.

Вывод

Тест POCT обладает необходимыми чувствительностью и специфичностью для проведения теста на диагностирование кори в полевых условиях. Вместе с тем, его роль в глобальных программах борьбы против кори требует дальнейшей оценки.


