What assay is optimal for the diagnosis of measles virus infection? An evaluation of the performance of a measles virus real-time reverse transcriptase PCR using the Cepheid SmartCycler® and antigen detection by immunofluorescence

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Article history:
Received 30 April 2015
Received in revised form 1 July 2015
Accepted 5 July 2015

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Abstract

Background: Despite the World Health Organization (WHO)-reported elimination of measles in Australia, importation of cases especially in travellers from Asia continues in Sydney, Australia’s largest city. Laboratory confirmation supports clinico-epidemiological evidence of measles virus infection, and is needed to establish elimination.

Objectives: To evaluate the performance of a random access real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay using the moderate complexity SmartCycler® platform, and measles antigen detection by immunofluorescence (IFA), for the detection of measles virus in patient samples.

Study design: One hundred samples comprising nose and throat swabs, nasopharyngeal aspirates and urine, collected from patients with suspected measles were tested in parallel using IFA and nucleic acid testing using the SmartCycler® and LightCycler® RT-PCR platforms. The LightCycler® RT-PCR was used as the reference assay against which the SmartCycler® RT-PCR and IFA were compared.

Results: Using the LightCycler® RT-PCR, measles virus was detected in 35 clinical samples. There was 100% concordance between the results of the SmartCycler® and the LightCycler®-based RT-PCR. Measles genotypes detected included B3, D8, and D9. Testing urine in addition to NTS did not improve diagnostic yield. In contrast, the sensitivity and specificity of IFA compared to the reference LightCycler® RT-PCR was 34.3% and 96.7%, respectively.

Conclusion: The performance of the SmartCycler® is comparable to the LightCycler® for the detection of measles virus. However, IFA had poor sensitivity and should not be used to confirm measles virus infection where nucleic acid testing is available.

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1. Background

In March 2014, the World Health Organization (WHO) announced that measles elimination had been achieved in Australia [22]. However, the importation of measles by foreign visitors or returned travelers from areas of high prevalence continues to result in multiple localized outbreaks [3,11,18,19]. Laboratory confirmation is essential to accurately monitor the epidemiology of measles and to implement effective control strategies. Detection of measles virus-specific IgM antibody on acute-phase sera is the most widely available test, but is not without limitations. Testing sera collected <72 h after rash onset has a false negative rate of 33% [7]. Measles virus-specific IgM testing in a highly vaccinated low prevalence population has a 4% false positive rate; cross-reactivity

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http://dx.doi.org/10.1016/j.jcv.2015.07.004
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from other viruses including human parvovirus, rubella and human herpesvirus 6 has been described [1,21]. The IgM response in measles vaccine failures may also be absent [8].

Due to these limitations with measles virus-specific serology, detection of measles virus antigen by immunofluorescence (IFA) or by nucleic acid testing (NAT) is increasingly used. In our laboratory, an immunofluorescent monoclonal antibody against measles virus hemagglutinin and matrix protein (EMD Millipore Corp, Temecula, CA, USA) is used as the first line test for direct detection of measles. The in-house real-time reverse transcriptase polymerase chain reaction (RT-PCR) using the LightCycler® 480 System (LightCycler; Roche Diagnostics, Mannheim, Germany) is not generally performed, unless there is a strong clinical suspicion for infection despite a negative IFA. However, the acquisition of measles in non-travellers within the local community suggested that not all index patients with measles infection were being identified using this diagnostic algorithm.

We therefore developed and evaluated a Taqman-based RT-PCR on the SmartCycler® (SmartCycler; Cepheid, Sunnyvale, CA, USA) platform for the detection of measles virus. The SmartCycler platform was chosen because it allows for random access and the assay is categorized as moderate complexity in terms of test performance and result interpretation. Non-molecular biology trained staff at our 24 h, 7 day a week laboratory were already familiar with the SmartCycler.

2. Objectives

To evaluate the performance of a random access real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay using the SmartCycler® platform and measles antigen detection by immunofluorescence for the detection of measles virus. Each assay was compared against the LightCycler NAT as the reference assay.

3. Study design

3.1. Samples used to determine the analytical sensitivity and specificity of the SmartCycler NAT

For analysis of the lower limit of detection (LoD) of the SmartCycler assay, RNA extracted from 5 μL of the M–M–R II vaccine (CSL Limited/Merck & Co., Inc., Parkville, Victoria, Australia). Each 0.5 mL vial of M–M–R II vaccine contains >10^5 50% tissue culture infectious dose (TCID50) of the Edmonton strain of measles [5]. A solitary vial was tested, and the amount of RNA extracted from the vaccine was not quantified. Serial dilutions (neat to 10^-7) of RNA extracted were then used as template nucleic acid extract for the RT-PCR. Experiments were performed in duplicate to determine the LoD of the assay. To determine specificity of the RT-PCR assay, nucleic acid extracts of stored clinical samples of enterovirus/rhinovirus, varicella zoster virus, herpes simplex virus-1 and -2, human herpesvirus 6, human parvovirus, adenovirus, influenza A, influenza B, respiratory syncytial virus, human metapneumovirus, and parainfluenza viruses 1–3 were tested. To determine specificity against mumps and rubella, commercially sourced mumps and rubella RNA (Vircell AmpliRun Mumps RNA control and Vircell AmpliRun Rubella RNA control, Abacus Diagnostics, Granada, Spain) were also tested on the measles RT-PCR assay.

3.2. Patient specimens

One hundred specimens (combined nose and throat swabs [NTS], n = 48; nasopharyngeal aspirate [NPA], n = 7; and urine, n = 45) from 86 patients, previously submitted for measles antigen detection by IFA over a 20-month period from August 2012 to March 2014 were retrieved from storage at −80 °C. Included in this collection were five archived pediatric specimens where measles antigen was detected by IFA but no corresponding epidemiological information was available. The age of patients ranged from 4 months to 73 years. Where available, all specimens from different sites for each patient were tested. In 14 patients, NTS and urine samples were tested concurrently. NTS samples were collected and submitted to the laboratory in viral transport media (Copan Italia, Brescia, Italy).

3.3. RNA extraction

RNA was extracted using the BioRobot EZ1 and EZ1 Virus Mini Kit v2.0 according to the manufacturer’s instructions (Qiagen, Valencia, CA, USA). For NTS samples, swabs were vortexed in 1 mL of HBSS (Gibco, Life Technologies, Grand Island, NY, USA). For all samples, 200 μL of specimen was used for RNA extraction.

3.4. SmartCycler and LightCycler assays

The primers and probes against measles virus N-gene were designed based on previously published assays [9], with minor modifications, with the inclusion of degenerate nucleotides at positions 585, 590, 599, 600 and 606 (forward primer); 687 (reverse primer) and 657 (probe). The sequences were as follows: forward primer MVF2, 5′TCCTGGGATTACGATGATGCT; reverse primer MVR2, 5′ATCCACTCTCTAWGCTCGAACATC; Taqman probe, 5′FAM (TCTTGCTCGAAAGGCGGTTACKG) BHQ-1. This produced a 114 bp product. An internal positive control to detect the human β-globin gene (BGL) was used: forward primer, 5′ACACACTGTGGTCTACAGC; reverse primer, 5′CAACTTATCCACGTTCACC; Taqman probe 5′Quasar (TCAAAA-GACGACCATGGTCACCTGA) BHQ-2. The PCR master mix contained 0.4 μM of each measles primer, 0.2 μM of each BGL primer, 0.32 μM of measles probe MeaP, 0.16 μM of BGL probe, AgPath-ID enzyme mix (Ambion, Applied Biosystems, Foster City, CA, USA), AgPath-ID buffer, and 5 μL of nucleic acid template in a final 25 μL reaction. The PCR cycling conditions on the SmartCycler were as follows: reverse transcription, 50 °C for 30 min; followed by 95 °C for 15 min; followed by 3 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 40 s; followed by 40 cycles of 95 °C for 15 s, 60 °C for 15 s, 72 °C for 20 s.

3.5. Measles virus genotyping

Amplification and sequencing of the measles virus N gene was used to genotype the RT-PCR positive RNA extracts at the Measles Reference Laboratory, Victorian Infectious Diseases Reference Laboratory, Australia [4]. Specimens were referred for genotyping as directed by local public health unit investigations. All specimens from sporadic cases of measles infection and at least one case from any documented chain of transmission were genotyped.

3.6. Measles immunofluorescence antigen test

Briefly, NTS were vortexed in 1 mL of HBSS and then centrifuged at 2000 rpm for 10 min. The supernatant was removed, and the cell pellet resuspended in phosphate-buffered saline with 10% fetal calf serum. This suspension was then placed onto a slide, fixed in acetone and stained using mouse anti-measles monoclonal antibody directed against measles hemagglutinin and matrix protein (EMD Millipore Corp). Anti-mouse IgG-FITC conjugate was added to the slide, and incubated at 37 °C for 30 min. Slides were then read using a fluorescence microscope. Urines were processed in a similar manner, but the cell pellet was obtained without the addition of HBSS.
3.7. Measles virus-specific serology

Where available, data on measles virus-specific IgG and IgM detection were collected. Various serology platforms were used, including the WHO LabNet recommended Enzygnost® anti-measles virus IgG and IgM (Siemens, Erlangen, Germany) [13] in our laboratory, and the BioPlex® 2200 (Biorad, Berkeley, CA, USA) and LIAISON® (DiaSorin, Stillwater, MN, USA) assays in laboratories that referred specimens for IFA and/or NAT.

3.8. Public health unit investigations

In the state of New South Wales (NSW), Australia, measles virus infection is notifiable by doctors and laboratories to the local public health unit. Notified cases were assessed against the national case definition and investigated in accordance with national guidelines [12].

4. Results

4.1. Limit of detection of measles virus and analytical specificity on the SmartCycler RT-PCR

The mean cycle threshold (Ct) values of RNA extracted from the M–M–R II vaccine tested neat, $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$ was 22.7, 26.3, 30.3, 34.3 and 40.9, respectively. For clinical samples tested, the Ct values were noted to be marginally higher on the SmartCycler compared to the LightCycler with a mean difference of 0.89 cycles (range $-1.88$ to 3.38). No PCR products were obtained when nucleic acid extracts of other non-measles viruses were tested on both the LightCycler and SmartCycler RT-PCR platforms.

4.2. The SmartCycler RT-PCR was concordant with the LightCycler RT-PCR

As shown in Table 1, there was 100% concordance between positive and negative SmartCycler and LightCycler NAT results. However, using the SmartCycler, there were nine indeterminate results, defined as failure to amplify the human β-globin gene (BGL) internal control due to the presence of RT-PCR inhibitors, or insufficient human cellular material. Of these nine specimens, five were also indeterminate using the LightCycler. All of these specimens were urine. Nucleic acid extracts were therefore diluted 1:10 and the assay repeated. This resulted in amplification of the BGL internal control for six of the nine indeterminate samples on the SmartCycler assay, suggesting that the reason for non-amplification for the remaining three samples was insufficient extracted nucleic acid due to inadequate specimen quality. This also resolved four of the five indeterminate results on the LightCycler assay. Measles antigen was not detected by immunofluorescence in any specimens with indeterminate results, and none of these patients had a clinical syndrome compatible with measles. When the indeterminate results were excluded, the sensitivity and specificity of the SmartCycler compared to the LightCycler RT-PCR were both 100% (Table 1). The mean Ct value for positives on the SmartCycler was 29.6 (range 23.8–40.2).

4.3. Testing urine in addition to NTS or NPA by RT-PCR did not improve laboratory diagnosis of measles infection

There were 14 patients who had both NTS and urine tested; in six of these patients, measles RNA was not detected by NAT in both specimens; in seven patients, measles RNA was detected in both specimens; and in one patient, measles RNA was detected in NTS but not in urine. For seven samples where measles RNA was detected in both samples, the Ct values for NTS were lower than the urine samples (mean Ct difference $-4.6$, range $-16.2$ to $-2.7$), indicating a higher viral burden in the upper respiratory tract specimens.

4.4. Positive RT-PCR results were clinically significant

All patients apart from one patient ($n=27$), with samples that were measles virus RT-PCR positive, had a clinico-epidemiological syndrome compatible with measles (Table 3). The solitary patient where measles virus was detected by RT-PCR but not IFA had exudative tonsillitis. This patient had been vaccinated against measles 93 days prior to NTS collection. Measles virus genotyping demonstrated genotype A, consistent with the vaccine genotype.

4.5. Genotyping supports clinical and epidemiological investigations by public health units

Thirteen samples where measles virus was detected by RT-PCR were genotyped for epidemiological purposes. Genotypes B3 ($n=10$), D8 ($n=2$), and D9 ($n=1$) were identified. Public health investigations identified four distinct epidemiological clusters linking patients where measles virus was detected (Table 3). Outbreak 1 (O1) was associated with an imported case of measles from the Philippines who attended a large music festival during the incubation period. Local secondary transmission to 15 others occurred—eight are reported here. Outbreak 2 (O2) occurred in a family where the index case acquired infection during interstate travel outside NSW. Outbreak 3 (O3) was in a high school group resulting in seven cases, four of which are included here. The single case in Outbreak 4 (O4) was part of a larger cluster in which

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Table 1

<table>
<thead>
<tr>
<th>Measles virus RT-PCR on SmartCycler® platform</th>
<th>Detected</th>
<th>Not detected</th>
<th>Indeterminate</th>
</tr>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>LightCycler®</td>
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<td>62</td>
<td>0</td>
</tr>
<tr>
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<td>1</td>
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Table 2

<table>
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<th>Measles virus antigen detection by IFA</th>
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<th>Not detected</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
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<td>2</td>
<td>0</td>
</tr>
<tr>
<td>LightCycler®</td>
<td>23</td>
<td>59</td>
<td>1</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

---
Table 3
Clinical and epidemiological characteristics of patients with measles RNA detected by nucleic acid testing.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years, except where otherwise stated)/Sex</th>
<th>Day of collection of specimens after rash onset</th>
<th>Clinical specimen</th>
<th>Measles virus nucleic acid testing (NAT)</th>
<th>Measles virus antigen detection by immunofluorescence (IFA)</th>
<th>Measles virus-specific IgG</th>
<th>Measles virus-specific IgM</th>
<th>Genotype</th>
<th>Clinical criteria met</th>
<th>Epidemiological information</th>
<th>Vaccination status</th>
<th>Outbreak number (O#) or sporadic (S) case</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19/M</td>
<td>Unknown</td>
<td>NTS</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>B3</td>
<td>Y</td>
<td>Attended music festival</td>
<td>Unknown</td>
<td>O1</td>
</tr>
<tr>
<td>2</td>
<td>19/M</td>
<td>0</td>
<td>NTS</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>B3</td>
<td>Y</td>
<td>Attended music festival</td>
<td>Fully vaccinated</td>
<td>O1</td>
</tr>
<tr>
<td>3</td>
<td>19/M</td>
<td>1</td>
<td>NTS and U</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>NP</td>
<td>Y</td>
<td>Attended music festival</td>
<td>Unvaccinated</td>
<td>O1</td>
</tr>
<tr>
<td>4</td>
<td>21/M</td>
<td>1</td>
<td>NTS</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>NP</td>
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<td>Attended music festival</td>
<td>Unvaccinated</td>
<td>O1</td>
</tr>
<tr>
<td>5</td>
<td>20/M</td>
<td>0</td>
<td>U</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>B3</td>
<td>Y</td>
<td>Attended music festival</td>
<td>Partially vaccinated</td>
<td>O1</td>
</tr>
<tr>
<td>6</td>
<td>18/M</td>
<td>3</td>
<td>U</td>
<td>+</td>
<td>−</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>Y</td>
<td>Attended music festival</td>
<td>Unvaccinated</td>
<td>O1</td>
</tr>
<tr>
<td>7</td>
<td>23/M</td>
<td>1</td>
<td>NTS and U</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>NP</td>
<td>Y</td>
<td>Attended music festival</td>
<td>Unvaccinated</td>
<td>O1</td>
</tr>
<tr>
<td>8</td>
<td>20/F</td>
<td>Serum collected on day 3; NTS and U collected on day 4</td>
<td>NTS and U</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>NP</td>
<td>Partial (Fever and rash only)</td>
<td>Unvaccinated music festival</td>
<td>Unvaccinated</td>
<td>O1</td>
</tr>
<tr>
<td>9</td>
<td>23/F</td>
<td>3</td>
<td>U</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>NP</td>
<td>Y</td>
<td>Infection acquired interstate; index case</td>
<td>Partially vaccinated</td>
<td>O2</td>
</tr>
<tr>
<td>10</td>
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<td>1</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>B3</td>
<td>Y</td>
<td>Household contact of case 9</td>
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<td>O2</td>
</tr>
<tr>
<td>11</td>
<td>13/M</td>
<td>0</td>
<td>NTS and U</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>B3</td>
<td>Y</td>
<td>High school cluster; index case</td>
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<td>O3</td>
</tr>
<tr>
<td>12</td>
<td>13/F</td>
<td>Serum collected on day 2; NTS collected on day 3</td>
<td>NTS</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>NP</td>
<td>Y</td>
<td>High school cluster, contact of case 11</td>
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<td>O3</td>
</tr>
<tr>
<td>13</td>
<td>30/F</td>
<td>0</td>
<td>U</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>B3</td>
<td>Y</td>
<td>Contact of case 12—attended the same birthday party</td>
<td>Partially vaccinated</td>
<td>O3</td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>Age (years, except where otherwise stated)/Sex</td>
<td>Day of collection of specimens after rash onset</td>
<td>Clinical specimen</td>
<td>Measles virus nucleic acid testing (NAT)</td>
<td>Measles virus antigen detection by immunofluorescence (IFA)</td>
<td>Measles virus-specific IgG</td>
<td>Measles virus-specific IgM</td>
<td>Genotype</td>
<td>Clinical criteria met</td>
<td>Epidemiological information</td>
<td>Vaccination status</td>
<td>Outbreak number (O#) or sporadic (S) case</td>
</tr>
<tr>
<td>------</td>
<td>-----------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>------------------</td>
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<td>-----------------------------</td>
</tr>
<tr>
<td>14</td>
<td>2/M</td>
<td>2</td>
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<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>B3</td>
<td>Y</td>
<td>Contact of case 13—attended the same birthday party</td>
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<td>O3</td>
</tr>
<tr>
<td>15</td>
<td>30/F</td>
<td>7</td>
<td>U</td>
<td>+</td>
<td>−</td>
<td>Equivocal</td>
<td>+</td>
<td>D8</td>
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<tr>
<td>16</td>
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<td>NTS was +; U was −</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>B3</td>
<td>Y</td>
<td>Exposed to a measles case at a medical practice</td>
<td>Fully vaccinated</td>
<td>O5</td>
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<tr>
<td>17</td>
<td>1/F</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>F</td>
<td>Y</td>
<td>Infection acquired overseas; Philippines</td>
<td>Unvaccinated</td>
<td>S</td>
</tr>
<tr>
<td>18</td>
<td>8/F</td>
<td>1</td>
<td>U</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>F</td>
<td>Y</td>
<td>Infection acquired overseas; Indonesia</td>
<td>Unvaccinated</td>
<td>S</td>
</tr>
<tr>
<td>19</td>
<td>20/F</td>
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<td>U</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>D8</td>
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<td>Infection acquired overseas; United Kingdom</td>
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<td>S</td>
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<td>20</td>
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<td>−</td>
<td>+</td>
<td>+</td>
<td>B3</td>
<td>Y</td>
<td>Infection acquired overseas; Philippines</td>
<td>Fully vaccinated</td>
<td>S</td>
</tr>
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<td>NTS and U</td>
<td>NTS was +; U was −</td>
<td>−</td>
<td>+</td>
<td>B3</td>
<td>Y</td>
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<td>−</td>
<td>−</td>
<td>+</td>
<td>D9</td>
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<td>Unvaccinated</td>
<td>S</td>
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<td>NP</td>
<td>A</td>
<td>N</td>
<td>Vaccinated 93 days prior to testing</td>
<td>Unknown</td>
<td>NR</td>
</tr>
</tbody>
</table>

Note: M—male; F—female; NTS—combined nose and throat swabs; U—urine; +—detected; −—not detected; NP—not performed; F—genotyping failed or unsuccessful due to low viral load or insufficient specimen; Y—yes; N—no; O—outbreak; S—sporadic; NR—not relevant. Where both combined nose and throat swabs and urine specimens are collected in the one patient and, results of testing for NAT and IFA are concordant, results are presented as detected (+) or not detected (−).
acquisition of infection occurred interstate. Genotyping from at least one of the cases from outbreaks O1, O2, O3 and O5 identified the responsible genotype as B3; the genotype of O4 was D8.

In addition to the clusters, six sporadic cases were identified. Four were imported from overseas—two from the Philippines and one each from Indonesia and the United Kingdom. No epidemiological links could be determined in two locally acquired sporadic cases (cases 21 and 22). Where successfully performed, genotypes B3, D8, and D9 were identified in the sporadic cases.

Only laboratory diagnostic data was available on the patients who provided the five archived specimens (Cases 24–28).

4.6. Measles virus antigen detection by immunofluorescence

Results of measles virus detection by IFA are shown in Table 2. An indeterminate IFA result was reported when there was a paucity of cells visualized on the slide. There were 23 specimens (from 20 patients) where measles virus was detected by RT-PCR but not by IFA. The Ct values for these specimens on the reference LightCycler RT-PCR ranged between 23.9 and 40. Measles virus was detected in two specimens by IFA but not by RT-PCR. Neither of these patients was measles virus-specific IgM seropositive, nor did they have clinico-epidemiological features consistent with measles. When the indeterminate results were excluded from analysis, the sensitivity and specificity of IFA versus RT-PCR was 34.3% and 96.7%, respectively. The positive and negative predictive value of IFA was 85.7% and 72%, respectively.

4.7. Measles virus-specific serology

Only 43 out of 86 patients had serology performed, perhaps reflecting the many pediatric patients in this cohort where it is less convenient to obtain a serum sample. Of the 28 patients where measles virus was detected by RT-PCR, 24 had measles virus-specific serology performed (Table 3). Of these, 16 had measles-specific IgM detected, two had an equivocal IgM result, and six were negative. In these six patients where measles-specific IgM was not detected, 5 serum specimens were collected <72 h after rash onset; in one patient, information to determine the timing of the serological test in relation to rash onset was unavailable.

Clinical information was available on all 17 patients where measles virus-specific IgM was detected. Sixteen patients had clinical and epidemiological features consistent with measles infection. The remaining patient was diagnosed with parvovirus infection and had been vaccinated with the first dose of a measles-containing vaccine 3 weeks prior to presentation, likely accounting for IgM detected. Measles virus RNA was detected by RT-PCR in this patient.

When all these diagnostic modalities were assessed in combination, there were five patients where measles virus was detected by NAT, but who were measles-specific IgM and IFA not detected. Laboratory confirmation of measles infection was missed in four of these patients until this study was performed (Table 3).

5. Discussion

In the present study, the SmartCycler assay was developed using the M–M–R II vaccine as a positive control in the absence of cell cultures. Other investigators have developed RT-PCR assays for the detection of measles virus using synthetic measles virus generated from recombinant plasmids, clinical material or viral culture supernatant [9,10]. Although the vaccine contains both attenuated mumps and rubella virus strains, these viruses are unlikely to have affected the LoD of measles virus as the assay was shown to be measles virus specific when extracts of mumps and rubella viruses were tested.

In conclusion, this study provides compelling reasons for laboratories to use NAT for the detection of measles virus. The accurate diagnosis of measles virus infection is critical in facilitating prompt and appropriate public health responses to prevent further transmission.

Funding

None.

Conflict of interest

None.
Ethical approval

The investigation of individual cases of measles infection was conducted as part of public health investigations of suspected or confirmed cases of measles notified under the legal authority conferred by the New South Wales Public Health Act 2010. Research ethics approval was not required.

Acknowledgements

We thank Justin Ellem, Gordana Nedeljkovic, Neisha Jeoffreys and Ian Carter from CIDMLS for technical advice. Referring laboratories and their staff, in particular Prof. Alison Kesson from The Children’s Hospital at Westmead, kindly submitted samples and provided measles-specific serology data. We thank staff from the Victorian Infectious Diseases Reference Laboratory (VIDRL) for providing measles virus genotyping data. VIDRL is the regional reference laboratory for the WHO Measles and Rubella Laboratory Network (LabNet) in the Western Pacific Region.

References