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VIROLOGY

Simultaneous co-detection of wild-type and vaccine strain measles virus using the BD MAX system

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Summary

Despite the reported elimination of measles virus in Australia, importation of cases from endemic countries continues to lead to secondary local transmission and outbreaks. Rapid laboratory confirmation of measles is paramount for individual patient management and outbreak responses. Further, it is important to rapidly distinguish infection from wild-type virus or vaccine strains to guide public health responses. We developed a high throughput, TaqMan-based multiplex reverse-transcription-polymerase chain reaction (PCR) assay using the BD MAX platform (Becton Dickinson) that simultaneously detects measles virus and differentiates between wild-type and vaccine strains without the need for sequencing.

Key words: Measles; genotype; wild-type; vaccine.

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INTRODUCTION

As a result of an extended period of high childhood vaccination coverage and the absence of outbreaks, the World Health Organization (WHO) declared measles to be eliminated from Australia in March 2014.[1,2] There has been a two dose live attenuated measles virus (MeV) vaccination program in Australia since 1998, and the National Immunisation program currently provides for doses at 12 months of age as measles-mumps-rubella vaccine and at 18 months of age as the measles-rubella-varicella vaccine.[2] However, there remain susceptible individuals, either adults born during or after 1966 in Australia, and prior to the national measles control campaign in 1988, or immigrants born overseas that are unvaccinated or not fully vaccinated. Importation of MeV by this susceptible population or foreign visitors from countries where measles is endemic leads to secondary local transmission and outbreaks.[3,4]

Laboratory confirmation of MeV infection is essential to trigger the public health response to prevent further transmission and limit potential outbreaks. In addition, confirmation of infection allows for surveillance of MeV, including circulating genotypes and monitoring of vaccine uptake and effectiveness. Typical diagnostic methods of MeV infection include measles-specific serology and nucleic acid detection. These diagnostic methods are useful under different circumstances. The presence of immunoglobulin G is useful in that it indicates past infection or vaccination. On the other hand, MeV-specific immunoglobulin M may be negative in up to 23% of cases in the first 72 hours following the onset of rash.[5,6] Nucleic acid testing (NAT) is the most sensitive and specific method for the diagnosis of acute MeV infection, and it can be performed on upper respiratory tract and/or urine samples. Although the MeV nucleoprotein gene is the most commonly used target for nucleic acid detection, it does not differentiate between wild-type and vaccine MeV strains. The measles vaccine contains live attenuated virus that can result in a systemic measles-like illness with fever and rash in up to 5% of recipients approximately 5–12 days following vaccination that spontaneously resolves.[6]

Measles virus can be genetically characterised or genotyped by detecting sequence differences in the 450-nucleotide region coding for the COOH-terminal 150 amino acids of the nucleoprotein gene.[7] There are currently 24 genotype reference strains recognised by the WHO, including the genotype A vaccine strain (MeVA), which is not currently considered transmissible under normal circumstances.[7] Rapid and accurate differentiation between wild-type and vaccine strains is useful to guide individual patient and public health management including contact tracing, and to inform strategies for post-exposure vaccination and normal human immunoglobulin use. As sequencing of the nucleoprotein gene is labour intensive, time consuming and expensive, we developed and evaluated a rapid one-step, single-tube multiplex TaqMan assay using the BD MAX (Becton Dickinson, USA) platform that simultaneously detects MeV and differentiates between wild-type and MeVA vaccine strains.

MATERIALS AND METHODS

Ethics approval

Research ethics approval was not required as investigations of suspected or confirmed cases of measles was conducted under the legal authority conferred by the New South Wales Public Health Act 2010.

Primers, probes and controls

The primers and probes used to detect all MeV variants were as previously published,[3] whilst those used specifically to detect MeVA were adapted

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from Roy *et al.*[8] Briefly, MeVA primers and probes were designed following the analysis of 31 sequences available on GenBank from Edmondston- and non-Edmonston-derived vaccine strains. Similar to Roy *et al.*, our assay also uses a locked nucleic acid (LNA) probe, which targets a 23-base sequence similar to MeVA strains, but with four nucleotide differences from sequences of wild-type strains within the highly conserved amino terminus of the N gene. Oligonucleotides targeting the human β-globulin (HβG) gene were used as internal control. The primer sequences used in this study are listed in Table 1.[7] A serial dilution series of 10^{-1} to 10^{-4} of the measles-mumps-rubella (MMR) vaccine (PRIORIX; GlaxoSmithKline Biologicals, Belgium) was used as positive control for the MeVA assay. Each 0.5 mL dose of the MMR vaccine contains no less than 10^3 cell culture infectious dose 50% ($CCID_{50}$) of the attenuated Schwarz measles virus strain.[8]

BD MAX MeV and MeVA assay

The MeV and MeVA assay was developed on the BD MAX, an integrated and fully automated platform that combines extraction of nucleic acid, thermocycling and detection of amplified products in a single platform. This open-system can run multiple specimen types and assays in a single run.

Two hundred microliters of sample were added into a BD Max TNA-3 sample buffer tube and vortexed for 20 s prior to loading into the BD MAX for extraction and real-time polymerase chain reaction (PCR). In a 12.5 μL PCR reaction, 3.5 μL of extract was reverse transcribed and amplified using 4.25 μL of SensiFAST One-Step Real-Time RT-PCR (Bioline, United Kingdom), 0.4 μM of each primer and HβG probe and 0.8 μM of MeV and MeVA probes all in the BD Max PCR cartridge. Cycling conditions were: cDNA synthesis step of 10 min at 45°C, denaturation step of 2 min at 98°C and 50 cycles of 8 s at 95°C and annealing and acquisition of 1 min at 60°C. The amplification products of MeV, MeVA and HβG were analysed at excitation/detection wavelengths of 475/520 nm, 530/565 nm and 630/665 nm, respectively.

Sensitivity and specificity

The analytical sensitivity of the MeV and MeVA multiplex assay was determined using a 10-fold MMR vaccine dilution series that ranged from 10^{-2} to 10^{-6}. Assay specificity was determined by testing both MeV and MeVA assays against other viruses that may cause a similar clinical illness including mumps, rubella, herpes simplex virus type 1 and 2, human cytomegalovirus, varicella zoster, Epstein–Barr virus, enterovirus, and parvoviruses.[9] For specificity testing, a combination of clinical specimens and synthetic positive controls were used.

Samples

Fifty-eight clinical specimens where MeV had been previously detected by NAT (combined nose and/or throat swabs, nasopharyngeal aspirates and urines) were available for testing; 48 samples collected between January 2015 and June 2017 (including 16 samples from the most recent MeV outbreak in Sydney during April 2017)[10] that were stored at −80°C and 10 samples (of known genotypes) that were provided by the Victorian Infectious Diseases Reference Laboratory (VIDRL). MeV samples from VIDRL were retested using our laboratory's existing MeV PCR and genotyping protocols.

RESULTS

Analytical sensitivity and specificity of MeV and MeVA PCR on MMR vaccine and clinical specimens

The cycle threshold (Ct) values of the MMR vaccine dilution series that ranged from 10^{-2} to 10^{-6} for MeV/MeVA were 21.9/23.8, 25.5/27.2, 29.4/32.2 and 33.3/36.7, respectively. Therefore, the limit of detection of the MeVA assay was 10^{-6} of $10^{3.0}$ $CCID_{50}$ measles virus.

Measles virus was detected in 58 clinical samples using the multiplex PCR assay, of which 22 were identified as MeVA, with Ct values between 20.8 and 34.5. This was 100% concordant with the genotype results obtained by N-450 sequencing. These results are summarised in Table 2. The MeV and MeVA assay was also negative when tested against non-measles viruses as outlined above.

Measles virus genotypes during the April 2017 outbreak in Sydney, New South Wales

Of 522 clinical specimens submitted to our laboratory for MeV testing during the period of April to May 2017, 16 samples were positive for MeV. Using N-450 sequencing, 13 were genotyped as D8, and the remaining three were genotype A. The phylogenetic tree of the cases is illustrated in Fig. 1. All three MeVA positive samples were collected from children aged between 12 and 14 months, vaccinated with MMR 11–30 days prior. Utilising MEGA 6.06 software, the phylogenetic tree was constructed using maximum parsimony; to further assess the relatedness of samples, analysis with next generation sequencing would be required.

Turnaround times and costs of MeV and MeVA PCR

The turnaround time for the simultaneous detection of MeV and MeVA is 2 hours. This compares favourably with the

Table 2 Results of clinical samples tested for MeV and MeVA using a TaqMan-based multiplex reverse-transcription-PCR assay

Measles virus genotype	No. samples	MeV detected	MeVA detected
A	22	22	22
B3	4	4	0
D4	1	1	0
D8	24	24	0
D9	2	2	0
H1	3	3	0
G3	2	2	0
Total	58	58	22

Table 1 Primers and probes for detection of measles virus (MeV) and vaccine strains (MeVA)

Targets	Direction	Sequence (5′– 3′)
MeV	Forward	YCCTGMGGGATTCARYATGATYCT
	Reverse	ATCCACCTTCTWAGCTCCGAATC
	Probe	6FAM-TCTTGCTCGCAAAGGCGGTTAC*K*G-BHQ-1
MeVA	Forward	AGGATGAGGCGGACCAATACTT
	Reverse	GAACCATCCGAACCTGGAT
	LNA Probe[a]	TxRd-CATG[+A]TG[+A]TCCAA[+T]TAGTA[+G]TGA-BHQ2
HβG	Forward	ACACAACTGTGTTCACTAGC
	Reverse	CAACTTCATCCACGTTCACC
	Probe	Quasar 670-TCAAACAGACACCATGGTGCACCTGA-BHQ-2

[a] Indicates a locked nucleic acid nucleotide.

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