

Co-detection of the measles vaccine and wild-type virus by real-time PCR: public health laboratory protocol

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Abstract

In rare cases vaccination with the measles virus vaccine genotype A (MeVA) may cause a vaccine reaction with clinical signs similar to infection with wild-type measles virus (MeVwt). Rapid differentiation between MeVA and MeVwt infection is important for taking adequate public health measures. Recently, a few MeVA real-time reverse-transcription quantitative PCR methods (RT-qPCRs) were described that can distinguish between MeVA and MeVwt. However, detection of MeVA does in theory not exclude infection with MeVwt. In the present study, we established a protocol for determination of co-infections with MeVA and MeVwt. To this end, MeVA RT-qPCRs were used in combination with the routine measles virus (MeV) RT-qPCR, and the results suggested that the differences between the RT-qPCR Ct values (delta Ct, Δ Ct) could be used as criteria. Subsequently, we tested samples from vaccine-associated measles cases that were confirmed by genotyping. In addition, experimental mixtures of MeVA and MeVwt were tested in different concentrations. All tested MeVA clinical samples had Δ Ct \leq 3.6. The results of experimental mixtures showed a mean Δ Ct \leq 2.8 for genotype A alone and $>$ 3.2 when combined with either genotype B3 or D8. The results of a receiver operator characteristic analysis indicated that the optimum Δ Ct for use as a cut-off value was 3.5, while with Δ Ct values of 2.9 and 3.7 sensitivity and specificity were respectively 1.00. Thus, Δ Ct could be used to exclude the presence of MeVwt if MeVA is detected and Δ Ct is $<$ 2.9, while Δ Ct $>$ 3.7 were highly suggestive of co-infection and \geq 2.9 Δ Ct $<$ 3.7 warranted additional confirmation, such as next-generation sequencing. This RT-qPCR-based protocol could be used for the exclusion of infection with MeVwt in cases with vaccine-associated measles reaction, crucial for the timely implementation of public health prevention and control measures.

INTRODUCTION

Measles viruses (MeV) are single-stranded, negative-sense RNA viruses belonging to the genus *Morbillivirus*, family *Paramyxoviridae* [1]. MeV are the causative agent of measles, a highly contagious disease characterized by coryza, conjunctivitis, fever, malaise, cough and exanthema [2]. However, there is a safe and effective vaccine available [3], that has prevented an estimated 23.2 million deaths between 2000 and 2018 [4]. Despite various elimination efforts [5, 6], MeV continue to circulate and cause outbreaks in various parts of the world, including in the European region [7, 8].

Occasionally, vaccination with the live attenuated measles virus vaccine genotype A (MeVA) causes a rash, fever and

overall clinical presentation like infection with wild-type measles virus (MeVwt) [3, 9, 10]. Distinguishing between vaccine-associated measles symptoms and MeVwt infection can be difficult in these cases, while this differentiation is important from a public health perspective, including in countries with a relatively high vaccination coverage [11, 12]. In many countries with measles elimination goals, the routine handling of a case with MeVwt infection includes various public health measures, such as contact tracing, case isolation and possible post-exposure prophylaxis with immunoglobulins in very young contacts [13]. In contrast, no strict measures are needed for cases with a vaccine reaction since there is no evidence of human-to-human transmission of MeVA [14].

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Abbreviations: Δ Ct, delta Ct; Ct, cycle threshold; hSLAM, human signaling lymphocytic activation molecule; MeV, measles virus; MeVA, measles virus vaccine genotype A; MeVwt, wild-type measles virus; NGS, next-generation sequencing; PCR, polymerase chain reaction; pmol, picomole; RIVM, National Institute for Public Health and the Environment; ROC, receiver operator characteristic; RT-qPCR, real-time reverse-transcription quantitative PCR method; SD, standard deviation.

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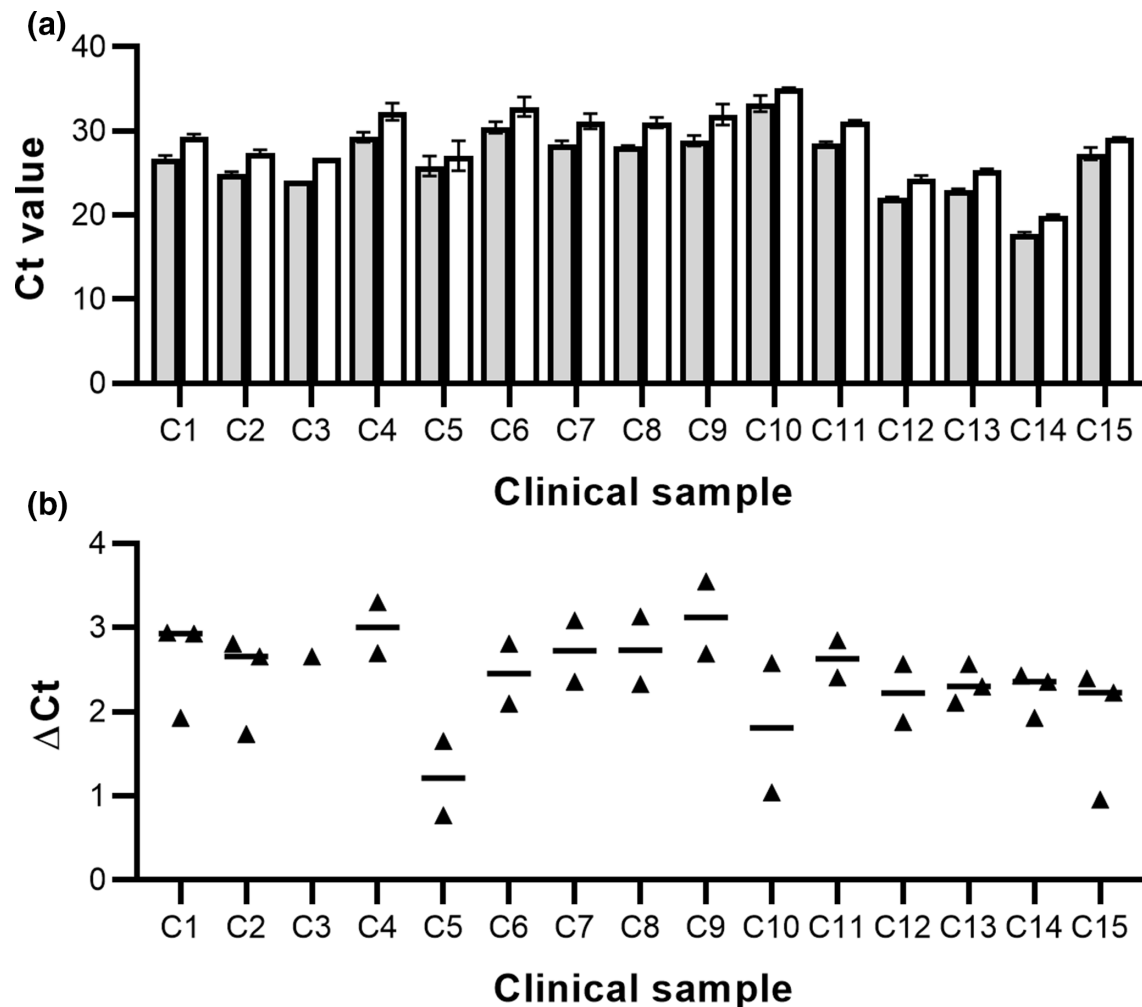


Fig. 1. Results of MeV and MeVA RT-qPCR performed on clinical samples with MeVA confirmed by genotyping. (a) Mean Ct values (with SD) obtained by MeV RT-qPCR (grey bars) and MeVA RT-qPCR (white bars) of 15 clinical samples. (b) Calculated individual (triangles) and mean (bars) Δ Ct values of 15 clinical samples. The number of experiments performed on each sample ranged from one to three, dependent on the available clinical material.

Genotyping by PCR and Sanger sequencing following the protocol of the World Health Organization [15] is a useful approach to distinguish between MeVA and MeVwt, but it is relatively slow. Next-generation sequencing (NGS) can also provide information about the presence of one or multiple measles virus strains, but is costly, labour-intensive and often not available in regional laboratories [16–18]. Thus, simpler techniques, like real-time reverse-transcription quantitative PCR (RT-qPCR), are preferable from a public health perspective, as they could differentiate rapidly between MeVA and MeVwt [19–21]. RT-qPCR results could eventually be confirmed by Sanger sequencing or NGS.

A few years ago, two very useful MeVA RT-qPCR assays were described that allowed to rapidly distinguish between MeVA and MeVwt [19, 20]. However, the detection of MeVA by these assays does not exclude the presence of MeVwt, and thus measles co-infection could theoretically occur in case of vaccination and exposure to MeVwt within a short timeframe.

For example, measles virus vaccination is recommended in various countries for unvaccinated children that have been exposed to measles virus [22–25]. Of interest, another multiplex RT-qPCR was developed recently that allows detection and differentiation of both MeVA and MeVwt [21]. However, this assay's use is limited to detection of Moraten and Schwarz vaccine strains based on a unique single nucleotide polymorphism. Considering the variety of measles vaccine strains used worldwide [26], this RT-qPCR would not differentiate between MeVwt and MeVA strains used in various regions, including Europe.

The aim of this study was to establish a protocol for determination of (theoretical) co-infections with MeVA and MeVwt. The objectives of this study were as follows: (1) to implement and validate the MeVA RT-qPCR assays described by Roy and colleagues and Tran and colleagues [19, 20] in our laboratory; (2) to evaluate the use of MeVA RT-qPCR in combination with MeV RT-qPCR, including

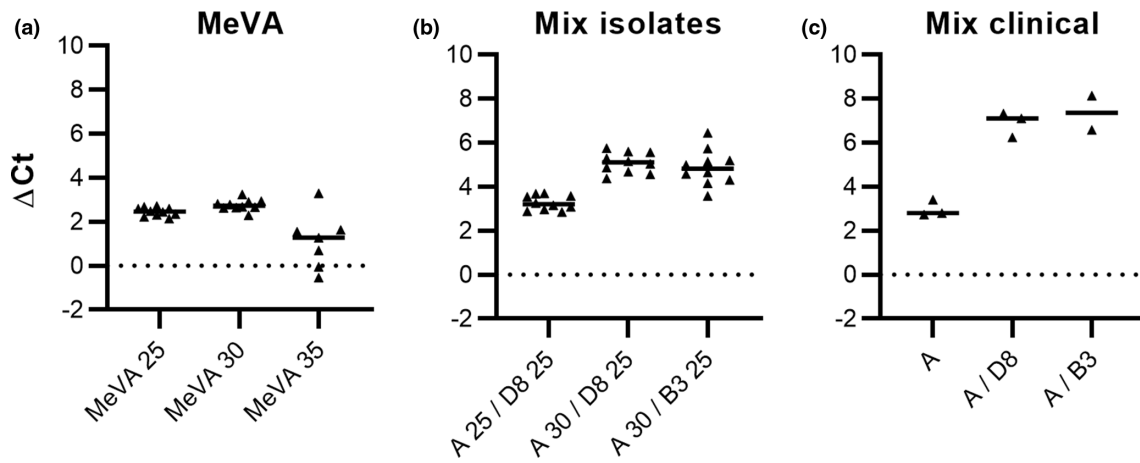


Fig. 2. Differences between RT-qPCR Ct values (Δ Ct) of experiments with MeV isolates and experimental mixtures of MeVwt and MeVA tested with MeV and MeVA RT-qPCR. (a) Three dilutions of a MeVA isolate were tested in tenfold and mean (bars) and individual (circles) Δ Ct values were calculated. The highest dilution was detected in only seven out of ten experiments with the MeVA RT-qPCR. (b) Mixes of isolates of MeVA and MeVwt (D8 or B3) were tested in tenfold and mean (bars) and individual (circles) Δ Ct values were calculated. (c) A clinical sample with MeVA was mixed with MeVwt B3 or D8 clinical samples (all previously confirmed by sequencing) and Δ Ct values were calculated. Experiments were performed in triplicates (A and A/D8) or duplicates (A/ B3).

the use of the differences between the Ct values (delta Ct, Δ Ct) as criteria; (3) to test the protocol workflow with clinical samples and if unavailable with simulated mixes of MeVA and MeVwt isolates.

METHODS

Clinical specimens

Clinical specimens (throat swabs, oral fluid and urine specimens) used in this study were submitted from 2013 to 2021 to the National Institute for Public Health and the Environment (RIVM) for molecular diagnostics and/or genotyping. Samples were processed for molecular diagnostics and/or genotyping using the required volume, and the remaining clinical material was stored at -70°C .

Viruses

Measles virus isolates MVi/Hetauda.NEP/2.99[D8] [27], MVi/Luxembourg.Lux/80[A] [28] and MVi/Khartoum.SUD/34.97/2[B3] [29] were kindly provided by Dr Muller and Dr de Swart. Virus isolates were propagated in Vero/hSLAM cells [30], aliquoted and stored at -70°C until use.

Measles virus detection and genotyping

Sample processing, detection of MeV by the routine MeV RT-qPCR and genotyping was performed as described previously [31, 32]. Sanger sequencing was performed at BaseClear (Leiden, The Netherlands).

Table 1. Mean Ct and differences between RT-qPCR Ct values (Δ Ct) results for mixtures containing measles virus genotype A (MeVA) and wild-type measles virus (MeVwt) isolates or clinical samples

Samples	Replicates	MeV RT-qPCR		MeVA RT-qPCR		Δ Ct	SD
		Mean Ct	SD	Mean Ct	SD		
Experimental virus isolate mixtures*							
A 25/D8 25	10	25.1	0.3	28.4	0.3	3.3	0.3
A 30/D8 25	10	26.8	0.1	31.9	0.4	5.1	0.5
A 30/B3 25	10	26.6	0.1	31.5	0.8	4.9	0.8
Clinical sample mixtures							
A / D8	3	25.3	0.3	32.2	0.9	6.9	0.6
A / B3	2	23.0	0.1	30.4	1.2	7.4	1.1

*Sample dilutions calculated to correspond approximately to a target Ct value of 25 or 30 and then mixed.

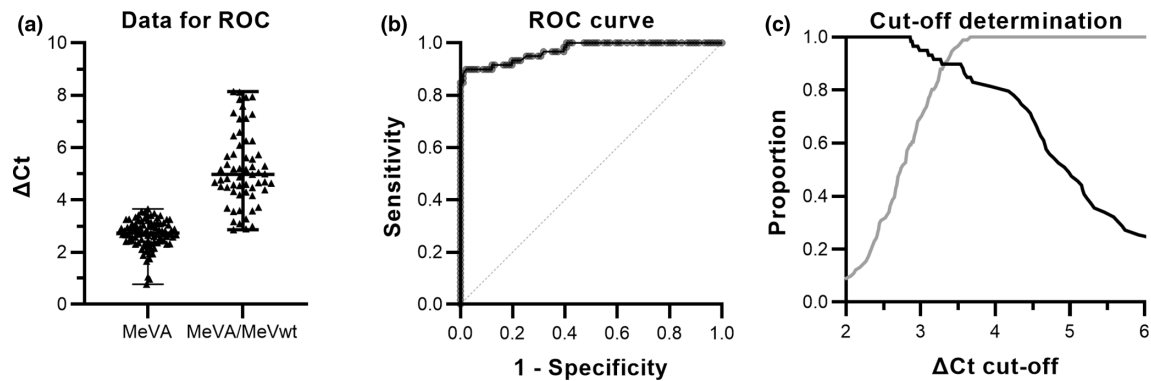


Fig. 3. ROC analysis using differences between MeV and MeVA RT-qPCR (ΔCt) values calculated by multiple MeVA and MeVA/MeVwt experiments. (a) ΔCt values obtained from experiments with MeVA and mixes of MeVA and MeVwt that were included in the ROC analysis. (b) ROC curve based on ΔCt values. (c) Plot of the sensitivity (black line) and specificity (grey line) as a function of the ΔCt cut-off value.

Validation of MeVA RT-qPCR assays

MeVA RT-qPCR assays [19, 20] were implemented and a complete validation was performed (including analysis of specificity, sensitivity, efficiency, correctness, limit of detection) in our laboratory for use in confirmed MeV RT-qPCR measles cases with a history of recent vaccination. The assays were performed on a LightCycler 480 platform (Roche) using TaqMan Fast Virus 1-step Master mix (Applied Biosystems, ThermoFischer). For the assay described by Roy and colleagues, 10 pmol forward primer, 30 pmol reverse primer, and 20 pmol probe per 15 μ l total reaction volume were used as optimal concentrations, while for the assay described by Tran and colleagues 40 pmol forward primer, 60 pmol reverse primer, and 15 pmol probe per 15 μ l total reaction volume were used as optimal concentrations [19, 20]. Primers and probes were purchased from Eurogentec. For both assays an annealing temperature of 60 $^{\circ}$ C was used in the RT-qPCR. Validation experiments were performed with a selection of historical clinical samples that tested positive for the presence of MeVA or MeVwt by genotyping. Experiments with historical clinical samples were performed in triplicate unless the sample volume was limited.

Experimental setup with mixtures of MeVwt and MeVA

To evaluate the use of the MeV and MeVA RT-qPCR for the detection of co-infection cases with MeVA and MeVwt, experimental mixes of MeVwt and MeVA RNA extracts were prepared in multiple ratios (1:1, 1:10, 1:50, 1:100) and then in dilutions corresponding to Cycle threshold (Ct) values 25–35. Experimental mixes were prepared with measles virus isolates MVi/Khartoum.SUD/34.97/2[B3] and D8 MVi/Hetauda.NEP/2.99[D8] in combination with MVi/Luxembourg.Lux/80[A]. Ct values used were based on the expectations for a case with acute measles (co-)infection. Each condition was tested in tenfold and by two operators on different and on the same plates. In addition to experimental mixes performed with measles virus isolates, experimental mixes were also performed with MeVwt genotype B3 (MV/Eindhoven.

NLD/5.18) and D8 (MV/Noord-Holland.NLD/46.19/2) and MeVA (MV/Zuid-Holland.NLD/48.19) detected in recent clinical samples. Experiments with clinical samples were performed in triplicate, unless the available clinical material was limited.

Statistical analysis, receiver operating characteristic analysis and visualisation

Mean values, SD and ΔCt values were calculated in Excel version 2002 (Microsoft). The mean Ct value calculated from the repetitions of a sample in the MeV RT-qPCR was subtracted from the mean Ct value of the repetitions of a sample in the MeVA RT-qPCR in order to calculate the ΔCt . Based on ΔCt data from experimental mixtures and clinical samples, a receiver operating characteristic (ROC) analysis was performed with default parameters in GraphPad Prism version 9.1.0. (GraphPad Software) and a plot of the diagnostic sensitivity and specificity of the ΔCt was prepared as described previously [33]. A protocol flowchart was created using draw.io/diagrams.net online software version 14.9.3 (JGraph).

RESULTS

Validation of MeVA RT-qPCR assays

Both the MeVA RT-qPCR assays from Roy and colleagues and Tran and colleagues [19, 20] were validated in our lab. The experiments in our laboratory confirmed the results described in both published studies: the presence of MeVA was detected in all clinical samples in which MeVA was confirmed by genotyping, while no MeVA positive result was obtained in clinical samples that tested positive for MeV genotype B3 and D8 by genotyping. Additional analysis indicated that the validation results in our laboratory were somewhat in favour of the MeVA assay described by Roy and colleagues with a higher sensitivity and higher efficiency (data not shown). Therefore, this assay was used for all subsequent experiments.

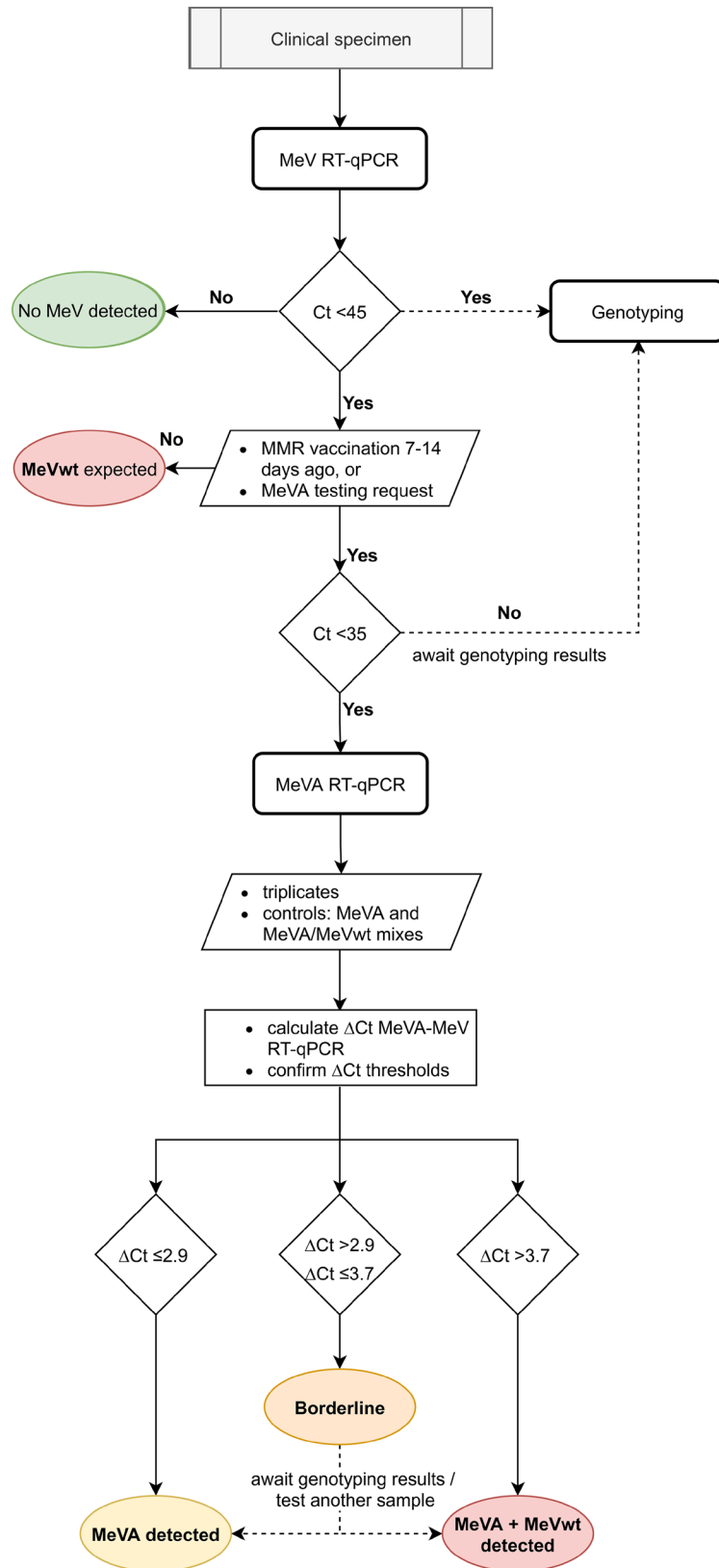


Fig. 4. Flowchart for detection of MeV, MeVA and MeVA/MeVwt co-infections via MeV and MeVA RT-qPCR. All RT-qPCR assays should be performed according to standard quality-assurance considerations.

Detection of MeVA by MeV and MeVA RT-qPCR

Ct values detected by MeV RT-qPCR in clinical samples that tested positive for the presence of MeVA by genotyping ranged from 17.6 to 33.9, while Ct values detected by MeVA RT-qPCR ranged from 19.9 to 35.2. Subtracting the Ct value of the MeVA RT-qPCR of individual samples from the MeVwt RT-qPCR resulted in Δ Ct values ranging from 0.8 to 3.6. Due to the limited available clinical sample volumes, only five samples in which MeVA was detected by genotyping could be tested in triplicate, while nine samples could be tested in duplicates and one sample could be tested only in a single experiment (Fig. 1).

In tenfold experiments with MeVA only in three different dilutions (MeVA 25, MeVA 30, MeVA 35), the mean Ct values in the MeV RT-qPCR were respectively 26.5 (SD, 0.2), 29.4 (SD 0.2) and 35.3 (SD 1.2). In one out of ten experiments in the highest dilution, no positive signal was detected in the MeV RT-qPCR. The mean Ct values in the MeVA RT-qPCR were respectively 28.9 (SD 0.1), 32.2 (SD 0.2) and 36.6 (SD 0.6). In three out of ten experiments in the highest dilution, no positive signal was detected in the MeVA RT-qPCR. The mean Δ Ct values of the two lowest dilutions were respectively 2.5 (SD 0.2) and 2.8 (SD 0.3), while the mean Δ Ct of the highest dilution was 1.3 (SD 1.3), using results from seven out of ten experiments (Fig. 2a). Based on these results, experiments with mixtures were performed only with the two lowest concentrations.

Detection of MeVA and MeVwt in experimental mixtures

In tenfold experiments with mixtures of MVi/Luxembourg. Lux/80[A] and MVi/Hetauda.NEP/2.99 [D8] and in two different concentrations (A 25/D8 25 and A 30/D8 25), the mean Δ Ct were 3.3 (SD 0.3) and 5.1 (SD 0.5), respectively (Fig. 2a, Table 1). Similar results were obtained when experiments were performed by another operator (data not shown). With mixes of MVi/Khartoum. SUD/34.97/2 [B3] and MVi/Luxembourg.Lux/80 [A] (B3 25/A 30), the mean Δ Ct was 4.9 (SD 0.8) (Fig. 2b, Table 1). Furthermore, similar results were obtained with experimental mixtures of recent clinical samples: a Δ Ct value of 6.9 (SD 0.6) and 7.4 (SD 1.1) for MeV D8/A and B3/A respectively, while the mean Δ Ct value of MeVA only was 3.0 (SD 0.4) (Fig. 2c, Table 1).

Selection of cut-off values for use of the Δ Ct

The calculated Δ Ct values of all individual experiments of both clinical samples and experimental mixtures were used in the ROC analysis, resulting in 91 MeVA only and 59 MeVA/MeVwt mix datapoints (Fig. 3a). The area under the ROC curve was 0.97 with a P -value < 0.0001 (Fig. 3b). The highest cut-off value of the Δ Ct with a sensitivity of 1.00 was 2.86 (specificity 0.59), while the lowest cut-off value of the Δ Ct with a specificity of 1.00 was 3.67 (sensitivity 0.85). The optimum of both specificity and sensitivity was at Δ Ct=3.54 with a sensitivity of 0.90 and specificity of 0.98 (Fig. 3c).

DISCUSSION

In the present study, we demonstrated that the method described by Roy and colleagues [19] can not only be used to differentiate MeVA from MeVwt, but also to a certain extent to exclude the presence of MeVwt if MeVA is detected. The results of these experiments indicated that Δ Ct values between the MeV RT-qPCR and the MeVA RT-qPCR were up to 2.85 for genotype A alone and above 3.67 when mixed with MeV genotype B3 or D8. Between 2.86 and 3.67, co-infection cannot be excluded although at 3.54 the sensitivity was still 0.90 according to ROC analysis.

Based on results of our study, we propose the following protocol that could be used for laboratory testing of measles cases with a history of recent vaccination that have also been exposed to measles virus: (i) screen for measles using the routine MeV RT-qPCR and if positive with a Ct value <35, (ii) run MeVA RT-qPCR in triplicate on the clinical sample including positive control samples of genotype A and genotype A mixed with two commonly circulating strains in The Netherlands (MeVA 30, MeVA 30/MeVwt B3 25, MeVA 30/MeVwt D8 25), (iii) determine the Δ Ct values comparing the two assays and use the controls to confirm the Δ Ct thresholds (Fig. 4).

Ideally, there would be a clear, single cut-off value between the Δ Ct of samples with MeVA only and with both MeVA and MeVwt. However, in mixtures with a relatively high viral load of both MeVA and MeVwt (MeVA 25/MeVwt D8 25), Δ Ct values were relatively low compared to mixtures with a lower viral load of MeVA. Although it can be expected that in most clinical cases, the viral loads of MeVA will be lower than MeVwt, it cannot be excluded that both have a similar and high viral load and therefore two cut-off values and a grey zone were included in our protocol.

To our knowledge, no measles cases have been reported in which co-infection occurred with MeVA and MeVwt. Likewise, there was no indication of the presence of both MeVA and MeVwt in historical samples available at the RIVM in which MeVA was confirmed by genotyping. Therefore, we could not validate our protocol on clinical samples with both MeVA and MeVwt, but had to prepare experimental mixtures with concentrations that we might expect in clinical samples.

In conclusion, the assay described by Roy and colleagues could be expanded by calculating Δ Ct values and setting thresholds for determination of MeVA and MeVwt co-infections. Implementing this additional RT-qPCR-based protocol could be useful for quick and cost-saving laboratory diagnosis in recently vaccinated cases that might also have been exposed to MeVwt.

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Author contributions

K.R.S. methodology, investigation, formal analysis, visualisation, writing – original draft, writing – review and editing. R.H.G.K. methodology, investigation, validation, formal analysis, visualisation, writing – review and editing. R.B. conceptualization, methodology, data curation, formal analysis, visualisation, project administration, supervision, writing – original draft, writing – review and editing.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

Genotyping of measles viruses, including samples analyzed for this study, is part of the Public Health Act (BWBR0024705) in The Netherlands (<https://wetten.overheid.nl/BWBR0024705/2021-02-22/0/informatie>).

References

1. International Committee on Taxonomy of Viruses. Virus taxonomy: 2020 release [updated Mar 2021]. 2020. <https://talk.ictvonline.org/taxonomy/>
2. Moss WJ. Measles. *Lancet* 2017;390:2490–2502.
3. Di Pietrantonj C, Rivetti A, Marchione P, Debalini MG, Demicheli V. Vaccines for measles, mumps, rubella, and varicella in children. *Cochrane Database Syst Rev* 2020;4:CD004407.
4. World Health Organization. Measles: key facts 2019 [updated 5 Dec 2019]. 2019. <https://www.who.int/news-room/fact-sheets/detail/measles>
5. World Health Organization. Global vaccine action Plan 2011–2020. 2013. <https://www.who.int/publications/i/item/global-vaccine-action-plan-2011-2020>
6. World Health Organization. Measles and Rubella strategic framework 2021–2030. 2020. <https://www.who.int/publications/i/item/measles-and-rubella-strategic-framework-2021-2030>
7. World Health Organization. Surveillance for Vaccine Preventable Diseases (VPDs). Provisional monthly measles and rubella data 2021 [updated 2021]. . https://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/active/measles_monthlydata/en/
8. Datta SS, O'Connor PM, Jankovic D, Muscat M, Ben Mamou MC, et al. Progress and challenges in measles and rubella elimination in the WHO European Region. *Vaccine* 2018;36:S0264-410X(17)30829-0:5408–5415..
9. Berggren KL, Tharp M, Boyer KM. Vaccine-associated “wild-type” measles. *Pediatr Dermatol* 2005;22:130–132.
10. Sood SB, Suthar K, Martin K, Mather K. Vaccine-associated measles in an immunocompetent child. *Clin Case Rep* 2017;5:1765–1767.
11. Murti M, Krajdien M, Petric M, Hiebert J, Hemming F, et al. Case of vaccine-associated measles five weeks post-immunisation, British Columbia, Canada, October 2013. *Euro Surveill* 2013;18:20649.
12. Nestibo L, Lee BE, Fonseca K, Beirnes J, Johnson MM, et al. Differentiating the wild from the attenuated during a measles outbreak. *Paediatr Child Health* 2012;17:e32–3.
13. Gastañaduy PA, Banerjee E, DeBolt C, Bravo-Alcántara P, Samad SA, et al. Public health responses during measles outbreaks in elimination settings: Strategies and challenges. *Hum Vaccin Immunother* 2018;14:2222–2238.
14. Greenwood KP, Hafiz R, Ware RS, Lambert SB. A systematic review of human-to-human transmission of measles vaccine virus. *Vaccine* 2016;34:S0264-410X(16)30089-5:2531–2536..
15. World Health Organization. *Manual for the Laboratory-based Surveillance of Measles, Rubella, and Congenital Rubella Syndrome (Third edition)*. 2018.
16. Penedos AR, Myers R, Hadeef B, Aladin F, Brown KE. Assessment of the utility of whole genome sequencing of measles virus in the characterisation of outbreaks. *PLoS one* 2015;10:e0143081.
17. Jaafar R, Zandotti C, Grimaldier C, Etoundi M, Kadri I, et al. Epidemiological and genetic characterization of measles virus circulating strains at Marseille, France during 2017–2019 measles outbreak. *J Infect* 2021;83:S0163-4453(21)00352-2:361–370..
18. Gardy JL, Naus M, Amlani A, Chung W, Kim H, et al. Whole-Genome Sequencing of Measles Virus Genotypes H1 and D8 During Outbreaks of Infection Following the 2010 Olympic Winter Games Reveals Viral Transmission Routes. *J Infect Dis* 2015;212:1574–1578.
19. Roy F, Mendoza L, Hiebert J, McNall RJ, Bankamp B, et al. Rapid identification of Measles virus vaccine genotype by real-time PCR. *J Clin Microbiol* 2017;55:735–743.
20. Tran T, Kostecki R, Catton M, Druce J, Tang Y-W. Utility of a stressed single nucleotide polymorphism (SNP) real-time PCR assay for rapid identification of measles vaccine strains in patient samples. *J Clin Microbiol* 2018;56:e00360-18:18..
21. Pabbaraju K, Gill K, Wong AA, Tipples GA, Hiebert J, et al. Simultaneous detection and differentiation between wild-type and vaccine measles viruses by a multiplex real-time reverse transcription-pcr assay. *J Clin Microbiol* 2019;57:e01828-18:18..
22. European Centre for Disease Prevention and Control. Factsheet about measles. <https://www.ecdc.europa.eu/en/measles/facts>
23. Public Health England. Guidelines on post-exposure prophylaxis for measles [Last update 4 Jul 2019]. 2009. <https://www.gov.uk/government/publications/measles-post-exposure-prophylaxis>
24. National Institute for Public Health and the Environment (RIVM). LCI Mazelen Richtlijn 2020. 2020.
25. Australian Government Department of Health. Australian Immunisation Handbook [Last update 12 Apr 2019]. 2019. <https://immunisationhandbook.health.gov.au/resources/handbook-tables/table-post-exposure-prophylaxis-needed-within-72-hours-of-1st-exposure-for>
26. Bankamp B, Takeda M, Zhang Y, Xu W, Rota PA. Genetic characterization of measles vaccine strains. *J Infect Dis* 2011;204:S533–S548.
27. Truong AT, Mulders MN, Gautam DC, Ammerlaan W, de Swart RL, et al. Genetic analysis of Asian measles virus strains – new endemic genotype in Nepal. *Virus Res* 2001;76:71–78.
28. Klingele M, Hartter HK, Adu F, Ammerlaan W, Ikusika W, et al. Resistance of recent measles virus wild-type isolates to antibody-mediated neutralization by vaccinees with antibody. *J Med Virol* 2000;62:91–98.
29. el Mubarak HS, Van De Bildt MW, Mustafa OA, Vos HW, Mukhtar MM, et al. Serological and virological characterization of clinically diagnosed cases of measles in suburban Khartoum. *J Clin Microbiol* 2000;38:987–991.
30. Ono N, Tatsuo H, Hidaka Y, Aoki T, Minagawa H, et al. Measles viruses on throat swabs from Measles patients use signaling lymphocytic activation molecule (CDw150) but not CD46 as a cellular receptor. *J Virol* 2001;75:4399–4401.
31. Bodewes R, Reijnen L, Zwagemaker F, Kohl RHG, Kerkhof J, et al. An efficient molecular approach to distinguish chains of measles virus transmission in the elimination phase. *Infect Genet Evol* 2021;91:S1567-1348(21)00091-5:104794..
32. Hübschen JM, Kremer JR, De Landtsheer S, Muller CP. A multiplex TaqMan PCR assay for the detection of measles and rubella virus. *J Virol Methods* 2008;149:246–250.
33. Greiner M, Pfeiffer D, Smith RD. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. *Prev Vet Med* 2000;45:23–41.