Performance Assessment of a Lab Developed Multiplex RT-PCR-Based Test for Measles (Rubeola)

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INTRODUCTION

Measles Virus (MeV) is a highly infectious respiratory pathogen and causes systemic infections, especially in immunocompromised and unvaccinated populations. The Measles outbreak in 2025 was the highest in the last 25 years[1]. Multiplex PCR testing, due to its high specificity, sensitivity and rapid turnaround of results can play a key role in early and accurate detection of measles which becomes crucial due to the syndromic nature of respiratory infections, and diagnosis based on clinical presentation alone can be challenging[2,3]. We have developed a multiplex PCR assay for the detection of MeV and the discrimination of wild type and vaccine strains. This study presents the analytical and clinical performance of the assay.

MATERIAL & METHODS

Primer and probe sets were developed to detect pan-Measles virus (MeV), the MeV vaccine strain, and a human internal control within a single-well multiplex PCR reaction. Analytical validation was conducted using 180 clinical samples, including both spiked positive and negative patient specimens. For each specimen type— oropharyngeal/throat swabs, nasopharyngeal/nares swabs, and cough sputum—20 samples were spiked with the MeV wild-type strain, 20 with the vaccine strain, and 20 were left un-spiked as negatives. To evaluate potential assay interference, 18 different substances were tested. Performance characteristics assessed included the limit of detection (LOD), positive predictive agreement (PPA), negative predictive agreement (NPA), cross-reactivity, and the impact of interfering substances on the detection of both wild-type and vaccine strains of MeV. All samples were suspended in PrimeStore® MTM (Longhorn Diagnostics, Bethesda, MD) PCR reactions were performed using the QuantStudio™ 5 Real-Time PCR System (ThermoFisher Scientific) with TaqMan® chemistry.

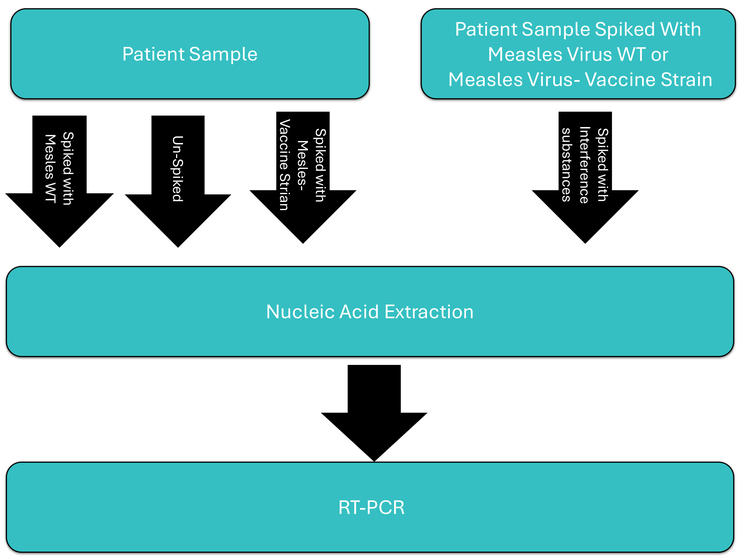

Figure 1. Study design

RESULTS

The LOD for both the pan-Measles and vaccine strain targets was established at 1.0 x 10^1 copies/µL. Clinical samples were spiked at 3x the LOD of wild-type Measles virus, and the observed detection rates were 100% across all specimen types—nasopharyngeal swabs, cough sputum, and oropharyngeal swabs—with 95% confidence intervals (CI) of 91.2%–100%. For clinical samples spiked with 3x the LOD of the Measles vaccine strain, all specimen types again showed 100% detection, with a 95% CI of 83.9%–100%. No cross-reactivity was observed from background pathogens present in the clinical matrix, and none of the 18 substances tested showed any interference with assay performance. The assay demonstrated 100% discrimination between MeV wild-type and vaccine strains.

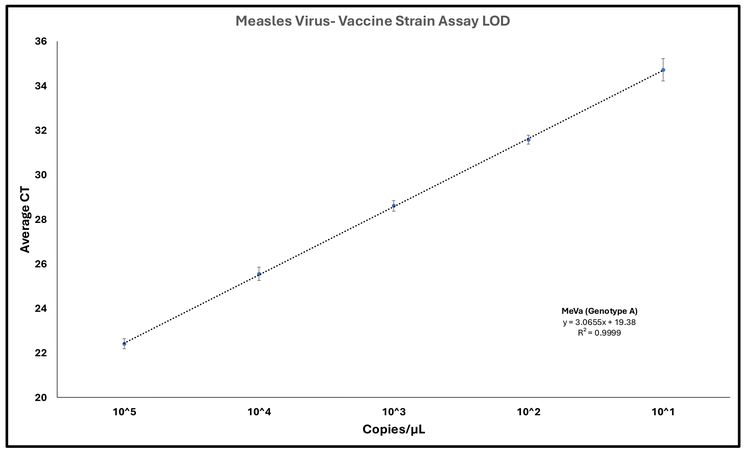

Figure 2. The Limit of Detection (LOD) for Measles Virus Vaccine Strain . The LOD for the Measles Virus Vaccine Strain LOD was 10^1 copies/µL.

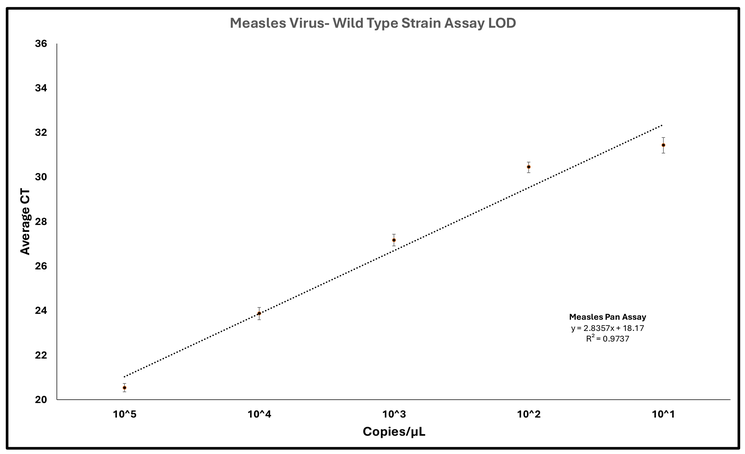

Figure 3. The Limit of Detection (LOD) for Measles Virus Wild Type Strain . The LOD for the Measles Virus Wild Type Strain LOD was 10^1 copies/µL.

Table 1. Pathogens present in samples screened for cross-reactivity. No cross-reactivity was observed in respect to these targets.

Target	Number of samples
Adenovirus	4
Bordetella spp.	1
Coronavirus 229E, NL63	3
Enterovirus D68	1
Epstein-Barr Virus	2
Fusobacterium spp.	2
Haemophilus Influenzae	4
Human metapneumovirus	1
Influenza A virus	1
Influenza B virus	2
Klebsiella pneumoniae, oxytoca	2
Moraxella catarrhalis	2
Mycoplasma pneumoniae	3
Parainfluenza virus (1-4)	1
Respiratory syncytial virus B	1
Rhinovirus	8
SARS-CoV-2	7
Staphylococcus aureus	3
Streptococcus pneumoniae	3

Table 2. Substances added to sample to test for interference. No interference was observed in respect to these substances.

Interfering Substance	Result
Matrix Alone	No change
Blood	No change
Chewing Gum	No change
Petroleum Jelly	No change
Vicks Inhaler	No change
Throat Lozenges	No change
Flonase Nasal Spray	No change
Chloraseptic Throat Spray	No change
Afrin Nasal Spray	No change
Nyquil	No change
Dayquil	No change
Benadryl	No change
Toothpaste	No change
Mouthwash	No change
Alcohol (5%)	No change
Orange Juice	No change
Emergencee	No change
Coffee	No change
NeilMed Sinus Rinse	No change

Table 3. Detection rate of Measles virus – wild type strain and Measles virus – vaccine strain in nasopharyngeal, oropharynx, and cough sputum swabs on the multiplex RT-PCR assay.

Target	Swab Type	Detection Rate
Measles virus – Wild Type	Nasopharyngeal/Nares	100% (95% CI: 91.2%-100%)
	Oropharynx/Throat	100% (95% CI: 91.2%-100%)
	Cough Sputum	100% (95% CI: 91.2%-100%)
Measles virus – Vaccine Strain	Nasopharyngeal/Nares	100% (95% CI: 83.9%-100%)
	Oropharynx/Throat	100% (95% CI: 83.9%-100%)
	Cough Sputum	100% (95% CI: 83.9%-100%)

CONCLUSIONS

In light of the recent and most severe measles outbreak in the United States since 2000, the availability of a rapid diagnostic test capable of distinguishing between wild-type infections and vaccine-related events is critical for effective outbreak surveillance and timely clinical intervention.

REFERENCES

1. CDC. Measles Cases and Outbreaks. Accessed 9-05-25.
2. Strebel PM, Orenstein WA. Measles. N Engl J Med. 2019;381(4):349-357.
3. CDC. Laboratory Testing for Measles. Accessed 9-05-25.