



## *I. Background*

The Colorado Wastewater Surveillance Program initiated a pilot phase for measles testing on May 1, 2025. During this period, results were restricted to internal teams while additional data were collected to support validation of the measles assays. The Colorado Department of Public Health and Environment (CDPHE) Laboratory brought on two assays to distinguish between wild-type and vaccine-derived measles strains. This is critical to ensure that detections are not attributable to shedding from the measles, mumps, and rubella (MMR) vaccine.

A primary objective of the pilot phase was to generate sufficient data to enable the laboratory to determine a more sensitive limit of detection. Instrument manufacturer-recommended limits of detection are often conservative; therefore, empirical data collected during the pilot allowed for refinement of this threshold. As a result, the limit of detection for the measles assay was lowered, optimizing the sensitivity of the testing approach and decreasing the threshold for public health response.

Following completion of the pilot phase, measles results were shared with local public health agencies (LPHAs) and subsequently made available on our public dashboard.

During the pilot period, wastewater surveillance data were also used to support a measles outbreak investigation in Colorado.<sup>1</sup> This experience underscored the need for clearly defined notification protocols and standardized communication materials to streamline and strengthen the public health response for future outbreaks.

## *II. Definitions*

**Limit of detection (LoD):** LoD is the lowest analyte concentration likely to be reliably distinguished from the Limit of Blank (LoB) and at which detection is feasible.<sup>2</sup>

**Assay:** A highly sensitive, end-point nucleic acid quantification method that partitions a polymerase chain reaction (PCR) mixture into thousands of individual micro reactions (droplets or wells) to detect target DNA/RNA.<sup>3</sup>

**Quantitative polymerase chain reaction (qPCR):** A laboratory technique that calculates the exact amount of target DNA or RNA by amplifying and



simultaneously detecting or quantifying a targeted DNA molecule by using fluorescent dyes or probes.<sup>3,4</sup>

**Digital polymerase chain reaction (dPCR):** A molecular technique for absolute quantification of DNA or RNA. It partitions a sample into thousands of individual micro reactions, amplifying the target, and using binary endpoints to calculate exact copy numbers without standards, offering higher precision and sensitivity than qPCR.<sup>3</sup>

**Wild-type measles:** The naturally occurring, circulating strain of the measles virus, distinct from the weakened (attenuated) vaccine strain.<sup>4</sup>

**Vaccine-derived measles:** Vaccine-derived measles in wastewater refers to the detection of the live attenuated virus (genotype A) used in the measles, mumps, and rubella (MMR) vaccine, which is shed in the urine and feces of individuals recently vaccinated (this can occur for up to four weeks after vaccine administration). This is distinct from wild-type measles, which causes active, transmissible infection.<sup>5</sup>

### *III. Communication tools*

- ❖ Measles response flowchart
  - The flowchart outlines the actions taken for measles wastewater detection. (Appendix A).
- ❖ LPHA measles notification template
  - A template used to notify LPHAs when measles is detected at a utility in their jurisdiction. (Appendix B).
- ❖ Utility communication template
  - A template used to notify utility staff when measles is detected at their utility. (Appendix C).
- ❖ LPHA measles provider messaging template
  - A template that LPHAs can send to local providers, alerting them of recent measles detections in the wastewater. (Appendix D).

### *IV. Conclusions*

The development of a standardized communication package for measles wastewater surveillance represents a critical step in strengthening public health preparedness and response. By translating complex environmental data into clear, actionable information, this plan supports timely decision-making across public health agencies, local providers, and other wastewater surveillance jurisdictions. By improving the standardization and



availability of communication materials/resources across jurisdictions, this plan also supports data interpretation for public stakeholders.

As wastewater surveillance continues to expand to new targets, the tools and frameworks established through this effort will provide a scalable foundation for future communication packages. Standardized templates, notification protocols, and data interpretation guidance will help ensure consistency, improve coordination among stakeholders, and prevent delays in response during emerging public health events.

## V. References

<sup>1</sup>Jensen GM, Gidfar C, Weisbeck K, et al. *Notes from the Field: Wastewater Surveillance for Measles Virus During a Measles Outbreak — Colorado, August 2025*. *MMWR Morb Mortal Wkly Rep* 2026;75:20–22. DOI:

<http://dx.doi.org/10.15585/mmwr.mm7502a2>

<sup>2</sup> Armbruster DA, Pry T. Limit of blank, limit of detection and limit of quantitation. *Clin Biochem Rev*. 2008;29(Suppl 1):S49–S52.

<https://pmc.ncbi.nlm.nih.gov/articles/PMC2556583/>

<sup>3</sup>Centers for Disease Control and Prevention. *Chapter 7: Measles*. In: *Manual for the Surveillance of Vaccine-Preventable Diseases*. Centers for Disease Control and Prevention Published June 3, 2025.

<https://www.cdc.gov/surv-manual/php/table-of-contents/chapter-7-measles.html>

<sup>4</sup>Dymond JS. Explanatory chapter: quantitative PCR. *Methods Enzymol*. 2013;529:279-89. doi: 10.1016/B978-0-12-418687-3.00023-9.

<https://pubmed.ncbi.nlm.nih.gov/24011054/>

<sup>5</sup>World Health Organization. *WES summary for measles*. World Health Organization Published December 1, 2023.

<https://cdn.who.int/media/docs/default-source/wash-documents/wash-related-diseases/wes-summary-for-measles---final-1-dec-v3.pdf>



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## Appendix A

- Measles response flowchart



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**Appendix B**

- [LPHA notification template \(for WWS programs to send to LPHAs\)](#)



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## Appendix C

- [Utility notification template \(for WWS programs to send to utilities\)](#)



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## Appendix D

- [LPHA provider templates \(for LPHAs to send to local clinicians about WWS data\)](#)