

# MLS Laboratory Update: *Vibrio* Speciation using Whole Genome Sequencing Method

JANUARY 18, 2024

## Purpose of this Message:

To inform clinical laboratories that MDH-PHL will begin to report the identification of *Vibrio* species using whole genome sequencing (WGS) as the primary method, replacing the conventional biochemical approach on January 18<sup>th</sup>, 2024. This change is accompanied by modifications in the report format for improved clarity and detailed insights.

## Action Item:

Please share this information with other laboratorians and clinical partners at your site.

## Background:

MDH-PHL has been sequencing various foodborne associated bacteria such as *Salmonella*, *Escherichia coli*, *Campylobacter* spp. and *Vibrio* spp. since 2019. The utilization of WGS analysis allows for precise determination of species, serotype, subtype and identification of virulence factors and antimicrobial resistance determinants.

- Starting January 18<sup>th</sup>, 2024, MDH-PHL will report *Vibrio* species results using WGS to laboratories submitting *Vibrio* spp. isolates or isolates that MDH has cultured from submitted specimens found to be positive for *Vibrio* spp. by a culture-independent diagnostic test (e.g, BioFire FilmArray GI panel or Verigene Enteric Pathogens test).
- The target turnaround time for reporting species information is 7-10 days from the receipt of isolates or isolates grown from culture.
- Clinical laboratories will receive an initial result indicating the confirmation or absence of *Vibrio* spp. using culture and MALDI-TOF mass spectrometry.
- When WGS analysis is completed, clinical laboratories will receive the final results (**Attachment1**), including genus and species information for the submitted *Vibrio* spp., along with serotype details for *Vibrio cholerae* (Vc), if identified. Serotyping method for Vc will be slide-agglutination (O1 and O139) not WGS.
- For *Vibrio cholerae*, MDH-PHL will send isolates to the CDC Enteric Reference Laboratory for toxin testing, with an estimated turnaround time of 13 weeks. Upon receipt of the CDC's results, the entire report from CDC will be sent to the submitting laboratories (**Attachment2**). On the CDC report, the Vc toxin (*ctxA*) results are listed under the Nucleic

acid-based test results. The information that contained in the CDC's report is explained below in the Additional Information section.

## Additional Information:

1. CDC reports will contain:
  - a. Phenotypic characterization
    - i. Serogroup: O1, O139, O141 and O75.
    - ii. Sucrose fermentation: Negative or positive
    - iii. TCBS agar: Green or Yellow colonies
    - iv. Hemolysis on TSA w/ 5% Sheep blood: Growth beta-hemolysis or no hemolysis or no growth
  - b. Nucleic acid-based test results
    - i. Identification using WGS and ANI: *Vibrio* spp.
    - ii. PCR results on *toxR*, *ompW*, *ctxA*, *tcpA* Classical and *tcpA* El Tor: positive or negative
      - \**toxR*, *ompW* : species-specific markers unique to Vc.
      - \**ctxA* : encoding cholerae toxin. This marker can differentiate between toxigenic and non-toxigenic Vc.
      - \**tcpA* Classical or *tcpA* El Tor: The O1 serogroup Vc has been classified into these two biotypes, distinguishable by *tcpA* alleles. While *tcpA* classical was associated with the first six pandemics, *tcpA* El Tor is responsible for the current seventh pandemic. \*\*Limitation: The CDC uses a molecular method, which implies that a positive PCR results for El Tor does not conclusively confirm the strain as an El Tor biotype. Additional phenotypic tests are essential for a conclusive determination of the El Tor biotype.
2. MDH Sequencing will be generated with a short-read based sequencing platform and analyzed using SPAdes<sup>a</sup> and ANI tool<sup>b</sup> for de-novo assembly and species determination, respectively.
3. References
  - a. [SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing - PMC \(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3342519/\)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3342519/).
  - b. [Genomic insights that advance the species definition for prokaryotes - PubMed \(https://pubmed.ncbi.nlm.nih.gov/15701695/\)](https://pubmed.ncbi.nlm.nih.gov/15701695/).

**Questions:** Please contact Jisun Haan, Ph.D, Enterics Unit Supervisor, at [jisun.haan@state.mn.us](mailto:jisun.haan@state.mn.us) or 651-201-5041 with any questions or concerns.

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