Investigation of potential false positivity for *Cryptosporidium* detected by the BioFire FilmArray Gastrointestinal Panel

CSTE Stories FROM THE Field

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CATEGORY: **Epidemiology and Laboratory Capacity (ELC)**

The Nebraska Public Health Laboratory used new interim guidance from the BioFire® Gastrointestinal Panel manufacturer to evaluate *Cryptosporidium* sequencing to ensure accurate test results.

The "What"

The BioFire® Gastrointestinal Panel (BioMérieux) uses multiplex polymerase chain reaction (PCR) technology to detect 22 pathogens in one assay including the parasite *Cryptosporidium*, which is detected using 2 molecular targets, Crypt 1 and Crypt 2. Recently, the manufacturer reported what appears to be non-specific amplification of the Crypt 2 target and now recommends that the user monitor the results of amplification for the 2 targets and confirm with another method if only the Crypt 2 target was positive, and a suspicion of a false-positive result was being considered.

The Nebraska Department of Health and Human Services State Public Health Laboratory (NPHL) uses Epidemiology and Laboratory Capacity for the Prevention and Control of Emerging Infectious Diseases (ELC) funding to purchase and maintain instruments and ensure staffing to perform testing. Proper use and understanding of results is necessary as laboratorians and epidemiologists collaborate to conduct disease surveillance and prevention activities.

All stool samples with *Cryptosporidium* detected are submitted to NPHL for sequencing for participation in the federal CryptoNet program. For this analysis, extracted DNA were amplified for 18S rRNA and subjected to nested PCR to produce a product of 800–850 bp. This product was separated by electrophoresis on an agarose gel, purified, and sequenced by Sanger sequencing. Trimmed sequences were subsequently compared against reference strains provided by the Centers for Disease Control and Prevention (CDC) to determine species. BioFire® FIREWORKS™ was used to evaluate melt curves and amplification plots for the two targets and correlate with sequencing data.

Sequence-negative stools had atypical melt curves, higher melt temperatures than sequence-positive stools, and/or late amplification. These results showed that the **Crypt 2 target was required to identify *C. felis*** and that **melt curve and amplification plot analysis can be useful in discerning suspected false-positive results** from the Crypt 2 target.

The "So What"

A total of 36 *Cryptosporidium*-positive stools were included in this evaluation. Twenty-one (21) stools were negative for the Crypt 1 target and positive for the Crypt 2 target. Of these, 12 did not sequence to identify species, and 9 were identified as C. *felis* following sequence analysis. Fifteen (15) stools were positive for both the Crypt 1 and Crypt 2 targets. These were identified as C. *parvum* (10), C. *hominis* (3), and C. *chipmunk*-genotype 1 (2) by sequencing. None (0) of the stools were positive for the Crypt 1 target only.

The "Now What"

Analysis using BioFire® FIREWORKS™ showed that sequence-negative stools had atypical melt curves, higher melt temperatures than sequence-positive stools, and/or late amplification. These results showed that the Crypt 2 target was required to identify C. *felis* and that melt curve and amplification plot analysis can be useful in discerning suspected false-positive results from the Crypt 2 target. As the manufacturer closely monitors this situation, all the various species noted as causes of cryptosporidiosis will be important to consider prior to developing a software update to this assay. ELC funding maintains NPHL's ability to monitor this situation, implement necessary changes, and communicate accurate results to public health partners working to prevent the spread of cryptosporidiosis and other communicable diseases.

Key contributors to this project include Amanda Bartling, Emily McCutchen, Amy Roden, all with Nebraska Public Health Laboratory; and Dr. Yolande Chan, University of Nebraska Medical Center.

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