Yersinia *enterocolitica* Detection by the BioFire® FilmArray® Gastrointestinal (GI) Panel

1. Introduction

Yersinia *enterocolitica* is among the 22 organisms detected by the BioFire GI Panel. The purpose of this technical note is (1) to provide background information about Y. *enterocolitica*, (2) to describe detection methods including the BioFire GI Panel and (3) to highlight the potential causes of discrepant test results.

2. Y. *enterocolitica*

Y. enterocolitica are gram-negative bacillus-shaped aerobic or facultative anaerobic bacteria, and members of the genus *Yersinia* that belong to the *Enterobacteriaceae* family. Of the approximately 11 *Yersinia* species, *Y. enterocolitica*, *Y. pseudotuberculosis,* and *Y. pestis* are considered the primary human pathogens[1]. Clinical symptoms of a yersiniosis infection range from self-limiting gastroenteritis to acute enteritis (particularly in young children). In older children and adults, the predominant symptoms are right-sided abdominal pain and fever, which may be confused with appendicitis[2]. Symptoms typically develop 4 to 7 days after exposure and may last 1 to 3 weeks or longer. In rare cases, complications include urinary and respiratory tract infections, skin rashes, and bacteremia[3,10].

Y. enterocolitica are psychrotrophic bacteria, which have the unusual ability to thrive at refrigeration temperatures and to survive extended periods of time in frozen foods even after repeated freezing and thawing cycles[4,5]. Due to this ability, *Y. enterocolitica* can be acquired as a result of ingestion of contaminated foods, primarily raw or undercooked pork, or by direct contact with a person who has recently prepared it [6,7]. Additional routes of transmission include consumption of contaminated water or unpasteurized lactose products[4,8], animal-to-human contact[9], blood transfusions[10], and as a hospital-acquired infection[11].

There is no effective method to differentiate pathogenic and non-pathogenic strains *of Y. enterocolitica. Y. enterocolitica* consists of a heterogeneous group of strains encompassing six biotypes: 1A, 1B, 2, 3, 4, and 5. Each biotype can have multiple O serotypes, leading to more than 50 distinct serotypes[12]. Biotypes 1B, 2, 3, 4, and 5 are generally considered pathogenic by two identified mechanisms: the presence of the virulence genes within plasmid pYV and/or by production of heat-stable enterotoxin, which is controlled by multiple chromosomal genes[13]. However, the pathogenic mechanism of yersiniosis is not completely understood and the distribution of these virulence-associated genes does not differ significantly between pathogenic and non-pathogenic strains[13-15]. Furthermore, biotype 1A, considered non-pathogenic due to the lack of such virulence genes, has been reported to cause clinical symptoms similar to those caused by pathogenic biotypes [13-15].

Y. *enterocolitica* Detection Methods

The most widely used method for the isolation of *Y. enterocolitica* (both pathogenic and non-pathogenic species) is the use of Cefsulodin-irgasan-novobiocin (CIN) selective agar developed by Schiemann[17]. However, this method still lacks specificity as other *Enterobacteriaceae* species (*Aeromonas, Pantoea, Morganella, Serratia,* and other

Yersinia spp.) may grow on the plate. This can cause a missed detection if any of these organisms are selected for identification instead of *Y. enterocolitica*[4,18]. More recently, the Chromagar Yersinia (CAY) method developed by Renaud[19] was found to be as sensitive but significantly more specific than CIN agar in detecting potentially pathogenic *Y. enterocolitica* species. Molecular methods (e.g. RT-PCR and MALDI-TOF) are also effective tools for rapid identification of *Y. enterocolitica*[4,21].

The BioFire GI Panel has one assay (Yent) designed for the specific detection of *Y. enterocolitica* but is not intended to differentiate non-pathogenic from pathogenic strains. Some potential for cross-reactivity exists for *Yersinia kristensenii* and *Yersinia frederiksenii* species when present at high concentrations (> 1 x 10^8 CFU/mL). Both species, also considered human pathogens, are part of the *Y. enterocolitica* group and are difficult to differentiate from *Y. enterocolitica* by phenotypic/culture methods[21].

Potential sources of discordant *Y. enterocolitica* results between the BioFire GI Panel and other identification methods include:

- Known cross-reactivity (described above)
- Low levels of *Y. enterocolitica* within the sample tested:
  - Present within the clinical stool
  - As a result of reagent or environmental contamination

PCR detection of low levels of *Y. enterocolitica* within the sample

Molecular methods are widely recognized to be more sensitive than culture for identification of pathogens from clinical specimens. *Y. enterocolitica* is fastidious and can be outgrown by other *Enterobacteriaceae*, making isolation from stool specimens difficult. Another drawback is the longer incubation time needed for growth and identification. The low isolation rate of *Y. enterocolitica* in clinical specimens may be due to the limited sensitivity of culture methods as these sometimes require a concentration up to 10^6 CFU/mL for detection[4,16].

The BioFire GI Panel detects *Y. enterocolitica* when its target nucleic acid is present in the sample tested. The reproducibility of results from the BioFire GI Panel (and all PCR methods) is dependent on the levels of nucleic acid available for amplification. Discrepant results with other PCR methods can also occur due to sequence variations in regions targeted by the assays or by differences in chemistry, methodology, and analysis of each method. Analytical testing established the limit of detection for *Y. enterocolitica* to be approximately 5x10^4 CFU/mL for the BioFire GI Panel[21]. Results from clinical samples near or below this concentration may not be reproducible across BioFire® FilmArray® Pouches and Instruments.

PCR methods will identify nucleic acids independently of the viability of cells/organism present in a clinical specimen, leading to potential discrepancies with culture methods. In some cases, pre-enrichment of clinical samples, specifically cold enrichment, has been successful in increasing the detection of viable *Y. enterocolitica* organism[4,7].

Through multiple investigations, BioFire Diagnostics has confirmed the presence of *Y. enterocolitica* in some clinical specimens by sequencing of DNA amplicons generated by an independent PCR test. In addition, some customer laboratories have reported success in isolating the organism following cold-enrichment of clinical samples in which *Y. enterocolitica* was detected by the BioFire GI Panel.

PCR detection of *Y. enterocolitica* due to low-level contamination

Sensitive molecular methods, such as PCR, can detect small numbers of organisms introduced into clinical specimens as contaminants. It is important to note that while reagents used in testing and sample collection may

be free of viable organisms, they can potentially contain background nucleic acid. Molecular test methods will be sensitive to these contaminants.

Contamination introduced from the testing process
Low-level contamination can be introduced during collection, handling, storage, sample setup, and testing and can lead to erroneous results. *Y. enterocolitica* is a significant food-borne pathogen and its transmission has been documented from multiple environmental surfaces[7,9,16]. False positive results due to contamination can be greatly minimized by following recommended cleaning protocols and by the inclusion of appropriate negative controls.

Contamination from Cary Blair media
Cary Blair media, used for dilution and processing of clinical stools, is screened by manufacturers for viable organisms but may not be generally tested for nucleic acid contamination. The presence of nucleic acids at levels that can be detected by the BioFire GI Panel may lead to false positive test results.

Contamination from BioFire GI Panel kit reagents
BioFire Diagnostics' quality control for the BioFire GI Panel kit reagents involves screening for organism and nucleic acid contamination using a high-confidence statistical sampling of each lot of reagents and other kit components. However, extremely low levels or sporadic contamination events may remain undetected.

Due to potential sources of false positive or false negative results, it is important to always consider results from the BioFire GI Panel in conjunction with other clinical, laboratory, and epidemiological data to effectively determine a diagnosis for the patient. Particular caution should be taken when molecular test results appear to be discrepant with the epidemiology and clinical presentations of the patient.

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Technical Support Contact Information

BioFire is dedicated to providing the best customer support available. If you have any questions or concerns about this process, please contact the BioFire Technical Support team for assistance.

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