Vibrio Detection by the BIOFIRE® FILMARRAY® Gastrointestinal (GI) Panel

1. Introduction

Vibrio and *Vibrio cholerae* are among the 22 organisms detected by the BIOFIRE® FILMARRAY® GI Panel. The purpose of this technical note is to provide background information about *Vibrio* and *V. cholerae* and to describe their detection by the BIOFIRE® FILMARRAY® GI Panel and gold standard methods.

2. *Vibrio* and *Vibrio Cholerae*

Vibrio are motile, gram-negative, comma-shaped bacteria typically found in marine environments. Several species are capable of causing illness in humans, both extraintestinal (soft tissue infection, septicemia, eye, and ear infections) and intestinal. Gastrointestinal illness is most commonly associated with *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. fluvialis*, *V.mimicus*, or *V. alginolyticus*, and infections are associated with consumption of contaminated food, particularly in coastal regions[1].

V. cholerae is the only *Vibrio* species that causes endemic, epidemic, and pandemic cholera. There are three major subgroups of *V. cholerae*: *V. cholerae* O1, *V. cholerae* O139, and *V. cholerae* non-O1/non-O139, of which the O1 and O139 are associated with epidemic or pandemic cholera. The BIOFIRE® FILMARRAY® GI Panel detects, but does not differentiate, all three subgroups. Cholera is endemic in many parts of the world and new outbreaks often follow natural disasters or social upheaval, where the disease remains a significant cause of morbidity and mortality.

Classic cholera is characterized by passing copious amounts of watery diarrhea leading to extreme dehydration and death; however, only about one in 20 infected people will have severe disease. In fact, *V. cholerae* infection is most often asymptomatic or results in mild gastroenteritis[2].

In the US and EU, sporadic cases of cholera are seen in travelers returning from endemic areas. Most cases and outbreaks have also occurred due to ingestion of raw oysters, other seafood, juices from shellfish, and seawater[3]. Strains producing cholera toxin are most often associated with cholera infections; however, non-toxigenic strains have also been found to cause sporadic vibriosis[4]. According to the CDC surveillance data from 2019[4] non-toxigenic strains of *V. cholerae* are reported more frequently in the U.S. than toxigenic strains. *V. cholerae* causing vibriosis can be food-borne or acquired through exposure to affected bodies of water, marine wildlife, or handling contaminated seafood[5,6,7].

3. *Vibrio* Detection

The gold standard method for detecting gastroenteritis caused by *Vibrio* is recovery by stool culture. Routine enteric media can be used, but recovery is enhanced by the use of specific media, such as thiosulfate-citrate-bile salts-sucrose (TCBS) agar plates. When bacterial concentration is potentially low, enrichment with alkaline peptone water (APW) is recommended prior to plating the sample[8].

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The BIOFIRE® FILMARRAY® GI Panel has two assays for the detection of *Vibrio* species. One assay (*Vibrio*) targets the gyrB gene and detects, but does not differentiate, the *Vibrio* species that are most commonly implicated in gastroenteritis (*V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae*). The second assay (*Vchol*) is designed for sensitive and specific detection of the toxR regulator gene present in all strains of *V. cholerae* (including all serogroups and both toxigenic and non-toxigenic strains). A positive result for either assay will give a '*Vibrio* Detected' result. A '*Vibrio cholerae* Detected' result will only be reported when the Vchol assay is positive. Potential sources of discordant *Vibrio cholerae* results between the BIOFIRE® FILMARRAY® GI Panel and culture include:

- PCR detection of low-level *Vibrio cholerae*

Molecular methods are widely recognized to be more sensitive than culture for identification of pathogens from clinical specimens. While culture is the gold standard, culture methods may lack sensitivity and therefore, PCR has gained popularity. Culture recovery can be reduced because *Vibrio* spp. are particularly susceptible to drying and *Vibrio* can enter into a viable but nonculturable state[9,10]. Multiple species of *Vibrio* may also grow together in a stool culture, thus complicating detection and identification of all organisms present. Alam et. al[1]. studied a 2009 cholera outbreak in Bangladesh and reported that only 64% of suspected cases of cholera were positive by conventional culture methods. Of the culture negative samples in the study, 73% were positive when tested by molecular methods. Similarly, authors such as Guillaume 2023 and Ontweka 2016 have also improved detection using molecular methods[11,12].

bioMérieux conducted comprehensive investigations into a limited number of unexpected *Vibrio* and *Vibrio cholerae* detections. In mostcases, the amplification signal is weak and the detection is not reproducible. This is likely the result of a low level of organism (e.g. transient presence fromingesting of contaminated food or water), unknown weak cross reactivity, or low-level contamination. In a few cases, *Vibrio* or *Vibrio cholerae* have been successfully cultured, or a toxR gene was identified in non-cholerae *Vibrio* spp. Other cross-reactivity has not been identified.

- Misidentification of *Vibrio* species carrying toxR homology

Horizontal transfer of toxR between *Vibrio* species has been recorded in rare instances. The BIOFIRE® FILMARRAY® GI Panel Vchol assay may detect rare isolates of other *Vibrio* species (e.g. *V. harveyi*, *V. mimicus*, *V. vulnificus*, and *V. alginolyticus*) that have acquired a homolog of toxR. See the BIOFIRE® FILMARRAY® GI Panel Instructions for Use and BIOFIRE® FILMARRAY® Operator's Manuals for limitations of the procedure[13].

- Unrecognized cross-reactivity with other organisms present in specimens

Over 175 off-panel organisms have been tested to confirm the analytical specificity of the BIOFIRE® FILMARRAY® GI Panel. The *Vibrio* assay (*V. parahaemolyticus*/*V. vulnificus*/*V. cholerae*) may cross-react with *V. alginolyticus*, *V. fluvialis*, *V. mimicus* and *Grimontia* (formerly *Vibrio*) *hollisae*. The Vchol assay did not react with any of the tested organisms. However, it is anticipated that the Vchol assay will react with other *Vibrio* spp. carrying homologs of toxR. In silico sequence analysis does not predict cross-reactivity with known sequences; however, cross-reactivity with untested organisms remains possible.

- Contamination of sample or reagents with *Vibrio* organisms or nucleic acid

Sensitive molecular methods, such as PCR, can detect small numbers of organisms or copies of nucleic acid introduced into specimens during collection or handling, sample setup, or the manufacturing process. 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.

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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® FILMARRAY® GI Panel may lead to false positive test results. bioMérieux quality control for the BIOFIRE® FILMARRAY® 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.

It is therefore important to use test results, such as those from the BIOFIRE® FILMARRAY® GI Panel in conjunction with other clinical, laboratory, and epidemiological data to determine a diagnosis for the patient. Particular caution should be taken when the molecular test results appear to be at odds with the characteristics of the patient and their disease state.

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