A quality assurance plan for the Copan FecalSwab™ for use in combination with Biofire® FilmArray® GI Panel

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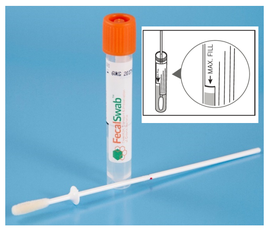
Background

Globally, *Vibrio* spp. and *Yersinia enterocolitica* are pathogens known to cause infectious gastroenteritis in humans. Historically, these pathogens have been detected by traditional culture in hospital laboratories. Recently, culture independent diagnostic tests (CIDTs) such as molecular diagnostics have been used to detect these pathogens. During 2018, a trend of false-positive *Vibrio* spp. and *Yersinia enterocolitica*, detected with CIDTs, occurred in stool samples stored in Cary-Blair media. A portion of this false positive trend was determined to originate from agar, a raw material used for the preparation of the media. While the Copan FecalSwab™ kit was not reported to be involved with false-positive cases related to this investigation, agar is still used as a raw material of the kit. The FecalSwab™ kit consists of a flocked swab for stool transfer or rectal swab collection and a tube with 2 mL of Cary-Blair medium for preservation, and can be used for both culture and nucleic acids-based assays.

Objective

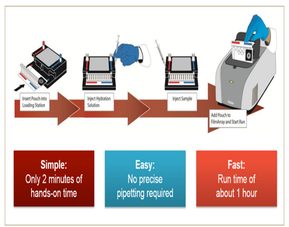
The objective of this study was to verify the feasibility of a molecular based quality assurance plan for the manufacturing process of the FecalSwab™. This quality assurance plan was designed to ensure with confidence that extraneous contaminant nucleic acids, often found in organic based raw materials like agar, is lower than detectable levels by CIDTs.

Materials



FecalSwab™

Seeweed: agar origin



Biofire® testing workflow

Methods

The quality assurance plan evaluated pilot lots of FecalSwab™ made with higher than nominal concentrations of agar. By testing lots made with higher concentrations of agar, the quality assurance testing plan can reduce the overall number of test replicates needed, while maintaining the statistical power to detect possible nucleic acid contaminants at levels lower than the typical limit of detection for CIDTs. Two pilot lots of FecalSwab™ were manufactured with increased concentrations of agar and tested with the BioFire GI Panel, a comprehensive CIDTs that detects both *Vibrio* spp. and *Yersinia enterocolitica* as well as 20 other common causes of gastroenteritis. One of the pilot lots was manufactured with 2.5X concentration of agar, and tested with 18 BioFire GI Panels. The other pilot lot was manufactured with concentration of 5X of agar, and tested with 13 BioFire GI Panels.
The test sampling plans provide 90% confidence that the CIDTs will detect contaminant nucleic acid less than 10% of the time. See Table 1. The test plan with increased concentrations reduces the number of samples required, making the quality assurance plan more feasible to implement.
If the lots made with higher concentrations of agar pass the proposed quality assurance plan, the lot of agar is confirmed to have lower than detectable levels of contaminant nucleic acid and can be used in the production of subsequent lots at the nominal concentration.

Results

Table 1: The sampling plans shown in the table below provide the minimum number of samples required to ensure with 90% confidence that the contamination rate of the lot of agar is less than 10% at the nominal concentration. The third column (Second round sampling plan) shows the minimum number of samples that need to be tested if one unexpected positive result is observed in the initial sampling. If no unexpected positive results are observed in the initial sampling plan, the lot of agar is qualified. If more than one unexpected positive result is observed in the initial sampling plan, or if a second unexpected positive result is observed in the second round sampling plan, the lot of agar is not qualified.

Concentration of Agar	Initial sampling plan	Second round sampling plan
Nominal	28	12
2.5X	18	6
5X	13	4

Results

The two pilot lots of FecalSwab™, even at higher concentrations, were able to be manufactured at the increased concentrations of agar (2.5X and 5X). Samples from these pilot lots were tested on the BioFire GI Panel according to the test plan. There were no positive results for all targets on the BioFire GI Panel observed in each test group, confirming these lots of agar has less than 10% contaminant nucleic acids with a 90% confidence.

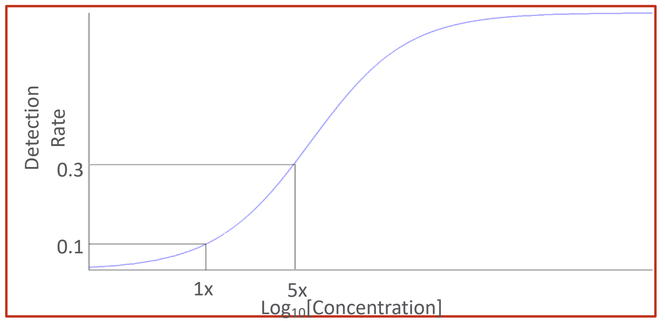


Figure 1: The sampling plans are calculated by using a binomial distribution. The example shown in the figure demonstrates that at the 5x concentration of agar, if we ensure the contamination rate is less than 30%, at the nominal (1x) concentration of agar, we ensure the contamination rate is less than 10%.

Conclusions

With increased usage of sensitive CIDTs for infectious gastroenteritis, the importance of media free of extraneous contaminant nucleic acids also increases. A quality assurance methodology has been investigated and shown to be feasible for ensuring that the raw materials going into Cary Blair medium have levels of contaminant nucleic acids below the limit of detection for common CIDTs, like the BioFire GI Panel.
Copan has implemented this quality assurance test plan in the manufacture of the FecalSwab™ product. This is the first Cary-Blair collection and preservation device certifying that testing was conducted to demonstrate that contamination due to extraneous nucleic acid is less than the limit of detection for CIDTs.

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