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Improved sensitivity and workflow utilising an modified pre-analytical methodology with the Serosep PCR EntericBio Gastro Panel 2

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Introduction

Gastrointestinal disease accounts for significant morbidity and mortality globally and may be caused by multiple agents, including bacterial, viral, and parasitic pathogens (Steiner *et al*., 2006). Rapid molecular multiplex testing has recently been introduced into enteric diagnostics and is positively impacting patient diagnosis and treatment for diarrhoeal diseases. However, one needs to take cognisance of the suboptimal performance of molecular assays for detecting Salmonella when compared to the typical selenite enrichment workflow (McAuliffe *et al*; 2017).

The enhancements of workflow direct from primary faecal collection devices which are “instrument ready” and require no further manipulation is the next logical progression. This coupled with pre-analytical automation could create a seamless workflow allowing for more efficiency gains in large diagnostic laboratories. The inability to obtain a stool specimen at the time of the patient visit can delay the diagnostic process and contribute to inappropriate treatment. The suitable of liquid collection devices for rectal sampling can tackle this issue particularly amongst children where a high incidence of morbidity and mortality exist.

The MWE Fecal Transwab® comes in a tube with 2 ml of modified Cary-Blair medium and a flocked or foam tip swab. While the Copan FLOQswab® is CE/IVD cleared for transport and use with the Serosep PCR EntericBio® Gastro Panel 2, neither the Copan Liquid collection device or the MWE Fecal Transwab® is. The objective of this study was to evaluate the MWE Fecal Transwab® for the simultaneous qualitative detection and identification of 6 GI pathogens, using the PCR EntericBio® Gastro Panel 2 (Serosep, Ireland) and the Roche LightCycler® 480 II system (Fig. 3).

Methods

A total of 38 known positive by culture (traditional culture methodology with a selenite enrichment for Salmonella) and by ELISA (Launch Crypto/Giardia) clinical stool samples were evaluated in this study. Twelve negative samples were included in the study. Samples were tested by the use of the EntericBio® Gastro Panel 2 and the Serosep work station. The panel consisted of targets for Salmonella, Shigella, and Campylobacter, verotoxin producing E.coli (VTEC), Giardia and Cryptosporidium (Launch Diagnostics Crypto/Giardia Combi EIA). Positive samples were selected in real time after following the standard protocol and re-tested utilising the modified protocol within 24 hrs.

System using two different protocols: the standard and the MWE Fecal Transwab® protocols.

Standard protocol with FLOQ swab

1. A FLOQ Swab is lightly coated with the stool/faecal sample, the swab is resuspended into a tube of SPS (Stool Preparation Solution), as recommended by the Serosep Entericbio manufacturer’s protocol.
2. Samples were inoculated onto an EntericBio Sample Processing Solution (S.P.S.) tube (Serosep) with a FLOQ Swab (Copan, Brescia, Italy), and the suspension was heated in a heating block (EntericBio heat station- QBD4; Grant Instruments, Shepreth, United Kingdom) at 103°C for 30 min to liberate the bacterial DNA.
3. The heat treated samples are placed on the EntericBio workstation for fully automated transfer of the processed samples directly to the lyophilised reaction wells using the EntericBio programme.
4. The wells are capped and transferred to real-time Roche Lightcycler® 480 instrument for automated amplification, detection and analysis with the EntericBio programme.

Alternative protocol with MWE Fecal Transwab®

1. A small amount of fresh stool was collected by insertion of the tip of the flocked or foam swab of the MWE Fecal Transwab® device into the stool sample and rotation of the swab.
2. The swab was carefully transferred into the MWE Fecal Transwab® device tube to ensure that the swab did not exceed the filling limit indicated on the label.
3. The vial was shaken until the sample appeared homogeneous and left for 12hrs at room temperature.
4. A pipette was used to transfer 100µL of homogenous suspension to EntericBio Sample Processing Solution (S.P.S.) tube (Serosep).
5. Samples were inoculated onto an EntericBio Sample Processing Solution (S.P.S.) tube (Serosep) with a FLOQ Swab (Copan, Brescia, Italy), and the suspension was heated in a heating block (EntericBio heat station- QBD4; Grant Instruments, Shepreth, United Kingdom) at 103°C for 30 min to liberate the bacterial DNA.
6. The heat treated samples are placed on the EntericBio workstation for fully automated transfer of the processed samples directly to the lyophilised reaction wells using the EntericBio programme.
7. The wells are capped and transferred to real-time Roche Lightcycler® 480 instrument for automated amplification, detection and analysis with the EntericBio programme.

Fig 1: EntericBio Heat Station

Fig 2: EntericBio Work Station

Fig 3: Roche Lightcycler® 480

Fig 4: MWE Fecal Transwab®

Results

Table 1: Summary of results comparing different pre-analytical methodology.

Culture Result	Result of culture	Copan Flog swab successfully detected	Medical Wire Flocked successfully detected	Medical Wire Foam successfully detected
Campylobacter sp	12	11	12	12
Salmonella sp	17	13	16	16
Shigella sp	5	5	5	5
Cryptococcus	1	1	1	1
Giardia	3	3	3	3
No pathogens	12	12	12	12
Totals	50	45	49	49

* zero inhibition

Fig 5: Image of Roche 480 Lightcycler® curves for a Salmonella run

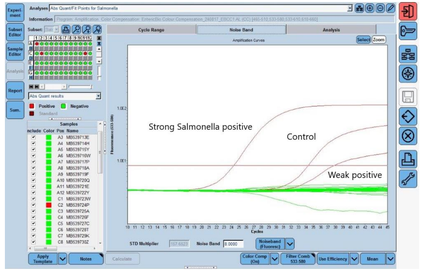

Table 2: Different sampling methodologies and associated reslt for the EntericBio system

Count	Culture Result	Copan Flog Swab Result	Copan Flog Swab CT	Medical Wire Flocked Alternative result	Medical Wire Flocked Alternative C	Medical Wire Foam Alternative resul	Medical Wire Foam Alternative CT
1	Campylobacter sp	Detected	41.63	Detected	36.35	Detected	38.92
2	Campylobacter sp	Detected	33.54	Detected	32.16	Detected	33.15
3	Salmonella sp	Detected	27.78	Detected	26.89	Detected	27.19
4	No pathogens	No pathogens detected		No pathogens detected		No pathogens detected	
5	No pathogens	No pathogens detected		No pathogens detected		No pathogens detected	
6	No pathogens	No pathogens detected		No pathogens detected		No pathogens detected	
7	Campylobacter sp	Detected	31.77	Detected	31.24	Detected	31.14
8	Campylobacter sp	Detected	27.3	Detected	26.93	Detected	27.11
9	Salmonella sp	No pathogens detected		No pathogens detected		No pathogens detected	
10	Salmonella sp	Detected	19.74	Detected	19.19	Detected	20.16
11	No pathogens	No pathogens detected		No pathogens detected		No pathogens detected	
12	No pathogens	No pathogens detected		No pathogens detected		No pathogens detected	
13	Campylobacter sp	No pathogens detected		Detected	37.5	No pathogens detected	
14	Campylobacter sp	Detected	29.96	Detected	29.5	Detected	28.9
15	Salmonella sp	Detected	19.79	Detected	18.29	Detected	18.98
16	No pathogens	No pathogens detected		No pathogens detected		No pathogens detected	
17	No pathogens	No pathogens detected		No pathogens detected		No pathogens detected	
18	Campylobacter sp	Detected	32.37	Detected	31.88	Detected	32.48
19	Campylobacter sp	Detected	27.18	Detected	25.91	Detected	25.11
20	Salmonella sp	Detected	20.41	Detected	22.65	Detected	21.61
21	Salmonella sp	Detected	19.64	Detected	19.68	Detected	20.38
22	No pathogens	No pathogens detected		No pathogens detected		No pathogens detected	
23	Salmonella sp	No pathogens detected		Detected	33.08	Detected	31.89
24	Salmonella sp	Detected	19.95	Detected	19.74	Detected	19.95
25	Salmonella sp	Detected	23.45	Detected	23.56	Detected	23.92
26	Salmonella sp	Detected	21.89	Detected	20.76	Detected	21.5
27	No pathogens	No pathogens detected		No pathogens detected		No pathogens detected	
28	No pathogens	No pathogens detected		No pathogens detected		No pathogens detected	
29	No pathogens	No pathogens detected		No pathogens detected		No pathogens detected	
30	No pathogens	No pathogens detected		No pathogens detected		No pathogens detected	
31	Campylobacter sp	Detected	27.83	Detected	26.8	Detected	27.15
32	Campylobacter sp	Detected	30.34	Detected	29.9	Detected	31.18
33	Shigella sp	Detected	22.29	Detected	23.1	Detected	22.5
34	Salmonella sp	Detected	26.54	Detected	25.12	Detected	26.73
35	Shigella sp	Detected	29.95	Detected	28.78	Detected	29.36
36	Shigella sp	Detected	27.28	Detected	25.23	Detected	26.36
37	Shigella sp	Detected	20.17	Detected	18.9	Detected	18.1
38	Shigella sp	Detected	28.56	Detected	29.15	Detected	30.1
39	Campylobacter sp	Detected	31.1	Detected	30.45	Detected	31.33
40	Campylobacter sp	Detected	29.24	Detected	28.84	Detected	29.12
41	Salmonella sp	No pathogens detected		Detected	26.79	Detected	27.39
42	Salmonella sp	Detected	27.58	Detected	26.7	Detected	27.17
43	Salmonella sp	Detected	23.37	Detected	24.1	Detected	24.84
44	Salmonella sp	Detected	24.35	Detected	23.89	Detected	23.1
45	Salmonella sp	No pathogens detected		Detected	37.07	Detected	38.33
46	Salmonella sp	Detected	33.85	Detected	32.62	Detected	33.19
47	Crypto (ELISA)	Detected	30.68	Detected	31.86	Detected	33.15
48	Giardia (ELISA)	Detected	28.6	Detected	31.1	Detected	34.19
49	Giardia (ELISA)	Detected	34.72	Detected	34.65	Detected	35.19
50	Giardia (ELISA)	Detected	29.9	Detected	30.73	Detected	30.78

Concordant results were observed between the various methodologies with the exception of Salmonella and Campylobacter. The majority of discordant results were observed on the Serosep packet insert method utilising a Copan FLOQ Swab when compared to the traditional culture enrichment methodology. The MWE Fecal Transwab® demonstrated the highest comparability with the “gold standard”

Conclusions

Given the excellent sensitivity and negative predicative value of molecular testing as reported by many authors this study focused on the possible improvements in the pre-analytical phase of the analysis and any other potential benefits. The results did highlight reduced sensitivity for Salmonella in comparison to culture which has been previously reported for the standard extraction method as recommended in the Serosep kit insert. However, of note was that when the MWE Fecal Transwab® was utilised an improvement in detection for Salmonella did occur reducing the number of false negatives from 4 to 1. Cunningham *et al*. (2010) attributed reduced sensitivity to the enhancement of the culture method with a selenite enrichment broth. The evaluation of the benefit of a selenite broth enrichment step prior to multiplex PCR for enteric pathogens may be warranted. One can hypothesise that the bacterial load was greater when utilising the MWE Fecal Transwab® protocol as highlighted in this study which improved the detection limits of the assay for Salmonella.

The MWE Fecal Transwab® is a convenient system for transporting faecal samples in small instrument-ready tubes saving space and making it easier to transport to the laboratory. It enables rectal sampling which has benefits particularly in paediatric patients. In addition, it simplifies and standardizes stool sample collection, transport and processing by converting solid or semi-solid specimens into liquid phase, in instrument ready tubes, to facilitate automated faecal sample processing. Further enhancements of the Serosep system should incorporate automated de-capping, sampling, inoculation of the SPS tube through and re-capping of the primary tube systems. Although parts of this pre-analytical automation is available currently further development is required to create a seamless front-end system. Additional benefits could incorporate the simultaneous inoculation of a selenite liquid collection device in selected cases. Furthermore, it would allow for enhanced process traceability and standardization.

This study indicates that MWE Fecal Transwab® MW168S can provide improved test results particular with Salmonella when used for molecular testing of enteric pathogens utilising the Serosep EntericBio system. The discordant results between the CE and “non CE” method need to be investigated further to fully understand the cause. The MWE Fecal Transwab® system optimizes the collection and transport of gastrointestinal pathogens coupled with the rapid molecular diagnosis of gastrointestinal diseases.

References:

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St George's had recently installed a Serosep PCR EntericBio work station to assist with the processing of enteric specimens. They were concerned that SeroSep only endorsed the use of particular Copan swabs for inoculation of specimens into the system, and they had a suspicion they were missing some important pathogens. They also wanted to be able to have Fecal Transwabs as an option for the collection of specimens.

In this study they compared the performance of the system on a number of known positive samples, previously confirmed by conventional culture methods (including selenite enrichment), and by ELISA, when retested using Serosep's procedure with Copan swabs, and with Fecal Transwabs (flock and foam).

From the 38 samples tested, the MWE swabs (flock and foam) correctly identified Salmonella where it was missed when the Copan swabs. There were no cases of MWE missing specimens that Copan swabs detected. There was also one case in which the MWE flock swab picked up a Campylobacter that Copan swab had missed. Campylobacter specimens are notoriously fickle, but it is interesting that at least one of the MWE swabs allowed it to be detected. There was one Salmonella that none of the swabs (Copan or MWE) detected – which may have been because the concentration was very low.

The study clearly demonstrates that Fecal Transwabs (foam or flock) are completely compatible with the SeroSep system, a fact which they have previously acknowledged in personal communications, but refuse for commercial reasons to refer to in their Instructions for Use. St George's are concerned because of the risk of missing genuine cases of Salmonella, and have taken it up with the company.