Comparison of two Swab Transport Systems to Maintain Viability of Clinically Relevant Enteric Pathogenic Bacteria

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ABSTRACT

Background: Studies have indicated that rectal swab specimens can provide accurate test results when used with stool culture and molecular testing of enteric pathogens. The aim of this study was to evaluate two fecal swab systems to transport and maintain clinically relevant enteric pathogenic bacteria: FecalSwab™ Transport System (Copan, USA) and Fecal Opti-Swab® (Puritan, USA).

Methods: The following ATCC enteric pathogenic bacteria strains were evaluated for survival after incubation at room temperature (25°C) using the two transport swab systems described above: Escherichia coli ATCC 25922, Shigella flexneri ATCC 12022, Salmonella typhimurium ATCC 14028, Shigella sonnei ATCC 9290 and Yersinia enterocolitica ATCC 9610. A vortex elution protocol proposed by the CLSI standard M40-A2 was performed. This CLSI standard document provides a method of quality control testing, together with acceptance criteria not only for viability but also for overgrowth of bacteria. Briefly, an initial 0.5 MacFarland suspension of each strain was prepared, followed by six 10 fold dilutions (1.5 x 10^7 to 10^2 CFU/mL). The last three suspensions with concentrations of 1.5 x 10^7 to 10^5 CFU/mL were used as the working dilutions for each strain.

Vortex Elution Method from the CLSI M40-A2 document:

- An initial 0.5 McFarland suspension of each bacterial strain was prepared, followed by six 10 fold dilutions (1.5 x 10^7 to 10^2 CFU/mL) of each microorganism tested.
- The last three suspensions with concentrations of 1.5 x 10^7 to 10^5 CFU/mL were used as the working dilutions for each strain.
- Both swab systems were inoculated in triplicate with 100µL of each working suspension.
- After inoculations, swabs were placed into transport device and incubated for 0h, 24h and 48h. Swabs were incubated at 0h, 24h and 48h.
- Time 0h (T0h) swabs were used to determine initial microorganism concentration by spreading 100µL of swab transport media onto TSA with 5% sheep blood agar. Plates were incubated at 35°C for 24-48h.
- Time 24h (T24h) and Time 48h (T48h) swabs were incubated at 25°C for 24 and 48 hours, respectively and cultured after incubation time. Plates were incubated at 35°C for 24-48h and bacterial survival was assessed after 24-48h incubation at 35°C.

Results: Cultures from all swabs were averaged and results were compared with the M40-A2 CLSI document acceptance criteria. Bacterial recovery from swabs held at 0h were within the limits accepted by the M40-A2 CLSI document for both, Copan and Puritan fecal transport devices. Additionally, all five strains tested were recovered from all swab systems after 24h and 48h of incubation. Copan FecalSwab was able not only to maintain the organisms viable, but to keep the growth stable for up to 48h of incubation. On the other hand, organisms’ overgrowth were observed after 24h and 48h of incubation in all five species inoculated on the Puritan Fecal Opti-Swab. These overgrowth cultures didn’t remain within the 2 log10 of the initial microorganism concentration, as required by the CLSI M40-A2 document.

CONCLUSIONS

- Microorganism growth from T0h swabs were similar in both swab systems evaluated.
- The Puritan Fecal Opti-Swab showed overgrowth of all five microorganism strains tested, after incubation at room temperature for 24h and 48h. Copan FecalSwab cultures, however, remained within 2 log10 of the initial microorganism concentration.
- The risk of overgrowth is very high for stool samples and if cultures are not processed within the first two hours, this overgrowth can lead to normal flora obscuring pathogens, making the diagnostic more challenging.
- For this reason and in order to be in compliance with the CLSI M40-A2 document acceptance criteria, cultures from the T24h and T48h must remain within 2 log10 of the initial microorganism concentration.
- In the end, only Copan FecalSwab transport system was able to comply with all criteria for each enteric pathogenic organism’s survival tested at all time points, as described by the CLSI standard M40-A2.