Speedy Breedy -Lab Memo 8

Quantitative determination of low level *Escherichia coli* contamination in surface water using Speedy Breedy

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Principle & background:

Escherichia coli bacteria are a commonly used indicator of sanitary quality of foods and water. Following addition of a test sample to a selective culture medium, MacConkey broth, acid and gas production within 48 hours when incubated at 44°C (the fecal coliform test) is practically specific* for *E. coli* and indicative of fecal pollution in the sample. In surface water, a semi-quantitative (equivalent to using agar plates) determination of the degree of contamination is useful to assess risk due to fecal contamination.

In this study we investigated the correlation between Time to Detection (TTD) in Speedy Breedy and numbers of *E. coli* organisms present in a surface water sample in order to determine whether Speedy Breedy could provide a rapid, portable solution to semi-quantitation of low level *E. coli* contamination. The second goal was to determine whether a correlation for E. *coli* could be achieved in the presence of large numbers of other organisms such as non *E. coli* coliforms and pseudomonads.

Speedy Breedy determines contamination by measuring sensitive pressure changes within a closed vessel due to microbial respiration and assigns a Time to Detection using an internal algorithm that defines a significant pressure event. A colour change (from purple to yellow) of the bromocresol purple (dye) within the medium is used to provide a sensitive and definite indication of acid formation.

Experiment:

A surface water sample was taken from a small urban stream and diluted using sterile RO (reverse osmosis) water to create a set of four sub-samples of varying microbial content. 10 ml of each of the resultant sub-samples were cultured in MacConkey broth at 44 Deg C in Speedy Breedy while parallel samples were applied to chromogenic "Brilliance" coliforms/ E coli) agar plates (Oxoid). Coloured colonies were counted in accordance with the manufacturer's instructions to determine the cfu values inoculated in the Speedy Breedy test for three groups of organisms, *E coli*, other Coliforms and "other non-coliforms" (such as pseudomonas).

The organisms cultured in Speedy Breedy were confirmed as *E. coli* by plating onto Chromogenic agar plates and noting the colour of the resulting colonies after 24 hours.

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Results:

E. coli cfu value per test	Time to Detection (minutes)	Final Colour	Confirmation of Species cultured	Other coliforms cfu	Other non-coliforms cfu
18	477	Yellow	E. coli only	68	366
92	446	Yellow	E. coli only	287	High
183	417	Yellow	E. coli only	High	High
365	379	Yellow	E. coli only	High	High
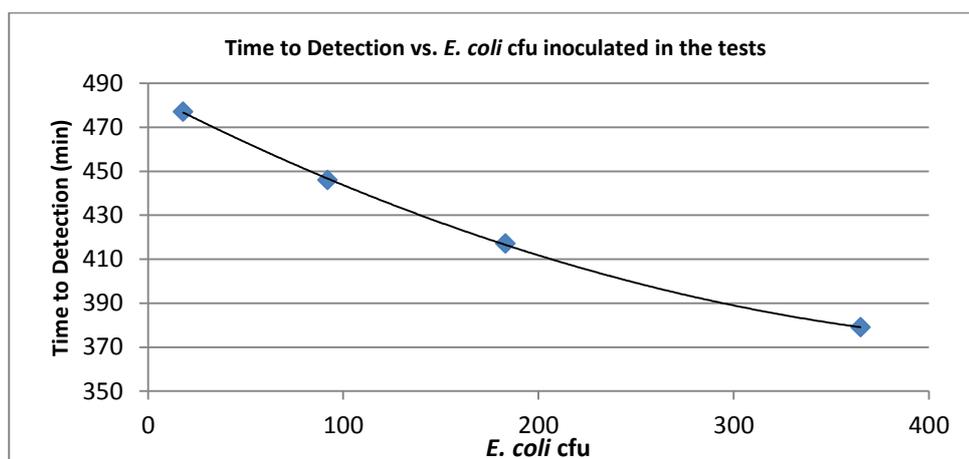
Conclusions:

- Speedy Breedy demonstrates a high degree of correlation between the Time to Detection and the number of *E. coli* organisms present in a test sample.

- The correlation works at low numbers of *E. coli* organisms present in the sample.

- Organisms cultured from surface water sources in Speedy Breedy were demonstrated to be only *E. coli* in the course of this study.

- Speedy Breedy *E. coli* test and correlation was not affected by large numbers of non-*E coli* coliforms and other environmental organisms in the test samples.

- Speedy Breedy represents a sensitive, rapid method for the semi-quantitative (equivalent to using agar plates) determination of *E. coli* for use in an operational capacity to assess operational risk due to fecal contamination.

- Speedy Breedy can be used in remote locations, field stations, portable units or other situations where rapid access to a laboratory is difficult.

Speedy Breedy
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