# Speedy Breedy- Lab Memo 9

## Quantitative determination of low microbial numbers in samples spiked with Escherichia *coli* using Speedy Breedy

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### Principle & background:

In this study we investigated the correlation between the Time to Detection (TTD) in Speedy Breedy and the numbers of *E. coli* organisms present in a test sample in order to demonstrate that Speedy Breedy provides a rapid, portable solution to semi-quantitation (equivalent to agar plates) of low level *E. coli* contamination.

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.

MacConkey broth and a culture temperature of 44° C were used to mimic the selective conditions that would be applied in an operational test situation. Growth under these conditions and a colour change in the medium from purple to yellow is indicative of *E. coli* contamination.

The investigation aimed to show that the Time to Detection is closely linked to the bacterial numbers in the sample since each generation in exponential growth will take a measurable duration (doubling time).

### Experiment:

Vessels filled with 50 ml of MacConkey's broth were inoculated with *E. coli (NCTC 9001)* at a range of cfu values following serial dilution from stock culture and grown at 44°C in Speedy Breedy. The number of organisms added to the test vessel was determined by plating samples onto agar plates in parallel and counting the colonies after 24 hours. In this experiment, organisms were considered to be stressed having been recovered from frozen stocks of "Lenticule" discs only 45 minutes before the tests began.
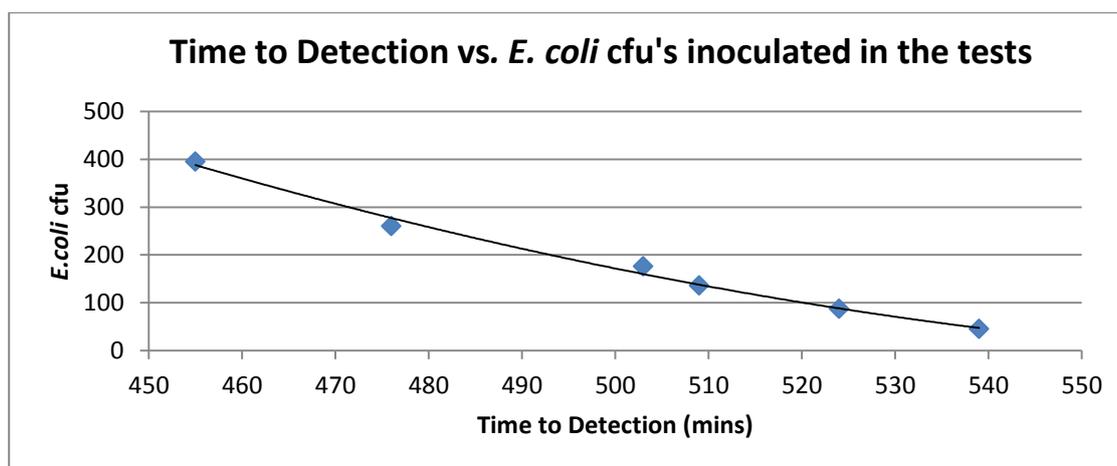
### Typical Growth Curve:
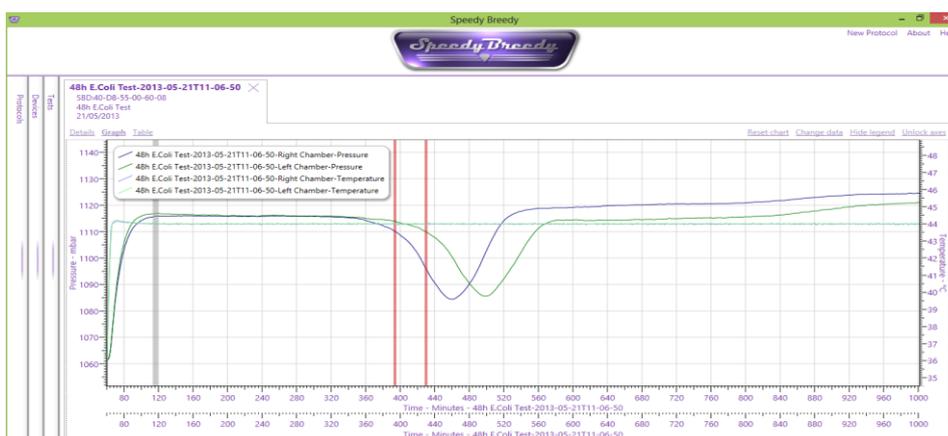
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Results:

| E. coli cfu in Sample | Time to Detection (minutes) | Final Colour of pH indicator |
|---|---|---|
| | | |
| 45 | 539 | Yellow |
| 87 | 524 | Yellow |
| 136 | 509 | Yellow |
| 176 | 503 | Yellow |
| 260 | 476 | Yellow |
| 395 | 455 | Yellow |

**Time to Detection vs. *E. coli* cfu's inoculated in the tests**

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.

- Allowing for any variances in Lag phase due to the presentation of the sample, Speedy Breedy has significant potential for the semi-quantitative determination of *E. coli* (equivalent to agar plates) in a wide range of test applications

- Speedy Breedy is a sensitive, rapid method to identify the presence of *E. coli* in remote locations, field stations, portable units or other situations where access to a laboratory is difficult.

- With Speedy Breedy tests can be started immediately, at any time of the day, a feature that enables even more rapid results.

*Speedy Breedy*

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