



Speedy Breedy - Lab Memo 2

Evaluation of Speedy Breedy for the Detection of Coliform Organisms.

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Principle & Background

Coliform bacteria are a commonly used bacterial indicator of sanitary quality of foods and water. These rod-shaped Gram-negative bacteria typically ferment lactose with the production of acid and gas when incubated at 35-37°C and a number of tests have been developed to utilize these characteristics as a means of identification.

One member of the coliform group, *Escherichia coli* can be distinguished from most other coliforms by its ability to ferment lactose at 44°C (the faecal coliform test), and formation of gas within 48 hours is practically specific for *Escherichia coli* and indicative of faecal pollution of the original sample. Some strains of *E. coli* can cause serious illness in humans.

In this study rapid culture and detection of coliforms was investigated in Speedy Breedy while differentiation of those of faecal origin was determined using established culture principles and media.

Medium Procedure and Results

MacConkey Broth has been used for over 50 years as a presumptive medium for the detection of the coli-aerogenes organisms in water and milk products. Growth was determined by pressure Transients within Speedy Breedy's closed vessel and turbidity of the final medium while large positive pressure transients indicated lactose fermentation to produce gas. Colour change (from purple to yellow) of the bromocresol purple within the medium was used to provide a sensitive and definite indication of acid formation.

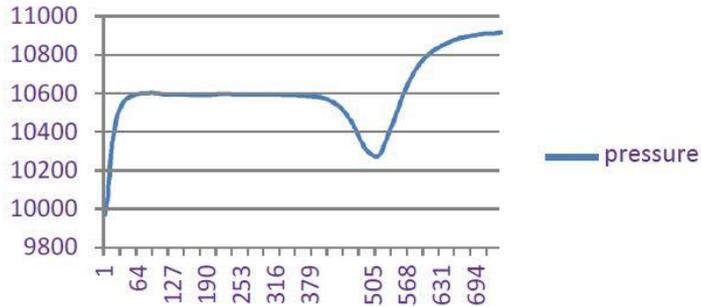
Three microbial species were cultured at different temperatures:

1. Faecal coliform positive control. *E. coli* – NCTC 9001
2. Representative coliform. *Klebsiella aerogenes* (*Raoultella planticola*) – NCTC 9528
3. Non-coliform control. *Staphylococcus aureus* – NCTC 6571

	<i>Escherichia coli</i>		<i>Klebsiella aerogenes</i>		<i>Staph aureus</i>	
	36° C	44° C	36° C	44° C	36° C	44° C
cfu microbes / sample	1	1	4	4	31	31
Speedy Breedy result	positive	positive	positive	negative	positive	negative
Time to detection (hr)	10:26	12:40	13:27	-	12:57	-
Turbidity	yes	yes	yes	no	yes	no
Colour	yellow	yellow	yellow	purple	purple/red	purple

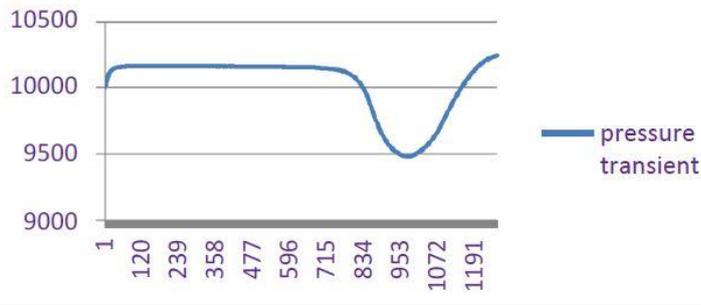


Coliform growth



E coli at 36° C and 44° C and *K. aerogenes* at 36° C, exhibiting gas production from lactose fermentation after time point 530.

Staph aureus



S. aureus at 36° C, exhibiting growth with some gas transients but returning to background pressure at time point 1200. Very little additional gas production suggesting no lactose fermentation.

Conclusions

- Speedy Breedy can detect and differentiate faecal coliforms from other coliforms using a simple, rapid protocol utilising the selective medium, MacConkey's broth and a culture temperature of 44 degrees Celsius. This combination is practically specific for *Escherichia coli* and indicative of faecal pollution of the original sample.
- The basic microbiology is well established and understood in many applications such as food, hygiene, water and milk testing.
- Speedy Breedy is extremely sensitive and can detect single organisms within a test volume of 50 ml.
- By visualising pressure curves, differentiation of coliform from non-lactose fermenters can be determined.
- Speedy Breedy represents a suitable, sensitive, rapid method for the detection of faecal coliforms for use in remote locations, field stations, portable units or other situations where rapid access to a laboratory is difficult.
- Speedy Breedy requires little user training and no interpretation so can be used by unskilled operators and can start the test immediately, at any time of the day, a feature that enables more rapid results than laboratory tests in many cases.