Speedy Breedy - Lab Memo 38

Experiment to Investigate the Ability of Speedy Breedy to Detect Contamination in Tissue Culture Medium

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Background

Speedy Breedy detects microbial contamination by the sensitive monitoring of pressure changes within a closed vessel. Samples are added to a vessel containing culture medium, which promotes rapid replication if micro-organisms are present. Any microbial respiration leads to changes in gas composition in the vessel, which can be monitored using Speedy Breedy. An internal algorithm defines a significant pressure event associated with detection of contamination and the length of time from inoculation of sample to pressure event is indicative of the degree of contamination. This length of time is referred to in this study as the Time to Detection.

Tissue Culture medium supports the rapid growth of contaminating bacteria which can be highly destructive to valuable cell lines and research work. It is essential then to be vigilant in monitoring for microbial contamination at various stages during the tissue culture process.
Speedy Breedy is an ideal method to use for the rapid testing of bacterial contamination in Tissue Culture medium.

Traditional Microbiology often involves the practice of filtering liquid samples. Filtering can introduce sheer stress to the micro-organisms present in the sample and also remove the natural environment of the bacteria from the sample. This can encourage any bacteria present to lose viability or behave in a manner atypical of the tissue culture environment, thereby providing the potential for an inaccurate assessment of the actual microbiological content of the sample.

Traditional Microbiological analysis often also involves shipping samples to a Microbiology lab.
Shipping samples can cause a loss of viability for certain bacteria, or they may grow in the sample, during shipment; both scenarios can lead to a wholly inaccurate Microbiological test result for shipped samples, in relation to their actual bacterial content at the time of sampling.

Tissue Culture medium can be decanted directly into Speedy Breedy culture vessels and tested immediately on-site, enabling an accurate and immediate assessment of the bacterial content of the medium without a shipping delay in testing the sample and without manipulation of the sample prior to testing through techniques such as filtration.

Hypothesis

Our hypothesis was that a low level of E. coli in Tissue Culture medium would be detected rapidly, reliably and successfully in Tissue Culture medium, using Speedy Breedy TSB culture vessels and the 24hr General Contamination Protocol.

Aim of Study

The aim of this study was to test neat and dilute Tissue Culture medium (with and without horse serum), using a 102 cfu/vessel (low inoculum) of E. coli, to determine whether or not the presence of the E. coli could be detected by Speedy Breedy using TSB culture vessels and the 24hr General Contamination Protocol.

Materials & Methods

The following materials were used for the experiment:

- E. coli (Fluka disc Cat # RQC01708-10EA, ATCC 11775)
- TSB culture vessels (Batches 100008 and 100009)
- TSA plates (prepared at Bactest)
- Spectrophotometer
- Sterile 1.5ml and 5ml tubes, sterile 1ml filter tips and sterile 200ul filter tips
- Sterile dH20
- Dulbecco's Modified Eagle Medium (Sigma D5796)
- Horse Serum (Sigma H0146)
- 37°C incubator
- 6 Speedy Breedy machines

The following method was used:

1. Inoculate 3ml TSB with an E. coli disc and incubate at 37°C until turbid
2. Measure the OD600 of the broth using a spectrophotometer (OD600 = 0.5 = 108 cfu/ml) and dilute the grown culture in TSB to give 100cfu/100ul
3. Add the following compositions of media and sterile dH2O to TSB culture vessels in duplicate:

- 50ml TC (Tissue Culture medium with 0% horse serum)
- 45ml TC + 5ml horse serum (Tissue Culture medium with 10% horse serum)
- 10ml TC + 40ml sterile water (diluted Tissue Culture medium with 0% horse serum)
- 9ml TC + 1ml horse serum + 40ml sterile water (diluted Tissue Culture medium with 10% horse serum)

1. Add 50ml sterile dH2O to 4 further TSB culture vessels
2. Inoculate each of the culture vessels, apart from 2 containing sterile water only negative controls), with 100ul of the diluted E. coli suspension
3. Run all vessels in Speedy Breedies, using the 24hr General Contamination Protocol
4. Spread-plate duplicate 100ul aliquots of the diluted E. coli suspension used for the inoculations onto TSA plates; incubate the plates overnight at 37°C and count the colonies in the morning.

Results

Inocula used

100ul spreads of the dilution of E. coli prepared gave 220 & 235 colonies on TSA plates =

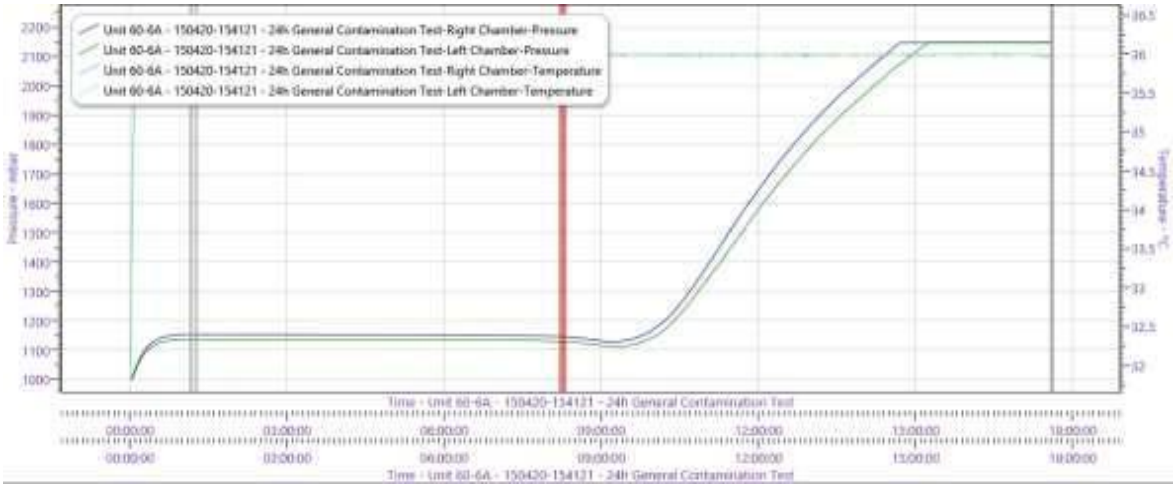
227.5 cfu/vessel inoculum.

Speedy Breedy Detection times

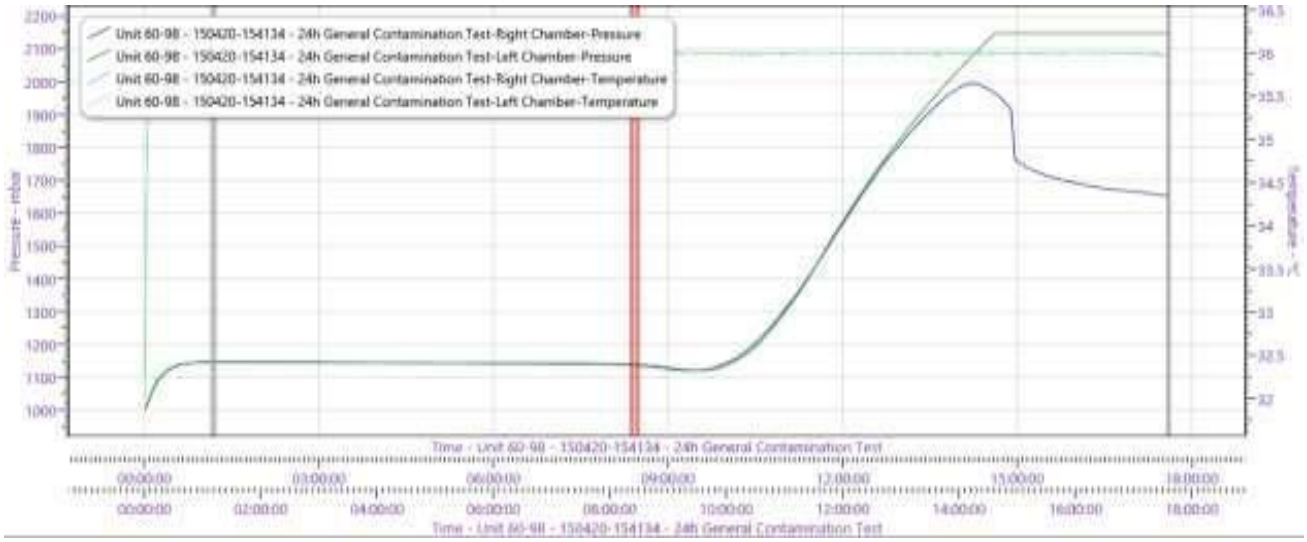
Media	Replicate	Time to Detection	Colour of medium	Turbidity
Tissue Culture medium with 0% horse serum	1	08:18	Yellow	Turbid
	2	08:14	Yellow	Turbid
Tissue Culture medium with 10% horse serum	1	08:23	Yellow	Turbid
	2	08:29	Yellow	Turbid
Diluted Tissue Culture medium with 0% horse serum	1	07:19	Yellow	Turbid
	2	07:28	Yellow	Turbid
Diluted Tissue Culture medium with 10% horse serum	1	07:26	Yellow	Turbid
	2	07:33	Yellow	Turbid
5ml sterile water with inoculum (positive control)	1	07:17	Yellow	Turbid
	2	07:22	Yellow	Turbid
5ml sterile water, no inoculum (negative control)	1	none	Yellow	Clear
	2	none	Yellow	Clear

Speedy Breedy Curves

Tissue Culture medium + 0% horse serum

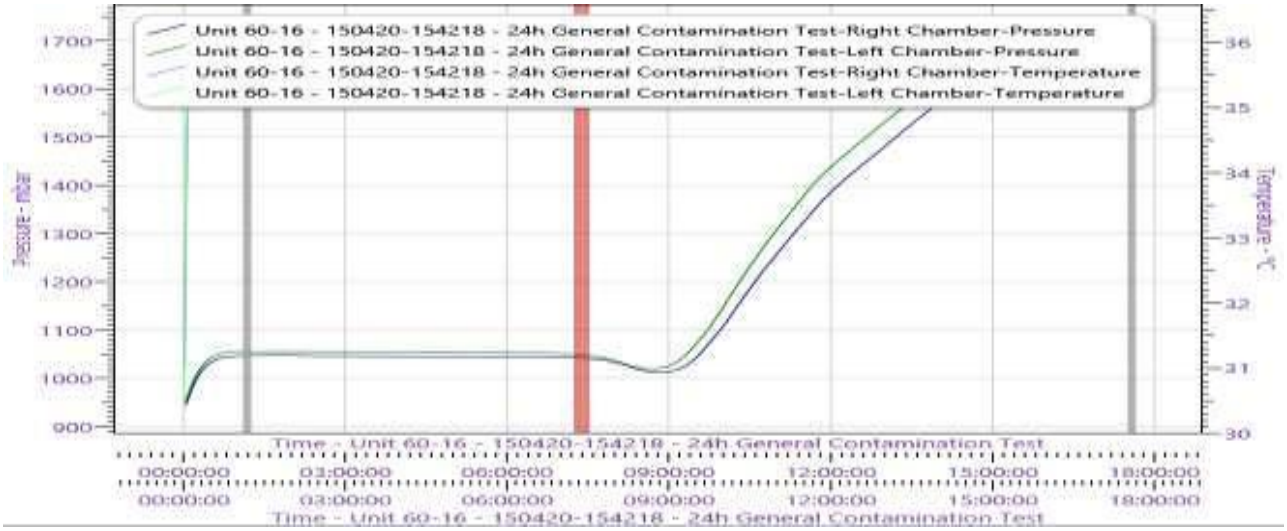


Tissue Culture medium + 10% horse serum

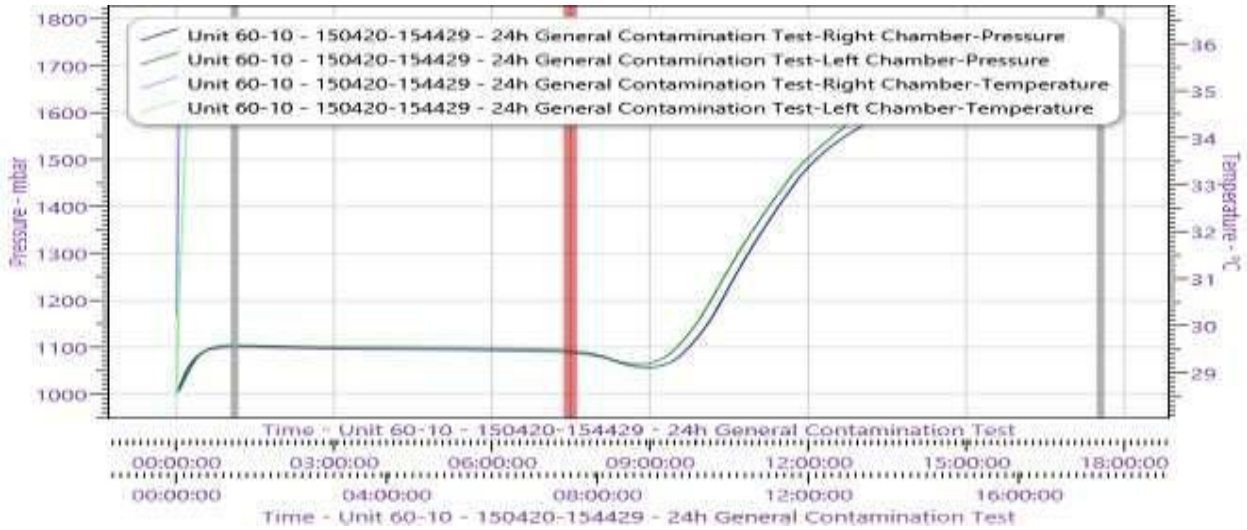


Speedy Breedy from BACTEST, St John's Innovation Centre, Cowley Road, Cambridge, CB4 0WS, UK
www.speedybreedy.com +44 01223 422312

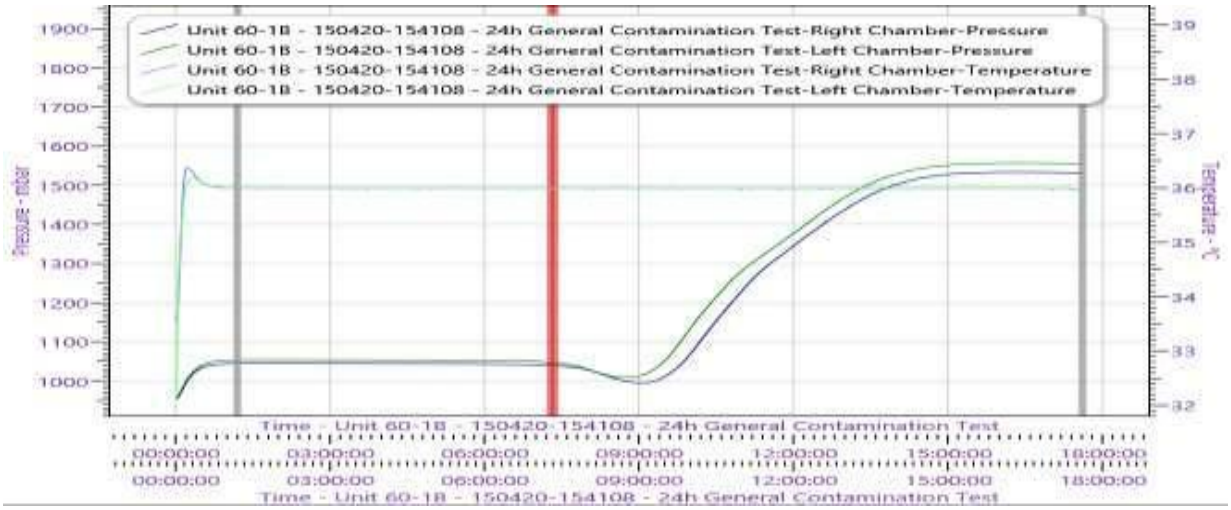
Diluted Tissue Culture medium + 0% horse serum



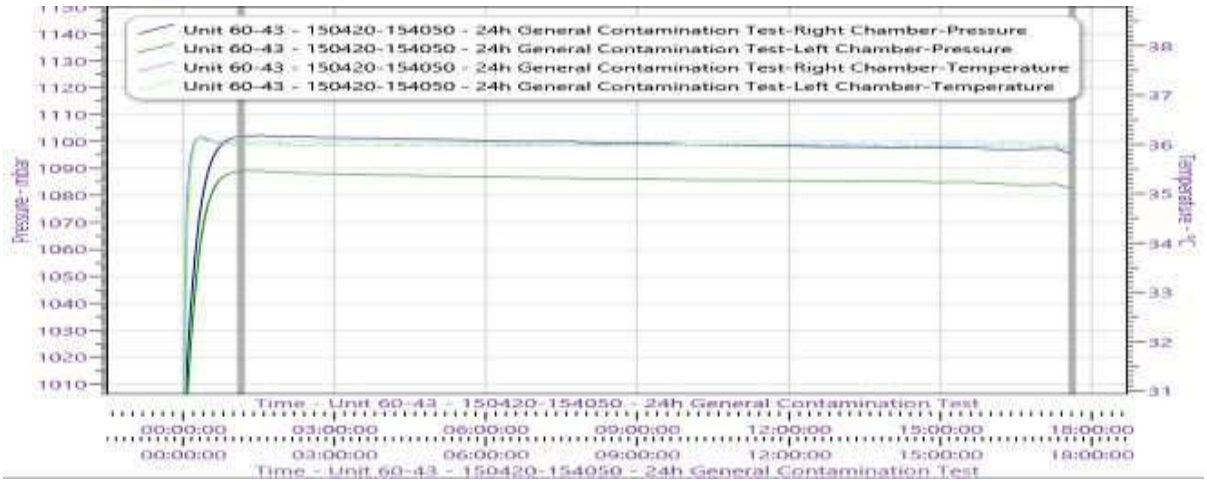
Diluted Tissue Culture medium + 10% horse serum



H2O with inoculum (positive control)



H2O, no inoculum (negative control)



Interpretation

The results show that Speedy Breedy successfully, rapidly and reliably detects the presence of a low level of E. coli contamination in Tissue Culture medium and diluted Tissue Culture medium (with and without horse serum), using Speedy Breedy TSB culture vessels and the Speedy Breedy 24hr General Contamination Protocol. 102 cfu of E. coli were detected at approximately 7.5hr in diluted Tissue Culture medium and 8.5hr in neat Tissue Culture medium. Heavier contamination should be detected more rapidly.

Conclusions & Observations

As per our hypothesis, Speedy Breedy can be used for the rapid detection of E. coli contamination in Tissue Culture medium, using the 24hr General Contamination Protocol. This detection method is quicker and easier than shipping samples to a Microbiology laboratory, where shipping times and destructive laboratory techniques, such as filtration, could skew the Microbiological results to be less accurate than those obtained using Speedy Breedy. This method also offers quicker times to detection than traditional, direct plating methods.

Neat Tissue Culture medium can be tested directly in culture vessels, however, neat medium can give rise to high pressures in contaminated vessels, triggering an automatic Speedy Breedy pasteurisation protocol to run after the detection of contamination. If growth in a vessel is required for identification purposes, Tissue Culture medium should be diluted (at least 1 in 5) in the vessel prior to carrying out the Speedy Breedy contamination check.
Vessels are a totally enclosed culture system so there is no risk of cross contamination by positive cultures grown in Speedy Breedy. This means that Speedy Breedy can be used closely associated to the tissue culture laboratory.