Speedy Breedy - Lab Memo 39

Experiment to evaluate the use of Tissue Culture Medium in Speedy Breedy

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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.

Speedy Breedy is an ideal method to use for the rapid testing of liquids such as Tissue Culture medium. A question has been raised whether the bicarbonate buffering system used in tissue culture media and the absence of a carbon dioxide overlay would affect the pH, possibly create a pressure event due to equilibration of the buffering system, and therefore affect and contamination results in Speedy Breedy. This study evaluates the suitability of Speedy Breedy for the testing of Tissue Culture medium, by confirming that pressure events are only caused in the presence of contamination and that the pH of the TSB culture vessels is not affected by Tissue Culture medium; it is important to maintain the pH of TSB medium in order to provide suitable conditions to support the growth and detection of contaminants.

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

1. Tissue Culture medium (with and without horse serum) can be used in Speedy Breedy culture vessels and does not cause pressure events in the absence of contamination.

2. Tissue Culture medium (with and without horse serum) does not affect the pH of Speedy Breedy TSB culture vessels.

Aims of the Study

1. To determine whether or not sterile Tissue Culture medium (with and without horse serum) creates a pressure event in Speedy Breedy

2. To determine whether or not sterile Tissue Culture medium (with and without horse serum) affects the pH of TSB culture vessels.

Materials & Methods

The following materials were used for the experiment:

- TSB culture vessels (Batches 100008 and 100009)
- Empty culture vessels
- Pipetter and sterile 1ml filter tips Sterile dH20
- Sterile, plastic 50ml tubes and syringes
- Dulbecco's Modified Eagle Medium (Sigma D5796)
- Horse Serum (Sigma H0146)
- Speedy Breedy machines

The following methods were used:

Pressure Tests

1. Add 50ml Dulbecco's Modified Eagle Medium (Sigma D5796) to each of two empty Speedy Breedy culture vessels and run in Speedy Breedy using the Tissue Culture protocol (24h, 60rpm, 37°C)

2. Add 10% and 20% Horse Serum (Sigma H0146) to culture vessels containing Eagle Medium (50ml total volume) in duplicate and run in Speedy Breedies using the Tissue Culture protocol (24h, 60rpm, 37°C)

3. Run duplicate controls of sterile dH2O (50ml volume) added to empty culture vessels, using the Tissue Culture protocol (24h, 60rpm, 37°C)

pH tests

- Test the pH of the following media using Macherey-Nagel pH strips (921 10, pH-Fix 0-14): - Dulbecco's Modified Eagle Medium (Sigma D5796)

- Dulbecco's Modified Eagle Medium (Sigma D5796) + 5% Horse Serum (Sigma H0146) - Dulbecco's Modified Eagle Medium (Sigma D5796) + 10% Horse Serum (Sigma H0146) - Dulbecco's Modified Eagle Medium (Sigma D5796) + 15% Horse Serum (Sigma H0146) - Dulbecco's Modified Eagle Medium (Sigma D5796) + 20% Horse Serum (Sigma H0146)

1. Add 50ml Dulbecco's Modified Eagle Medium (Sigma D5796) to each of two TSB Speedy Breedy culture vessels. Record any colour change and aseptically extract 100ul for testing on a pH strip after the capsules of TSB have completely dissolved

2. Run the vessels in Speedy Breedy for 30min using the Tissue Culture Protocol (37°C, 60rpm).

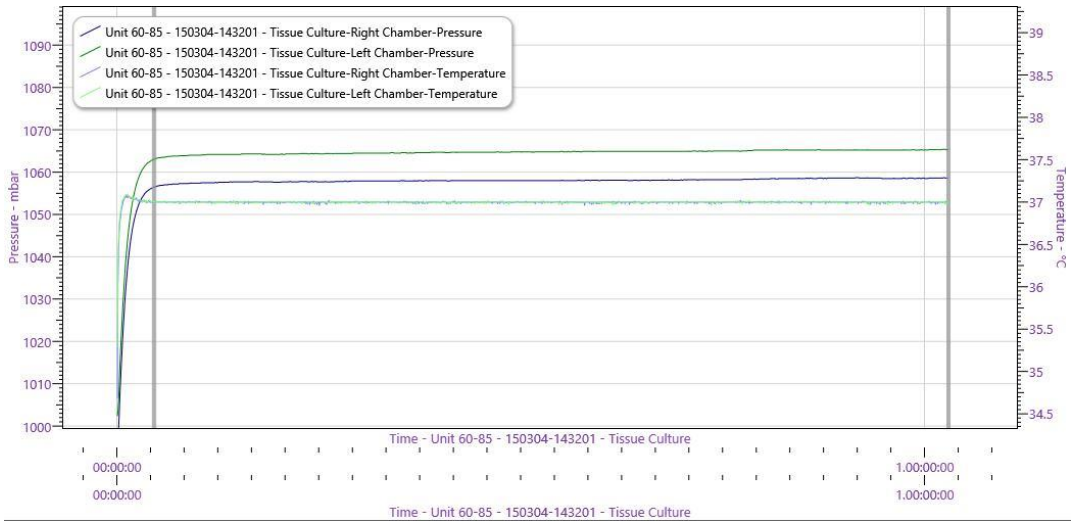
3. Repeat 2 & 3 using Eagle Medium + 5, 10, 15 & 20% horse serum.

Results

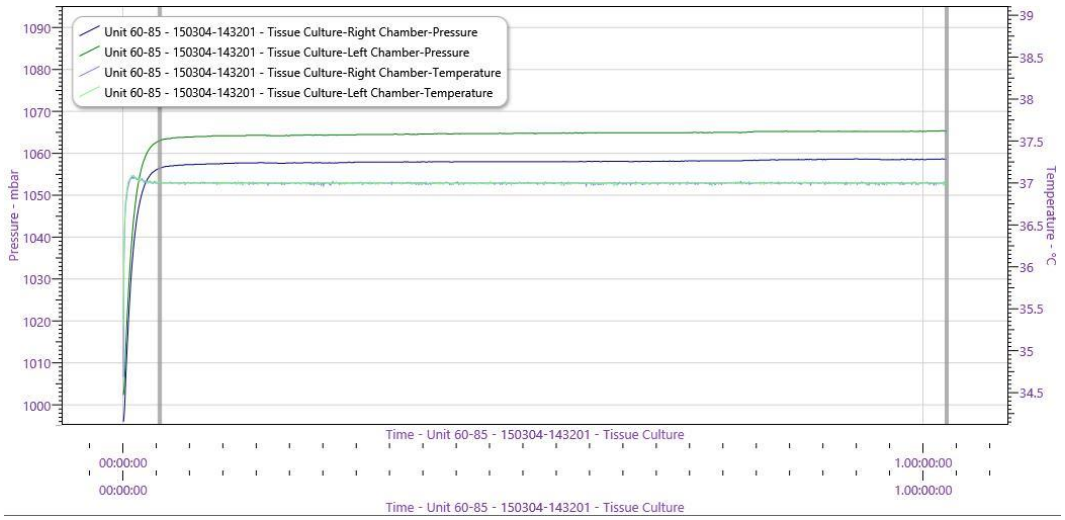
No pressure events were detected in the pressure tests and Tissue Culture medium (with and without horse serum) did not affect the pH in TSB culture vessels.

Speedy Breedy Curves (pressure tests)

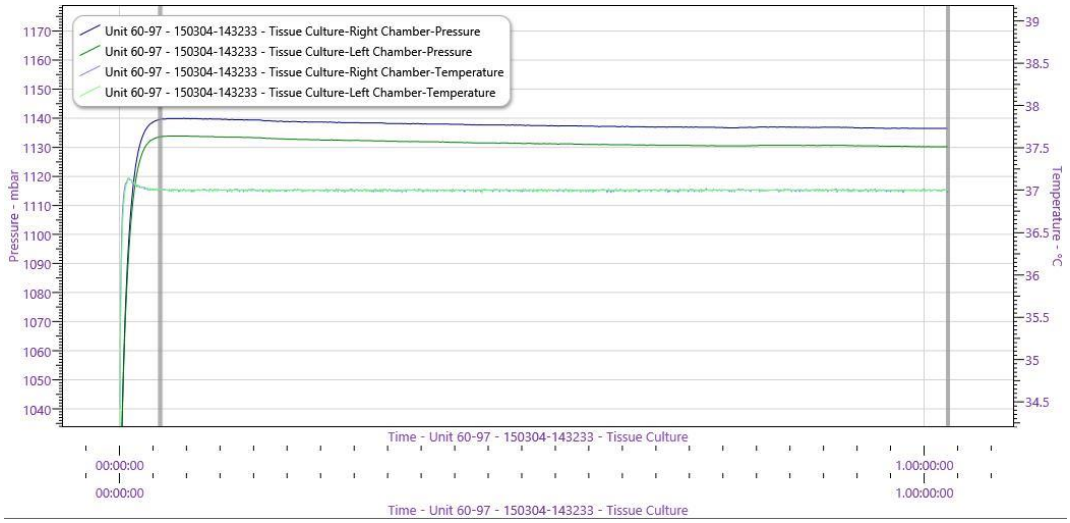
Sterile water



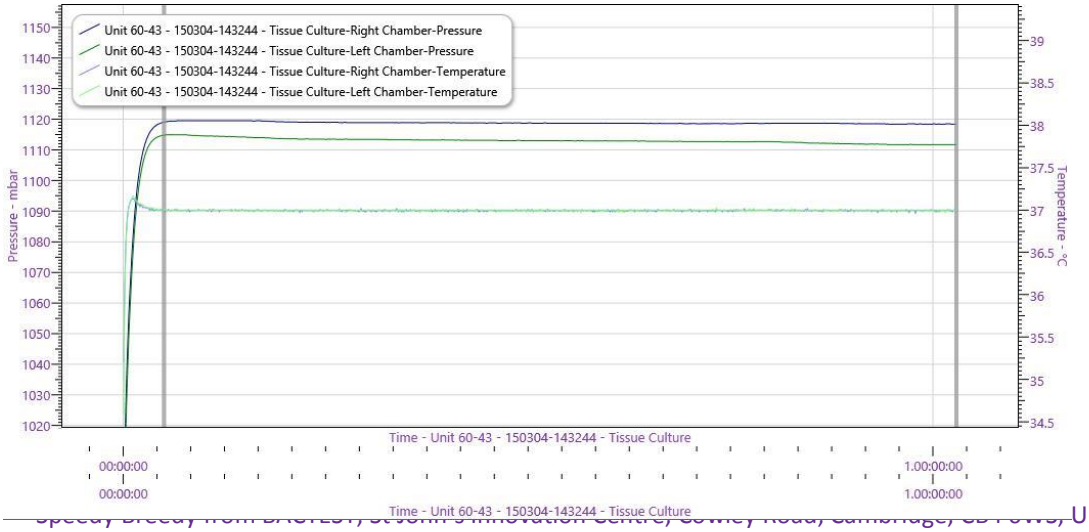
Tissue Culture medium (no horse serum)



Tissue Culture medium + 10% horse serum



Tissue Culture medium + 20% horse serum



pH tests

		Medium pH after adding to TSB vessel	TSB vessel after Speedy Breedy	Medium	Colour after	Medium Colour in TSB vessel after mixing in
Eagle	7.5	7.5	7.5	Red	Red	Red
Eagle + 5% Serum	7.5	7.5	7.5	Red	Red	Red
Eagle + 10% Serum	7.5	7.5	7.5	Red	Red	Red
Eagle + 15% Serum	7.5	7.5	7.5	Red	Red	Red
Eagle + 20% Serum	7.5	7.5	7.5	Red	Red	Red

Interpretation

Tissue Culture medium (with and without horse serum) does not cause pressure events in the absence of contamination.
Tissue Culture medium (with and without horse serum) does not affect the pH of Speedy Breedy TSB culture vessels.

Conclusions & Observations

As per our hypothesis, Speedy Breedy can be used to test Tissue Culture medium (with and without horse serum). 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.

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