A comparative study of two microbial detection systems

Speedy Breedy and bioMérieux BacT/ALERT

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ABSTRACT

Septicaemia is a major cause of mortality and speed of detection is vital to the patients' outcome.

Speedy Breedy (Bactest Ltd, Cambridge, UK, CB4 0WS) is a new instrument for the detection of microbial contamination in blood and other liquids which has the advantage of being able to commence testing at the point of care, therefore reducing the time to detection. Speedy Breedy utilises established culture media and measures pressure transients (as a consequence of microbial respiration) as a means of determining contamination. Speedy Breedy was compared with the bioMérieux BacT/ALERT machine currently used for routine testing of clinical specimens at the Public Health England (PHE) Specialist Microbiology Services laboratory, located at Cambridge University Hospitals NHS Foundation Trust Addenbrooke's site. Speedy Breedy was run using two different media, the BacT/ALERT medium (BM) to enable direct comparison of the technologies, and Tryptone Soy Broth (TSB), a widely available alternative.

The results demonstrate a high degree of correlation between the two instruments and on average Speedy Breedy was approximately 20% faster than the BacT/ALERT when the same medium was used in both instruments. Speedy Breedy is designed as a point of care product and further speeds results by eliminating the time it takes to transport samples to a laboratory and await the return of results.

INTRODUCTION

Septicaemia is a potentially life threatening condition where time to detection is a key factor in morbidity[1]. Quickly determining microbial infection in an emergency blood sample could be vital for a patient's treatment and prognosis so speed is of paramount importance for any new test method. Culture techniques are routinely used to test a patient's blood sample as this enables rapid detection of a small but significant number of organism, therefore amplifying the available test signal.

The current product of choice for routine testing of clinical isolates at the PHE Specialist Microbiology Services Laboratory in Cambridge is the bioMérieux BacT/ALERT machine.

In this study we investigated a new instrument called Speedy Breedy, a portable point-of-care microbial respirometer with a number of design features for the rapid growth and detection of bacteria and yeasts.

The predicate device for comparison in this study was the BacT/ALERT by bioMérieux, an automated, non-portable, laboratory based microbial detection system based on the colorimetric detection of CO2 produced by growing microorganisms[2].

MATERIALS AND METHODS

Six Speedy Breedy units were run over 3 weeks in parallel with the BacT/ALERT diagnostic laboratory Instrument**.**

Description of Speedy Breedy

Speedy Breedy consists of three elements, a small portable instrument, disposable plastic culture vessels and MICROSOFT Windows compatible software installed on a PC. User generated on board protocols control culture conditions and detection parameters. Speedy Breedy can operate as a stand-alone device once culture protocols have been installed from a PC.
Speedy Breedy is a microbial respirometer which utilises sensitive pressure sensors to detect microbial activity within a sealed culture vessel. A positive contamination event triggers when the metabolic gas utilisation or production reaches a critical level that is recognised as a pressure transient in the headspace of the culture vessel. Typically, detection is achieved following exponential growth of organisms from small numbers and a critical mass has been reached where respiration rate within the

vessel creates a significant pressure event. The mixing system enhances this signal. Sensitive pressure sensors continuously monitor the culture and are themselves protected, as is the operator, by a flexible diaphragm that isolates the lumen of the vessel from the sensors. Speedy Breedy applies an internal algorithm, smoothing the data and allowing accurate pressure event analysis.

Significant pressure events are communicated through coloured LEDs and a visual display message and accompanied by the Time to Detection. Speedy Breedy can operate independently and saves data onto an internal SD card. When connected to a laptop or PC Speedy Breedy can output to proprietary software every minute. The data is plotted as pressure versus temperature, but smoothed differential pressure and rotor speed can also be plotted.

Within the instrument, two independently heated chambers house the culture vessels during the course of a test run and quickly reach the pre-programmed temperature. The single-use culture vessels with a patented non-invasive magnetic paddle mechanism continuously agitate the sample, therefore rapidly creating optimal homogenous growth conditions.

Speedy Breedy was originally developed for rapid detection of septicaemia in human blood samples, but is now used for the rapid detection of microbial contamination across a range of industries and disciplines.

Methods

A panel of six organisms' representative of those commonly found in septicaemia was used for the study in order to investigate the speed and efficacy of Speedy Breedy. These organisms were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Klebsiella pneumoniae* and *Streptococcus pneumoniae.*

Test organisms were provided by the PHE Specialist Microbiology Services Laboratory from stock. Each species was cultured and colonies or confluent growth re-suspended in sterile physiological saline solution. Concentration was determined by optical density (OD) measurement and serial dilutions made to give a suitable number of Colony Forming Units (CFU) in 20 ml to achieve the target range required when inoculated in the final sample.

The actual CFU count per millilitre for each inoculum was confirmed by plating onto Colombia Blood Agar and counting colonies after 24 hours. Samples were prepared by spiking the highest concentration of organisms in 20 ml of saline into 380 ml of blood and then by 1 in 10 serial dilution in blood. Samples were then inoculated into each of three test systems (Speedy Breedy, BacT/ALERT and Colombia Blood Agar plate) to give the following final predicted CFU count for each sample, therefore representing the detection number in each 10 ml sample of:

10^0 tested in quadruplicate
10^1 tested in duplicate
10^2 tested in duplicate
10^3 tested in duplicate
10^4 tested in duplicate

To test the limit of detection it was aimed to include tests for single organisms in the sample, this required multiple tests as dilution to single organisms is difficult and location of those organisms is always random. To achieve a positive test result for a single organism inoculum the lowest concentration (expected to be single organism inoculum) was run in quadruplicate.

In duplicate (or quadruplicate in the case of single organisms), 10ml of spiked blood sample was inoculated into BacT/ALERT bottles (aerobic medium) and placed into the BacT/ALERT instrument and run according to the manufacturer's instructions. Two further 10 ml spiked blood samples were inoculated into Speedy Breedy culture vessels containing each of two different broths:

1. 40 ml of Tryptone Soya Broth (Oxoid Ltd, Basingstoke, UK).
2. 40 ml of BacT/ALERT aerobic medium (bioMérieux)

Each vessel was then placed in a Speedy Breedy instrument.

Manufacturers recommended protocols were run for each instrument, with temperature set at 37°C for 2 days or for a reduced time if a positive result was detected. For Speedy Breedy, the paddle rotation speed was set at 60 rpm. Tests were performed in aerobic conditions only.

Any negative samples were removed after 2 days and incubation continued in a 37° C incubator. Cultures were examined for turbidity after 5 days.

The actual number of organisms inoculated into each test was confirmed by a plate count using the remaining sample from the 400 ml starting material.

Both Speedy Breedy and the BacT/ALERT were run over a 3 week period with the samples being run simultaneously in both machines for each culture.

NOTES REGARDING MEDIA

Two different media were used in the vessels for Speedy Breedy, the first being Oxoid's Tryptone Soy Broth as it is one of the most commonly available and widely known general medium suitable for fastidious organisms. The study aimed to determine the efficacy of Speedy Breedy for commonly encountered organisms using a non-proprietary medium and compare results with a highly developed medium.

Secondly, both Speedy Breedy and BacT/ALERT were run with the proprietary bioMérieux aerobic medium to enable a better comparison between the two technology platforms. The bioMérieux medium is also based on TSB but supplemented with complex amino acids and carbohydrates.

TIME TO DETECTION

Time to detection in both instruments is defined as the time from when the tests were started to the time they were reported as positive by the instrument i.e. in the case of Speedy Breedy a positive pressure event exceeding the set threshold was detected. In the BacT/ALERT this was the time a consistent change in colorimetric measurement of $C0^2$ was observed.

RESULTS

Speedy Breedy demonstrated a high degree of correlation with BacT/ALERT and detected 100% of the samples that were positive by BacT/ALERT.

Further to this, Speedy Breedy detected the lowest concentration of *S. pneumoniae,* whereas there was no value for the BacT/ALERT.

For both instruments the detection time increased when the concentration of bacteria decreased, in a linear fashion.

Table 1. Shows how Speedy Breedy tests with either TSB or BM medium compare with the BacT/ALERT tests with BM medium. For most bacteria (except *S. pneumoniae*) the type of medium used did not greatly influence the time to detection, however time to detection tended to be a little lower for all organisms except *S. pneumoniae* when the test was run with TSB. The decreasing concentrations seemed to correlate with the time to detection in a more linear fashion when only one type of medium (either TSB or BM) was taken into account.

TABLE 1. TIME TO DETECTION WITH BLOOD SPIKED AT DIFFERENT CONCENTRATIONS.(54000-0 CFU) PERFORMED ON DIFFERENT DAYS (DAY1 AND DAY2)

	Mean Time to Detection (hours)		
Organism	Speedy Breedy with TSB medium	Speedy Breedy with BM medium	**BacT/ALERT** with BM medium
Staphylococcus aureus 1	6.7	6.7	9.7
Staphylococcus aureus 2	8.9	9.0	12.1
Escherichia coli 1	8.2	8.4	6.38
Escherichia coli 2	6.8	7.4	5.9
Pseudomonas aeruginosa 1	10.1	10.1	13.7
Pseudomonas aeruginosa 2	10.1	10.3	13.0
Candida albicans	18.0	17.1	19.44
Klebsiella pneumoniae 1	6.9	7.15	9.6
Klebsiella pneumoniae 2	6.7	7.6	9.7
Staphylocuccus pneumoniae 1	13.3	16.1	21.6
Staphylocuccus pneumoniae 2 **	12.4	13.4	17.9

*Mean taken from 4 instead of 5 results as the BacT-Alert did not find bacterial growth at the lowest concentration.

Figures 1-6 below, graphically represent a direct comparison between Speedy Breedy and the BacT/Alert, both using BM medium for each organism.

Figures 7-12 below, compare BM and TSB media with Speedy Breedy for each of the 6 organisms.

Figure 1. shows how the time to detection increased as the inoculum of *S. aureus* decreased for both Speedy Breedy and BacT/ALERT. Both were run with the same medium to enable direct comparison.

The detection of *S. aureus* took an average 31.14% longer with BacT/ALERT when compared with Speedy Breedy using BM medium. Similar results were found using TSB medium in Speedy Breedy where no significant difference (average difference 31.34%) was noted in the time to detection. (See figure 7)

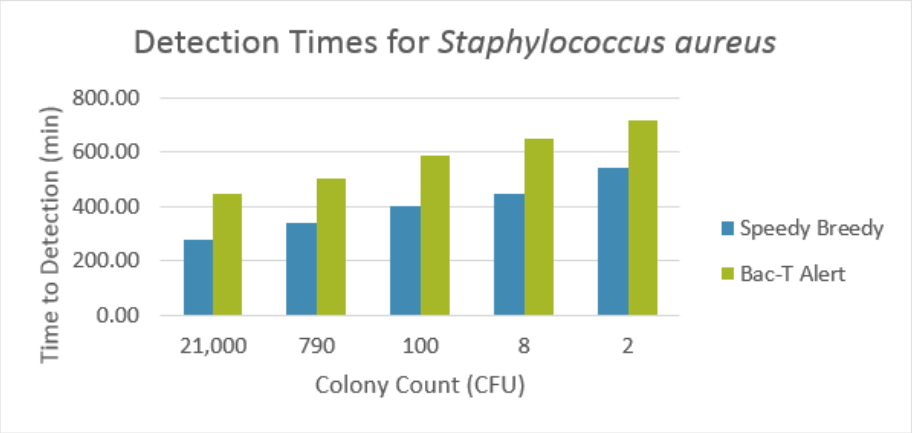

FIGURE 1. COMPARISON BETWEEN DETECTION TIMES FOR S. AUREUS FOR BACT/ALERT AND SPEEDY BREEDY USING BM MEDIUM

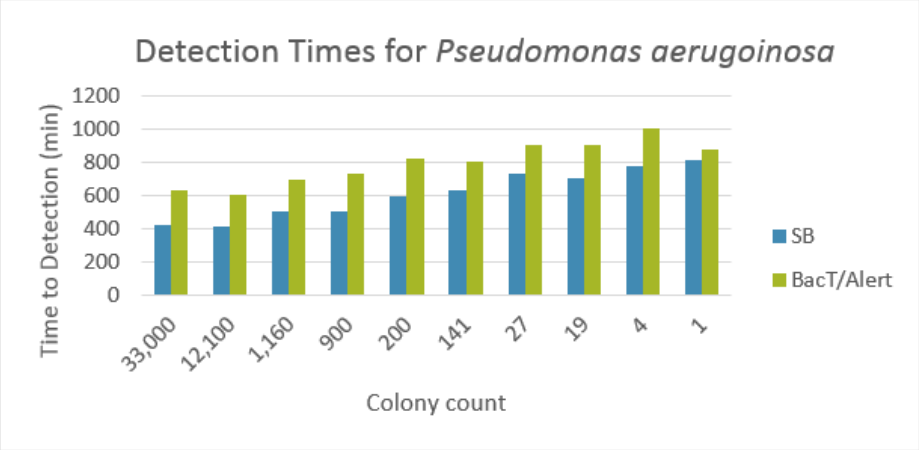

FIGURE 2. COMPARISON BETWEEN DETECTION TIMES FOR P. AERUGINOSA FOR BACT/ALERT AND SPEEDY BREEDY USING BM MEDIUM

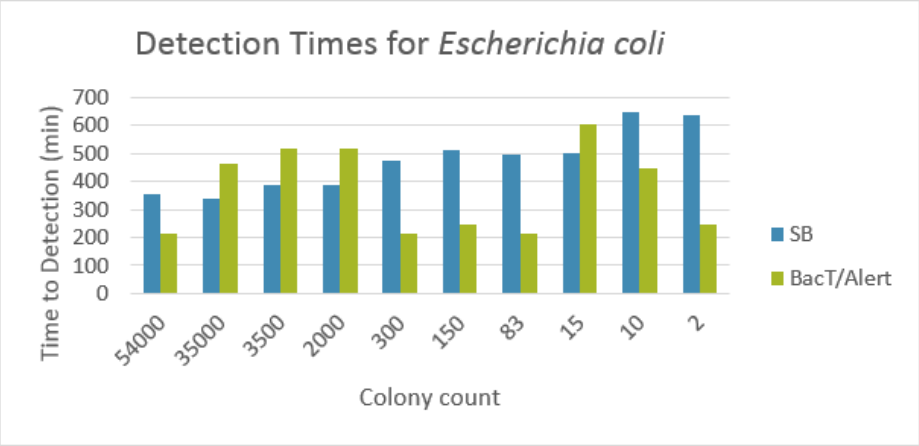

FIGURE 3. COMPARISON BETWEEN DETECTION TIMES FOR E. COLI FOR BACT/ALERT AND SPEEDY BREEDY USING BM MEDIUM

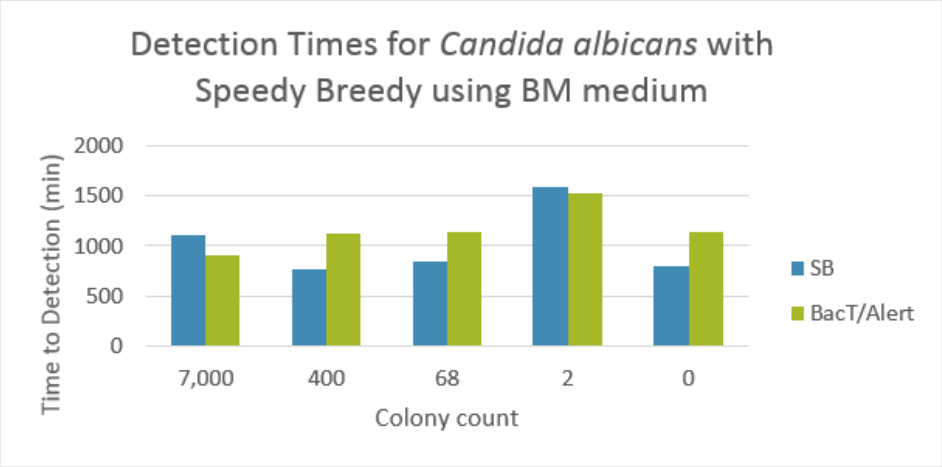

FIGURE 4. COMPARISON BETWEEN DETECTION TIMES FOR C. ALBICANS FOR BACT/ALERT AND SPEEDY BREEDY USING BM MEDIUM

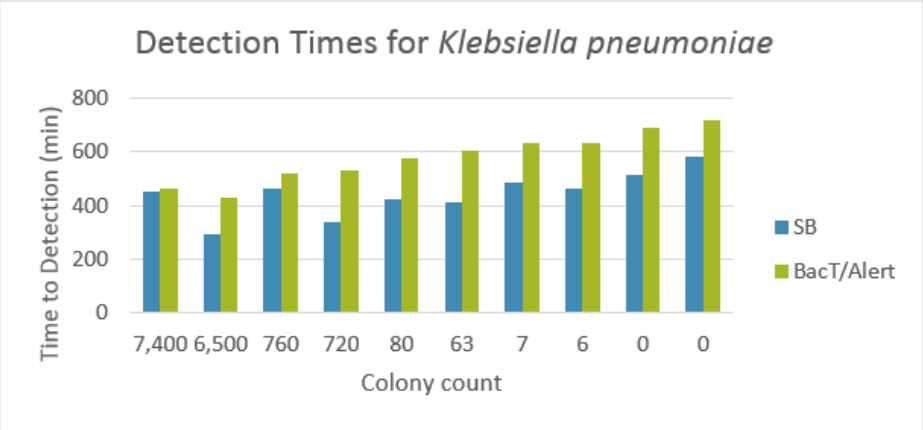

FIGURE 5 COMPARISON BETWEEN DETECTION TIMES FOR K. PNEUMONIAE FOR BACT/ALERT AND SPEEDY BREEDY USING BM MEDIUM

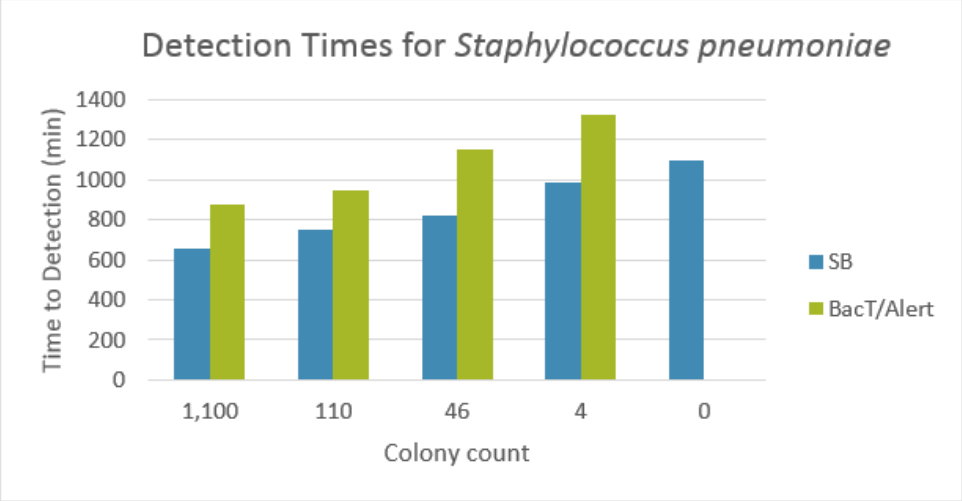

FIGURE 6. COMPARISON BETWEEN DETECTION TIMES FOR S. PNEUMONIAE FOR BACT/ALERT AND SPEEDY BREEDY USING BM MEDIUM

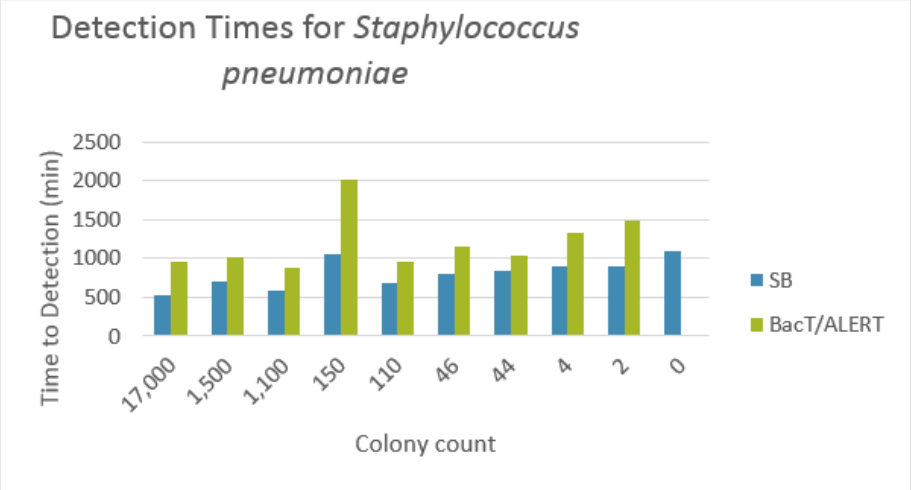

FIGURE 7. COMPARISON BETWEEN DETECTION TIMES FOR S. PNEUMONIAE FOR BACT/ALERT AND SPEEDY BREEDY USING TSB MEDIUM

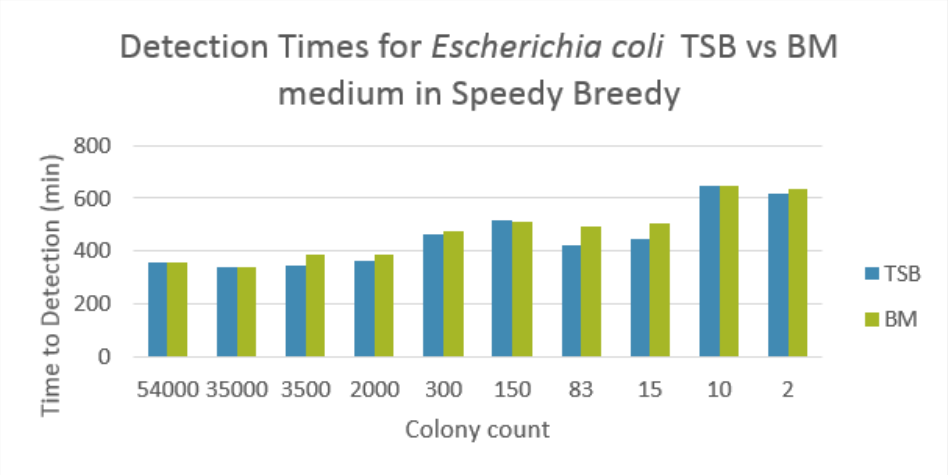

FIGURE 8 COMPARISON BETWEEN TSB AND BM MEDIUM FOR E. COLI WITH SPEEDY BREEDY

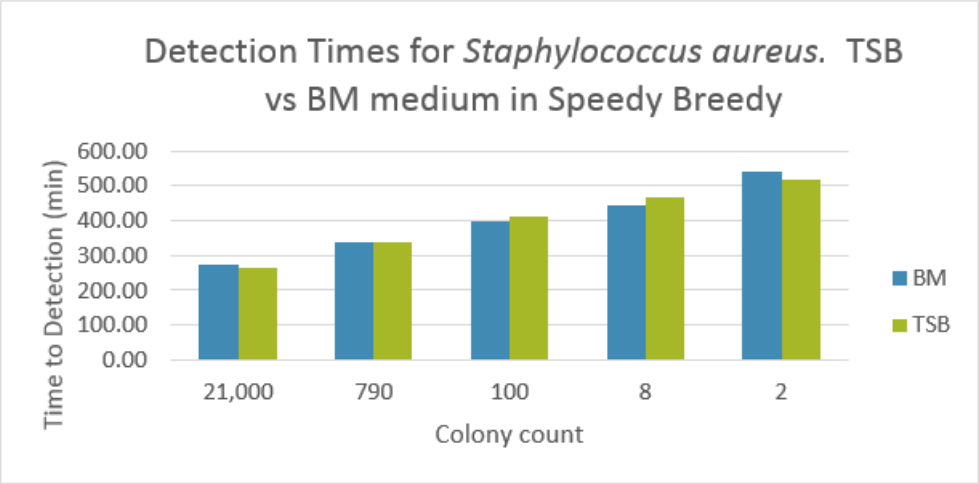

FIGURE 9 COMPARISON BETWEEN TSB AND BM MEDIUM FOR S. AUREUS WITH SPEEDY BREEDY

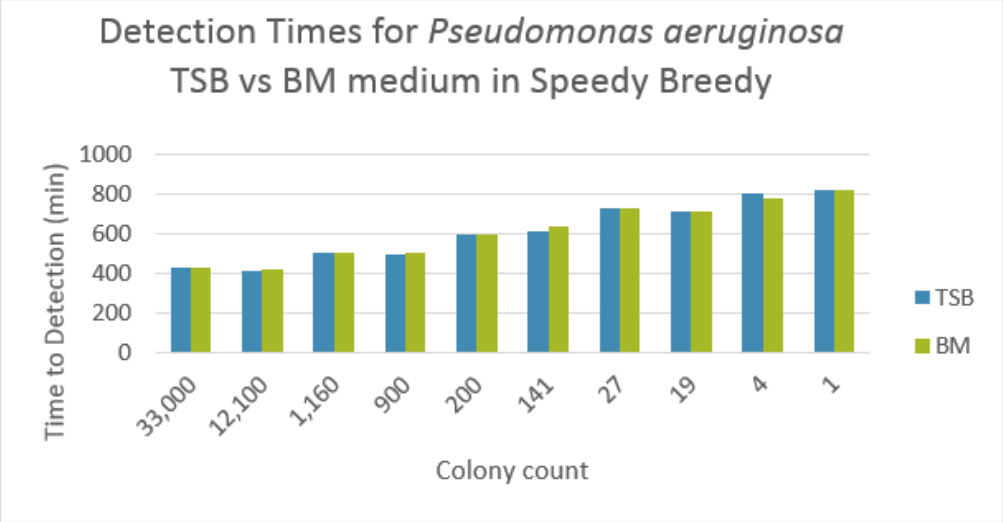

FIGURE 10 COMPARISON BETWEEN TSB AND BM MEDIUM FOR P. AERUGINOSA WITH SPEEDY BREEDY

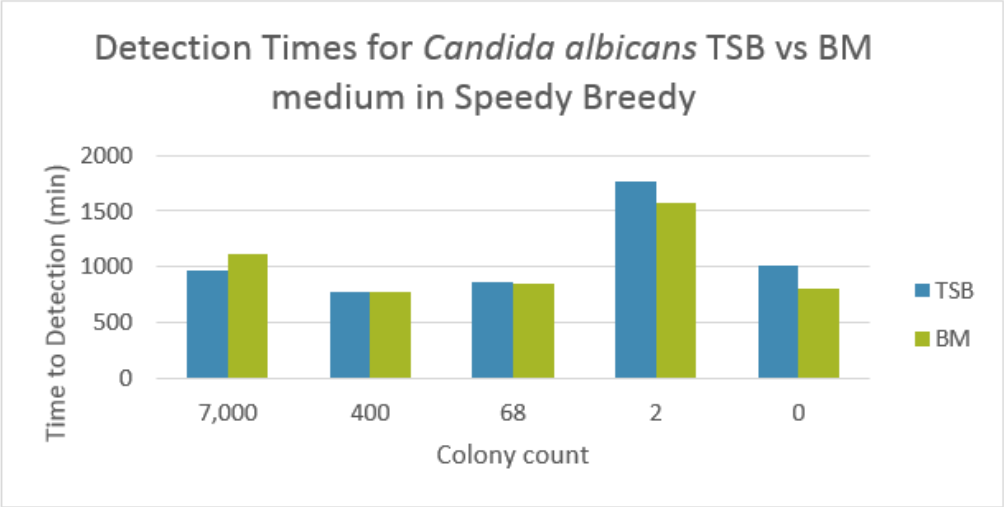

FIGURE 11 COMPARISON BETWEEN TSB AND BM MEDIUM FOR C. ALBICANS WITH SPEEDY BREEDY

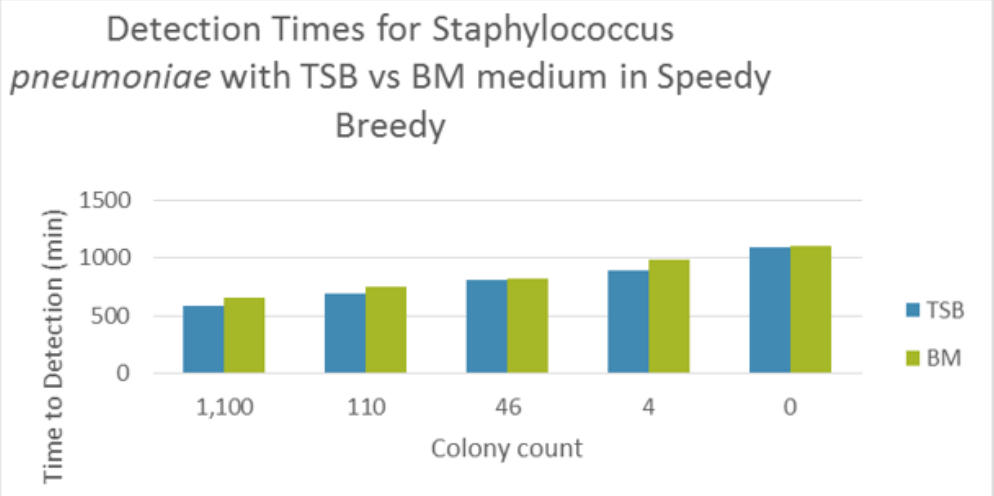

FIGURE 12 COMPARISON BETWEEN TSB AND BM MEDIUM FOR S. PNEUMONIAE WITH SPEEDY BREEDY

CONCLUSIONS

Speedy Breedy shows a high degree of correlation to BacT/ALERT in terms of its ability to detect microbial contamination both at high and low cell concentrations. Speedy Breedy is on average 20.35% faster than BacT/ALERT for the organisms tested taking in account both TSB and BM media for Speedy Breedy. When BM medium was used in Speedy Breedy and across the whole study Speedy Breedy was 18.5% faster, when TSB was used Speedy Breedy proved to be 22.2% faster. Speedy Breedy detected contamination in all samples which BacT/ALERT identified as positive and also detected two samples with very low concentrations of *S. pneumoniae*, when BacT/ALERT marked the samples as negative.

The faster time to detection is particularly noticeable with *S. pneumoniae* where Speedy Breedy is on average 8.3 hours (38.4%) faster when TSB is used as a medium and 5.5 hours (25%) faster when BM medium is used.

The contamination detection time in Speedy Breedy is shorter than BacT/ALERT when using either the BM medium or the TSB medium for all organisms except *E. coli*. However it must be taken into account that the results for *E. coli* on BacT/ALERT are questionable as the times to detection appear to be exactly the same for different concentrations, whereas for all other organisms the time to detection increases as the bacterial concentration decreases.

In this study Speedy Breedy demonstrated efficacy as a rapid instrument for microbial contamination testing in blood samples and compares favourably with the bioMérieux BacT/ALERT machine currently used for routine testing of clinical isolates at the Clinical Microbiology and Public Health laboratory of Cambridge University's Addenbrookes Hospital site.

Speedy Breedy is designed as a point of care product to reduce time to detection both in speed of microbial growth and detection as well as eliminating the time it takes to transport samples to a laboratory and await the return of results. This study supports that intent in a clinical environment.

References cited

[1] BacT/ALERT: *an automated colorimetric microbial detection system*. TC Thorpe. J Clin Microbiol. 1990 July; 28(7): 1608–1612.

[1] *Surviving the first hours in sepsis: getting the basics right (an intensivist's perspective)*. Daniels, R. J. Antimicrob. Chemother. (2011)66 (suppl 2): ii11-ii23.