The effects of sensor colour change in negative blood culture bottles; are terminal sub-cultures necessary in some cases?

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THE EFFECTS OF SENSOR COLOUR CHANGE IN NEGATIVE BLOOD CULTURE BOTTLES; ARE TERMINAL SUB-CULTURES NECESSARY IN SOME CASES?

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ABSTRACT

FACULTY OF SCIENCE & TECHNOLOGY

MASTER OF SCIENCE

THE EFFECTS OF SENSOR COLOUR CHANGE IN NEGATIVE BLOOD CULTURE BOTTLES; ARE TERMINAL SUB-CULTURES NECESSARY IN SOME CASES?

By CRAIG ANTHONY POWNALL

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Blood culture bottles are seen as the 'Gold Standard' of the diagnosis of bloodstream infections. However, it has been noted that bottles that have been designated as negative after incubation may share some of the characteristics of positive bottles, for example comparable sensor colours. This study set out to verify that bottles indicated as negative by the BacT/ALERT 3D (bioMérieux) analyser do not contain microorganisms. Additionally, the sensor colour was investigated as a means to aid the interpretation by the users of this system.

This study was achieved by sub-culturing negative bottles that had been incubated for 5 days on the BacT/ALERT 3D analyser onto a range of agar types in various atmospheric conditions for different lengths of time in order to ensure conditions that are favourable for a wide range of organisms that may have been present in the bottle, but that had not triggered the analyser algorithm. A small number of samples were also subjected to molecular-based testing in order to account for factors that may have restricted organisms present from growing on sub-culture, such as inactivation by antibiotics given to the patient prior to inoculation of the bottles.

The results of this study showed that the BacT/ALERT 3D system in use at Nobles Hospital on the Isle of Man can be seen as highly reliable with regards to the reporting of negative blood cultures. Furthermore, it was also shown that the bottle sensors are not a dependable indicator as to the requirement to sub-culture negative bottles due to the fact that other factors, such as blood volume in the bottles, may contribute to the observed sensor colour variations.

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Introduction

The Medical Microbiology department at Nobles Hospital on the Isle of Man uses the BacT/ALERT 3D™ (bioMérieux) system for the analysis of blood cultures. This system employs a sensor in the bottom of culture bottles which changes colour in the presence of carbon dioxide produced by the microbial metabolism of substrates in the culture medium. This change is detected by the analyser, and the bottle flagged as positive depending on certain parameters programmed into the analyser algorithm. It has, however, been anecdotally noted by Biomedical Scientists involved in the routine use of this system that negative blood culture bottles are removed from the analyser with varying degrees of sensor colour intensity; some negative bottles having an intensity of colour comparable to that of positive bottles.

In light of this, the primary objective of this study is to determine the significance of blood culture bottle sensor colour change in negative bottles, as visually inspected by a Biomedical Scientist. The hypothesis being tested in this research is that some negative bottles showing a large sensor colour intensity change are not genuinely negative, as the analyser algorithm may miss slower-growing organisms. To this end, bottles that have been reported as negative for growth after 5 days incubation in the analyser are to be sub-cultured onto a range of agar types and incubated in a variety of environmental conditions for various time periods. A secondary objective of this study is to investigate the necessity of further study into the algorithms used to determine positivity of blood culture bottles in this system should the primary objective reveal shortcomings in the current laboratory protocols regarding negative bottles.

This research is important as the results may be used to determine a review of policy in the Medical Microbiology department at Nobles Hospital on the Isle of Man. If it is shown that a statistically significant number of isolates are missed through not taking into account a manual examination of the

blood culture bottle sensor in negative bottles, then it may be advantageous to incorporate this process into the routine blood culture work in order to provide better support to service users and patients.

There is, however, an ethical risk in this study as there may be a revelation of the presence of organisms in the blood of patients that had already been reported as negative. In cases such as this the Laboratory Number of the patient involved shall be reported to the Consultant Microbiologist, who may use this to access the demographics of the patient, thereby enabling an amended report to be sent out detailing the organism grown. This is especially problematic, however, if the organism isolated is a probable skin flora contaminant, for example coagulase-negative *Staphylococcus* species, *Micrococcus* species. A decision is therefore required to be made by the Consultant Microbiologist along with requesting consultant as to whether these organisms should be reported, as based on the patient's clinical details and case history. As far as this study is concerned, however, only anonymous Laboratory Numbers are to be recorded and reported.

Literature review

Sepsis

Sepsis is a syndrome in which signs of inflammation may be detected in tissues remote from a site of infection due to the products of bacterial proliferation and/or the inflammatory response, potentially leading to distributive shock and organ failure (Tierney, McPhee and Papadakis, 2008; MacFie, 2013; Public Health England, 2014a). The respiratory and cardiovascular systems are most commonly affected in sepsis, with possible renal and neuronal complications (Angus and van der Poll, 2013). Singer *et al* (2016) point to the early activation of both pro- and anti-inflammatory responses of the immune system in addition to major modifications of other pathways, for example hormonal, metabolic and coagulation pathways, as the causation of organ dysfunction and homeostasis dysregulation. Endogenous factors include bacterial toxins, for example the Gram-negative structural Lipopolysaccharide (LPS) endotoxin, or secreted exotoxins such as Type I exotoxins, for example Toxic Shock Syndrome Toxin-1 (TSST-1), Type II exotoxins, for example haemolysins, and Type III exotoxins, for example Shiga toxin (Ramachandran, 2014). These structural molecules and toxins are able to elicit an immune response whereby a massive proliferation of pro-inflammatory cytokines such as TNF-α, INF-γ and IL-2 are released into the surrounding tissues and bloodstream leading to cellular apoptosis and associated tissue damage (Ramachandran, 2014). However, the cellular components of this inflammation can vary depending on the type of organism involved; Gram-negative sepsis associated with an increased proportion of monocytic myeloid-derived suppressor cells in relation to $CD14^{+}$ mononuclear cells, and Gram-positive sepsis associated with an increased proportion of $CD14^{LOW}$ Low Density Granulocytes (Janols *et al*, 2014). This is most likely due to the different Toll-like receptor (TLR) ligands that recognise Gram-negative and Gram-positive antigens; TLR-2 and TLR-4 respectively (Janols *et al*, 2014). Conversely, the immune response can become negatively affected during sepsis, due to a possible decreased

responsiveness of blood leucocytes and enhanced apoptosis of B-cells, $CD4^+$ T-cells and follicular dendritic cells (Angus and van der Poll, 2013), with Gram-negative sepsis in particular demonstrating massive intravascular erythrophagocytosis as well as haemolysis (Haak *et al*, 2016). Furthermore, Huson, Grobusch and van der Poll (2015) have pointed out that patients with pre-existing immunosuppressive disorders such as HIV infection show evidence of up-regulation of TLRs on immunological cells, thereby contributing to a more aberrant inflammatory response, and therefore more disturbed homeostasis, in such patients.

Sepsis has been reported variously as having a mortality rate between 30% and 87% (Tierney, McPhee and Papadakis, 2008; Public Health England, 2014a), and has been labelled as the primary cause of death from infection (Singer *et al*, 2016). It has been estimated that sepsis occurs in approximately 2% of all hospitalisations in developed countries, increasing to up to 30% in intensive care patients (Martin, 2012), with the UK Health Protection Agency submitting that approximately 20% of sepsis cases are associated with bloodstream infection (Public Health England, 2014a). Indeed, 99,191 patient episodes of bacteraemia were reported in England, Wales and Northern Ireland in 2014, of which 91,186 were monomicrobial infections (Public Health England, 2015). However, it is important to note that these numbers are obtained by voluntary surveillance, and do not distinguish between true pathogens and blood culture contamination. Despite all efforts, totally eliminating all skin flora and environmental organisms from the potentially heavily colonised skin surface is not possible, thereby conceivably inoculating such organisms during phlebotomy (Hossain *et al*, 2016). Regardless, Angus and van der Poll (2013) and Opota *et al* (2015) are in agreement that Gram-negative organisms are predominantly isolated from the blood, with 62% of isolates belonging to this morphotype. Of these, *Escherichia coli* and *Klebsiella* species are the predominant Gram-negative organisms isolated (Angus and van der Poll, 2013). These studies are, however, in disagreement as to the prevalence of the isolation of Gram-

positive organisms, with Angus and van der Poll (2013) suggesting a 47% Gram-positive isolation rate, and Opota *et al* (2015) submitting a lower isolation rate of 36%. Of Gram-positive organisms isolated from blood, *Staphylococcus aureus* and *Streptococcus pneumoniae* are the most commonly isolated (Angus and van der Poll, 2013).

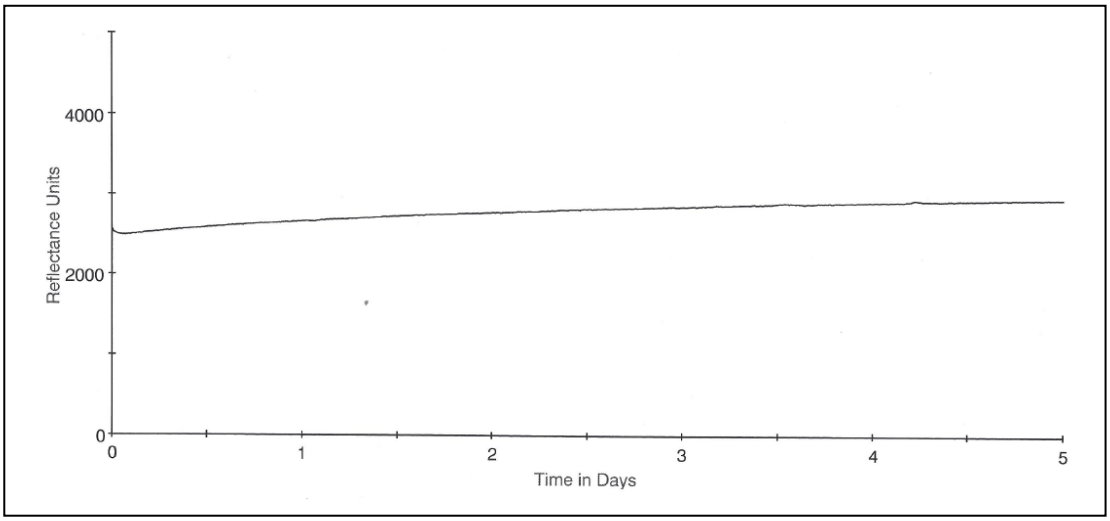
It is currently accepted that the time window for admission of appropriate therapy is less than six hours once septic shock, the sequelae of sepsis, is recognised, with every hour of delay increasing mortality by 7.6% (Jordana-Lluch *et al*, 2014). It is therefore clear that sepsis is a major concern for patients, with source identification and treatment options required as soon as possible. Indeed, Stoneking *et al* (2013) surmise that therapy would remain the same in only 23% patients, had the causative organism been known to emergency physicians.

Blood culture analysis

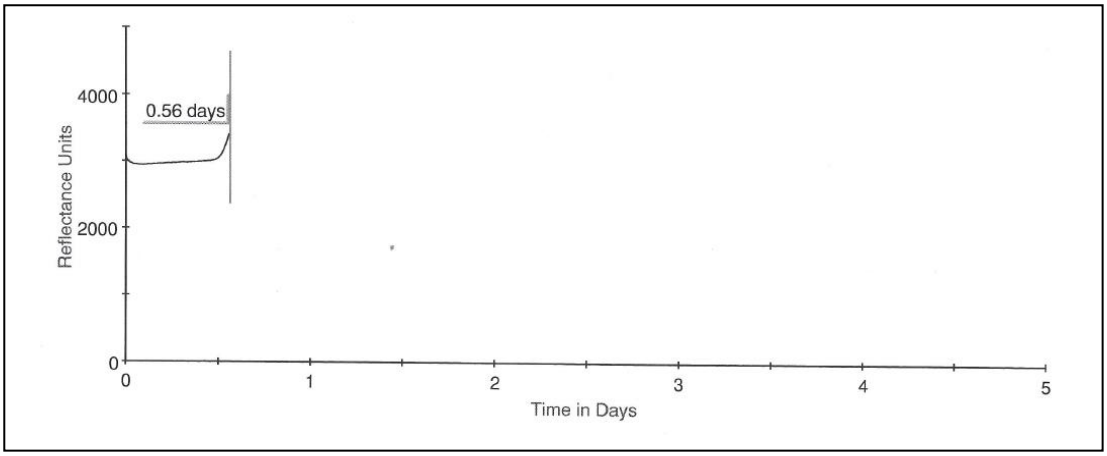
The "Gold Standard" for the detection of microorganisms in the blood is through the use of a blood culture system (Mancini *et al*, 2010; Public Health England, 2014a). The first blood culture systems were manual, a highly labour intensive process of daily examinations for macroscopic evidence of microbial growth such as haemolysis, turbidity, gas production or colony formation over the course of seven days coupled with a terminal sub-culture (Weinstein, 1996). Current systems are automated, therefore requiring much less time manipulating bottles. Many of these systems are based on creating optimal conditions for microbial growth in aerobic and anaerobic environments, with the detection of microbial metabolic products and gases the cornerstone of the methodology (Smith *et al*, 2008). Presently, there are three main analysers on the market for the analysis of blood cultures; BD BACTEC (BD Diagnostics) utilising a fluorescent sensor to detect CO_2 production, BacT/ALERT 3D (bioMérieux) utilising a colorimetric sensor to detect CO_2 production, and Versa TREK (TREK Diagnostic Systems) utilising the monitoring of redox variations (Opota *et al*, 2015). All of these systems show comparable performance for the isolation of various microbes from

blood samples, although there are individual differences. For example, Horvath *et al* (2004) reported suboptimal *Candida* species isolation in both the BacT/ALERT and BACTEC systems; 70% and 10% respectively. This was improved in both systems by the use of specialised mycology media bottles, although these were reported as being three times more expensive than standard bottles, therefore cost-effectiveness dictates the use only where there is high clinical suspicion of candidaemia (Horvath *et al*, 2004). The Medical Microbiology department at Nobles Hospital on the Isle of Man uses the BacT/ALERT 3D (bioMérieux) system for the analysis of blood cultures. This system employs a sensor in the bottom of a variety of different blood culture bottle types which changes from dark to a yellow colour in the presence of CO_2 produced by the bacterial metabolism of substrates in the culture medium (Thorpe *et al*, 1990; bioMérieux, Inc., 2010). Specifically, the sensor bonded to the bottom of each bottle is separated from the broth by a semi-permeable membrane which allows CO_2 to diffuse across and into a water layer, whereby the resulting drop in pH causes a colour change in the bottle sensor (Thorpe *et al*, 1990; bioMérieux, Inc., 2010). This sensor colour, measured as Relative Fluorescent Units (RFUs), is monitored by the BacT/ALERT 3D analyser at 10 minute intervals and signalled as positive based upon the rate of colour change (increase in RFUs) and therefore CO_2 concentration established by a growth curve algorithm for each bottle type (Thorpe *et al*, 1990; bioMérieux, Inc., 2010). The instrument analyses the growth curve for each bottle either for an increasing rate of change, or a sustained increase in CO_2 concentration (Wilson *et al*, 1992). Figure 1 depicts the growth curve of a typical example of a bottle that showed no growth after incubation in the BacT/ALERT 3D analyser after 5 days alongside those of two different organisms isolated from positive blood culture bottles.

(a)



(b)



(c)

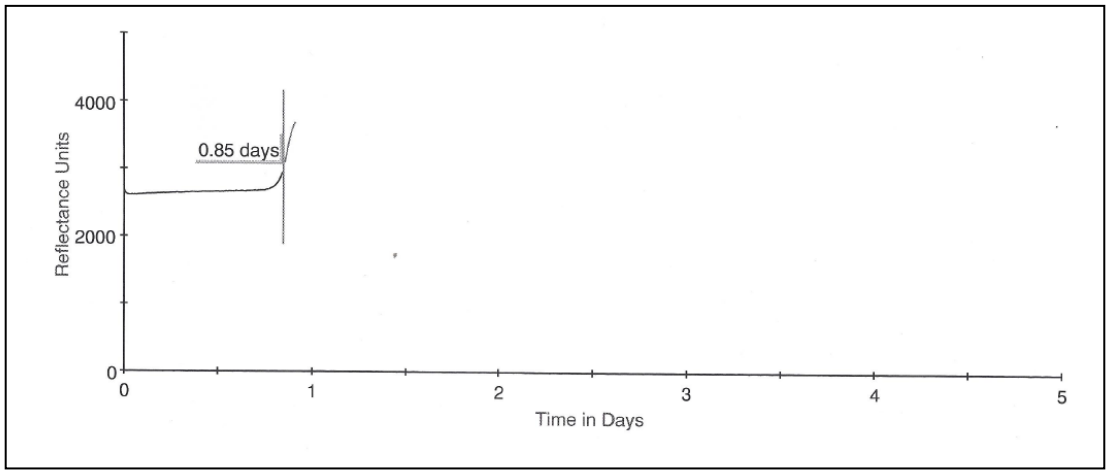


Figure 1. Growth curves of a typical negative bottle (a), *Escherichia coli* growth (b) and *Staphylococcus aureus* growth (c). Note, (b) and (c) curves are shortened due to the positive bottle being removed from the analyser, with the vertical line crossing the curve at the point that the bottle was flagged as positive.

Limitations of the Blood Culture method

Detection of bacteraemia by the blood culture method, however, has a number of potential limitations. Whilst Tattevin *et al* (2015) claim that 98.7% of clinically significant organisms are detected within the standard time limit of 5 days using the blood culture method, it has also been suggested that 20-55% bloodstream infections are not identified by routine blood culture methods (Stoneking *et al*, 2013). Murray and Masur (2012) assert that the value of blood cultures are sub-optimal, with only around 30% of patients with sepsis and/or septic shock deriving from bacterial pneumonia and intra-abdominal infections having positive blood cultures. Loffler *et al* (2013) give a higher figure, arguing that blood cultures are negative in over 50% of cases where true bacterial- or yeast-derived sepsis is believed to exist based upon other measurements and clinical presentation. Indeed, soon after the advent and refining of this technology, Weinstein (1996) warned that no one media or system is capable of detecting all microbes. In the ensuing years since this assertation, a number of species have been identified as failing to reach the threshold algorithm of a blood culture analyser, due to slow growing thereby giving only weak signals, or being present in blood culture media without showing visible signs of growth. Examples of species fitting this criteria include *Neisseria spp.*, *Brucella spp.*, *Francisella spp.*, *Haemophilus influenzae*, *Legionella spp.*, *Pseudomonas aeruginosa*, and *Candida spp*. (Murray and Masur, 2012; Public Health England, 2014a). The ways of addressing the problems posed by more fastidious or slower growing species used by diagnostic microbiology laboratories is to lengthen the amount of time that a blood culture bottle is incubated inside the analyser, or to perform a terminal sub-culture of the blood culture bottle subsequent to removal from the analyser after the standard incubation is complete. This would in itself, however, be undesirable due to a longer total detection time leading to a later antibiotic switch if so required (Rönnberg *et al*, 2013; Jordana-Lluch, *et al*, 2014), and is therefore to the detriment of the patient. In support of this, Public Health England (2014a) state that terminal subcultures are not routinely

recommended if automated systems are used and manufacturer instructions are followed, whilst the current standard recommended by the College of American Pathologists is that the 5 day incubation period need not be exceeded, even for more fastidious organisms such as the HACEK group of organisms (*Haemophilus*, *Aggregatibacter*, *Cardiobacterium*, *Eikenella*, *Kingella*), and *Brucella spp*. (Potula, Dadhania and Truant, 2015). Regarding *Brucella* spp., Solomon and Jackson (1992) proposed that the CO_2 pattern was recognised by the blood culture algorithm in 2-8 days with a standard aerobic bottle. However, in the early period of this technology, Weinstein (1996) suggested that greater than seven days may be useful when fungaemia or bacteraemia due to fastidious organisms such as HAECK group, *Legionella spp*. or *Brucella spp*. are suspected. This has been more recently confirmed by Public Health England contradicting the above guidance by suggesting the necessity of terminal sub-culture if clinical presentation or history is indicative of these organisms (Public Health England, 2014a). Although fastidious and anaerobic organisms are rarely implicated in clinical practice, the consequences of such organisms for the patient are very serious when they are isolated (Kirn and Weinstein, 2013; Almuhayawi *et al*, 2015). It is therefore imperative to get the diagnosis correct. Cohen *et al* (2015) are in favour of extra time spent on blood cultures if fastidious organisms are suspected, stating that sensitivity for blood culture continues to be suboptimal for fastidious organisms, and is significantly reduced in patients on antibiotic therapy at time of blood draw. A study by Potula, Dadhania and Truant (2015) found that although 95% of causative organisms were detected within 3 days, greater than 10% of yeasts were isolated after day 3 of incubation in the blood culture analyser (compared to less than 5% bacteria) thereby possibly warranting further incubation or work done on these blood cultures if yeasts are suspected. Kirn and Weinstein (2013) confirm this by revealing that moulds and dimorphic fungi often grow poorly in typical instrumented blood culture systems, with some of these organisms requiring highly specialised medium supplements, for example lipid supplementation for *Malassezia furfur*.

Additionally, Klaerner *et al*, (2000) found 27.3% of *Candida* species to be undetected by blood culture systems, along with 40.5% non-fermenter Gram-negative species, of which 46.9% of these were *Pseudomonas aeruginosa*. The same study submitted that *Pseudomonas aeruginosa* and *Acinetobacter baumanii* may grow only weakly in blood culture systems, and reach steady state concentration whereby CO_2 acceleration occurs at a relatively low basis, thereby not triggering the analyser algorithm.

Further factors affecting isolation of causative organisms in blood cultures include method of collection, number/timing of samples, previous antibiotic therapy and neutralisation of antimicrobial agents, volume of blood, media used, and incubation time and temperature (Public Health England, 2014a). This has led to the proposal that as many as four sets of culture bottles may be required to achieve a detection rate of greater than 99%, especially if infection by above mentioned species such as *Pseudomonas aeruginosa* or *Candida albicans* is suspected (Lee *et al*, 2007; Stoneking *et al*, 2013). These limitations are confounded by the fact that the number of microbes present in patient blood is usually less than 10 colony forming units per millilitre (cfu/ml) of blood, and sometimes less than 1 cfu/ml (Lin *et al*, 2013).

Study objectives and hypotheses

The primary objective of this study is to determine the significance of blood culture bottle sensor colour change in negative bottles. The hypothesis being tested is that some bottles may contain organisms that have not been sufficiently metabolically active to trigger the analyser algorithm for a variety of potential reasons, but have affected the bottle sensor to such an extent that an experienced Biomedical Scientist may use this colour change to aid in interpreting and reporting a result to the requesting clinician. If this hypothesis is proved to be correct, further study into the algorithms used to determine positivity of blood culture bottles in this system may be necessary. It can be seen that this is the first study of this kind, outside of the manufacturer development, production and validation processes. No

other studies can be found by the author that examine sensor colour change and associated algorithmic mechanisms with regards to false negative results. Further, no other study has been found that attempts to quantify the subjective appearance of the blood culture sensor in order to aid in interpretation when removing and reporting negative bottles, either using the BacT/ALERT 3D system, or any other blood culture system.

Material & Methods

Selection criteria

Data for this research was collected by taking a random selection of blood culture bottles that tested negative after incubation in the BacT/ALERT 3D analyser. Randomisation was achieved on the basis of selecting bottles in an indiscriminate manner, only accepting bottles on the basis of fulfilling the following criteria:

- A set of both anaerobic and aerobic blood culture bottles. No paediatric blood culture bottles were used in this study.
- Both bottles must be negative
- Both bottles must be the newer "PLUS" range containing polymeric beads (as opposed to older, charcoal containing bottles that may still be in stock on some wards)
- Blood culture bottles are to have been incubated for 5 days only (no extended incubation bottles to be used)
- Blood culture bottles are to have been incubated in the BacT/ALERT 3D analyser for the full 5 day incubation without interruption, for example being removed and manipulated due to a false-positive result
- Blood culture bottles from patients younger than 18 years old were excluded from this study

These strict criteria were designed to limit variability/confounding factors from influencing the results of the study.

Inoculation

An aliquot (5ml) of the blood/broth mixture was taken aseptically from the bottles via sterile syringe and needle sets and added to sterile universal containers, with 30µl of this mixture being added to each agar type for culturing. This serves as a standard volume in order to account for potential variability in the data due to quantity of sub-cultured material, and was determined based upon manufacturer instructions. However, these instructions were somewhat ambiguous, with examples including "smear and

subculture all positive bottles" (bioMérieux, 2013a; bioMérieux, 2013b) with no indication of volume for subculture, and "place 1-2 drops of broth onto surface of plated medium" (bioMérieux, 2006), giving only a general, non-quantified measure of volume. A small experiment was therefore necessary in order to decide upon a set volume with which to subculture blood culture bottles in this study. Deionised H_2O was dropped out onto a balance using the manufacturer recommended bioMérieux subculture unit and a sterile syringe; the rationale being that 1g of deionised H_2O is equal to 1000µl. Using this method it was found that 60 drops of deionised H_2O passing through the subculture unit achieved the weight of precisely 1g. Dividing 1000µl by 60 drops gave the volume as 16.7µl per drop from the official subculture unit. It was therefore determined that 30µl as an inoculum in this study would fit the manufacturer instruction of "1-2 drops", being at the higher end of this volume. Subsequent to inoculation onto each agar type, the inoculum was streaked across the plate in order to give semi-quantitative enumeration of any organisms that may have been isolated. This was achieved by using a sterilised flamed loop for each separate plate in order to eliminate cross-contamination that may occur from plate contaminants. Aseptic technique was observed at all times during the extraction and inoculation process.

Media

Terminal sub-cultures were subsequently performed on a variety of agar types in a variety of conditions for set periods of time, as follows:

- Blood Agar, incubated in 7% CO_2 at 37°C for 72 hours - this allows the growth of most bacterial types, with CO_2 enrichment being required for some more fastidious organisms, e.g. *Capnocytophaga canimorsus*
- Chocolate Agar, incubated in 7% CO_2 at 37°C for 120 hours - this allows the growth of fastidious organisms that require growth factors found within Red Blood Cells, but are unable to lyse these cells. 5 day incubation has been selected for these plates as slow growing organisms may take several days to become detectable

- Fastidious Anaerobic Agar, incubated in an anaerobic environment at 37°C for 120 hours - this enables the growth of fastidious anaerobes when incubated in an anoxic environment
- Blood Agar, incubated in a reduced O_2 (5%) and an increased CO_2 (5%) environment at 42°C for 72 hours - this enables the growth of microaerophilic organisms that are able to grow at higher temperatures than that found in the human body, e.g. *Campylobacter* species

Bottle sensor colour differentiation

In order to test the significance of colour change intensity of bottle sensors, and to attempt to put a quantitative figure to an inherently qualitative aspect of this study, the following scale was utilised:

- 1 = no colour change
- 2 = intermediate colour change
- 3 = large colour change (equivalent to that of positive bottles)

Figure 2 shows the colour intensity range of the blood culture bottle sensors.

Figure 2. The colour intensity range of BacT/ALERT 3D (bioMérieux) blood culture bottle sensors, left to right: Positive bottle (score = 3), intermediately coloured bottle sensor (score = 2), uninoculated bottle (score = 1)

Data collection

All data regarding selected bottles and results was entered into a table bearing the following headings:

- **Date**
- **Bottle Number** - the Laboratory Number assigned to the bottle
- **Sensor colour intensity rating** - Score as based upon the criteria given above
- **Relative Fluorescence Unit value at start of incubation** - RFU value of the bottle sensor when the bottle was loaded, as found on the BacT/ALERT 3D analyser
- **Relative Fluorescence Unit value at end of incubation** - RFU value of the bottle sensor when the bottle was unloaded, again found on the BacT/ALERT 3D analyser
- **Increase in Relative Fluorescence Unit value** - the difference in RFU value upon loading and unloading of the bottle, this was noted in order to give the subjective sensor colour rating as described above an objective, measurable value upon collation of the final results
- **BA batch no./exp** - Batch number and expiry date of Blood Agar, used to ensure Quality Control audit
- **Growth BA CO_2 (72 hours)** - Identity of any isolate(s) grown on Blood Agar in 7% CO_2; "NG" if no growth
- **Growth BA μO_2 (72 hours)** - Identity of any isolate(s) grown on Blood Agar in microaerophilic conditions; "NG" if no growth
- **Choc batch no./exp** - Batch number and expiry date of Chocolate Agar, used to ensure Quality Control audit
- **Growth Choc (120 hours)** - Identity of any isolate(s) grown on Chocolate Agar; "NG" if no growth
- **AN0 batch no./exp** - Batch number and expiry date of Fastidious Anaerobic Agar, used to ensure Quality Control audit
- **Growth AN0 (120 hours)** - Identity of any isolate(s) grown on Fastidious Anaerobic Agar; "NG" if no growth

Two record tables were set up; one for each bottle type. This allows the different bottles in each set to be compared at the end of the study, as the aerobic bottle number will correspond to the same anaerobic bottle number.

Additional testing

Supplementary to the main group of blood culture bottles being tested, a small sample of bottles that may be suspected of containing organisms, for example due to an unusual appearance or RFU activity, underwent further molecular testing via the Polymerase Chain Reaction (PCR) based FilmArray (BioFire Diagnostics) analyser. This tested for the nucleic acids of known pathogens that may not be viable, for example due to antibiotic activity, or that may be non-cultivable *in vitro* under certain conditions, thereby bypassing the limitations of culture.

Results

Samples tested and confidence intervals

A population of 225 blood culture sets were analysed in this study, with a total of 450 blood culture bottles being sub-cultured onto 1,800 agar plates. Table 1 shows how this sample population relates to the total population of possible candidate samples for selection on the dates that sampling took place, and the Confidence Interval measurement this subsequently establishes.

Table 1. Sample size Vs. Population size, and associated confidence.

Sample size	Population size	Confidence interval*
225	403	0.043

*Confidence interval calculated using two independent web-based statistical calculators; Decision Support Systems (2016), National Statistical Service (2016)

This table shows that there is a confidence level of 95.7% that results are correct, based on parameters such as sample and population sizes, and estimation of proportional variance (Decision Support Systems, 2016; National Statistical Service, 2016). This indicates that there is a probability of less than 5% ($P<0.05$) that the results obtained in this study could have arisen by chance, and can therefore be seen as statistically significant.

Data analysis

The information gained from the study of this sample population is illustrated in Tables 2-4 and Figures 3-6, with all raw data provided in Appendix 1. These Tables and Figures are subjected to an in-depth analysis in the Discussion section of this report. In addition, temperature check and media production record sheets are also available on request to the author in order to show Quality Control measures have been taken to ensure all results are valid.

Table 2. RFU values upon entry and removal from the BacT/ALERT 3D analyser, and total RFU increase for each bottle type.

		Bottle type	
		Aerobic (n=224)	Anaerobic (n=225)
Start RFU	Minimum	2455	1906
	Maximum	3404	3487
	Mean	2825	2811
	SD	192	227
End RFU	Minimum	2656	2062
	Maximum	4150	4061
	Mean	3290	3237
	SD	290	373
Total RFU increase	Minimum	72	41
	Maximum	922	1017
	Mean	464	425
	SD	209	277

The number of aerobic bottles in Table 2 is 224 due to one bottle (sample number 669007) having a final RFU value of 5732, leading to an RFU value increase of 2932. It was therefore decided that this outlier sample should be omitted from the results as it skews the data too much. However, to analyse the reasons behind the high RFU increase value, this sample was subjected to molecular testing method, as described later in this section.

Table 3. RFU values upon entry and removal from the BacT/ALERT 3D analyser, and total RFU increase for each score number for aerobic bottles.

		Sensor colour score - Aerobic		
		1	2	3
	Proportion	0.9%	9.4%	89.7%
Start RFU	Min	2511	2455	2478
	Max	3247	3260	3404
	Mean	2879	2772	2830
	SD	520	206	188
End RFU	Min	2659	2656	2678
	Max	3379	3458	4150
	Mean	3019	2980	3325
	SD	509	219	275
Total RFU increase	Min	132	72	119
	Max	148	470	922
	Mean	140	208	494
	SD	11	89	197

Again, the data in Table 3 omits the outlying sample, as described above.

Table 4. RFU values upon entry and removal from the BacT/ALERT 3D analyser, and total RFU increase for each score number for anaerobic bottles.

		Sensor colour score - Anaerobic		
		1	2	3
	Proportion	8.9%	28.0%	63.1%
Start RFU	Min	2232	1906	2363
	Max	3155	3149	3487
	Mean	2663	2734	2866
	SD	277	217	205
End RFU	Min	2308	2062	2631
	Max	3658	3650	4061
	Mean	2824	3008	3396
	SD	351	251	265
Total RFU increase	Min	42	41	112
	Max	503	606	1017
	Mean	161	274	529
	SD	114	134	173

Graphical representation of the data in tables 2-4 can be found in Figures 3-6.

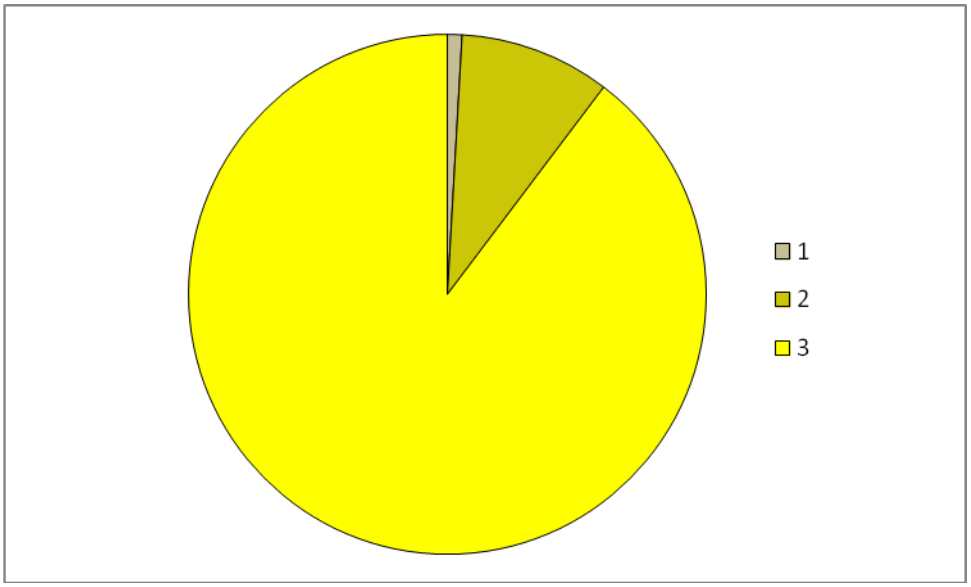


Figure 3. Proportions of Aerobic bottles scored 1, 2 or 3 in this study

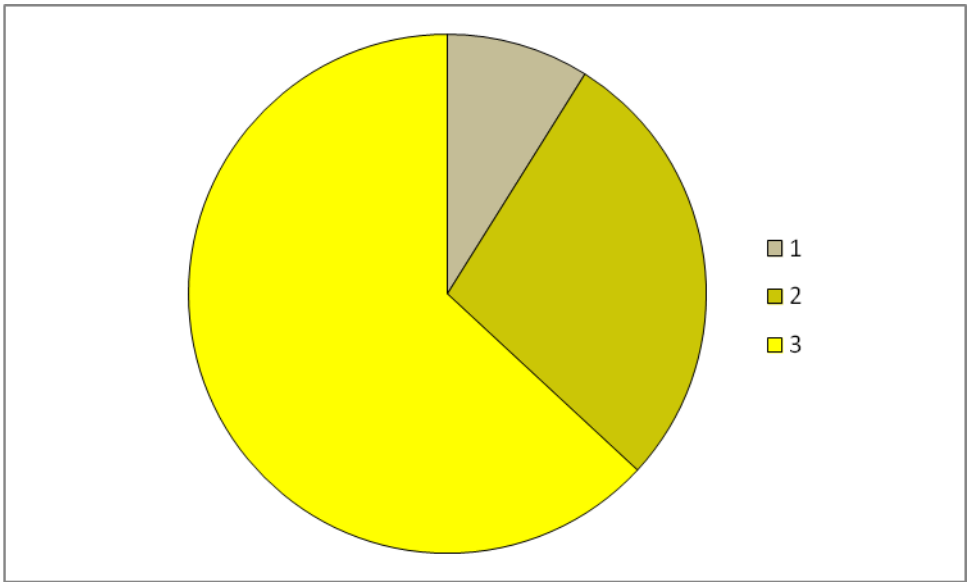


Figure 4. Proportions of Anaerobic bottles scored 1, 2 or 3 in this study

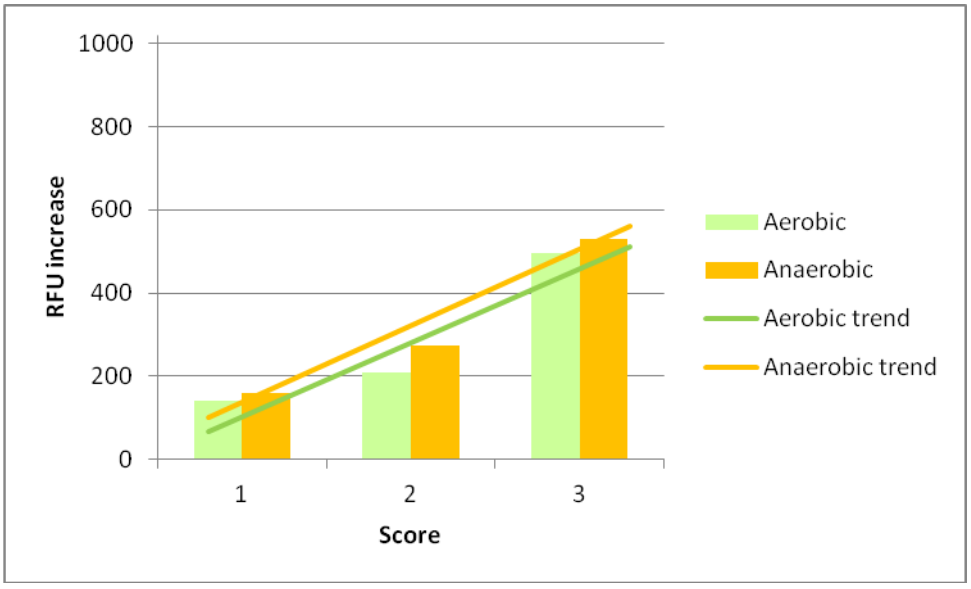


Figure 5. A graph showing the mean increase in RFU value for observational scores for both bottle types.

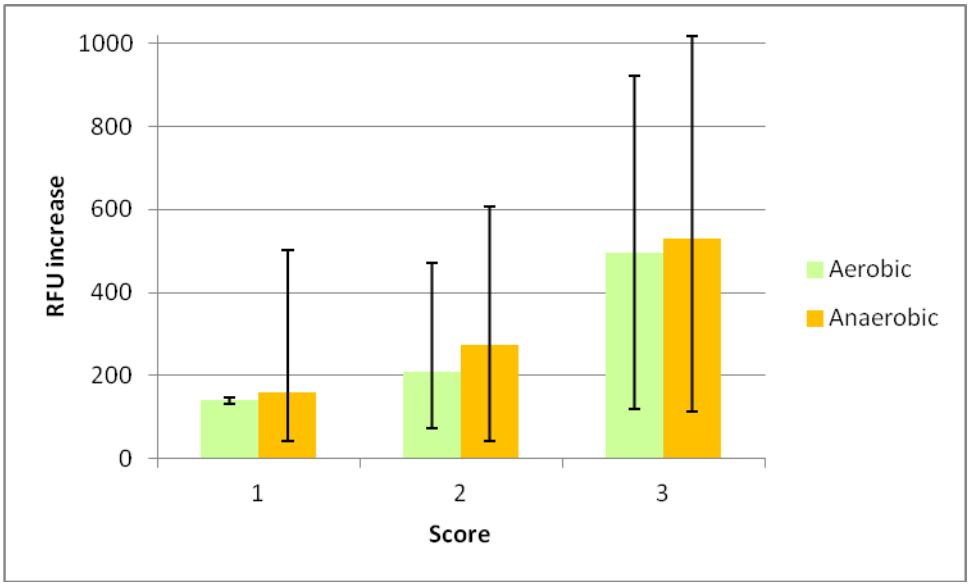


Figure 6. A graph showing the mean increase in RFU value for observational scores for both bottle types in conjunction with the range of respective minimum and maximum values for that bottle type/score.

Samples showing growth on sub-culture

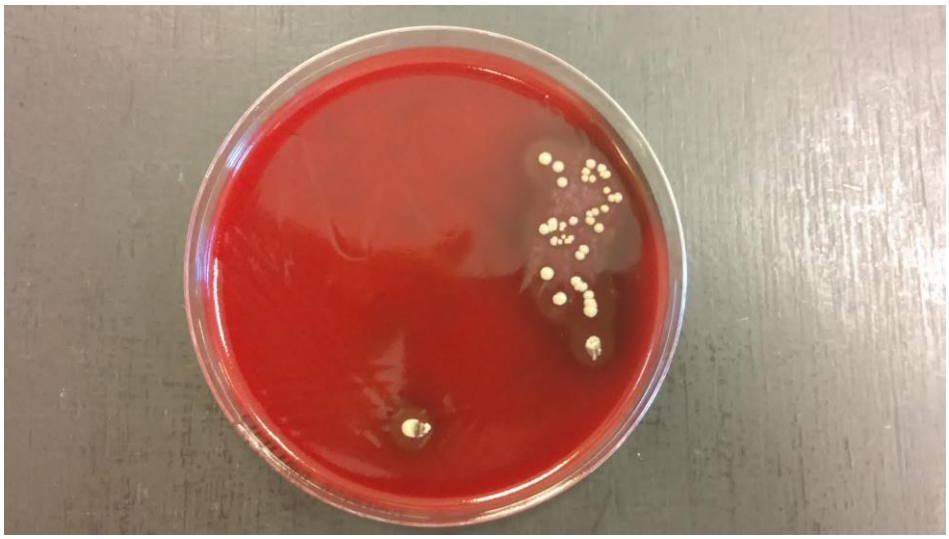
Of the sample population investigated in this study, 5 sets showed growth upon sub-culture in one bottle, comprising 1.1% of the bottles under scrutiny in this investigation. Table 5 presents a summary of the samples that yielded growth, detailing the organism grown and total RFU increase therein.

Table 5. Sample bottles showing growth upon sub-culture in this study

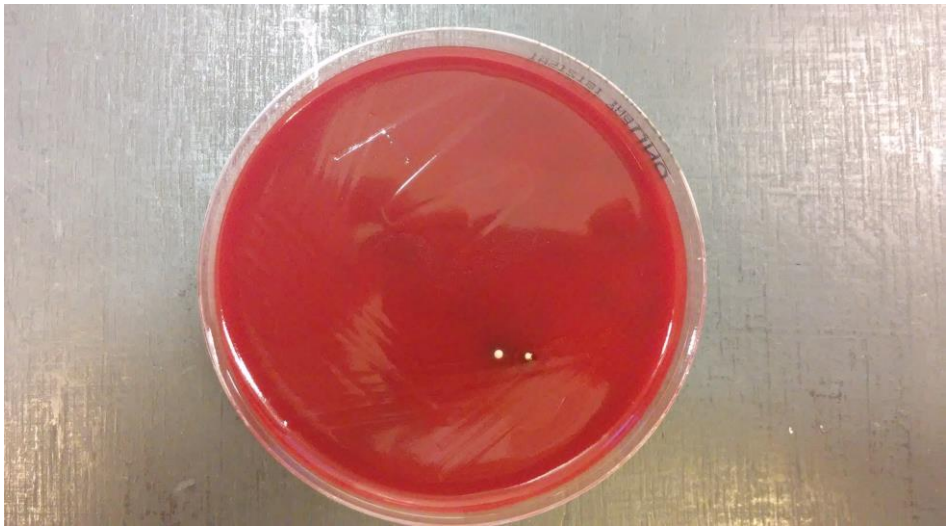
Bottle Number	Bottle type	Organism	Total RFU increase
663598	Aerobic	*P.acnes*	687
665255	Anaerobic	*P.acnes*	482
666942	Anaerobic	*M.catarrhalis*	595
672156	Aerobic	*P.acnes*	611
673980	Aerobic	*P.acnes*	632

Four blood culture sets in this study (663598 - Aerobic; 665255 - Anaerobic; 672156 – Aerobic; 673980 - Aerobic) produced growth of *Propionibacterium acnes* on the Anaerobic Agar plate (See Figure 7). These organisms were identified by way of the Vitek® 2 analyser (bioMérieux) using an ANC ID card (Appendix 2).

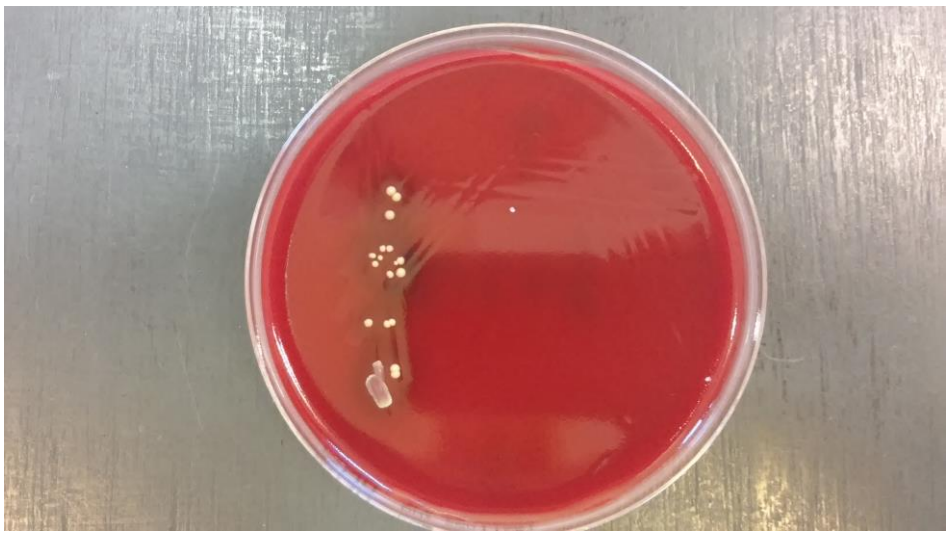
(a)



(b)



(c)



(d)

Figure 7. Plates of sample numbers 663598 – Aerobic (a) 665255 – Anaerobic (b) 672156 – Aerobic (c) and 673980 – Aerobic (d) showing growth of *Propionibacterium acnes*

One anaerobic bottle (666942) produced growth of *Moraxella catarrhalis* on the Chocolate Agar plate (See Figure 8).

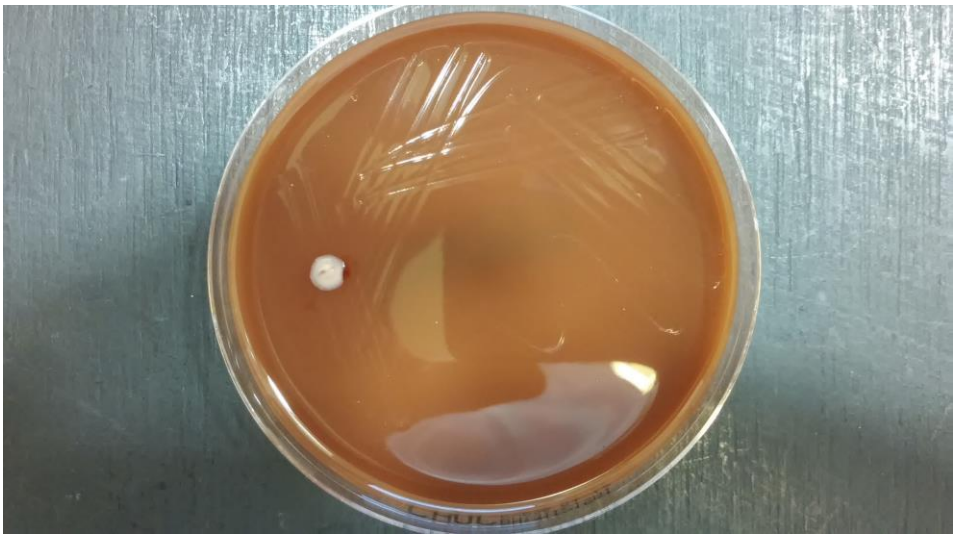


Figure 8. Plate of sample number 666942 – Anaerobic, showing growth of *Moraxella catarrhalis*

This organism was also identified by way of the Vitek® 2 analyser using an NH ID card (Appendix 2). However, further tests were required as a confirmation, as the analyser was not able to fully discriminate between this organism and a further two organisms; *Campylobacter coli* and *Campylobacter fetus ssp fetus*. Confirmation of *Moraxella catarrhalis* identification was based upon the morphology of cells on Gram stain (Figure 9) and the use of the Catarrhalis Test Disc (Remel^{TM}), a simple test used to

differentiate this organism from other Gram-negative cocci. This test uses the production of indigo, an insoluble blue pigment, to confirm the presence of the enzyme butyrate esterase in order to give an indication of *Moraxella catarrhalis* (Remel, 2014; Shields, 2014).

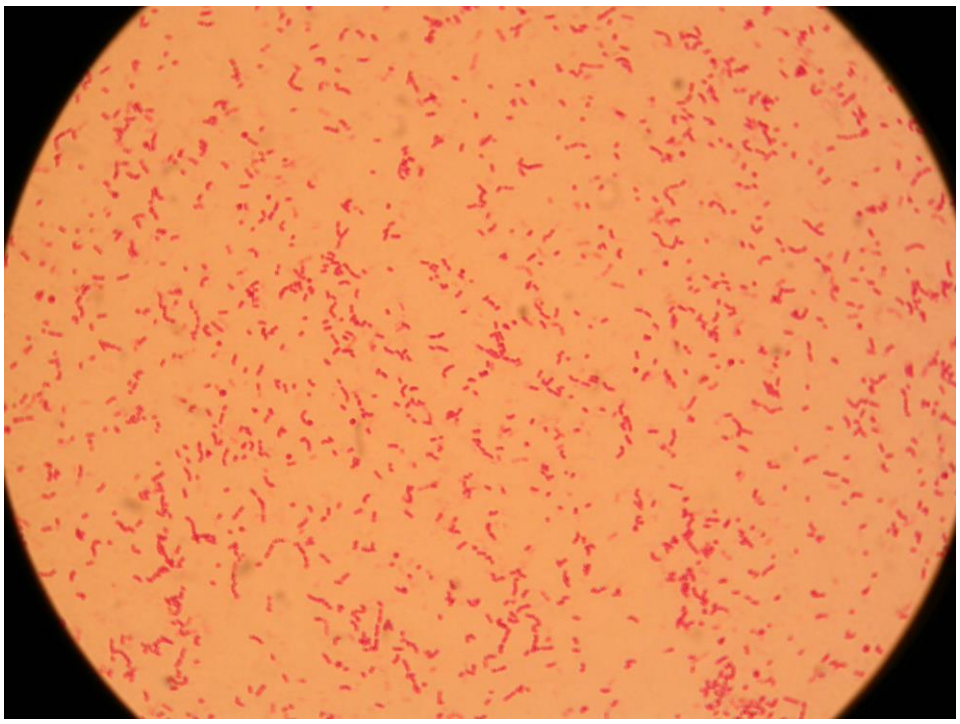


Figure 9. Gram stain of *Moraxella catarrhalis* from sample number 666942

As this organism represents a potentially serious result in terms of patient health repercussions, it was decided that it was prudent to inoculate it back into sterile blood culture bottles in order to confirm the growth characteristics in this system, and therefore give an indication as to the validity of this organism being legitimately isolated from the blood of the patient, as opposed to being a plate/environmental contaminant. To this end, a quantity of cells of this isolate comparable to what would reasonably be found in a bloodstream infection was inoculated into sterile bottles, and inserted into the BacT/ALERT 3D analyser. The quantity of cells was determined by using the premise that a 1.0 McFarland density equates to approximately 3.0×10^8 cfu/ml (Pro-Lab Diagnostics, 2012). A serial dilution of this density of the isolate in question was performed by performing a 1:10 dilution of each successive solution to obtain a cell count of approximately 300 (3.0×10^2) cfu/ml (Figure 10).

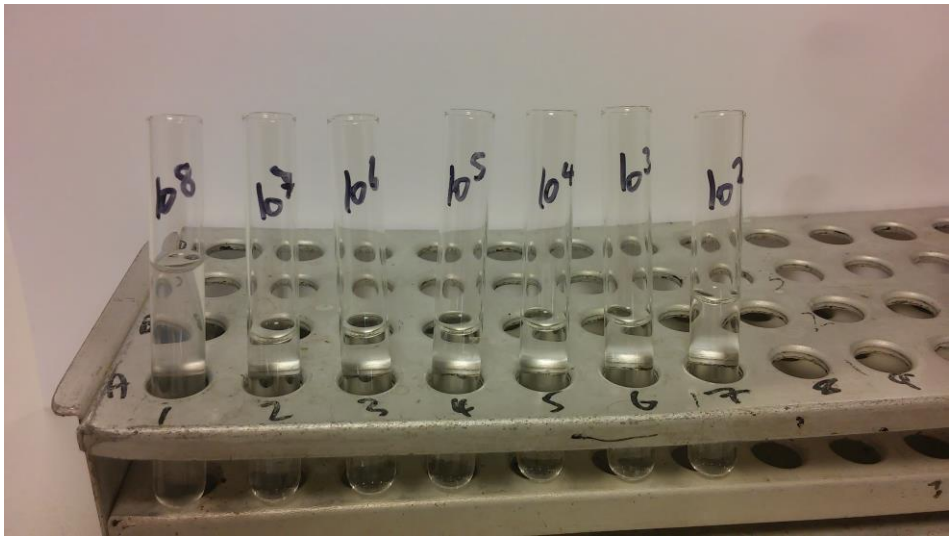


Figure 10. Serial dilution of *Moraxella catarrhalis* from sample number 666942

An aliquot of 167µl of this final solution was inoculated into blood culture bottles to provide a figure of approximately 50 cfu per bottle, thereby being consistent with the numbers that may be found in recently inoculated true-positive bottles, assuming a count of approximately 1-10cfu/ml in the bloodstream of patients with bacteraemia (Smith *et al*, 2008; Cohen *et al*, 2015). The aerobic bottle of this set was flagged as positive after 0.67 days with an increase of 902 RFU, whereas the anaerobic bottle remained negative even after a prolonged incubation period of 10 days, showing an increase of 75 RFU only during this incubation.

Additional small-scale studies

In order to ensure full comprehension of the data collected in this investigation, supplemental small-scale studies were also performed using both negative and positive blood culture bottles as a basis of comparison. For the comparison of known sterile blood culture bottles, ten sets of bottles were entered into the BacT/ALERT 3D analyser without having been inoculated. The results of this are shown in Tables 6-7.

Table 6. Increase in RFU values for sterile aerobic bottles incubated for 5 days in the BacT/ALERT 3D analyser.

Sterile Aerobic Bottles				
Lot number	**Expiry date**	**Start RFU**	**Final RFU**	**Total RFU increase**
3043902	06/05/2016	2632	2761	129
3043902	06/05/2016	2496	2651	155
3043902	06/05/2016	2679	2842	163
3043902	06/05/2016	2719	2845	126
3043902	06/05/2016	2745	2871	126
3043902	06/05/2016	2974	3108	134
3044334	16/06/2016	2466	2651	185
3044334	16/06/2016	2685	2835	150
3044334	16/06/2016	2904	3071	167
3044334	16/06/2016	2645	2870	225

Table 7. Increase in RFU values for sterile anaerobic bottles incubated for 5 days in the BacT/ALERT 3D analyser.

Sterile Anaerobic Bottles				
Lot number	**Expiry date**	**Start RFU**	**Final RFU**	**Total RFU increase**
3043519	24/03/2016	2522	2562	40
3043907	08/05/2016	2527	2585	58
3043907	08/05/2016	2473	2523	50
3043907	08/05/2016	2314	2378	64
3043907	08/05/2016	2345	2398	53
3043907	08/05/2016	2463	2518	55
3043907	08/05/2016	2536	2606	70
3043907	08/05/2016	2436	2486	50
3044236	10/06/2016	2493	2549	56
3044236	10/06/2016	2600	2687	87

Based on the above data, a sterile aerobic bottle can expect to see an increase of 156 RFU on average after incubation for 5 days, whereas a sterile anaerobic bottle increases by 58 RFU on average. However, it is important to note that this is a small study, with larger sample numbers required for this data to be fully valid. Nevertheless, it can be seen that this data does have a use as a basis of comparison to the obtained results.

Conversely to the above results for sterile bottles, throughout this study the results of a range of different organisms obtained from true positive bottles were noted down in order to demonstrate and compare the growth characteristics of positive bottles, as shown in Tables 8-9.

Table 8. A range of organisms and associated BacT/ALERT 3D parameters obtained from positive aerobic bottles.

Positive Aerobic bottles					
Laboratory Number	**Start RFU**	**Final RFU**	**Total RFU increase**	**Time to positive (hours)**	**Organism(s) isolated**
660803	3275	4348	1073	22.8	*Staphylococcus epidermidis*
661029	2767	3772	1005	19.2	*Streptococcus sanguinis*
662015	2786	4044	1258	16.8	Mixed Coagulase-negative *Staphylococci*
664638	2680	3516	836	25.4	*Staphylococcus capitis*
665071	3053	4353	1300	12.0	*Escherichia coli*
665246	2570	3601	1031	13.9	*Enterobacter cloacae*
665356	2771	3280	509	122.9	*Actinomyces odontolyticus*
670588	2685	3708	1023	13.4	Unidentified Alpha-haemolytic *Streptococcus*
674310	2849	3383	534	15.4	*Streptococcus gordonii*
675035	2717	3699	982	11.8	*Streptococcus pneumonia*
675288	2609	3690	1081	20.4	*Staphylococcus aureus* (MRSA)

Table 9. A range of organisms and associated BacT/ALERT 3D parameters obtained from positive anaerobic bottles.

Positive Anaerobic bottles					
Laboratory Number	**Start RFU**	**Final RFU**	**Total RFU increase**	**Time to positive (hours)**	**Organism(s) isolated**
660562	3037	5087	2050	16.8	*Clostridium perfringens*
660803	2844	3609	765	30.2	*Staphylococcus epidermidis*
661029	2546	3680	1134	14.4	*Streptococcus sanguinis*
662099	2826	3004	178	27.4	*Staphylococcus hominis*
662015	2976	4528	1552	16.8	Mixed Coagulase-negative *Staphylococci*
665071	3066	4822	1756	11.8	*Escherichia coli*
665413	2685	3797	1112	22.1	*Streptococcus pyogenes*
665756	2637	3716	1079	12.0	*Streptococcus equisimilis*
667460	2958	3248	290	85.7	*Staphylococcus urealyticum*
669519	2839	4044	1205	118.3	*Propionibacterium acnes*
670359	2771	3534	763	17.8	*Serratia marcescens* & *Citrobacter freundii*
669737	2722	4005	1283	128.2	*Propionbacterium acnes*
674310	2527	2813	286	23.0	*Streptococcus gordonii*
673986	3056	4410	1354	76.6	Unidentified Anaerobe Gram-positive Cocci
675206	2829	3418	589	79.9	Capnoaerophilic *Streptococcus*
676781	2386	3099	713	42.0	*Actinomyces naeslundii*
676566	2689	3404	715	129.4	*Propionibacterium acnes*

With regards to the use of molecular diagnostic methodology by the PCR-based FilmArray analyser, funding issues necessitated only a very limited number of samples to be subjected to this testing method. Table 10 shows

the results of the four samples tested using the FilmArray analyser, with all results sheets being available in Appendix 3.

Table 10. Samples tested using the PCR-based FilmArray (BioFire Diagnostics) blood culture panel.

Sample number	Bottle Type	Reason for PCR analysis	Result*
662714	Aerobic	Blood haemolysed in bottle	NEGATIVE
665909	Aerobic	High starting RFU value (3301)	NEGATIVE
668449	Aerobic	Bottle media very frothy	NEGATIVE
669007	Aerobic	High RFU increase (2932)	NEGATIVE

*Note, Limit of Detection not established by either literature search of peer-reviewed papers or official manufacturer guides

As can be seen from the above, all bottles chosen for this analysis were of the aerobic variety. This was done as the FilmArray Blood Culture panel does not contain any obligate anaerobic organisms, but does contain organisms that are strictly aerobic under normal conditions, such as *Acinetobacter baumanii* and *Pseudomonas aeruginosa*. If both bottles shared the trait that resulted in being chosen for PCR analysis, the aerobic bottle was therefore chosen due to this rationale.

Discussion

Data analysis

As demonstrated by Tables 3 and 4 and Figures 3 and 4, the sample population in both of the bottle types were dominated by a score of 3. Interestingly, there is an increase in the start RFU value as the score increases from 1 through 3 for the anaerobic bottles, an occurrence that is absent in the aerobic bottle data. This may not, however, be truly indicative of the population of samples as a whole. Whilst the randomisation of samples to be included in this study was attempted, there is a possibility of selector bias due to the perceived increase in the chance of detecting organisms in bottles that have higher RFU values, and therefore a higher associated score. Any further extrapolation of this study should ensure randomisation is accordingly ensured. Additionally, it may be advantageous for any study that wishes to continue this work to record bottle scores for the entire population, which would result in a comparison of the proportions of study population scores with sample population scores.

With regards to the attempt to apply a quantitative figure to a qualitative concept in this study by relating the sensor colour score to the RFU value of the sensor, Figure 5 shows that, on average, there is an increase in RFU value with each score increment with an upward trend clearly apparent for each bottle type. However, this does not follow any kind of pattern, for example a linear or logarithmic relationship. Furthermore, Table 4 and Figure 6 show a great deal of overlap of the RFU ranges, with the maximum RFU increase value at a score of 1 being much higher than the minimum RFU increase value at a score of 3, to use the anaerobic bottles as an example (RFU values at 503 and 112 respectively). It can, however, be argued that the aerobic bottle data is flawed due to having only a very small sample of data (n=2) at the score of 1. Nevertheless, it can be demonstrated that, whilst there is an average trend towards a higher RFU value with each assigned score increase, on an individual level the correlation is unfounded, thereby showing a critical deficiency in this part of the study. This is also

confounded by the phenomenon whereby the colour perception of different people may be quite different, especially in the subjective impression of similar hues of the same colour (Cropper, Kvansakul and Little, 2013), thus preventing further application of this to multiple members of staff in the routine laboratory. Whilst this may be remedied somewhat by a "paint colours chart" being set up, or some kind of mobile RFU reader, the above conclusions regarding the scores of individual bottles renders this point moot.

Volume of inoculum in blood culture bottles

The small sample of sterile bottles that were scrutinised as to the increase in RFU values over a 5 day incubation period in this study showed an approximate increase in the mean RFU value three times higher in the aerobic bottles than the anaerobic bottles (156 and 58 respectively). However, the mean RFU value increase for each bottle type was roughly equal after 5 days incubation post-inoculation with patient blood (464 for aerobic bottles, 425 for anaerobic bottles). This may, however, be due to several factors, such as the proportion of scores assigned each bottle type being different, or differences in the volume of inoculum in each bottle type. It is recommended by the bottle manufacturer that aerobic bottles are inoculated first, so as to minimise the risk of inoculating air bubbles that may be present in the tubing into the anaerobic bottle (bioMérieux, Inc., 2013a; bioMérieux, Inc., 2013b). The order of bottle inoculation may therefore influence the amount of blood present in each bottle type. To this end, one impression gained whilst collecting data for this study was that without noting the volume of blood present in all blood culture bottles concurrently with the reflectance unit values, the data generated can be seen to be meaningless, as white blood cells and platelets also capable of respiration (Smith *et al*, 2008), thus blood volume can affect these values. This can be seen as a methodological error in this study, with any further research progressing this study ensuring that inoculum volume is included into the data analysis to account for increasing RFU levels. This is based upon the

premise whereby Thorpe *et al* (1990) noted that the addition of sterile blood generated detectable CO_2 which slowly rises over time, although this did not interfere with ability of algorithm to interpret CO_2 production by microbes. This is due to bottles necessarily attaining either an increase in rate of change, or a sustained linear increase in CO_2 to be classed as positive, of which the background increase produced by leucocytes usually does not meet these criteria (Wilson *et al*, 1992). Typically, microbes dominate CO_2 production due to increased metabolic respiration rates, although in cases of leukaemia or thrombocytosis, this trend may be present in leukocytes or platelets respectively (Smith *et al*, 2008). It can therefore be seen that blood volume is an important factor in determining the significance in a rise in RFU value, a factor not taken into account during the conception of this study. Any further study of this nature should therefore seek to find a correlation between blood volume in blood culture bottles and increase in RFU values as a baseline prior to making any conclusions regarding significance of such increases, and associated sensor colour change. This also further highlights the importance of clinical details in determining action taken with samples upon receipt, as a history of leukaemia and/or thrombocytosis would be a significant factor in the decision process regarding not only length of time the blood culture bottles are incubated in the analyser, but also the importance of sensor colour change if said bottles were to be considered negative at the end of this period.

Microbial population concentrations in blood culture bottles

Relating to the significance of blood volume in blood culture bottles is the concept that rate of detection depends on initial concentration of organisms in addition to incubation conditions, with blood culture bottles typically requiring a load of 10^8 - 10^9 cfu/ml for the algorithm to detect required CO_2 levels and/or rate change (Smith *et al*, 2008). In most cases bloodstream infections are intermittent, and circulating microbial loads are low, typically being 1-10cfu/ml (Smith *et al*, 2008; Cohen *et al*, 2015). Indeed, Lin *et al* (2013) found that the yield of blood cultures in adults increase by

approximately 3% per millilitre of blood collected, as well as resulting in earlier detection. This same study, however, found that recovery rate decreases at volumes greater than 10ml of blood added to bottles, most probably due to the inhibitory effect of blood compounds and/or antimicrobial agents that may be present. Zadroga *et al* (2013) support this view by stating concern that initiation of therapy prior to blood draw may artificially sterilise blood cultures. The blood culture bottles used in this study, however, contain polymeric resin beads that it is claimed has the ability to effectively sequester antimicrobial agents and inactivate toxic compounds in blood (bioMérieux, 2013a; bioMérieux 2013b; Kirn *et al*, 2014), thereby rendering this argument potentially erroneous. It can be seen that this counter argument is nullified, however, if certain conditions are met; for example the addition of too much blood, or taking the sample downstream of an antibiotic IV line which would exceed the neutralising capacity of the beads.

Microbes in a patient sample generally start growing in blood culture bottles prior to incubation in the blood culture system (Rönnberg *et al*, 2013), therefore if a large amount of CO_2 production occurs prior to the entry of the bottle, any further increase may go unnoticed (van der Velden *et al*, 2011). Both delayed entry and pre-incubation has been shown to be responsible for failures of the computer algorithm to detect certain positive cultures (Klaerner *et al*, 2000). Following logarithmic expansion, organisms in the stationary phase would not register as positive on the analyser (Klaerner, *et al*, 2000; Public Health England, 2014a). Public Health England (2014a) recommends that delayed cultures should be inspected upon receipt for signs of growth, including yellowing of the sensor, haemolysis, gas production and turbidity, as storage of inoculated blood culture bottles at room temperature doubles or triples the time-to-positive for many organisms. Conversely, Klaerner *et al* (2000) suggests that pre-incubation at room temperature may give an enhanced yield of *Pseudomonas aeruginosa*, although there is uncertainty in that study as to whether fastidious

organisms would tolerate this. It is therefore preferable to get the inoculated blood culture bottles to the analyser as quickly as possible, as the potential increased yield of one organism is outweighed by the detrimental effects this may have upon other organisms. Some laboratories also routinely pre-incubate blood cultures at 37°C prior to insertion into the analyser if there is a delay in order to maintain viability of organisms, a practice that van der Velden *et al* (2011) suggests increases the incidence of false-negative results compared to storage at room temperature. Regardless of effects on organisms, the clinical benefits of improvements in speed of diagnostic methods such as are limited if a blood culture takes a long time to get to the laboratory (Rönnberg *et al*, 2013). In theory, this should not present an issue for this study, with Nobles Hospital being a relatively small hospital whereby all wards are only a short walk away from the centralised laboratory specimen reception area. However, it is clear from the results of this study that some bottles had a high RFU value prior to being entered into the analyser; sometimes having a higher value than bottles that had been removed from the analyser as negative, or even those that had been flagged as positive.

Isolation of *Propionibacterium acnes*

In this study, four bottles produced growth of a *Propionibacterium acnes* isolate; three originating from an aerobic bottle, and one from an anaerobic bottle in different sets. The only plate that showed growth in all situations was the AN0 plate, being incubated in an anaerobic atmosphere conducive to the growth of this anaerobic, though aerotolerant, organism (Barnard *et al*, 2015). Whilst these bottles show an RFU rise of slightly above the average increase compared to their respective bottle types, the RFU increase of all isolates is well within the ranges shown by Figure 6. Additionally, a comparison with the true positive bottles in which *Propionibacterium acnes* was isolated, as in Table 9, show that the true positive isolates showed a much greater RFU value increase, with two of the three bottles achieving an RFU value increase of >1200 within approximately the same time-scale of

120 hours as the study bottles were incubated. This may suggest a low bacterial load of the original blood sample, indicating a contamination issue rather than an actual blood infection, of which further evidence is presented. Although a study by Almuhayawi *et al* (2015) found 6/400 false negative bottles, 5 of which were *Propionibacterium* species, the significance of this organism is in doubt in this situation. In the first instance, this study used simulated blood cultures, and may therefore not be truly representative of what occurs in a natural setting. Secondly, *Propionibacterium acnes* is a common skin organism which sometimes contaminates blood cultures due to samples being obtained by penetrating the skin (Brooks *et al*, 2013). To this end, a study by Damgaard *et al* (2015) reported *Propionibacterium acnes* as being the one of the main contaminants of blood donor blood, second only to Coagulase-Negative *Staphylococci*. This is based on a small study, however, and therefore requires corroboration from larger scale research. Further, Murray and Masur (2012) suggest that true pathogens tend to be detected on a timescale of less than 24-48 hours, with greater than 48-72 hours suggestive of low numbers, and therefore probable contaminants. As the *Propionibacterium acnes* isolates in this study were not detected in the original 5 day incubation period, this can be shown to imply contamination as opposed to true pathogenesis. However, Achermann *et al* (2014) report the mean times to detection with *Propionibacterium* species as 6.4 days in anaerobic bottles, and 6.1 days in aerobic blood culture bottles. Though these species grow better in anaerobic bottles, they are able to grow in aerobic bottles due to the development of an anaerobic microenvironment at the bottom of non-shaken bottles (Achermann *et al*, 2014). This should not apply to the BacT/ALERT 3D analyser, due to the bottles being continually agitated during incubation (Thorpe *et al*, 1990). Although this organism rarely causes significant bacteraemia, there is nosocomial infection potential, with infections mostly due to predisposing factors, for example the presence of a foreign device such as a Central Venous Catheter, or prosthetics (Park *et al*, 2011; Ramirez, *et al*, 2015). It is therefore crucial that the isolates in this study are considered contaminants based on solid evidence, in order to

both not omit treatment for infection, and to not administer unnecessary antibiotics for a case of contamination. One way in which to distinguish between contamination and infection is that more than one sample is often positive in the case of infection (Achermann *et al*, 2014). An important factor to take into account here, however, is that bacteraemia is often asymptomatic in immunocompetent individuals, with daily activities such as chewing and tooth brushing facilitating translocation of bacteria into the bloodstream (Damgaard *et al*, 2015). Conversely, there is an increased incidence of infection in patients who are immunocompromised caused by organisms that are non-virulent in the normal host and form part of the normal flora, thereby usually considered contaminants in immunocompetent host bloodstream infection (Gale, 2016). Additional tests/incubation time should only be considered if clinically indicated, for example IV drug use, prolonged central venous catheter, cancer, or contact with cats (*Bartonella henselae*) (Tattevin *et al*, 2015). In the case of samples from immunocompromised patients at Nobles Hospital, blood cultures are incubated for 10 days (Gale, 2016), thereby excluding such bottles from this study. It is therefore clear that much of the interpretation of Medical Microbiology is based upon the clinical details provided by clinicians being complete and relevant. Without sufficient information regarding a patient's medical history, a bacterial species such as *Propionibacterium acnes* may be incorrectly deemed insignificant. In this study, the four isolates were deemed contaminants. This was decided upon based upon the above evidence, and through deliberation with the Consultant Microbiologist at Nobles Hospital.

Isolation of *Moraxella catarrhalis*

One anaerobic bottle in this study revealed growth of *Moraxella catarrhalis*, a non-motile, non-fermenter, oxidase positive species considered both normal biota and a potentially significant pathogen of the upper respiratory tract since the mid-1980s (Ioannidis *et al*, 1995; Brooks *et al*, 2013). This bottle showed an overall RFU value increase of 595; a 140% increase on the mean

RFU increase for that bottle type overall, and a 112% increase on the mean RFU increase for the respective sensor score of 3. There was, however, some doubt from the author about this isolate being truly representative of an organism inoculated into the bottle as a part of a bloodstream infection, with a plate contaminant being more likely. Although there have been reports of severe bacteraemia, endocarditis and meningitis in imunocompetent, otherwise healthy patients (Ioannidis *et al*, 1995; Brooks *et al*, 2013), Bernhard, Spaniol and Aebi (2012) state that invasive infection of this kind is very rare with less than 80 cases reported during the previous 30 years. Whilst Ioannidis *et al* (1995) suggested that it is unclear as to whether the isolation of *Moraxella catarrhalis* can be considered contamination in any situation, one colony of this organism was detected on one plate sub-cultured from the anaerobic bottle of the set only. Though long thought of as a strict aerobe, some strains of *Moraxella catarrhalis* are now known to be capable of anaerobic respiration via a nitrate reductase pathway, though this requires specialist media for anaerobic growth (Plotkin, Hatakeyama and Ma, 2015). However, Shoji *et al* (2013) reported 50% of cases of *Moraxella catarrhalis* isolated from both bottles in blood culture systems, with 50% of cases from the aerobic bottle only, and none from the anaerobic bottle only. This may indicate that there is scope in the blood culture system for the isolation of *Moraxella catarrhalis* in anaerobic bottles, although this requires confirmation and validation, due to the small numbers of samples involved in that study. Upon re-inoculation of this organism into a sterile set of blood culture bottles, as described in the results section of this paper, the aerobic bottle was flagged as positive after 0.67 days, whereas the anaerobic bottle showed no growth after an extended incubation period of 10 days. Official manufacturer guidelines recommend the addition of blood such as sterile defibrinated horse blood (10% v/v) to aid in the recovery of some species where there may be a risk of less than 100.0% recovery, i.e. *Capnocytophaga ochracea*, *Cardiobacterium hominis* and *Haemophilus parainfluenzae* (bioMérieux, 2013a; bioMérieux 2013b). The isolate uncovered in this study, however, grew well when passaged to CLED

media, an agar lacking in any blood addition. It can therefore be seen that due to the absence of growth of the organism after being reintroduced into an anaerobic blood culture bottle, correlated with the lack of evidence of invasive disease of this organism, this isolate indeed represented a plate contaminant.

Molecular testing

One aspect that was not taken into account in this study was the use of antimicrobial agents prior to inoculating blood culture bottles. As discussed above, this could affect the viability of organisms present, thereby giving a false-negative result. Newly devised technologies are emerging, however, that may address both this and the requirement for specialised media for organisms that are hard to culture, such as *Legionella* species, or those that are unable to grow on any media types at all, such as spirochetes. Such emerging technology may also reduce the time required for an organism identification and/or antimicrobial sensitivity profile to be generated. For example, whilst current culture based methods can take up to 72 hours or longer for identification and sensitivity (Southern *et al*, 2015), molecular assays have produced shorter turn-around times with larger number of positive results deemed clinically significant, especially if the patient has been on antibiotic therapy (Cohen *et al*, 2015). Several techniques are projected to be in routine use in the near future, such as Polymerase Chain Reaction (PCR), Mass Spectrometry (MS), and combinations of both, for example PCR amplicons analysed by MS (Jordana-Lluch *et al*, 2014). It has been claimed that pathogen DNA may be detected by combined PCR/MS in 7.7% of culture-negative samples (Loffler, *et al*, 2013), with the PCR-based FilmArray able to detect antibiotic resistance genes to compounds such as methicillin, vancomycin and carbapenems (Blaschke *et al*, 2012). Indeed, the FilmArray BCID panel identifies a wide range of bacterial and fungal pathogens, accounting for 93% orgs isolated from clinical specimens in just over an hour (Blaschke *et al*, 2012). It can therefore be seen that this emerging technology is set to revolutionise the way in which blood cultures

are processed and interpreted, although there are certain drawbacks to this technology. Cohen *et al* (2015) assert that technological improvements are required in order to enhance DNA extraction procedures, decrease contamination rates and decrease detection thresholds for difficult-to-grow, slow growing or non-growing microorganisms. Polymicrobial culture is also cited as the most common reason for detection failure in PCR (Blaschke *et al*, 2012), although multiplex diagnostic platforms allowing the identification of several pathogens in a single specimen may be used to negate this problem (Cohen *et al*, 2015). As well as problems with polymicrobial cultures, FilmArray PCR limitations include examples of identifying *Salmonella sp*. as *Klebsiella oxytoca*, and Coagulase-Negative *Staphylococcus* as *Staphylococcus aureus* (Southern *et al*, 2015). Murray and Masur (2012) express the complexities of PCR and MS, as non-microbial cells, serum proteins and culture nutrients must be removed before microbial cells are evaluated, whilst Jordana-Lluch *et al* (2014) describe the use of whole blood PCR assay as challenging due to excess human DNA and haemoglobin potentially hampering detection, or inhibiting the reaction. Further, MS as a discrete methodology without the use of PCR still relies on culture, therefore a 12-17 hour delay is unavoidable (Jordana-Lluch *et al*, 2014). It can therefore be seen that at present, molecular methods are useful in complementing culture methods, rather than being used as a replacement for those methods. In this study a small number of samples were subjected to PCR-based FilmArray analysis (Table 10). All samples tested using this PCR-based method were chosen due to the physical properties of the sample being related to a possible bacterial load in the blood culture bottle, for example frothy blood culture broth or a high RFU increase indicating copious gas production. Table 11 shows the organisms detected by the FimArray blood culture panel.

Table 11. Organisms detected by the FilmArray analyser (BioFire Diagnostics, 2016)

Gram negative bacteria	**Gram positive bacteria**	**Yeasts**
Acinetobacter baumanii *Enterobacteriaceae* family -*Enterobacter cloacae* -*Escherichia coli* -*Klebsiella oxytoca* -*Klebsiella pneumoniae* -*Proteus spp*. -*Serratia marcescens* *Haemophilus influenzae* *Neisseria meningitidis* *Pseudomonas aeruginosa*	*Enterococcus spp*. *Listeria monocytogenes* *Staphylococcus* genus -*S.aureus* *Streptococcus* genus -*S.agalactiae* -*S.pneumoniae* -*S.pyogenes*	*Candida albicans* *Candida glabrata* *Candida krusei* *Candida parasilopsis* *Candida tropicalis*

Further to the drawbacks listed above, Table 11 shows that no organisms requiring specialised media, for example *Legionella spp*., or those that are unable to be cultivated *in vitro*, e.g. spirochetes, are detected by this method. Whilst the use of other panels, for example the respiratory panel, may be able to detect a limited range of other organisms, there are several reasons why this is unreasonable as a diagnostic test in this situation. Firstly, these specific organisms would necessarily be suspected due to clinical interpretation of the signs, symptoms and other diagnostic test results of the patient and, of greater importance, other panels are not validated for testing on blood samples (BioFire Diagnostics, 2015).

Laboratory quality management

Even though it can be seen that this study has not revealed anything new or innovative about the BacT/ALERT 3D blood culture analyser, or local and national procedures that are used in conjunction with this asset, the study may still be used in a positive way in the Medical Microbiology at Nobles Hospital on the Isle of Man. Validation is a Clinical Pathology Accreditation (CPA), United Kingdom Accreditation Service (UKAS) and International Organisation for Standardization (ISO) requirement, with examination procedures validated for intended use prior to adoption, and methods and results obtained recorded (Public Health England, 2014b). For methods that

are in use for which no specific existing validation is in place, documentary evidence which supports reasons for their use must be provided to adhere to various standards (Public Health England, 2014b). This is addressed in the following ISO 15189:2012 extracts.

> "The laboratory shall verify upon installation and before use that the equipment is capable of achieving the necessary performance and that it complies with requirements relevant to any examinations concerned" (ISO 15189:2012 - 5.3.1.2)

> "The independent verification by the laboratory shall confirm, through obtaining objective evidence (in the form of performance characteristics) that the performance claims for the examination procedure have been met. The performance claims for the examination procedure confirmed during the verification process shall be those relevant to the intended use of the examination results" (ISO 15189:2012 - 5.5.1.2)

Validation examines whole process that is being used to check that results are correct (Public Health England, 2014b). This study may therefore be used as an internal validation report to confirm that the laboratory is justified in sending out negative reports on blood cultures when the BacT/ALERT 3D analyser has indicated that this is the case.

Further study

Whilst many factors were taken into account during this study in order to ensure equal treatment of samples so that confounding elements do not influence the results, it can be argued that the timings of sub-culturing bottles is methodologically unsound. For example, negative blood cultures are flagged as negative throughout the day, as the laboratory receives and processes these at all times of the day. Furthermore, bottles may be removed and processed as negative several times a day. This may present a situation where a bottle may have been sitting on the analyser for several hours and then sub-cultured several hours more after being removed from

the analyser, alongside a bottle that may have been relatively recently been flagged as negative. Under ideal conditions, a bottle would be taken off of the analyser as soon as it is determined to be negative, and sub-cultured immediately. This would be a very labour intensive process, however, thereby rendering it operationally impractical. As this study stands, it has been decided that after being incubated on the BacT/ALERT 3D analyser for 5 days, small differences in time delay between bottles prior to sub-culture should not introduce significant consequences. Any further research emulating and progressing this study, however, should be set up in such a manner that this is as tightly controlled as is practical.

Conclusion

This study set out to test the efficacy of the BacT/ALERT 3D analyser in the Medical Microbiology Laboratory at Nobles Hospital on the Isle of Man; specifically, the competence of the analyser to assign a negative value to a blood culture bottle after 5 days incubation. To this end, it has been conclusively proven that bottles are indeed sterile, as claimed by the analyser, with 98.9% of sample bottles in this study showing no growth on terminal sub-culture. It has also been shown that organisms isolated upon terminal sub-culture in the remaining number of bottles were either skin contamination of the inoculation process of the bottle, or environmental contamination of the agar plate. Accordingly, negative blood culture results sent out by the Medical Microbiology Laboratory at Nobles Hospital can be seen to show a true representation of the health status of the patient in question at the time of blood sampling.

An objective of this study was also to ascertain if the sensor colour of the blood culture bottles may be used to inform the operator of the need to perform terminal sub-cultures. This can be seen as being proven to not be the case, both due to the reasoning above, and also due to colour changes not being a reliable predictor of Relative Fluorescence Unit values on an individual level. It can be argued that sensor colour changes are more closely tied to volume of blood inoculated, as opposed to bacterial metabolic activity. Unfortunately, this was not taken into account in this study, and is therefore a critical component that should be investigated if this study was to be taken further in the future.

It can be seen that any subsequent research that uses this study as a starting point should also consider methodology that can be seen to reduce confounding factors that were not as strict in this study as they perhaps should have been, for example bottles being sub-cultured as soon as they are removed from the analyser, rather than being batched. Additionally, whilst this study only utilised the minimum of molecular methods, further

research in this area should consider this of paramount importance in order to be able to obtain a holistic picture of the reporting of negative blood cultures.

Although this study showed no real novel results, it can be seen as being useful as an internal validation exercise that may be used as a quality control and accreditation tool. It has been shown here that the performance claims of the manufacturer have been verified in the routine laboratory and, although done retrospectively, this can be considered valid documentary evidence to this end.

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Appendix 1 – Raw data

Aerobic bottles: 1

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
01/12/15	660071	3	3212	3817	605	20111SB1 18/12/15	NG	NG	10111SA1 08/12/15	NG	20111SA1 18/12/15	NG
01/12/15	660197	3	2875	3537	662	↓	NG	NG	↓	NG	↓	NG
01/12/15	660256	3	2720	3262	542	↓	NG	NG	↓	NG	↓	NG
01/12/15	660262	3	2667	3411	744	↓	NG	NG	↓	NG	↓	NG
01/12/15	660265	1	3247	3379	132	↓	NG	NG	↓	NG	↓	NG
02/12/15	660441	2	2710	2861.	151	26111SB2 24/12/15	NG	NG	↓	NG	↓	NG
↓	660491	3	2698	3409	711	↓	NG	NG	↓	NG	↓	NG
↓	660495	2	3163	3639	276	↓	NG	NG	↓	NG	↓	NG
04/12/15	660768	3	3149	3577	428	↓	NG	NG	26111SA1 24/12/15	~~NG~~ NG	↓	NG
↓	660772	3	2877	3302	425	↓	NG	NG	↓	~~NG~~ NG	↓	NG
↓	660797	3	2679	3226.	547	↓	NG	NG	↓	~~NG~~ NG	↓	NG
05/12/15	660804	3	3239	3827	588	↓	NG	NG	↓	NG	↓	NG
↓	660830	3	3244	3600	356	↓	NG	NG	↓	NG	↓	NG
↓	660831	3	2714	3101	387	↓	NG	NG	↓	NG	↓	NG
08/12/15	661021	3	3085	3826	741	↓	NG	NG	↓	NG	↓	NG

Anaerobic bottles: 1

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
01/12/15	660071	3	2710	3480	770.	20111SB1 18/12/15	NG	NG	10111SA1 08/12/15	NG	20111SA1 18/12/15	NG
01/12/15	660197	3	2942	3543	601	↓	NG	NG	↓	NG	↓	NG
01/12/15	660256	2	2621	3160	539	↓	NG	NG	↓	NG	↓	NG
01/12/15	660262	1	2937	3166.	229	↓	NG	NG	↓	NG	↓	NG
01/12/15	660265	2	3034	3399	365	↓	NG	NG	↓	NG	↓	NG
02/12/15	660441	2	2520	2741	221	26111SB2 24/12/15	NG	NG	↓	~~NG~~ NG	↓	NG
↓	660491	1	3155	3658.	503	↓	NG	NG	↓	~~NG~~ NG	↓	NG
↓	660495	2	2721	2856.	135	↓	NG	NG	↓	~~NG~~ NG	↓	NG
04/12/15	660768	3	3119	3522	403	↓	NG	NG	26111SA1 24/12/15	NG	↓	NG
↓	660772	2	2827	3231	404	↓	NG	NG	↓	NG	↓	NG
↓	660797	3	2939	3460.	521	↓	NG	NG	↓	NG	↓	NG
05/12/15	660804	3	2754	3159	405	↓	NG	NG	↓	NG	↓	NG
↓	660830	2	3102	3424	322	↓	NG	NG	↓	NG	↓	NG
↓	660831	2	3034	3290	256	↓	NG	NG	↓	NG	↓	NG
08/12/15	661021	3	3148	3827	679	↓	NG	NG	↓	NG	↓	NG

Aerobic bottles: 2

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
04/12/15	661022	3	2803	3468	665	26111582 24/12/15	NG	NG	26111541 24/12/15	NG	20111541 18/12/15	NG
↓	661023	3	2645	3168	523	↓	NG	NG	↓	NG	↓	NG
↓	661027	3	2851	3527	676	↓	NG	NG	↓	NG	↓	NG
↓	661028	3	2960	3513	553	↓	NG	NG	↓	NG	↓	NG
07/12/15	661033	3	2826	3443	617	07121541 30/12/15	NG	NG	↓	NG	↓	NG
↓	661285	3	2554	2868	314	↓	NG	NG	↓	NG	↓	NG
↓	661286	3	2735	3036	301	↓	NG	NG	↓	NG	↓	NG
08/12/15	661450	3	2703	3001	298	↓	NG	NG	↓	NG	↓	NG
↓	661489	2	2925	3076	151	↓	NG	NG	↓	NG	↓	NG
↓	661507	3	2603	2784	181	↓	NG	NG	↓	NG	↓	NG
↓	661509	3	2525	3235	710	↓	NG	NG	↓	NG	↓	NG
↓	661564	3	2727	3017	290	↓	NG	NG	↓	NG	↓	NG
09/12/15	661695	3	2452	3076	424	↓	NG	NG	↓	NG	07121541 04/01/16	NG
↓	661696	2	2754	2826	72	↓	NG	NG	↓	NG	↓	NG
↓	661699	3	3088	3525	437	↓	NG	NG	↓	NG	↓	NG

Anaerobic bottles: 2

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
04/12/15	661022	3	3349	3987	638	26111582 24/12/15	NG	NG	26111541 24/12/15	NG	20111541 18/12/15	NG
↓	661023	2	2670	2831	161	↓	NG	NG	↓	NG	↓	NG
↓	661027	3	2803	3267	464	↓	NG	NG	↓	NG	↓	NG
↓	661028	3	2898	3475	577	↓	NG	NG	↓	NG	↓	NG
07/12/15	661033	3	3075	3625	550	07121541 30/12/15	NG	NG	↓	NG	↓	NG
↓	661285	3	2988	3426	438	↓	NG	NG	↓	NG	↓	NG
↓	661286	2	2424	2802	378	↓	NG	NG	↓	NG	↓	NG
08/12/15	661450	3	2569	2888	319	↓	NG	NG	↓	NG	↓	NG
↓	661489	2	2827	3019	192	↓	NG	NG	↓	NG	↓	NG
↓	661507	2	2785	3019	234	↓	NG	NG	↓	NG	↓	NG
↓	661509	3	2560	3577	1,017	↓	NG	NG	↓	NG	↓	NG
↓	661564	3	2939	3350	416	↓	NG	NG	↓	NG	↓	NG
09/12/15	661695	3	2799	3230	431	↓	NG	NG	↓	NG	07121541 04/01/16	NG
↓	661696	1	2607	2649	42	↓	NG	NG	↓	NG	↓	NG
↓	661699	3	3065	3678	613	↓	NG	NG	↓	NG	↓	NG

Aerobic bottles: (3)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
10/12/15	661891	3	3083	3628	545	021215A1 30/12/15	NG	NG	261115A1 24/12/15	NG	071215A1 04/01/16	NG
↓	661892	3	3117	3857	740	↓	NG	NG	↓	NG	↓	NG
↓	661894	3	3019	3453	434	↓	NG	NG	↓	NG	↓	NG
11/12/15	661955	2	2657	2818	161	081215A1 05/01/16	NG	NG	↓	NG	↓	NG
↓	661976	3	2636	3055	419	↓	NG	NG	↓	NG	↓	NG
↓	662012	3	3404	3608	204	↓	NG	NG	↓	NG	↓	NG
↓	662013	3	3111	3747	636	↓	NG	NG	↓	NG	↓	NG
12/12/15	662014	3	3116	3683	567	↓	NG	NG	↓	NG	↓	NG
↓	662016	3	3030	3858	828	↓	NG	NG	↓	NG	↓	NG
13/12/15	662202	3	2667	3307	640	↓	NG	NG	↓	NG	↓	NG
↓	662262	3	2861	3634	773	↓	NG	NG	↓	NG	↓	NG
↓	662414	2	3260	3458	198	↓	NG	NG	↓	NG	↓	NG
14/12/15	662430	2	2673	2810	137	↓	NG	NG	071215B1 04/01/16	NG	↓	NG
↓	662498	3	2884	3154	270	↓	NG	NG	↓	NG	↓	NG
↓	662431	3	2773	3516	743	↓	NG	NG	↓	NG	↓	NG

Anaerobic bottles: (3)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
10/12/15	661891	2	2549	3085	536	021215A1 30/12/15	NG	NG	261115A1 24/12/15	NG	071215A1 04/01/16	NG
↓	661892	3	2961	3700	739	↓	NG	NG	↓	NG	↓	NG
↓	661894	3	2917	3371	454	↓	NG	NG	↓	NG	↓	NG
11/12/15	661955	2	2949	3118	169	081215A1 05/01/16	NG	NG	↓	NG	↓	NG
↓	661976	3	2768	3309	541	↓	NG	NG	↓	NG	↓	NG
↓	662012	2	3000	3197	197	↓	NG	NG	↓	NG	↓	NG
↓	662013	3	2845	3270	425	↓	NG	NG	↓	NG	↓	NG
12/12/15	662014	3	2777	3409	632	↓	NG	NG	↓	NG	↓	NG
↓	662016	3	3003	3772	769	↓	NG	NG	↓	NG	↓	NG
13/12/15	662202	3	2744	3229	485	↓	NG	NG	↓	NG	↓	NG
↓	662262	3	3246	3878	632	↓	NG	NG	↓	NG	↓	NG
↓	662414	3	3238	3436	198	↓	NG	NG	↓	NG	↓	NG
14/12/15	662430	2	3069	3146	77	↓	NG	NG	071215B1 04/01/16	NG	↓	NG
↓	662498	2	3149	3349	200	↓	NG	NG	↓	NG	↓	NG
↓	662431	3	3014	3388	374	↓	NG	NG	↓	NG	↓	NG

Aerobic bottles: (4)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
15/12/15	662505	3	3036	3587	551	08121SA1 05/01/16	NG	NG	07121SB1 04/01/2016	NG	07121SA1 04/01/16	NG
↓	662521	3	2560	3097	537	↓	NG	NG	↓	NG	↓	NG
↓	662647	3	2983	3605	622	↓	NG	NG	↓	NG	↓	NG
↓	662714	3	2779	3583	804	↓	NG	NG	↓	NG	↓	NG
↓	662716	3	2839	3313	474	↓	NG	NG	↓	NG	↓	NG
16/12/15	662851	3	2568	3131	563	14121SA1 11/01/16	NG	NG	↓	NG	↓	NG
↓	662852	3	2888	3194	306	↓	NG	NG	↓	NG	↓	NG
↓	662853	3	2939	3331	392	↓	NG	NG	↓	NG	↓	NG
↓	662855	3	2805	2942	137	↓	NG	NG	↓	NG	↓	NG
↓	662858	3	2566	3319	753	↓	NG	NG	↓	NG	↓	NG
18/12/15	663066	3	2888	3259	451	↓	NG	NG	↓	NG	↓	NG
↓	663071	3	2775	3179	404	↓	NG	NG	↓	NG	↓	NG
↓	663124	3	2656	3123	467	↓	NG	NG	↓	NG	↓	NG
19/12/15	663331	3	2597	3191	594	↓	NG	NG	↓	NG	↓	NG
↓	663352	3	2681	3371	690	↓	NG	NG	↓	NG	↓	NG

Anaerobic bottles: (4)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
15/12/15	662505	3	2743	3491	748	08121SA1 05/01/16	NG	NG	07121SB1 04/01/16	NG	07121SA1 04/01/16	NG
↓	662521	3	2761	3479	718	↓	NG	NG	↓	NG	↓	NG
↓	662647	3	3087	3615	528	↓	NG	NG	↓	NG	↓	NG
↓	662714	3	2725	3451	726.	↓	NG	NG	↓	NG	↓	NG
↓	662716	3	3123	3587	464	↓	NG	NG	↓	NG	↓	NG
16/12/15	662851	3	2710	3016.	306	14121SA1 11/01/16	NG	NG	↓	NG	↓	NG
↓	662852	3	3088	3492	404	↓	NG	NG	↓	NG	↓	NG
↓	662853	3	2919	3250	331	↓	NG	NG	↓	NG	↓	NG
↓	662855	2	2883	3008	125	↓	NG	NG	↓	NG	↓	NG
↓	662858	3	2616	3357	747	↓	NG	NG	↓	NG ~~NG~~	↓	NG
18/12/15	663066	3	2688	3014	326.	↓	NG	NG	↓	NG	↓	NG
↓	663071	3	2594	2941	347	↓	NG	NG	↓	NG	↓	NG
↓	663124	3	2953	3349	396	↓	NG	NG	↓	NG	↓	NG
19/12/15	663331	3	2705	3251	546	↓	NG	NG	↓	NG	↓	NG
↓	663352	3	2822	3466.	644	↓	NG	NG	↓	NG	↓	NG

Aerobic bottles: (5)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
21/12/15	663598	3	3022	3709.	687	141215A1 11/01/16	NG	NG	071215B1 04/01/16	NG	071215A1 04/01/16	Propionibacterium acnes.
↓	663646	3	2811	3591	780	↓	NG	NG	↓	NG	↓	NG
	663647	2	3015	3255	240		NG	NG		NG		NG
	663648	3	2819	3369	550		NG	NG		NG		NG
↓	663649	3	2860	3135	275	↓	NG	NG	↓	NG	↓	NG
29/12/15	665074	3	2705	2866.	161	171215A1 14/01/16	NG	NG	151215A1 12/01/16	NG	181215A1 15/01/16	NG
↓	665080	3	2762	2926.	164	↓	NG	NG	↓	NG	↓	NG
	665081	3	2752	2987	235		NG	NG		NG		NG
	665082	3	3179	3427	248		NG	NG		NG		NG
↓	665200	3	2714	3139	425	↓	NG	NG	↓	NG	↓	NG
30/12/15	665255	3	2985	3319	334	211215B2 18/01/16	NG	NG		NG		NG
↓	665256	3	2771	2913	142	↓	NG	NG	↓	NG	↓	NG
	665258	3	2537	3241	704		NG	NG		NG		NG
	665260	3	2704	2968	264		NG	NG		NG		NG
↓	665267	3	2948	3687	739	↓	NG	NG	↓	NG	↓	NG

Anaerobic bottles: (5)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
21/12/15	663598	3	2946	3600	654	141215A1 11/01/16	NG	NG	071215B1 04/01/16	NG	071215A1 04/01/16	NG
↓	663646	3	3105	3600	495	↓	NG	NG	↓	NG	↓	NG
	663647	2	2788	2829	41		NG	NG		NG		NG
	663648	3	3153	3265	112		NG	NG		NG		NG
↓	663649	3	3122	3485	363	↓	NG	NG	↓	NG	↓	NG
29/12/15	665074	3	2856	3459	605	171215A1 14/01/16	NG	NG	151215A1 12/01/16	NG	181215A1 15/01/16	NG
↓	665080	2	2700	2761	61	↓	NG	NG	↓	NG	↓	NG
	665081	2	2579	2766.	187		NG	NG		NG		NG
	665082	3	2854	3168	314		NG	NG		NG		NG
↓	665200	2	2737	3004	267	↓	NG	NG	↓	NG	↓	NG
30/12/15	665255	3	2839	3321	482	211215B2 18/01/16	NG	NG		NG		Propionibacterium acnes.
↓	665256	2	2795	2863	68	↓	NG	NG	↓	NG	↓	NG
	665258	3	2916	3439	523		NG	NG		NG		NG
	665260	1	2969	3060	91		NG	NG		NG		NG
↓	665267	3	2883	3585	702	↓	NG	NG	↓	NG	↓	NG

Aerobic bottles: (6)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
03/01/16	665479	3	3070	3231	161	21121SB2 18/01/16.	NG	NG	15121SA1 12/01/16	NG	18121SA1 15/01/16	~~18121SA1 15/01/16~~ NG
	665484	3	2900	3019	119		NG	NG		NG		NG
	665486	3	2812	3097	285		NG	NG		NG		NG
04/01/16	665574	3	2722	3213	491		NG	NG		NG		NG
	665582	3	2933	3286.	353		NG	NG		NG		NG
	665640	3	2831	3397	766.		NG	NG		NG		NG
	665689	3	2794	3177	383		NG	NG		NG		NG
	665691	3	2797	3204	407		NG	NG		NG		NG
05/01/16	665907	3	2796	3356	560	29121SA1 26/01/16	NG	NG		NG		NG
	665908	3	2696	3357	661		NG	NG		NG		NG
	665909	3	3301	4150.	849		NG	NG		NG		NG
	665911	2	2752	3002	250		NG	NG		NG		NG
	665914	3	3055	3374	319		NG	NG		NG		NG
08/01/16	666179	3	3048	3695	647	05011681 02/02/16	NG	NG		NG	31121SB1 28/01/16	NG
	666211	3	3345	3963	618.		NG	NG		NG		NG

Anaerobic bottles: (6)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
03/01/16	665479	3	2889	3200	311	21121SB2 18/01/16	NG	NG	15121SA1 12/01/16	NG	18121SA1 15/01/16	NG
	665484	2	2797	2913	116		NG	NG		NG		NG
	665486	2	2479	2671	192		NG	NG		NG		NG
04/01/16	665574	3	2972	3547	575		NG	NG		NG		NG
	665582	3	2627	3061	434		NG	NG		NG		NG
	665640	3	2961	3500	539		NG	NG		NG		NG
	665689	3	2515	2880	365		NG	NG		NG		NG
	665691	3	3094	3363	269		NG	NG		NG		NG
05/01/16	665907	3	2895	3566	671	29121SA1 26/01/16	NG	NG		NG		NG
	665908	3	2638	3245	607		NG	NG		NG		NG
	665909	3	3044	4010	966		NG	NG		NG		NG
	665911	3	2693	2937	244		NG	NG		NG		NG
	665914	3	2668	2977	309		NG	NG		NG		NG
08/01/16	666179	3	2693	3387	694	05011681 02/02/16	NG	NG		NG	31121SB1 28/01/16	NG
	666211	3	3150	3724	574		NG	NG		NG		NG

Aerobic bottles: (7)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	ANO batch/exp	ANO growth (120hrs)
08/01/16	666212	3	2769	3579	810	05011681 02/02/16	NG	NG	15121541 12/01/16	NG	31121581 28/01/16	NG
↓	666213	3	2742	3183	441	↓	NG	NG	↓	NG	↓	NG
↓	666214	3	2936	3583	647	↓	NG	NG	↓	NG	↓	NG
11/01/16	666728	3	2828	3003	175	↓	NG	NG	31121541 28/01/16	NG	↓	NG
↓	666912	3	3151	3544	393	↓	NG	NG	↓	NG	↓	NG
12/01/16	666941	3	2761	3547	786	↓	NG	NG	↓	NG	↓	NG
↓	666942	3	2925	3402	477	↓	NG	NG	↓	NG	↓	NG
↓	666943	3	27444	3058	314	↓	NG	NG	↓	NG	↓	NG
↓	666945	3	2960	3135	175	↓	NG	NG	↓	NG	↓	NG
↓	666948	3	2820	3411	591	↓	NG	NG	↓	NG	↓	NG
15/01/16	667459	3	3242	3904	662	05011681 05/02/16	NG	NG	↓	NG	↓	NG
↓	667461	3	2713	3334	621	↓	NG	NG	↓	NG	↓	NG
↓	667462	3	2918	3591	673	↓	NG	NG	↓	NG	↓	NG
↓	667446	3	2913	3210	297	↓	NG	NG	↓	NG	↓	NG
↓	667477	3	3010	3503	493	↓	NG	NG	↓	NG	↓	NG

Anaerobic bottles: (7)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	ANO batch/exp	ANO growth (120hrs)
08/01/16	666212	3	2972	3731	759	05011681 02/02/16	NG	NG	15121541 12/01/16	NG	31121581 28/01/16	NG
↓	666213	3	2865	3237	372	↓	NG	NG	↓	NG	↓	NG
↓	666214	3	2610	3432	822	↓	NG	NG	↓	NG	↓	NG
11/01/16	666728	3	2672	2885	213	↓	NG	NG	31121541 28/01/16	NG	↓	NG
↓	666912	3	2613	2995	382	↓	NG	NG	↓	NG	↓	NG
12/01/16	666941	3	2972	3600	628	↓	NG	NG	↓	NG	↓	NG
↓	666942	3	2861	3156	295	↓	NG	NG	↓	Moraxella catarrhalis	↓	NG
↓	666943	3	3204	3525	321	↓	NG	NG	↓	NG	↓	NG
↓	666945	3	2511	2631	120	↓	NG	NG	↓	NG	↓	NG
↓	666948	3	2687	3019	332	↓	NG	NG	↓	NG	↓	NG
15/01/16	667459	3	3362	4061	699	05011681 05/02/16	NG	NG	↓	NG	↓	NG
↓	667461	3	2982	3552	570	↓	NG	NG	↓	NG	↓	NG
↓	667462	3	2924	3598	674	↓	NG	NG	↓	NG	↓	NG
↓	667446	2	2663	2930	267	↓	NG	NG	↓	NG	↓	NG
↓	667477	3	2774	3156	382	↓	NG	NG	↓	NG	↓	NG

Aerobic bottles: 8

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
19/01/16	668257	3	2548	3270	722	14011681 11/02/16	NG	NG	31121541 28/01/116	NG	13011641 10/02/16	NG
↓	668274	3	2668	3357	689	↓	NG	NG	↓	NG	↓	NG
↓	668275	3	2983	3466	483	↓	NG	NG	↓	NG	↓	NG
↓	668281	3	2873	3228.	355	↓	NG	NG	↓	NG	↓	NG
↓	668282	3	3171	3790	619	↓	NG	NG	↓	NG	↓	NG
20/01/16	668449	3	2537	2678	141	↓	NG	NG	12011641 09/02/16	NG	↓	NG
↓	668551	3	2740	2907	167	↓	NG	NG	↓	NG	↓	NG
↓	668552	3	2693	3300	607	↓	NG	NG	↓	NG	↓	NG
21/01/16	668682	3	2890	3752	862	↓	NG	NG	↓	NG	↓	NG
↓	668683	3	2773	2985	212	↓	NG	NG	↓	NG	↓	NG
↓	668684	3	2691	3025	334	↓	NG	NG	↓	NG	↓	NG
↓	668741	3	2885	3294	409	↓	NG	NG	↓	NG	↓	NG
22/01/16	668745	3	3088	3632	544	↓	NG	NG	↓	NG	↓	NG
↓	668747	3	2917	3357	440	↓	NG	NG	↓	NG	↓	NG
↓	668749	3	2745	3464	719.	↓	NG	NG	↓	NG	↓	NG

Anaerobic bottles: 8

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
19/01/16	668257	3	2696	3343	647	14011681 11/02/2016	NG	NG	31121541 28/01/116	NG	13011641 10/02/2016	NG
↓	668274	3	2743	3410	667	↓	NG	NG	↓	NG	↓	NG
↓	668275	3	2601	3070	469	↓	NG	NG	↓	NG	↓	NG
↓	668281	3	2888	3257	369	↓	NG	NG	↓	NG	↓	NG
↓	668282	3	3097	3545	448	↓	NG	NG	↓	NG	↓	NG
20/01/16	668449	1	2232	2308	76	↓	NG	NG	12011641 09/02/16	NG	↓	NG
↓	668551	2	1906	2062	156	↓	NG	NG	↓	NG	↓	NG
↓	668552	3	2821	3291	470	↓	NG	NG	↓	NG	↓	NG
21/01/16	668682	3	2861	3587	726	↓	NG	NG	↓	NG	↓	NG
↓	668683	3	3004	3213	209	↓	NG	NG	↓	NG	↓	NG
↓	668684	2	2477	2769	292	↓	NG	NG	↓	NG	↓	NG
↓	668741	2	2826	3225	399	↓	NG	NG	↓	NG	↓	NG
22/01/16	668745	3	2952	3580	628	↓	NG	NG	↓	NG	↓	NG
↓	668747	3	2714	3073	359	↓	NG	NG	↓	NG	↓	NG
↓	668749	3	2764	3494	730.	↓	NG	NG	↓	NG	↓	NG

Aerobic bottles: (9)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
24/01/16	668967	3	2788	3289	501	19011681 16/02/16	NG	NG	12011641 09/02/16	NG	13011641 10/02/16	NG
↓	669007	3	2890	5732	2932	↓	NG	NG	↓	NG	↓	NG
	669026	3	2599	2922	323		NG	NG		NG		NG
	669038	3	2907	3754	847		NG	NG		NG		NG
↓	669040	3	2653	3066	413	↓	NG	NG	↓	NG	↓	NG
25/01/16	669245	3	2751	3552	801		NG	NG		NG		NG
↓	669281	3	2834	3071	237		NG	NG		NG		NG
	669282	3	2667	2997	330		NG	NG		NG		NG
	669286	3	2792	3022	230		NG	NG		NG		NG
↓	669287	3	3064	3516.	452	↓	NG	NG	↓	NG	↓	NG
27/01/16	669584	3	2775	3170.	395		NG	NG		NG		NG
↓	669756	3	2729	3276	547		NG	NG		NG		NG
	669757	3	2940	3199	259		NG	NG		NG		NG
	669758	3	2553	3235	682		NG	NG		NG		NG
↓	669829	3	2736	3231	495	↓	NG	NG	↓	NG	↓	NG

Anaerobic bottles: (9)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
24/01/16	668967	3	2983	3485	502	19011681 16/02/16	NG	NG	12011641 09/02/16	NG	13011641 10/02/16	NG
↓	669007	3	2941	3652	711	↓	NG	NG	↓	NG	↓	NG
	669026	2	2310	2577	267		NG	NG		NG		NG
	669038	3	2903	3548	645		NG	NG		NG		NG
↓	669040	2	2685	2817	132	↓	NG	NG	↓	NG	↓	NG
25/01/16	669245	3	2597	3299	702		NG	NG		NG		NG
↓	669281	3	2644	2880.	236		NG	NG		NG		NG
	669282	3	2978	3276	298		NG	NG		NG		NG
	669286	2	2940	3110.	170		NG	NG		NG		NG
↓	669287	3	3309	3810.	501	↓	NG	NG	↓	NG	↓	NG
27/01/16	669584	3	2622	3122	500		NG	NG		NG		NG
↓	669756	2	2632	2947	315		NG	NG		NG		NG
	669757	3	3009	3524	515		NG	NG		NG		NG
	669758	3	2986	3538	552		NG	NG		NG		NG
↓	669829	3	2767	3122	355	↓	NG	NG	↓	NG	↓	NG

Aerobic bottles: (10)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
28/01/16	669846	3	2642	2959	317	25011681 22/02/16	NG	NG	12011641 09/02/16	NG	26011681 23/02/16	NG
↓	670004	3	2802	3223	421	↓	NG	NG	↓	NG	↓	NG
↓	670006	3	2900	3393	493	↓	NG	NG	↓	NG	↓	NG
↓	670007	3	3196	3553	357	↓	NG	NG	↓	NG	↓	NG
↓	670008	3	2820	3330	510	↓	NG	NG	↓	NG	↓	NG
29/01/16	670069	3	3147	3536	389	↓	NG	NG	22011641 19/02/16	NG	↓	NG
↓	670073	3	2940	3752	812	↓	NG	NG	↓	NG	↓	NG
↓	670075	3	2877	3698	821	↓	NG	NG	↓	NG	↓	NG
↓	670078	3	3092	3225	133	↓	NG	NG	↓	NG	↓	NG
↓	670079	3	2695	3098	403	↓	NG	NG	↓	NG	↓	NG
06/02/16	671394	3	2718	3230	512	02021681 01/03/16	NG	NG	↓	NG	↓	NG
↓	671396	3	2803	3725	922	↓	NG	NG	↓	NG	↓	NG
↓	671398	3	3095	3600	505	↓	NG	NG	↓	NG	↓	NG
07/02/16	671502	3	2936	3296	560	↓	NG	NG	↓	NG	↓	NG
↓	671654	2	2901	3112	211	↓	NG	NG	↓	NG	↓	NG

Anaerobic bottles: (10)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
28/01/16	669846	3	2743	3388	645	25011681 22/02/16	NG	NG	12011641 09/02/16	NG	26011681 23/02/16	NG
↓	670004	3	2887	3224	337	↓	NG	NG	↓	NG	↓	NG
↓	670006	3	2835	3403	568	↓	NG	NG	↓	NG	↓	NG
↓	670007	3	2959	3353	394	↓	NG	NG	↓	NG	↓	NG
↓	670008	3	2906	3387	481	↓	NG	NG	↓	NG	↓	NG
29/01/16	670069	2	2885	3263	378	↓	NG	NG	22011641 19/02/16	NG	↓	NG
↓	670073	3	2760	3318	558	↓	NG	NG	↓	NG	↓	NG
↓	670075	3	3105	3878	773	↓	NG	NG	↓	NG	↓	NG
↓	670078	2	2967	3101	134	↓	NG	NG	↓	NG	↓	NG
↓	670079	2	2645	2954	309	↓	NG	NG	↓	NG	↓	NG
06/02/16	671394	2	2650	3037	387	02021681 01/03/16	NG	NG	↓	NG	↓	NG
↓	671396	3	2847	3631	784	↓	NG	NG	↓	NG	↓	NG
↓	671398	3	3066	3348	282	↓	NG	NG	↓	NG	↓	NG
07/02/16	671502	3	2556	3336	780	↓	NG	NG	↓	NG	↓	NG
↓	671654	1	2606	2741	135	↓	NG	NG	↓	NG	↓	NG

Aerobic bottles: (11)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
08/02/16	671762	3	2663	3235	572	02021681 01/03/16	NG	NG	01021681 29/02/16	NG	26011681 23/02/16	NG
↓	671763	3	2660	3233	573	↓	NG	NG	↓	NG	↓	NG
↓	671927	3	2597	3196	599	↓	NG	NG	↓	NG	↓	NG
09/02/16	672040	3	2676	3511	835	↓	NG	NG	↓	NG	↓	NG
↓	672156	3	2678	3289	611	↓	NG	NG	↓	NG	↓	Propionibacterium acnes.
↓	672236	3	2604	3305	701	↓	NG	NG	↓	NG	↓	NG
10/02/16	672310	3	2732	3522	790	↓	NG	NG	↓	NG	↓	NG
↓	672463	3	2928	3590	662	↓	NG	NG	↓	NG	↓	NG
↓	672474	3	2766	3587	821	↓	NG	NG	↓	NG	↓	NG
↓	672475	3	2794	3109	315	↓	NG	NG	↓	NG	↓	NG
↓	672479	3	2861	3462	601	↓	NG	NG	↓	NG	↓	NG
12/02/16	672714	3	3065	3452	387	08021681 07/03/16	NG	NG	↓	NG	03021681 02/03/16	NG
↓	672769	3	2678	2819	341	↓	NG	NG	↓	NG	↓	NG
↓	672773	2	2820	3007	187	↓	NG	NG	↓	NG	↓	NG
↓	672774	3	2646	2931	285	↓	NG	NG	↓	NG	↓	NG

Anaerobic bottles: (11)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
08/02/16	671762	3	2660	3100	440.	02021681 01/03/16	NG	NG	01021681 29/02/16	NG	26011681 23/02/16	NG
↓	671763	2	2659	3013	354	↓	NG	NG	↓	NG	↓	NG
↓	671927	3	2733	3397	664	↓	NG	NG	↓	NG	↓	NG
09/02/16	672040	3	2653	3172	519	↓	NG	NG	↓	NG	↓	NG
↓	672156	3	2363	2837	474.	↓	NG	NG	↓	NG	↓	NG
↓	672236	3	2537	3329	792	↓	NG	NG	↓	NG	↓	NG
10/02/16	672310	2	2886	3350	464	↓	NG	NG	↓	NG	↓	NG
↓	672463	3	2659	3282	623.	↓	NG	NG	↓	NG	↓	NG
↓	672474	3	2556	3254	698.	↓	NG	NG	↓	NG	↓	NG
↓	672475	3	2776	3171	395	↓	NG	NG	↓	NG	↓	NG
↓	672479	2	3022	3414	392	↓	NG	NG	↓	NG	↓	NG
12/02/16	672714	3	3022	3369	347	08021681 07/03/16	NG	NG	↓	NG	03021681 02/03/16	NG
↓	672769	1	3131	3271	140	↓	NG	NG	↓	NG	↓	NG
↓	672773	2	2724	2890	166.	↓	NG	NG	↓	NG	↓	NG
↓	672774	1	2581	2638	57.	↓	NG	NG	↓	NG	↓	NG

Aerobic bottles: 12

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
15/02/16	673595	3	2736	3085	349	08021681 07/03/16	NG	NG	01021681 29/02/16	NG	03021681 02/03/16	NG
↓	673597	3	2880	3331	451	↓	NG	NG	↓	NG	↓	NG
↓	673598	3	2726	2850	124	↓	NG	NG	↓	NG	↓	NG
16/02/16	673545	3	2701	3262	561	11021681 10/03/16	NG	NG	↓	NG	↓	NG
↓	673552	3	2627	3233	606	↓	NG	NG	↓	NG	↓	NG
↓	673558	3	2620	3081	461	↓	NG	NG	↓	NG	↓	NG
↓	673559	3	2837	3179	342	↓	NG	NG	↓	NG	↓	NG
↓	673560	3	2717	3155	438	↓	NG	NG	↓	NG	↓	NG
18/02/16	673780	3	2705	3453	748	↓	NG	NG	09021681 08/03/16	NG	11021681 10/03/16	NG
↓	673782	3	2718	3148	430	↓	NG	NG	↓	NG	↓	NG
↓	673785	3	2752	3151	399	↓	NG	NG	↓	NG	↓	NG
↓	673980	3	2878	3510	632	↓	NG	NG	↓	NG	↓	Propionibacterium acnes.
↓	673985	3	2768	3353	585	↓	NG	NG	↓	NG	↓	NG
21/02/16	674049	3	2795	3341	546	↓	NG	NG	↓	NG	↓	NG
↓	674068	3	2820	3071	251	↓	NG	NG	↓	NG	↓	NG

Anaerobic bottles: 12

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
15/02/16	673595	2	2782	3169	387	08021681 07/03/16	NG	NG	01021681 29/02/16	NG	03021681 02/03/16	NG
↓	673597	3	3192	3709	517	↓	NG	NG	↓	NG	↓	NG
↓	673598	2	2808	2861	53	↓	NG	NG	↓	NG	↓	NG
16/02/16	673545	3	2710	3214	504	11021681 10/03/16	NG	NG	↓	NG	↓	NG
↓	673552	2	2614	3220	606	↓	NG	NG	↓	NG	↓	NG
↓	673558	3	2687	3132	445	↓	NG	NG	↓	NG	↓	NG
↓	673559	2	2829	3221	392	↓	NG	NG	↓	NG	↓	NG
↓	673560	3	2688	3080	392	↓	NG	NG	↓	NG	↓	NG
18/02/16	673780	3	2735	3526	791	↓	NG	NG	09021681 08/03/16	NG	11021681 10/03/16	NG
↓	673782	2	2555	2756	201	↓	NG	NG	↓	NG	↓	NG
↓	673785	2	2560	3027	467	↓	NG	NG	↓	NG	↓	NG
↓	673980	3	2499	2844	345	↓	NG	NG	↓	NG	↓	NG
↓	673985	3	2841	3438	597	↓	NG	NG	↓	NG	↓	NG
21/02/16	674049	3	2696	3098	402	↓	NG	NG	↓	NG	↓	NG
↓	674068	2	2502	2637	135	↓	NG	NG	↓	NG	↓	NG

Aerobic bottles: (13)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
21/02/16	674672	3	2588	2758	170	1102168I 10/03/16	NG	NG	0902168I 08/03/16	NG	1102168I 10/03/16	NG
↓	674074	3	2622	3133	511	↓	NG	NG	↓	NG	↓	NG
↓	674115	3	3275	3916	641	↓	NG	NG	↓	NG	↓	NG
22/02/16	674509	3	2629	3334	705	1602164I 15/03/16	NG	NG	↓	NG	↓	NG
↓	674534	2	2705	2885	180	↓	NG	NG	↓	NG	↓	NG
↓	674535	2	2846	2947	101	↓	NG	NG	↓	NG	↓	NG
↓	674576	2	2659	2903	244	↓	NG	NG	↓	NG	↓	NG
↓	674703	3	2829	3206	377	↓	NG	NG	↓	NG	↓	NG
24/02/16	675023	3	2928	3484	556	↓	NG	NG	↓	NG	↓	NG
↓	675024	3	2845	3251	406	↓	NG	NG	↓	NG	↓	NG
↓	675027	3	2535	3178	643	↓	NG	NG	↓	NG	↓	NG
↓	675028	3	2846	3536	690	↓	NG	NG	↓	NG	↓	NG
↓	675029	2	3027	3325	298	↓	NG	NG	↓	NG	↓	NG
24/02/16	675343	2	2676	2828	152	↓	NG	NG	↓	NG	↓	NG
↓	675344	2	2492	2962	470	↓	NG	NG	↓	NG	↓	NG

Anaerobic bottles: (13)

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
21/02/16	674672	1	2524	2673	149	1102168I 10/03/16	NG	NG	0902168I 08/03/16	NG	1102168I 10/03/16	NG
↓	674074	1	2818	3199	381	↓	NG	NG	↓	NG	↓	NG
↓	674115	3	3220	3917	697	↓	NG	NG	↓	NG	↓	NG
22/02/16	674509	3	2697	3443	746	1602164I 15/03/16	NG	NG	↓	NG	↓	NG
↓	674534	1	2515	2615	100	↓	NG	NG	↓	NG	↓	NG
↓	674535	2	2466	2718	252	↓	NG	NG	↓	NG	↓	NG
↓	674576	1	2442	2652	210	↓	NG	NG	↓	NG	↓	NG
↓	674703	3	2737	3152	415	↓	NG	NG	↓	NG	↓	NG
24/02/16	675023	3	2929	3387	458	↓	NG	NG	↓	NG	↓	NG
↓	675024	3	2653	3038	385	↓	NG	NG	↓	NG	↓	NG
↓	675027	3	2903	3456	553	↓	NG	NG	↓	NG	↓	NG
↓	675028	3	3006	3391	385	↓	NG	NG	↓	NG	↓	NG
↓	675029	2	2920	3120	200	↓	NG	NG	↓	NG	↓	NG
24/02/16	675343	1	2844	2947	103	↓	NG	NG	↓	NG	↓	NG
↓	675344	2	2491	2847	356	↓	NG	NG	↓	NG	↓	NG

Aerobic bottles: 14

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
29/02/16	675873	3	3227	3846	619	22021681 21/03/16	NG	NG	19021641 18/03/16.	NG	23021641 22/03/16	NG
↓	675906	3	2559	3475	916	↓	NG	NG	↓	NG	↓	NG
↓	675909	3	2735	3516	781	↓	NG	NG	↓	NG	↓	NG
↓	675910	3	2675	3259	584	↓	NG	NG	↓	NG	↓	NG
↓	675913	2	2542	2685	143	↓	NG	NG	↓	NG	↓	NG
01/03/16	676155	3	2889	3379	490	↓	NG	NG	↓	NG	↓	NG
↓	676174	2	2455	2656	201	↓	NG	NG	↓	NG	↓	NG
↓	676177	3	3204	3497	293	↓	NG	NG	↓	NG	↓	NG
↓	676178	3	26916	2893	197	↓	NG	NG	↓	NG	↓	NG
↓	676180	3	2688	3206	518	↓	NG	NG	↓	NG	↓	NG
04/03/16	676633	3	3002	3316	314	↓	NG	NG	↓	NG	↓	NG
↓	676634	1	2813	3209	396	↓	NG	NG	↓	NG	↓	NG
↓	676637	3	2969	3577	608	↓	NG	NG	↓	NG	↓	NG
↓	676640	3	2555	2878	323	↓	NG	NG	↓	NG	↓	NG
↓	676641	3	2722	3214	492	↓	NG	NG	↓	NG	↓	NG

Anaerobic bottles: 14

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
29/02/16	675873	3	3487	3978	491	22021681 21/03/16	NG	NG	19021641 18/03/16	NG	23021641 22/03/16	NG
↓	675906	3	2946	3834	888	↓	NG	NG	↓	NG	↓	NG
↓	675909	3	2955	3488	533	↓	NG	NG	↓	NG	↓	NG
↓	675910	2	3133	3650	517	↓	NG	NG	↓	NG	↓	NG
↓	675913	1	2478	2527	49	↓	NG	NG	↓	NG	↓	NG
01/03/16	676155	2	2457	2861	424	↓	NG	NG	↓	NG	↓	NG
↓	676174	1	2410	2506	96	↓	NG	NG	↓	NG	↓	NG
↓	676177	2	2800	3195	395	↓	NG	NG	↓	NG	↓	NG
↓	676178	2	2880	3173	293	↓	NG	NG	↓	NG	↓	NG
↓	676180	1	2856	3026.	170	↓	NG	NG	↓	NG	↓	NG
04/03/16	676633	1	2703	2966	263	↓	NG	NG	↓	NG	↓	NG
↓	676636	3	2994	3409	415	↓	NG	NG	↓	NG	↓	NG
↓	676637	3	2916	3579	663	↓	NG	NG	↓	NG	↓	NG
↓	676640	3	2674	3139	465	↓	NG	NG	↓	NG	↓	NG
↓	676641	3	3025	3478	453.	↓	NG	NG	↓	NG	↓	NG

Aerobic bottles: 15

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
05/03/16	676681	2	2601	2937	336	29021681 28/03/16	NG	NG	12021641 18/03/16	NG	23021641 22/03/16	NG
	676682	3	2702	3571	669		NG	NG		NG		NG
	676683	3	3123	3924	801		NG	NG		NG		NG
	676684	3	3165	3887	722		NG	NG		NG		NG
	676686	3	2702	3242	540		NG	NG		NG		NG
07/03/16	677129	3	2841	2981	140		NG	NG	19021641 18/03/16	NG		NG
	677130	3	3006	3309	303		NG	NG		NG		NG
	677217	3	2567	2688	121		NG	NG		NG		NG
	677283	2	2687	2873	186		NG	NG		NG		NG
	677317	3	2683	2837	154		NG	NG		NG		NG
08/03/16	677339	3	2708	3156	448	03031641 31/03/16	NG	NG		NG		NG
	677340	3	2860	3294	434		NG	NG		NG		NG
	677341	3	2710	3235	525		NG	NG		NG		NG
	677342	2	2913	3178	265		NG	NG		NG		NG
	677346	1	2511	2659	148.		NG	NG		NG		NG

Anaerobic bottles: 15

Date	Bottle number	Sensor colour intensity	Start reflectance	Final reflectance	Total reflectance increase	BA batch/exp	Growth BA CO_2 (72hrs)	Growth BA μO_2 (72hrs)	Choc batch/exp	Growth Choc (120hrs)	AN0 batch/exp	AN0 growth (120hrs)
05/03/16	676681	2	2595	2969	374	29021681 28/03/16	NG	NG	12021641 18/03/16	NG	23021641 22/03/16	NG
	676687	3	2810	3536	726		NG	NG		NG		NG
	676683	3	2713	3566	853		NG	NG		NG		NG
	676684	3	3257	3972	715		NG	NG		NG		NG
	676686	3	3248	3968	720		NG	NG		NG		NG
07/03/16	677129	2	2676	2790	114		NG	NG	19021641 18/03/16.	NG		NG
	677130	3	2757	3271	514		NG	NG		NG		NG
	677217	2	2757	2990	233		NG	NG		NG		NG
	677283	1	2427	2572	149		NG	NG		NG		NG
	677317	2	2735	2926	191		NG	NG		NG		NG
08/03/16	677339	2	2760	3126	366	03031641 31/03/16	NG	NG		NG		NG
	677340	1	3082	3240	158		NG	NG		NG		NG
	677341	2	2949	3243	294		NG	NG		NG		NG
	677342	2	2676	2971	295		NG	NG		NG		NG
	677346	1	2449	2561	112		NG	NG		NG.		NG

Nobles Hospital

bioMerieux Customer:
System #:

Laboratory Report

Printed Jan 5, 2016 20:41 GMT
Printed by: nplpowc
Report Version: 3 of 3
Patient ID:

Patient Name:
Isolate Group: craig msc-2
Last Updated: Jan 5, 2016 20:39 GMT By: nplpowc
Card Type: ANC Testing Instrument: 000015F25C09 (2529)

Bionumber: 7703040200411
Organism Quantity:

Comments:	

Identification Information	Card:	ANC	Lot Number:	244362210	Expires:	Nov 13, 2016 12:00 GMT
	Completed:	Jan 5, 2016 17:35 GMT	Status:	Final	Analysis Time:	6.25 hours
Selected Organism	97% Probability		Propionibacterium acnes			
	Bionumber:	7703040200411			Confidence:	Excellent identification
SRF Organism						
Analysis Organisms and Tests to Separate:						
Analysis Messages:						
Contraindicating Typical Biopattern(s)						
Propionibacterium acnes BGURi(12),						

Biochemical Details

4	dGAL	(+)	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	-
28	SAC	-	30	ARB	-	33	NAG	(-)	34	BGLUi	-	36	URE	-	37	BGURi	+
39	BGALi	-	41	AARA	-	42	AGALi	-	43	BMAN	-	44	ARG	+	45	PVATE	-
51	MTE	-	53	ESC	-	54	BdFUC	-	55	BNAGi	-	56	AMANi	-	57	AlFUC	-
59	PHOS	-	60	IARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	-
	GRAM	+		MORPH	-		AERO	-									

Installed VITEK 2 Systems Version: 07.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Nobles Hospital

bioMerieux Customer:
System #:

Laboratory Report

Printed Dec 30, 2015 09:05 GMT
Printed by: nplchel
Report Version: 1 of 1
Patient ID:

Patient Name:
Isolate Group: craig msc-1

Card Type: ANC Testing Instrument: 000015F25C09 (2529)

Bionumber: 6703040202001
Organism Quantity:

Comments:	

Identification Information	Card:	ANC	Lot Number:	244362210	Expires:	Nov 13, 2016 12:00 GMT
	Completed:	Dec 29, 2015 17:33 GMT	Status:	Final	Analysis Time:	6.00 hours
Selected Organism	97% Probability		Propionibacterium acnes			
	Bionumber:	6703040202001			Confidence:	Excellent identification
SRF Organism						
Analysis Organisms and Tests to Separate:						
Analysis Messages:						
Contraindicating Typical Biopattern(s)						
Propionibacterium acnes BGURi(12),						

Biochemical Details

4	dGAL	-	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	-
28	SAC	-	30	ARB	-	33	NAG	-	34	BGLUi	-	36	URE	-	37	BGURi	+
39	BGALi	-	41	AARA	-	42	AGALi	-	43	BMAN	-	44	ARG	+	45	PVATE	(-)
51	MTE	-	53	ESC	-	54	BdFUC	-	55	BNAGi	-	56	AMANi	+	57	AlFUC	-
59	PHOS	-	60	IARA	-	61	dRIB2	-	62	OPS	-	63	AARAF	-	64	dXYL	-
	GRAM	+		MORPH	-		AERO	-									

Installed VITEK 2 Systems Version: 07.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Appendix 2 – VITEK 2 identification of isolates

Nobles Hospital

bioMerieux Customer:
System #:

Laboratory Report

Printed Jan 21, 2016 15:10 GMT
Printed by: nplpowc
Report Version: 2 of 2
Patient ID:

Patient Name:
Isolate Group: craigs msc-3
Last Updated: Jan 21, 2016 15:09 GMT By: nplpowc
Card Type: NH Testing Instrument: 000015F25C09 (2529)

Bionumber: 0211000100
Organism Quantity:

Biochemical Details																
1	ArgA	-	2	GGT	-	3	LysA	-	4	dGAL	-	5	LeuA	+	6	ELLM	-
7	PheA	+	8	ProA	-	10	PyrA	-	13	TyrA	(+)	15	APPA	-	18	dGLU	-
19	GLYG	-	20	dMNE	-	22	dMAL	-	28	SAC	-	33	NAG	-	36	URE	-
39	BGALi	-	40	ODC	-	41	AARA	-	45	PVATE	+	46	PHC	-	47	dMLT	-
51	MTE	-	52	IGLM	-	59	PHOS	-	61	dRIB2	-	62	OPS	-	64	dXYL	-

Installed VITEK 2 Systems Version: 07.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Nobles Hospital

bioMerieux Customer:
System #:

Laboratory Report

Printed Jan 21, 2016 15:10 GMT
Printed by: nplpowc
Report Version: 2 of 2
Patient ID:

Patient Name:
Isolate Group: craigs msc-3
Last Updated: Jan 21, 2016 15:09 GMT By: nplpowc
Card Type: NH Testing Instrument: 000015F25C09 (2529)

Bionumber: 0211000100
Organism Quantity:

Comments:	

Identification Information	Card:	NH	Lot Number:	245354120	Expires:	Aug 24, 2016 13:00 BST
	Completed:	Jan 20, 2016 22:52 GMT	Status:	Final	Analysis Time:	6.00 hours
Selected Organism	91% Probability		Moraxella (Branhamella) catarrhalis			
	Bionumber:	0211000100			Confidence:	Low discrimination

SRF Organism	

Analysis Organisms and Tests to Separate:	
Low Discrimination Organism	
Campylobacter coli	COCCI(0),25C(1),
Campylobacter fetus ssp fetus	COCCI(0),25C(99),
Moraxella (Branhamella) catarrhalis	COCCI(100),

Analysis Messages:

Contraindicating Typical Biopattern(s)	
Campylobacter coli	PheA(2),dMLT(85),
Campylobacter fetus ssp fetus	PheA(12),dMLT(96),
Moraxella (Branhamella) catarrhalis	LysA(88),ArgA(81),PVATE(4),

Installed VITEK 2 Systems Version: 07.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Nobles Hospital

bioMerieux Customer:
System #:

Laboratory Report

Printed Feb 27, 2016 01:54 GMT
Printed by: nplpowc
Report Version: 1 of 1
Patient ID:

Patient Name:
Isolate Group: craig msc-5

Card Type: ANC Testing Instrument: 000015F25C09 (2529)

Bionumber: 6723040602001
Organism Quantity:

Comments:	

Identification Information	Card:	ANC	Lot Number:	244366910	Expires:	Dec 30, 2016 12:00 GMT
	Completed:	Feb 26, 2016 22:05 GMT	Status:	Final	Analysis Time:	6.00 hours
Selected Organism	97% Probability		Propionibacterium acnes			
	Bionumber:	6723040602001			Confidence:	Excellent identification
SRF Organism						
Analysis Organisms and Tests to Separate:						
Analysis Messages:						
Contraindicating Typical Biopattern(s)						
Propionibacterium acnes	BGURi(12),					

Biochemical Details

4	dGAL	-	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	-	13	TyrA	(+)	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	-
28	SAC	-	30	ARB	-	33	NAG	-	34	BGLUi	-	36	URE	-	37	BGURi	+
39	BGALi	-	41	AARA	-	42	AGALi	-	43	BMAN	-	44	ARG	+	45	PVATE	+
51	MTE	-	53	ESC	-	54	BdFUC	-	55	BNAGi	-	56	AMANi	+	57	AlFUC	-
59	PHOS	-	60	IARA	-	61	dRIB2	-	62	OPS	-	63	AARAF	-	64	dXYL	-
	GRAM	+		MORPH	-		AERO	-									

Installed VITEK 2 Systems Version: 07.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Nobles Hospital

bioMerieux Customer:
System #:

Laboratory Report

Printed Feb 16, 2016 16:17 GMT
Printed by: nplpowc
Report Version: 1 of 1
Patient ID:

Patient Name:
Isolate Group: craig msc-4

Card Type: ANC Testing Instrument: 000015F25C09 (2529)

Bionumber: 6703040600001
Organism Quantity:

Comments:	

Identification Information	Card:	ANC	Lot Number:	244366910	Expires:	Dec 30, 2016 12:00 GMT
	Completed:	Feb 15, 2016 23:14 GMT	Status:	Final	Analysis Time:	6.00 hours
Selected Organism	96% Probability		Propionibacterium acnes			
	Bionumber:	6703040600001			Confidence:	Excellent identification
SRF Organism						
Analysis Organisms and Tests to Separate:						
Analysis Messages:						
Contraindicating Typical Biopattern(s)						
Propionibacterium acnes	BGURi(12),					

Biochemical Details

4	dGAL	-	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	-
28	SAC	-	30	ARB	-	33	NAG	-	34	BGLUi	-	36	URE	-	37	BGURi	+
39	BGALi	-	41	AARA	-	42	AGALi	-	43	BMAN	-	44	ARG	+	45	PVATE	+
51	MTE	-	53	ESC	-	54	BdFUC	-	55	BNAGi	-	56	AMANi	-	57	AlFUC	-
59	PHOS	-	60	IARA	-	61	dRIB2	-	62	OPS	-	63	AARAF	-	64	dXYL	-
	GRAM	+		MORPH	-		AERO	-									

Installed VITEK 2 Systems Version: 07.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Page 1 of 1

Appendix 3 – FilmArray results

FilmArray™ BCID Panel

BIOFIRE

www.BioFireDx.com

Run Summary			
Sample ID:	665909 O2 (Craig MSc)	**Run Date:**	02 Apr 2016 7:39 PM
Organisms Detected:	None	**Controls:**	Passed
Applicable Antimicrobial Resistance Genes:			

Result Summary - Interpretations	
Antimicrobial Resistance Genes	
⊘ N/A	KPC (carbapenem-resistance gene)
⊘ N/A	*mecA* (methicillin-resistance gene)
⊘ N/A	*vanA/B* (vancomycin-resistance genes)
⚠ **NOTE:** Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for the FilmArray antimicrobial resistance gene assays does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.	
Gram Positive Bacteria	
Not Detected	*Enterococcus*
Not Detected	*Listeria monocytogenes*
Not Detected	*Staphylococcus*
Not Detected	*Staphylococcus aureus*
Not Detected	*Streptococcus*
Not Detected	*Streptococcus agalactiae* (Group B)
Not Detected	*Streptococcus pneumoniae*
Not Detected	*Streptococcus pyogenes* (Group A)
Gram Negative Bacteria	
Not Detected	*Acinetobacter baumannii*
Not Detected	*Enterobacteriaceae*
Not Detected	*Enterobacter cloacae* complex
Not Detected	*Escherichia coli*
Not Detected	*Klebsiella oxytoca*
Not Detected	*Klebsiella pneumoniae*
Not Detected	*Proteus*
Not Detected	*Serratia marcescens*
Not Detected	*Haemophilus influenzae*
Not Detected	*Neisseria meningitidis*
Not Detected	*Pseudomonas aeruginosa*
Yeast	
Not Detected	*Candida albicans*
Not Detected	*Candida glabrata*
Not Detected	*Candida krusei*
Not Detected	*Candida parapsilosis*
Not Detected	*Candida tropicalis*

Run Details			
Pouch:	BCID Panel v2.0	**Protocol:**	BC v3.1
Run Status:	Completed	**Operator:**	Craig Pownall (Craig)
Serial No.:	03187122	**Instrument:**	2FA01066
Lot No.:	223815		

FilmArray™ BCID Panel

BIOFIRE

www.BioFireDx.com

Run Summary			
Sample ID:	662714 O2 (Craig MSc)	**Run Date:**	05 Apr 2016 5:40 PM
Organisms Detected:	None	**Controls:**	Passed
Applicable Antimicrobial Resistance Genes:			

Result Summary - Interpretations	
Antimicrobial Resistance Genes	
⊘ N/A	KPC (carbapenem-resistance gene)
⊘ N/A	*mecA* (methicillin-resistance gene)
⊘ N/A	*vanA/B* (vancomycin-resistance genes)
⚠ **NOTE:** Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for the FilmArray antimicrobial resistance gene assays does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.	
Gram Positive Bacteria	
Not Detected	*Enterococcus*
Not Detected	*Listeria monocytogenes*
Not Detected	*Staphylococcus*
Not Detected	*Staphylococcus aureus*
Not Detected	*Streptococcus*
Not Detected	*Streptococcus agalactiae* (Group B)
Not Detected	*Streptococcus pneumoniae*
Not Detected	*Streptococcus pyogenes* (Group A)
Gram Negative Bacteria	
Not Detected	*Acinetobacter baumannii*
Not Detected	*Enterobacteriaceae*
Not Detected	*Enterobacter cloacae* complex
Not Detected	*Escherichia coli*
Not Detected	*Klebsiella oxytoca*
Not Detected	*Klebsiella pneumoniae*
Not Detected	*Proteus*
Not Detected	*Serratia marcescens*
Not Detected	*Haemophilus influenzae*
Not Detected	*Neisseria meningitidis*
Not Detected	*Pseudomonas aeruginosa*
Yeast	
Not Detected	*Candida albicans*
Not Detected	*Candida glabrata*
Not Detected	*Candida krusei*
Not Detected	*Candida parapsilosis*
Not Detected	*Candida tropicalis*

Run Details			
Pouch:	BCID Panel v2.0	**Protocol:**	BC v3.1
Run Status:	Completed	**Operator:**	Craig Pownall (Craig)
Serial No.:	03187099	**Instrument:**	2FA01066
Lot No.:	223815		

FilmArray™
BCID Panel

BIOFIRE

www.BioFireDx.com

Run Summary

Sample ID:	669007 O2 (Craig MSc)	**Run Date:**	23 Mar 2016 2:16 PM
Organisms Detected:	None	**Controls:**	Passed
Applicable Antimicrobial Resistance Genes:			

Result Summary - Interpretations

Antimicrobial Resistance Genes

N/A	KPC (carbapenem-resistance gene)
N/A	*mecA* (methicillin-resistance gene)
N/A	*vanA/B* (vancomycin-resistance genes)

NOTE: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for the FilmArray antimicrobial resistance gene assays does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.

Gram Positive Bacteria

Not Detected	*Enterococcus*
Not Detected	*Listeria monocytogenes*
Not Detected	*Staphylococcus*
Not Detected	*Staphylococcus aureus*
Not Detected	*Streptococcus*
Not Detected	*Streptococcus agalactiae* (Group B)
Not Detected	*Streptococcus pneumoniae*
Not Detected	*Streptococcus pyogenes* (Group A)

Gram Negative Bacteria

Not Detected	*Acinetobacter baumannii*
Not Detected	*Enterobacteriaceae*
Not Detected	*Enterobacter cloacae* complex
Not Detected	*Escherichia coli*
Not Detected	*Klebsiella oxytoca*
Not Detected	*Klebsiella pneumoniae*
Not Detected	*Proteus*
Not Detected	*Serratia marcescens*
Not Detected	*Haemophilus influenzae*
Not Detected	*Neisseria meningitidis*
Not Detected	*Pseudomonas aeruginosa*

Yeast

Not Detected	*Candida albicans*
Not Detected	*Candida glabrata*
Not Detected	*Candida krusei*
Not Detected	*Candida parapsilosis*
Not Detected	*Candida tropicalis*

Run Details

Pouch:	BCID Panel v2.0	**Protocol:**	BC v3.1
Run Status:	Completed	**Operator:**	Craig Pownall (Craig)
Serial No.:	03187127	**Instrument:**	2FA01066
Lot No.:	223815		

FilmArray™
BCID Panel

BIOFIRE

www.BioFireDx.com

Run Summary

Sample ID:	668449 O2 (Craig MSc)	**Run Date:**	28 Mar 2016 1:36 PM
Organisms Detected:	None	**Controls:**	Passed
Applicable Antimicrobial Resistance Genes:			

Result Summary - Interpretations

Antimicrobial Resistance Genes

N/A	KPC (carbapenem-resistance gene)
N/A	*mecA* (methicillin-resistance gene)
N/A	*vanA/B* (vancomycin-resistance genes)

NOTE: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for the FilmArray antimicrobial resistance gene assays does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.

Gram Positive Bacteria

Not Detected	*Enterococcus*
Not Detected	*Listeria monocytogenes*
Not Detected	*Staphylococcus*
Not Detected	*Staphylococcus aureus*
Not Detected	*Streptococcus*
Not Detected	*Streptococcus agalactiae* (Group B)
Not Detected	*Streptococcus pneumoniae*
Not Detected	*Streptococcus pyogenes* (Group A)

Gram Negative Bacteria

Not Detected	*Acinetobacter baumannii*
Not Detected	*Enterobacteriaceae*
Not Detected	*Enterobacter cloacae* complex
Not Detected	*Escherichia coli*
Not Detected	*Klebsiella oxytoca*
Not Detected	*Klebsiella pneumoniae*
Not Detected	*Proteus*
Not Detected	*Serratia marcescens*
Not Detected	*Haemophilus influenzae*
Not Detected	*Neisseria meningitidis*
Not Detected	*Pseudomonas aeruginosa*

Yeast

Not Detected	*Candida albicans*
Not Detected	*Candida glabrata*
Not Detected	*Candida krusei*
Not Detected	*Candida parapsilosis*
Not Detected	*Candida tropicalis*

Run Details

Pouch:	BCID Panel v2.0	**Protocol:**	BC v3.1
Run Status:	Completed	**Operator:**	Craig Pownall (Craig)
Serial No.:	03187116	**Instrument:**	2FA01066
Lot No.:	223815		

THE EFFECTS OF SENSOR COLOUR CHANGE IN NEGATIVE BLOOD CULTURE BOTTLES; ARE TERMINAL SUB-CULTURES NECESSARY IN SOME CASES?

CRAIG ANTHONY POWNALL

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