

# Evaluation of a new Rapid Antimicrobial Susceptibility system for Gram-negative and Gram-positive bloodstream infections: Speed and accuracy of Alfred 60 AST

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## Research article

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# Abstract

**Background:** Blood stream infections (BSIs) are a major cause of morbidity and mortality. The time from taking blood cultures to obtain results of antibiotic sensitivity can be up to five days which impacts patient care. The Alfred 60 AST™ can reduce laboratory time from positive culture bottle to susceptibility results from 16-25 hours to 5-6 hours, transforming patient care. **Objective:** To evaluate the diagnostic accuracy of a rapid antimicrobial susceptibility system, the Alfred 60 AST™, in clinical isolates from patients with BSIs and confirm time to results. **Methods:** 301 Gram-negative and 86 Gram-positive isolates were analysed directly from positive blood culture bottles following Gram staining. Antimicrobial susceptibility results and time-to-results obtained by rapid Alfred 60 AST system and BD Phoenix were compared. **Results :** A total of 2,196 antimicrobial susceptibility test results (AST) were performed: 1,863 Gram-negative and 333 Gram-positive. AST categorical agreement (CA) for Alfred 60 AST™ was 95% (1772/1863) for Gram-negative and 89% (295/333) for Gram-positive isolates. Gram-negative CA: ampicillin 96% (290/301); ciprofloxacin 95% (283/297); ceftriaxone 96% (75/78); meropenem 97% (288/297); piperacillin-tazobactam 95% (280/295); gentamicin 94% (279/297) and amikacin 93% (277/298). The median time to susceptibility results from blood culture flagging positive was 6.3 h vs 20 h (  $p < 0.01$  ) for Alfred system vs BD Phoenix™. **Conclusion:** Alfred 60 AST system greatly reduced time to antimicrobial susceptibility results in Gram-negative and Gram-positive BSIs with good performance and cost, particularly for Gram-negative bacteraemia. **Keywords:** Rapid diagnostics. Bloodstream infection. Bacteraemia. Antimicrobial susceptibility testing. Gram-negative bacteria. Gram-positive bacteria

## Background

Blood stream infections (BSIs) are worldwide, a major cause of morbidity and mortality (Adhikari, 2010). The clinical laboratory plays a key role in the diagnosis of BSIs and provides antimicrobial susceptibility results which are crucial in clinical decision-making. Blood cultures remain the main way to identify blood stream infection. Despite optimisation in laboratory workflows that have decreased time-to-result (Anderson, 2014), the time from taking a blood culture to obtaining a result of antimicrobial susceptibilities can be up to 5 days (Goto, 2013). The time to positivity, time from blood culture collection to bottle flagging positive, is typically 12 to 24 hours [1]. The subsequent mean time to identify bacterial species and obtain antimicrobial susceptibilities, using standard methods, is a further 36 hours (Maurer, 2017).

Phenotypic antimicrobial susceptibility tests (AST) generally relies on detecting bacterial growth in the presence of antibiotic. Standard methods of testing used in routine diagnostic laboratories for patient care include broth or agar micro-dilution, disc diffusion or antibiotic gradient strips, which require around 24-72 hours to complete (Meda, 2017). A number of automated systems for testing antimicrobial susceptibility are increasingly used such as BD Phoenix™ (Becton Dickinson, USA), VITEK-2™ (BioMerieux, France) and MicroScan™ (Beckman, USA) which have a time to results ranging between 12 and 24 hours after positive culture (Romero-Gomez, 2012).

The time to clinically useful antimicrobial susceptibility data has an impact on patient care either to change to an effective antibiotic in the case of a resistant isolates or to focus to a narrower spectrum antibiotic for susceptible isolate. The development of rapid diagnostics is a key aim in the control of the rise in antibiotic resistance (Buehler, 2015) . The use of genotypic methods such as nucleic acid amplification tests to detect antibiotic resistance in routine blood cultures has not been widely adopted due to high cost (Caliendo, 2013), lack of sensitivity when using blood direct from the patient (Warhurst, 2015) and inability to detect many resistance mechanisms and hence “rule-in” the use of antibiotics when no resistance marker is detected (Timbrook, 2017) . This highlights the need to increase the speed of phenotypic methods to shorten the time to obtain antimicrobial susceptibility results (Kerremans, 2008).

The Alfred 60 AST™ (Alifax, Italy) is a CE marked automated commercial laser-scattering based *in-vitro* diagnostic (IVD) providing antimicrobial susceptibility results directly from positive blood culture bottles within 4-6 hours. The Alfred 60 AST™ uses light scatter to detect bacterial growth in a liquid culture broth. In brief, 30 µL of blood from a positive blood culture is inoculated into a broth that is incubated, then light scatter is measured until it reaches a turbidity of 0.4 – 0.6 McFarland. A series of tubes containing antibiotics are then robotically inoculated and bacterial growth measured by light scatter providing real time bacterial growth curves. Growth curves of bacteria over 3-5 hours in the tested antibiotics are compared to growth a positive control (no antibiotic) and percentage of inhibition of growth (%PIC) is calculated. The %PIC is compared between control and antibiotic tubes and reported as resistant, intermediate and sensitive categories, according to the range of inhibition (Sensitive result: Inhibition of growth >65%; Intermediate: Inhibition of growth between 65% and 50%; Resistant: Inhibition of growth <50%) . To grant a reliable detection of the growth inhibition (keeping as reference the “Reference Vial” ) a proper growth must be detected ( $\geq 700.00$  CFU/ml) , if not the test is not considered valid.

The system is based on a light scattering technique, A light laser beam which is oriented trough a sample and two photo detectors placed at 30° and 90° receive signals generated by the light scattered by the bacteria present in the vial. Growth kinetic curves are obtained for each detector and displayed in two curves. Double reading channel (2 detectors) is important for samples with high initial turbidity level. If 1 detector (the 1st one) becomes saturated due to the high sample’s turbidity then the 2nd detector is able to detect signals. Finally, a mathematical algorithm selects the best reading detector channel for the calculation of the inhibition of growth. (Figure 1).

The panel of antibiotics tested can be predefined by users. Hands on time for each run is about 15 minutes. In addition, the system requires a daily set up of approximately 10 minutes and a weekly maintenance wash out of 30 minutes. Up to 60 individual tests can be performed in a run, the number of isolates tested depends on the number of antibiotics tested (for example 10 isolates may be tested for 6 antibiotics each).

The time to result is unaffected by the number of antibiotics or samples tested. Alfred 60 AST recognises each reference vial followed by its respective antibiotic vials as a single assay and has the capacity to initiate multiple single susceptibility assays at the same time for different isolates.

The purpose of this study was to compare the accuracy and speed of results of the Alfred 60 AST™ with the BD Phoenix™ (Becton Dickinson, USA) automated susceptibility testing system, a broth-based microdilution test that utilizes a redox indicator to enhance the detection of organism growth, which is the standard of care in our routine diagnostic laboratory.

## Methods

### Study Setting :

A prospective evaluation was conducted directly from blood cultures, between January and May 2018 in the microbiology laboratory of South West London Pathology in London. The diagnostic laboratory processes approximately 3,500 positive blood cultures per year. All blood culture samples were initially incubated on the BD BacTec FX400 (Becton Dickinson, USA). Blood culture bottles, including adult and paediatric samples, that flagged positive were then tested in parallel for antimicrobial susceptibility by Alfred 60 AST and by routine testing on the BD Phoenix™, after Gram staining performance to determine a Gram-positive or Gram-negative antibiotic panel required and exclude the polymicrobial samples.

Bacterial species were identified by Bruker Biotyper matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Germany), this technique was performed, as per routine laboratory practice, from isolated colonies obtained following 4 hours agar plate subculture, in order to fully interpret the results of the Alfred 60AST with species ID. This meant the species of the samples were identified before the Alfred 60AST result was available. (Figure 2.)

### Alfred 60 AST system:

The Alfred 60 ST was performed according to the manufacturer's instructions on blood culture bottle that had flagged positive on the BD BacTec FX system, this including running weekly controls with *Pseudomonas aeruginosa* 27853; *E.coli* 25922; *E.coli* 35218; *S.aureus* 29213; *E.faecalis* 29212; *E. faecalis* 51299. 30µL of the sample was inoculated into an enrichment broth and loaded onto the instrument. Each vial of antibiotic contains 45mg of a preparation of lyophilized antibiotic that requires to be dissolved in 2mL of regenerating solution to give a concentration of antibiotic at a specified breakpoint. Some antibiotics, including amikacin, piperacillin-tazobactam and meropenem, require different specific antibiotic vials for testing *Enterobacteriaceae* and *Pseudomonas Spp*. As the identity of organism is unknown when the Alfred 60 AST system was set up, a combined panel of antibiotics with both concentrations was used. When the identity of the organism was known the appropriate antibiotic result was reported.

### Antimicrobial agents:

The following antibiotic panel was performed on all Gram-negative organisms: ampicillin (8 mg/L), amikacin (8 or 16 mg/L), ciprofloxacin (0.5 or 1 mg/L), gentamicin (2 or 4 mg/L), meropenem (2 or 8 mg/L) and piperacillin-tazobactam (8/4 or 16/4 mg/L), concentrations for enterobacteriaceae and for

pseudomonas respectively. Ceftriaxone (2 mg/L) was included on the Gram-negative panel later in March 2018 and tested in 78 isolates. For Gram-positive organisms the antibiotic panel included: ampicillin (4 or 8 mg/L), ceftiofloxacin (4 or 8 mg/L), clindamycin (0.25 or 0.5 mg/L), teicoplanin (2 mg/L) and vancomycin (4 mg/L), concentrations for *Staphylococcus aureus* and for Coagulase negative staphylococci (*CoNS*) respectively. Purity plate was set up both on CLED agar and blood agar plates and examined the next day to confirm the inoculum was not mixed.

### **Comparator AST technique:**

The BD Phoenix™ automated susceptibility testing system CE marked IVD (BD Diagnostics, Sparks, MD, USA) (software version 5.02H/4.11B) was used as the routine laboratory method according to the manufacturer instructions, including running weekly controls with *Pseudomonas aeruginosa* 27853; *E.coli* 25922; *E.coli* 35218; *S.aureus* 29213; *E.faecalis* 29212; *E. faecalis* 51299 using cartridge PMIC-96 for Gram-positive and NMIC-417 for Gram-negative isolates.

### **Interpretation of results:**

Isolates were classified as susceptible, intermediate or resistant (S/I/R) according to interpreted reports provided by Alfred 60AST™ and BD Phoenix™. The final report interpretation of susceptibility results provided by Alfred 60 AST™ are based on bacterial growth curve analysis **Figure 1**.

Each sample result by Alfred 60 AST system was compared against BD Phoenix™. Categorical agreement (CA) was defined as agreement of test results interpreted within the same susceptibility category(S/I/R).

Discordant results were categorized as very major error (VME, reported susceptible on Alfred AST60™ when reported resistant Phoenix™), major error (ME, reported resistant when susceptible) or minor error (mE, reported intermediate when susceptible or resistant). The criteria for antimicrobial susceptibility testing proposed by Jorgensen has been used ([Jorgensen, 1993](#)) .

### **Time to AST results:**

The time to AST was defined as the time between the culture bottle flagging positive on the BacTec and the availability of the AST result by Alfred 60 AST system or BD Phoenix™. Wilcoxon test was performed for comparison of median AST time analyses, considering  $p < 0.05$  as a significant value.

SPSS (Statistical Package for the Social Sciences, IBM, USA) version 25.0 statistics software was used for all analyses.

### **Ethical approval**

This study reports the findings of the laboratory verification of the Alfred 60AST. No personal information was stored in the study database. Samples were collected as part of standard care and as is standard practice for a laboratory verification for the introduction of new equipment in the laboratory specific approval was not necessary under UK HRA decision tools.

# Results

## Antimicrobial susceptibility results of Alfred 60 system

Antimicrobial susceptibility results were performed for 387 clinical positive blood culture bottles that were monomicrobial on Gram stain (301 Gram-negative and 86 Gram-positive) with a total of 2,196 individual antimicrobial test results (1,863 Gram-negative and 333 Gram-positive). Eighteen samples (5%) were excluded from the analysis. Excluded samples comprised 3 polymicrobial samples that were not detected during Gram stain and 15 non valid results included 10 system technical failure before AST performance and 5 failures of no bacterial growth in the reference vial on Alfred 60 AST system, including: 1 *Acinetobacter baumannii*; 1 *Achromobacter spp*; 1 *Bacteroides fragilis*; 1 *Pseudomonas aeruginosa*; 1 *Klebsiella oxytoca*.

71% (213/301) of the Gram-negative and 43% (37/86) of the Gram-positive isolates were resistant to at least one antibiotic. 9% (28/301) of the Gram-negative organisms were identified as Extended-spectrum beta-lactamases (ESBLs) as defined by BD Phoenix™ expert rules for resistance. **Figure 3.**

There were a total of 301 valid Alfred 60 AST results for Gram-negative monomicrobial isolates. Data were available on the following organisms: 209 *E.coli*; 57 *Klebsiella spp*; 10 *P. aeruginosa*; 12 *Proteus spp*; 7 *Enterobacter spp*; 6 *Morganella morgani*. A total of 1,863 AST results were produced and compared with BD Phoenix™.

### Alfred 60 AST agreement for Gram-negative bacteria:

Overall categorical agreement for Alfred 60 AST was 95% (1772/1863) for Gram-negative isolates (55 major errors, 31 very major errors and 5 minor errors). The highest agreement was observed with meropenem 97% (288/297); ampicillin 96% (290/301); ciprofloxacin 95% (283/297); ceftriaxone 96% (75/78) and piperacillin-tazobactam 95% (280/295): Lower agreement was seen for amikacin 93% (277/298) and gentamicin 94% (279/297). Out of a total of 55 major errors, 65% (36/55) involved *E.coli* against amikacin 13; gentamicin 8; piperacillin-tazobactam 6; meropenem 3; ampicillin 4; ciprofloxacin 2 and 20% (11/55) involved *Klebsiella spp*. against ciprofloxacin 3; meropenem 3; amikacin 2; piperacillin/tazobactam 2. Very major errors were seen with ciprofloxacin 6; ampicillin 7; gentamicin 7; piperacillin/tazobactam 5; meropenem 3; ceftriaxone 2 and amikacin 1. **Table 1 and 2.**

### Alfred 60 AST agreement for Gram-positive bacteria:

A total of 86 valid Gram-positive organisms were analysed giving a total of 333 Alfred 60 AST results. The overall categorical agreement for Alfred 60 AST was 89% (295/333) for Gram-positive samples. The highest agreement was observed with ceftazidime 92% (69/75) and teicoplanin 92% (79/86) whilst clindamycin and vancomycin showed the lowest one 80% (60/75) and 90% (77/86) respectively. There were 29 major errors, 7 very major errors and 2 minor errors. Seventeen of the major errors (68%) involved *S. aureus* tested clindamycin 6, teicoplanin 6 and vancomycin 5. The remaining 12 major errors involved *CoNS* and *Enterococci Spp* against vancomycin and ceftazidime. Five of the very major errors involved *CoNS*

tested against clindamycin and two involved *S. aureus* and *Staphylococcus epidermidis* tested against cefoxitin. **Table 1 and 2.**

### **Time to results**

The median time to susceptibility results from flagging positive blood cultures was 6.3 h (IQR= 5.25 – 8.4) for Alfred 60 AST system compared to the 20 h (IQR= 16-24) for BD Phoenix™. As a result, a reduction in the turnaround time by 13 h when compared to BD Phoenix™ was observed.

In terms of “hands-on-time”, in our experience, an average time of 20 minutes were required to load the samples on the Alfred rapid system, comparable to 15 minutes for BD Phoenix™ system . **Table 3.**

## **Discussion**

This study shows the performance of Alfred 60 AST™ system, which significantly reduced the time to antimicrobial susceptibility results. The overall categorical agreement for Alfred 60 AST system was 96% for Gram-negative and 90% for Gram-positive organisms. These results are broadly comparable to an earlier smaller study reported by Barnini *et al* (Barnini, 2016), which compared the Alfred 60 AST™ with VITEK-2™, and reported a categorical agreement of 91% for enterobacteriaceae and 94% for staphylococci. Although similar, differences in the performances may be due to the choice of antibiotics tested, Barnini *et al* tested ceftazidime and gentamicin, but did not test amoxicillin and ciprofloxacin.

Compared to the BD Phoenix, the overall categorical agreement for Alfred 60 AST system was 96% for Gram-negative and 90% for Gram-positive organisms. Previous studies report the accuracy of the BD Phoenix™ system for susceptibility results compared against agar dilution and manual disc diffusion, showing categorical agreement of 97.5% (Carroll, 2006) and 95% (Donay, 2004) respectively.

Alfred 60AST™ system achieved a performance of over 95% CA compared to the BD Phoenix™ for all antibiotics except amikacin and gentamicin. However, , this new system showed slightly lower performance for Gram-positive isolates and for glycopeptides and clindamycin.

In our study, the average hands-on time per specimen was not different from routine testing, however the time to deliver susceptibility results has been significantly reduced compared to routine standard automated system (BD Phoenix™). The times to AST results were similar to the times noted in a previous study using the Alfred 60 AST™ system and other the Accelerate Pheno™ system (Accelerate Diagnostics, USA) (Barnini, 2016) (Descours, 2018) (Lutgring, 2018) (Brazelton de Cardenas, 2017). Given high categorical agreement between the BD Phoenix™ and Alfred 60 AST™ and the 16 hour reduction in time to results there are compelling clinical benefits of the Alfred AST 60™.

The Accelerate Pheno™ system is an alternative rapid phenotypic testing system. In studies using similar AST comparator techniques, an agreement of 93.3% (Marschal, 2017), 94.9% (Pantel, 2018) and 96.4%

respectively (Descours, 2018) has been reported. Showing very similar diagnostic performance to the Alfred AST 60™. The median time to AST results from positive culture bottle to AST result for the Accelerate Pheno™ system is reported as 10.7 [8.6-12.8] hours (Descours, 2018). It should be noted that an advantage of the Accelerate Pheno™ system is that it identifies the species of the isolate in addition to the sensitivity pattern. For this reason, we needed to perform a rapid identification with a MALDI-TOF MS at the same time as the Alfred 60 AST™.

A specific advantage of the Alfred 60 AST™ system versus other rapid phenotypic susceptibility methods is the capacity to accommodate between 7 and 9 isolates per run, the number of samples may be varied according to the customized antibiotic panel chosen by the user and a satellite module provides extra capacity to handle up to a total of 20 isolates per day. Furthermore, new samples can be loaded during an ongoing run without interfering previous loaded isolates.

The cost of performing rapid phenotypic sensitivity testing for some systems has been regarded as high and seen as a barrier to introducing these tests. We estimate the additional reagent costs for the Alfred 60 AST™ of €2-4 per antibiotic tested (Lahanas, 2013) or €30-40 per isolate, with relatively minimal laboratory hands on time. This cost is considerably lower than other rapid testing systems and we believe could be justified by the clinical benefits.

One of the limitations of the system is it will not perform antimicrobial susceptibility testing on polymicrobial samples, although Gram-staining before using the Alfred 60 AST™ meant polymicrobial samples were relatively uncommon in these samples. . Another possible drawback is the absence of MIC values, which are not provided on the susceptibility report, that may occasionally be relevant for specific therapeutic decisions (Roberts, 2014), however most clinical decisions can be made simply with an S/I/R result. Another limitation lies on the inability of performing susceptibility testing of *Acinetobacter baumannii* or *Stenotrophomonas maltophilia* which are emerging as a challenging to treat nosocomial pathogens (Coelho, 2004). Furthermore, there are inconsistencies with the concentration of ceftazidime in the Alfred 60 AST™ panel. The EUCAST breakpoint for ceftazidime is 4 mg/L for *S. aureus* and *S. lugdunensis* and 8 ug/L for *Staphylococcus saprophyticus*, whilst Alfred 60AST™ breakpoint for all coagulase negative Staphylococci is 8 ug/L.

Limitations of the study were that although broth microdilution is considered the “gold standard” technique for antimicrobial susceptibility evaluation, this is rarely routinely available in practice in clinical laboratories. The BD Phoenix™ system is a well validated automated AST system (Carroll, 2006). Similar methodologies of comparing rapid diagnostic tests with automated AST systems in place of broth or agar dilution has been used in majority of published studies (Pantel, 2018) (Lutgring, 2018) (Descours, 2018) (Brazelton 2017) (Marschal 2017) (Carrol, 2006).

We note the relatively small proportion of multi-resistant microorganisms in our particular setting and the fact that more than half of the susceptibility testing for Gram-negative isolates were performed for *E.coli*

blood stream infections. Furthermore, a smaller number of Gram-positive organisms were tested compared to the Gram-negative organisms.

Even though there is clinical utility in rapid antimicrobial sensitivity results we would still recommend isolates have further extensive testing with another system, such as the BD Phoenix™ or a VITEK™ 2, to give a complete sensitivity pattern to a wider range of antibiotics and give results for minimum inhibitory concentrations. Since, BD phoenix shows more accuracy specially for gram positive bacteria.

The implementation of the Alfred 60 AST™ system in the laboratory routine workflow, will provide rapid susceptibility results, that effectively communicated to clinicians might result in a prompt change to appropriate antimicrobial treatment, antimicrobial resistance reduction and improved infection control (Lee, 2017) (Nimmich, 2017) (Cain, 2015).

## **Conclusion**

In conclusion, Alfred 60 AST™ system has demonstrated an improvement in the turn-around time of antimicrobial phenotypic susceptibility results in Gram-negative and Gram-positive blood stream infections in comparison with automated AST techniques and conventional AST testing.

Whilst the system provided a notably good performance and high accuracy for Gram-negative bacteria, it still needs improvements in Gram-positive AST results and is less dependable for yeast detection (Lahanas 2013).

Prospective studies are needed to further analyse the impact and effects of the rapid diagnostic techniques among bacteraemia patients and consequences of the introduction of new available rapid automated systems on antibiotic prescription against BSI and on clinical patient pathways.

## **Declarations**

### **Ethics approval and consent to participate**

This study reports the findings of the laboratory verification of the Alfred 60AST. No personal information was stored in the study database. Samples were routinely sent to the laboratory as part of standard care and as is standard practice for a laboratory verification for the introduction of new equipment in the laboratory specific approval was not necessary under UK HRA decision tools.

### **Consent to publish**

Not applicable.

### **Availability of data and materials**

Raw data used in the study are provided in the form of excel spreadsheets on the SGUL data repository. <https://sgul.figshare.com/s/c8746f6e57980278bb4f>

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### **Competing interests**

Test reagents were provided by Alifax, Italy, for the study period. Alifax had no role in study design, data collection, data analysis or study submission for publication.

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### **Author's contributions**

VAV, SA, CS & TP designed the study. VAV & SA ran the assays. writing and analysis performed by VAV, SA, CS & TP.

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## **References**

(EUCAST), E. C. f. A. S. T. (2018). "Determination of antimicrobial susceptibility test breakpoints. European Society of Clinical Microbiology and Infectious Diseases(ESCMID).

Adhikari, N. K., et al. (2010). Critical care and the global burden of critical illness in adults. *The Lancet*. 376: 1339-1346.

Anderson, D. J., et al. (2014). "Bloodstream Infections in Community Hospitals in the 21 st Century : A Multicenter Cohort Study." 9(3): 1-10.

Barnini, S., et al. (2016). "A new rapid method for direct antimicrobial susceptibility testing of bacteria from positive blood cultures." *BMC Microbiology*.

Brazelton de Cardenas, J. N., et al. (2017). "Evaluation of rapid phenotypic identification and antimicrobial susceptibility testing in a pediatric oncology center." *Diagn Microbiol Infect Dis* 89(1): 52-57.

- Buehler, S. S., et al. (2015). "Effectiveness of practices to increase timeliness of providing targeted therapy for inpatients with bloodstream infections: A laboratory medicine best practices systematic review and meta-analysis." *Clinical Microbiology Reviews* 29(1): 59-103.
- Cain, S. E., et al. (2015). "Stratification of the impact of inappropriate empirical antimicrobial therapy for Gram-negative bloodstream infections by predicted prognosis." *Antimicrobial Agents and Chemotherapy*.
- Caliendo, A. M., et al. (2013). "Better tests, better care: improved diagnostics for infectious diseases." *Clin Infect Dis* 57 Suppl 3: S139-170.
- Carroll, K. C., et al. (2006). "Evaluation of the BD Phoenix automated microbiology system for identification and antimicrobial susceptibility testing of Enterobacteriaceae." *J Clin Microbiol* 44(10): 3506-3509.
- Coelho J, et al. 2004. Multiresistant acinetobacter in the UK: how big a threat? *J. Hosp. Infect.* 58:167-169.
- Descours, G., et al. (2018). "Evaluation of the Accelerate Pheno system for rapid identification and antimicrobial susceptibility testing of Gram-negative bacteria in bloodstream infections." *Eur J Clin Microbiol Infect Dis* 37(8): 1573-1583.
- Donay, J. L., et al. (2004). "Evaluation of the Automated Phoenix System for Potential Routine Use in the Clinical Microbiology Laboratory." *Journal of Clinical Microbiology* 42(4): 1542-1546.
- Goto, M. and M. N. Al-Hasan (2013). "Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe." *Clin Microbiol Infect* 19(6): 501-509.
- Hess, A.S, Johnson J.K, Thom A, Strassle P, Netzer G, Harris A.D (2012). "Methods and recommendations for evaluating and reporting a new diagnostic test". *European Journal of Clinical Microbiology & Infectious Diseases*. 31(9):2111-2116.
- J, O. N. (2016). "The Review on Antimicrobial Resistance. Tackling Drug-resistant Infections Globally: Final Report and Recommendations."
- Jorgensen, J. H. (1993). "Selection criteria for an antimicrobial susceptibility testing system." *J Clin Microbiol* 31: 2841–2844.
- Jorgensen, J. H. and M. J. Ferraro (2009). "Antimicrobial susceptibility testing: a review of general principles and contemporary practices." *Clin Infect Dis* 49(11): 1749-1755.
- Kerremans, J. J., et al. (2008). "Rapid identification and antimicrobial susceptibility testing reduce antibiotic use and accelerate pathogen-directed antibiotic use." *J Antimicrob Chemother* 61(2): 428-435.

Lahanas, S., G. Stathopoulos, R.C. Chan, and S.J. van Hal, Evaluation of the Alfred 60/AST device as a screening test for urinary tract infections. *J Clin Microbiol*, 2013. **51**(10): p. 3406-8.

Lee, C. C., et al. (2017). "Timing of appropriate empirical antimicrobial administration and outcome of adults with community-onset bacteremia." *Crit Care* 21(1): 119.

Lutgring, J. D. B., Cassiana ; Mcelvania Tekippe, Erin ; Cavuoti, Dominick ; Hollaway, Rita ; Burd, Eileen M (2018). "Evaluation of the Accelerate Pheno System: Results from Two Academic Medical Centers." *Journal of Clinical Microbiology* 56(4).

Marschal, M., et al. (2017). "Evaluation of the Accelerate Pheno System for Fast Identification and Antimicrobial Susceptibility Testing from Positive Blood Cultures in Bloodstream Infections Caused by Gram-Negative Pathogens." *Journal of Clinical Microbiology*.

Maurer, F. P., et al. (2017). "Advances in rapid identification and susceptibility testing of bacteria in the clinical microbiology laboratory: Implications for patient care and antimicrobial stewardship programs." *Infectious Disease Reports* 9(1): 18-27.

Meda, M. C., James ; Varghese, Reela ; Rangaiah, Jayakeerthi ; Grundy, Clive ; Dashti, Farnaz ; Garner, David ; Groves, Katherine ; Fitzmaurice, Karen ; Hutley, E (2017). "What are the critical steps in processing blood cultures? A prospective audit evaluating current practice of reporting blood cultures in a centralised laboratory serving secondary care hospitals." *Journal of Clinical Pathology* 70(4): 361.

Nimmich, E. B., et al. (2017). "Development of institutional guidelines for management of Gram-negative bloodstream infections: Incorporating local evidence." *Hospital Pharmacy* 52(10).

P, S. (2012). "Susceptibility testing methods, resistance and breakpoints: what do these terms really mean? ." *Rev. Sci. Tech.* 31(1): 33-41.

Pantel, A., et al. (2018). "Performance of the Accelerate Pheno system for identification and antimicrobial susceptibility testing of a panel of multidrug-resistant Gram-negative bacilli directly from positive blood cultures." *J Antimicrob Chemother* 73(6): 1546-1552.

Patel TS, K. R., Nagel JL, Newton DW, Stevenson JG (2017). "Cost analysis of implementing matrix-assisted laser desorption ionization–time of flight mass spectrometry plus real-time antimicrobial

stewardship intervention for bloodstream infections." *J Clin Microbiol* 55(1): 60-67.

Romero-Gomez, M. P., et al. (2012). "Identification and susceptibility testing of microorganism by direct inoculation from positive blood culture bottles by combining MALDI-TOF and Vitek-2 Compact is rapid and effective." *J Infect* 65(6): 513-520.

Timbrook, T. T., et al. (2017). "The Effect of Molecular Rapid Diagnostic Testing on Clinical Outcomes in Bloodstream Infections: A Systematic Review and Meta-analysis." *Clin Infect Dis* 64(1): 15-23.

Warhurst, G., et al. (2015). "Rapid detection of health-care-associated bloodstream infection in critical care using multipathogen real-time polymerase chain reaction technology: a diagnostic accuracy study and systematic review." *Health Technol Assess* 19(35): 1-142.

Weinbren, M. J. C., M. ; Heathcote, R. ; Umar, M. ; Nisar, M. ; Ainger, C. ; Masters, P (2018). "Optimization of the blood culture pathway: a template for improved sepsis management and diagnostic antimicrobial stewardship." 98 3: 232-235.

Lahanas S, Stathopoulos G, Chan RC, Van Hal SJ. Evaluation of the Alferd 60/AST device as a screening test for urinary tract infections. *Journal of clinical microbiology*. 2013 Oct 1;51(10):3406-8.

## Tables

microbial agent	No. of AST results	No. of category agreements					No. of discrepancies		
		S	R	I	CA Total	CA % (95% CI)	Minor	Major	Very major
<b>α-negative antimicrobials</b>									
ticillin	301	88	202	0	290	96 (94-98)	0	4	7
acin	298	273	2	2	277	93 (89-96)	3	17	1
ofloxacin	297	222	61	0	283	95 (92-97)	1	7	6
iaxone	78	64	11	0	75	96 (89-99)	0	1	2
amicin	297	240	38	1	279	94 (91-96)	1	10	7
racillin/Tazobactam	295	263	16	1	280	95 (92-97)	0	10	5
openem	297	284	4	0	288	97 (94-99)	0	6	3
<b>all agreement</b>	<b>1863</b>	<b>1434</b>	<b>334</b>	<b>4</b>	<b>1772</b>	<b>95 (94-96)</b>	<b>5 (0.3%)</b>	<b>55 (3%)</b>	<b>31 (2%)</b>
<b>α-positive antimicrobials</b>									
xitin	75	34	35	0	69	92 (83-97)	0	4	2
amycin	75	41	17	2	60	80 (69-88)	1	9	5
oplanin	86	79	0	1	79	92 (84-97)	1	6	0
omycin	86	76	1	0	77	90 (81-95)	0	9	0
icillin	11	9	1	0	10	90 (59-100)	0	1	0
<b>all agreement</b>	<b>333</b>	<b>239</b>	<b>54</b>	<b>3</b>	<b>295</b>	<b>89 (85-92)</b>	<b>2 (0.6%)</b>	<b>29 (9%)</b>	<b>7 (2%)</b>

**TABLE 1** AST results of the Alfred 60AST™ system compared to BD Phoenix™ system for each antibiotic. Column 2 shows the total number of susceptibility tests done with both methods; columns 3 to 6 the total number of tests for which there was agreement within each category and in total; columns 8 to 10 the number of discrepancies ; S = susceptible = intermediate, R=resistant, CA= Categorical agreement, 95 % CI = 95 % confidence interval

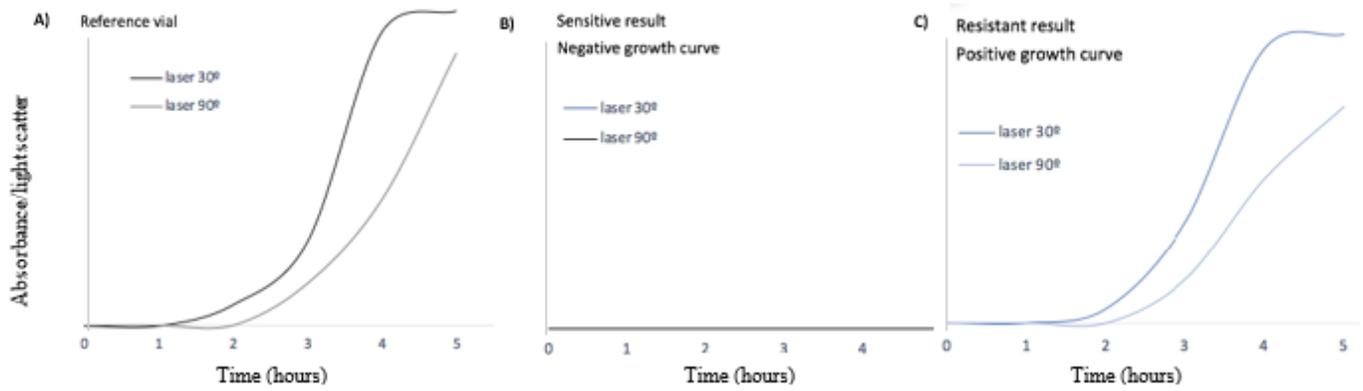
	No. of Isolates	No. of AST results	No. of CA	CA % (95% CI)	No. of discrepancies		
					Very major	Major	Minor
Gram-negative organisms							
<i>E.coli</i>	209	1291	1242	96 (95-97)	9	36	4
<i>Klebsiella spp</i>	57	351	330	94 (91-96)	10	11	1
<i>P.mirabilis</i>	12	79	69	87 (78-84)	6	3	0
<i>P.aeruginosa</i>	10	61	58	95 (86-91)	0	3	0
<i>E.cloacae</i>	7	43	37	86 (72-95)	0	6	0
<i>M.morgani</i>	6	38	36	95 (82-99)	0	2	0
<b>Overall Gram-negative</b>	301	1863	1772	95 (94-96)	31	55	5
Gram-positive organisms							
<i>S.epidermidis</i>	26	104	98	94 (88-98)	3	2	1
<i>S.aureus</i>	21	84	66	79 (68-87)	1	17	0
<i>S.hominis</i>	15	60	54	90 (79-96)	2	4	0
<i>CoNS other</i>	13	52	48	92 (81-97)	1	3	0
<i>E.faecalis</i>	10	30	27	93 (73-98)	0	3	0
<i>E.faecium</i>	1	3	2	67 (10-100)	0	0	1
<b>Overall Gram-positive</b>	86	333	295	89 (85-92)	7	29	2

**TABLE 2** AST results of the Alfred 60 AST™ system compared to BD Phoenix™ system for 301 Gram-negative and 86 Gram-positive organisms included in the study. Column 2 refers to the total number of susceptibility tests done with both methods; column 3 refers to the number of tests for which there was agreement between both tests. S=sensitive, I = intermediate, R=resistant, CA= Categorical agreement, 95% CI = 95% confidence interval. CoNS - Coagulase negative staphylococcus, 95%CI- 95% confidence interval

	Alfred 60 AST	BD Phoenix™ system	Alfred™ vs BD Phoenix™
<b>Time to results from positive blood culture (h)</b>	6.3 h (IQR, 5.25 - 8.25)	20 h (IQR, 16- 24)	p < 0.01
<b>Technician “hands-on” time (min)</b>	20 min (IQR, 10-30)	15 min (IQR, 10-20)	
<b>Technique Characteristics</b>	Direct culture bottle	Agar plate subculture (4 hours)	

**Table 3. Turnaround time for results from blood culture positivity:** Median Time (in hours) and Interquartile range from positive blood culture to results of Alfred vs BD Phoenix™. Time in hours from positive blood culture to susceptibility results.

## Figures



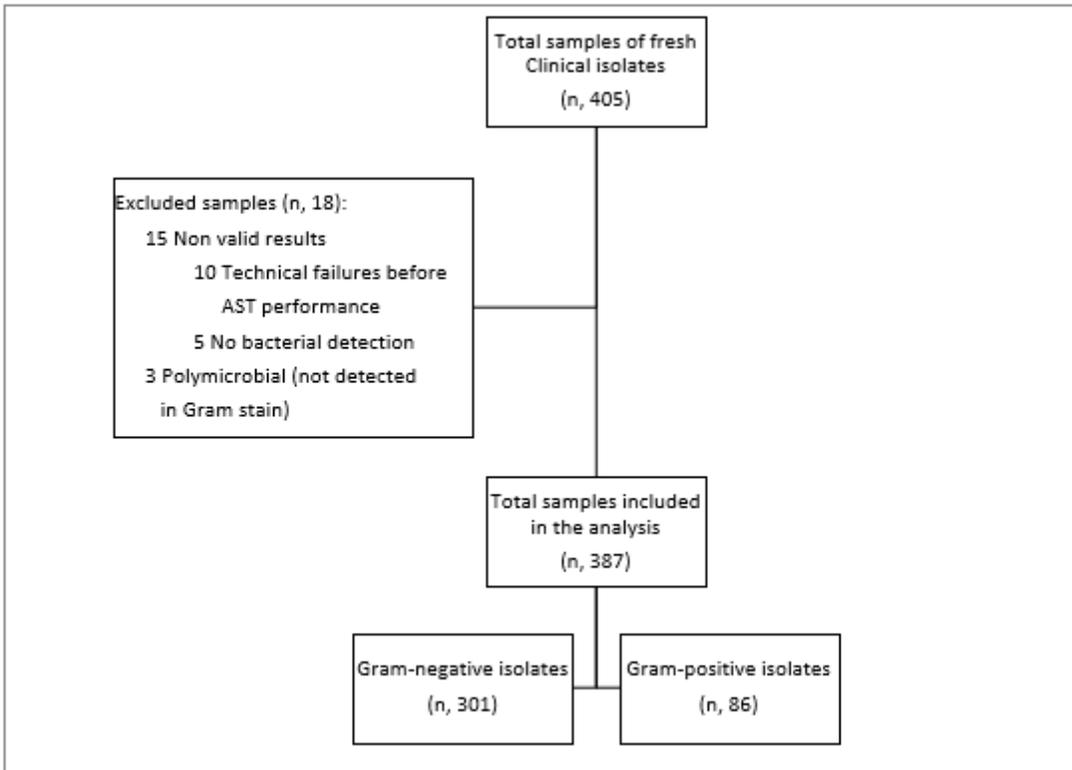
**Figure 1**

A) represents the standard growth curve without presence of antibiotic (curves are displayed representing the light scattered signals received by photodetectors placed at 30° and 90° from the laser beam). B) represents a sensitive or susceptible antimicrobial results. C) Represents a resistant result, in the presence of antibiotic the growth curve is positive , comparable to the reference vial.



**Figure 2**

Flowchart showing laboratory workflow followed along the rapid susceptibility system (Alfred 60 AST) evaluation. Once a blood culture bottle flagged positive following incubation, Gram-stain was performed in order to distinguish between Gram-positive and Gram-negative microorganism and exclude the polymicrobial ones, which will allow to select the appropriate antibiotic panel and a direct blood sample from the bottle was loaded onto the rapid system. In parallel, the identification results were obtained by MALDI-TOF, performed from isolated colonies obtained by 4h agar plate subculture. The mean total time from positive blood culture to identification and susceptibility results was 6 h (approx.).



**Figure 3**

Flowchart of study population. A total of 387 (301 Gram-negative and 86 Gram-positive) valid monomicrobial clinical isolates after 18 excluded samples. Excluded samples comprised 3 polymicrobial and 15 non valid results included 10 system technical failure and 5 no bacterial detection and subsequently no AST performance.