Q-LINEA

# The Importance of Controlled Inoculum

**Minimum inhibitory concentration (MIC) results as an output from antimicrobial susceptibility testing (AST), drive optimal treatment for patients by providing accurate information to clinicians regarding susceptibility. The inoculum effect is the attenuation of antimicrobial activity due to a high concentration of inoculated bacteria, which can cause unreliable MIC results. A fixed dilution sample preparation is inadequate at accounting for the inoculum effect. To overcome the inoculum effect a controlled inoculum is therefore important for the generation of accurate MIC results produced by AST. The ASTar® System utilises a controlled inoculum to generate accurate and reliable MIC results.**

## The current landscape of bloodstream infections

Bloodstream infections (BSIs) can be defined as the presence of viable bacteria, fungi, or viruses in the blood, associated with infection[1]. Globally, BSIs represent a significant burden of disease. BSI is often associated with sepsis, defined as a life-threatening organ dysfunction due to the dysregulation of the host immune response to an infection[2]. Septic shock is a subset of sepsis, in which the patient has further serious circulatory, cellular, and metabolic abnormalities. Septic shock is associated with a 50% risk of mortality[3]. In the US, sepsis is the leading cause of hospital deaths[4], with further studies suggesting this is a global trend[5]. Sepsis is one of the main causes of overall mortality globally, representing 1 in 5 of all deaths[4].

Antimicrobial therapy can improve patient outcomes significantly, particularly if initiated at an early stage[6]. Each hour of delay in antimicrobial administration from initial hypotension onset is associated with an average decrease in survival of 7.6% in patients with septic shock[7]. Prior to the identification of the pathogen, physicians rely upon empiric therapy to treat the infection. Empiric therapy centres on the administration of broad-spectrum antimicrobials, based on patient clinical presentation, history, and the epidemiologic setting[8]. One review found that the incidence of ineffective empiric therapy was reported in half of the included papers in severe cases of infection[9]. Ineffective empiric therapy has a significant effect on patient outcomes, represented by an increase in morbidity and mortality[10].

Antimicrobial susceptibility testing (AST) is needed to determine the optimal treatment. The current "gold standard" for phenotypic AST is minimum inhibitory concentration (MIC) determination by dilution methods. MIC demonstrates the lowest concentration of antimicrobial needed *in vitro* to prevent microbial growth. The standard of practice (SOP) test is performed by either broth microdilution, disk diffusion, or agar dilution. These methods traditionally have a turn-around time of approximately 24 hours but also require a prior step to isolate a pure culture for testing[11]. With the increasing burden of BSI, a need for faster turn-around times in AST has emerged. Rapid phenotypic AST is defined as capable of producing results in ≤8 hours[12].

There is currently a focus on rapid AST systems which increase automation, bypass the need for pure isolates, and with increased ease of use[13]. Rapid phenotypic AST has the potential to significantly improve the turn-around time, thereby reducing the duration of empiric therapy and the use of broad-spectrum antimicrobials. As part of a standard AST process, an inoculum must be prepared from the blood culture sample. Inoculum preparation requires bacteria in a state that is capable of inoculation into cell culture or media, for growth. The aim is usually to reach a high level of viable cells for use as an inoculum, to assess the concentration of bacteria in a sample[14].

## The inoculum effect issue

The generation of an accurate MIC value is vital for physicians to determine optimal therapy for BSI patients. However, some bacterial concentration ranges in a sample can generate misleading MIC values. This phenomenon is known as the inoculum effect and is caused by variation in the number of cells inoculated into the MIC assay[15]. The influence of the inoculum effect has led to guidelines dictating the defined bacterial concentrations for standard AST methods. Two governing bodies dictate inoculum concentration guidelines: the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The purpose of these guidelines is to standardize MIC determination and facilitate laboratory result reproducibility. CLSI defines inoculum effect testing as typically recorded at an inoculum concentration 100 times greater than the guideline. However, the inoculum effect has even been observed at lower concentrations of the CLSI recommended range, especially in strains resistant to meropenem and cefepime[16].

The inoculum effect is particularly pronounced in specific combinations of antimicrobials and bacteria. A variety of resistant strains of bacteria are likely to exhibit the inoculum effect[17,18]. A common example of such a combination includes β-lactam antimicrobials, in combination with Staphylococcus and Enterobacterales species. Currently, it is not fully understood what mechanisms lead to the inoculum effect. One proposed mechanism is that at a high density, bacteria reach the stationary phase more rapidly, and antimicrobials that target penicillin-binding proteins have a diminished effect. This would explain the inoculum effect seen in β-lactam antimicrobials[19]. Recent papers have found associations between an increased failure rate of treatment, and mortality, in patients who had an antimicrobial-bacteria combination that was prone to the inoculum effect[20,21].

The standard approach to creating an inoculum within the guideline concentration in AST is to use a fixed dilution. However, this process can result in a significant number of inoculum concentrations falling outside the EUCAST and CLSI guideline range[22]. As discussed, the inoculum effect has even been observed within the guideline concentrations[16]. Therefore, this can lead to erroneous MIC values and inappropriate antimicrobial treatment choices for patients.

## The fixed dilution issue of positive blood cultures

The viable count is the number of living cells present in a sample and allows technicians to correctly determine the number of actively dividing cells. Viable count in positive blood culture flasks varies based upon what species is present in the flask, the incubation method used, and whether the sample is left inside or outside an incubation cabinet[23]. Controlling the inoculum concentration relative to the initial concentration of bacteria in the blood flask has been highlighted as an improvement in method[23]. However, manual steps to standardize inoculum concentrations are time-consuming, which has prevented the widespread adoption of this procedure.

Most microbiology labs do not operate 24/7. Additionally, laboratories that do operate at night may operate with fewer staff. This means that there is an approximate 12-hour window in which samples can signal positive but when AST is less likely to be performed. Given an average time to positivity of 10–16 hours, many samples will signal positive whilst the lab is not fully staffed[24]. A fixed, pre-determined dilution of a positive blood culture can lead to the sample falling outside of the CLSI or EUCAST guideline inoculum range[25]. To solve this problem, an automated AST device would need to be able to process blood culture flasks in an approach that measured the concentration of bacteria in the specific flask, even if it has signalled positive many hours previously.

The viable count concentration of Gram-positive and Gram-negative species in positive blood culture flasks is shown in Figure 1. As shown, there is a wide range of viable count concentrations. This makes a fixed dilution method difficult, as it cannot achieve final inoculum concentrations within guidelines for all samples.

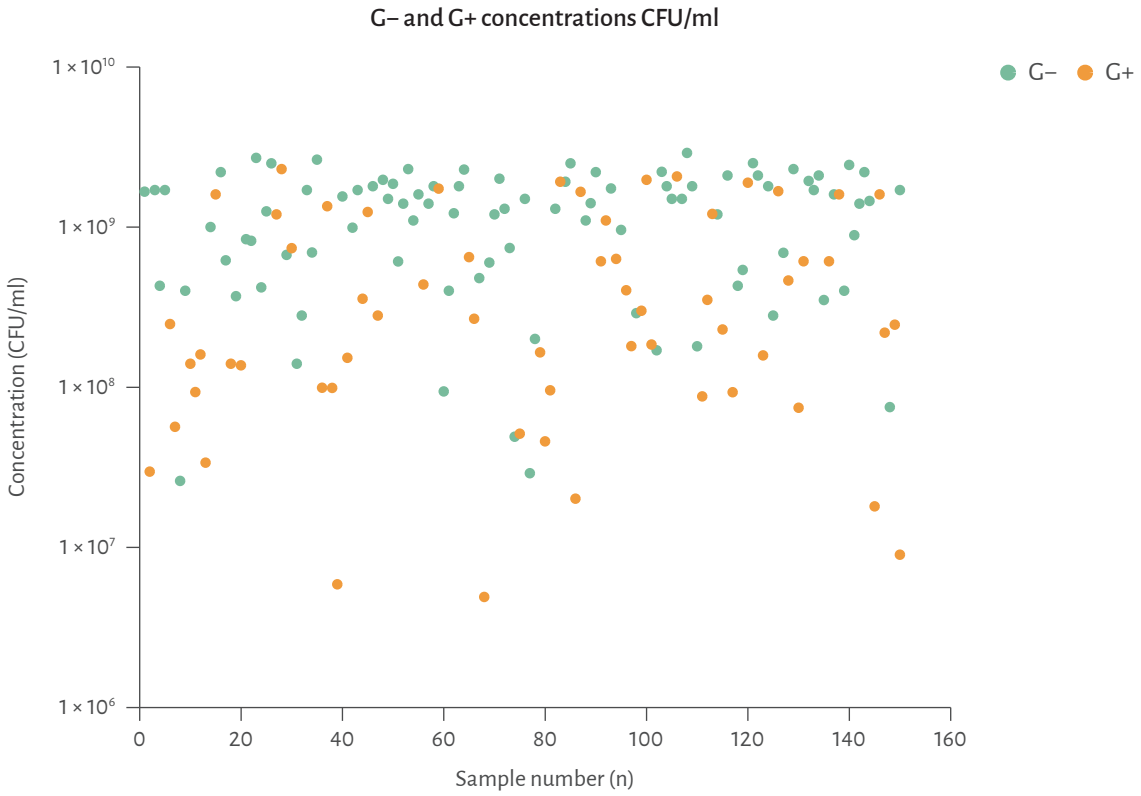


**Figure 1.** Viable count concentrations of Gram-positive and Gram-negative species present in positive BCFs. Species present: *A. baumannii, E. cloacae, E. coli, E. faecalis, H. influenzae, K. pneumoniae, L. grayi, L. monocyogenes, P. aeruginosa, P. mirabilis, S. aureus, S. capitis, S. epidermidis, S. hominis, S. lugdunensis, S. anginosus, S. mitis, S. pneumoniae, S. pyogenes*.

## The ASTar solution

The crude bacteria concentrations present in blood culture flasks (BCFs) are inherently variable, therefore a fixed dilution method risks generating an inoculum that is outside of the recommended guideline range. This could lead to an inoculum effect which would deliver erroneous MIC values. A fixed dilution method can also lead to a final inoculum concentration that is below the guidelines. A final inoculum that has a concentration below guidelines risks producing an inaccurate MIC value, likely showing a resistant strain as susceptible, as evidenced with regards to meropenem in a 2018 paper[16]. The ASTar® System solves this issue by generating a consistent and controlled inoculum from positive blood cultures to generate consistently accurate MIC values. A blood culture sample is loaded directly to the ASTar System, and the process of producing a controlled inoculum is fully automated. This capability saves significant lab work, as determining appropriate inoculum concentration is time-consuming. A summary of the automated method is shown in Figure 2.

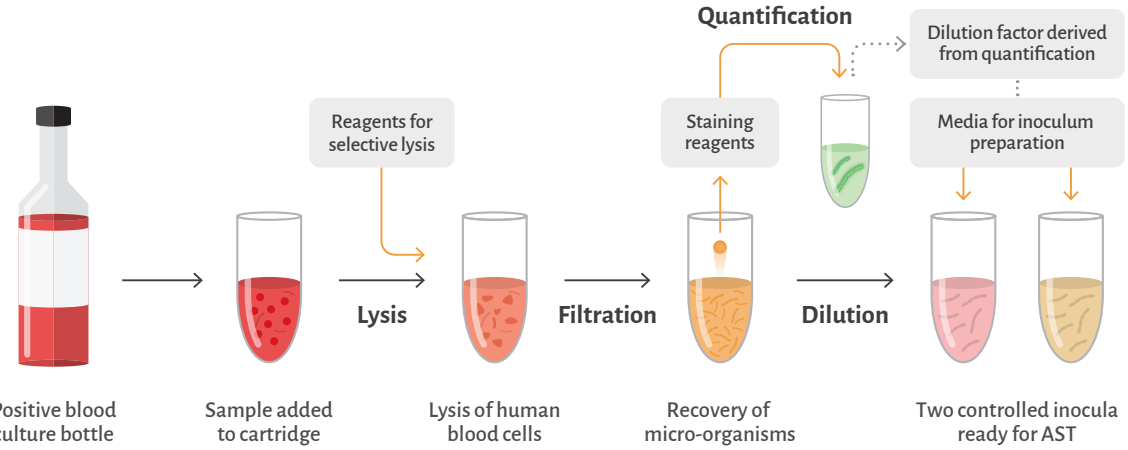


**Figure 2.** An overview of the sample preparation method used in the ASTar System.

The performance of the ASTar System in generating controlled inocula compared to a fixed dilution method is shown in Figure 3. Despite a range of initial bacteria species and concentrations, for Gram-negative samples, 93% were within the guidelines for the controlled inoculum used in ASTar. For a fixed dilution of the blood culture, 73% of Gram-negative samples would have been out of range. The strategy to prepare a controlled inoculum minimizes the risk of erroneous MICs for inoculum-dependent strains and pathogen-antimicrobial combinations[16]. ASTar includes this function to contribute to data stability and reproducibility. The generation of accurate MIC results can support physicians to determine the best treatment, and thereby improve patient outcomes.

The ability of the ASTar System to generate final inoculum concentrations within EUCAST and CLSI guidelines is shown in Figure 3. These inocula were prepared 0–8 hours after signalling positive. Even up to 8 hours after signalling positive, significant variations of concentration can be seen of bacteria in the blood flasks. This variation only increases as more time passes since signalling positive.

The controlled inoculum of the ASTar System allows accurate generation of stable MIC results up to 16 hours after the blood culture sample has signalled positive (Table 1). The inoculum preparation method can adapt to a range of viable count concentrations. Consequently, blood culture flasks that could previously not be used in AST can still be used with a high degree of reliability. This can decrease the need to collect new samples and therefore should reduce the time to MIC results for blood culture flasks that signal positive during inoperative laboratory hours.

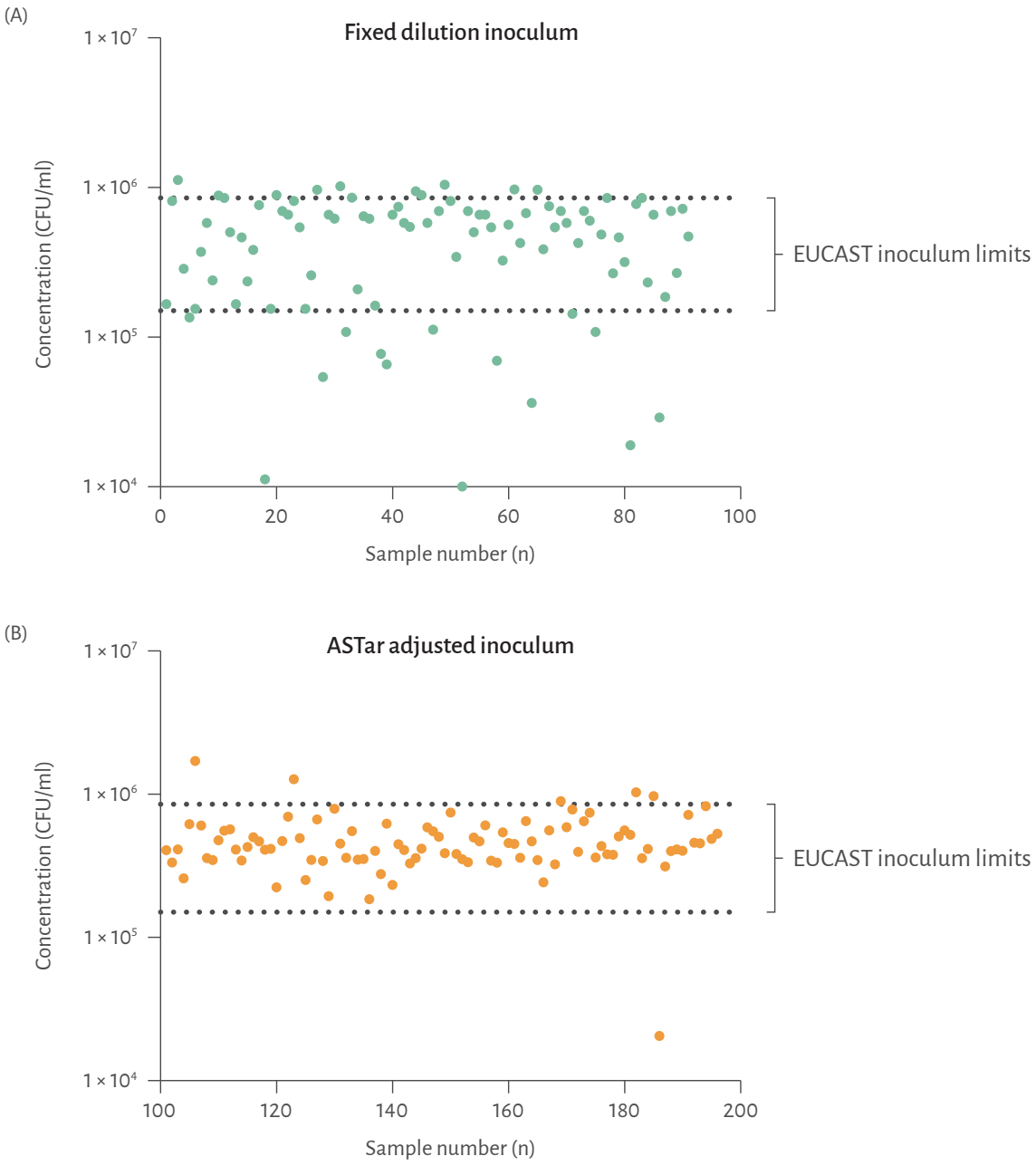


**Figure 3.** (A) Inoculum concentration of 100 samples following fixed dilution method. (B) Inoculum concentration of 100 samples following ASTar automated controlled sample preparation. Species present: *A. baumannii, C. freundii, C. koseri, E. asburiae, E.aerogenes, E.coli, K. aerogenes, K. oxytoca, K. pneumoniae, P. aeruginosa, P. mirabilis, S. marcescens.*

**Table 1.** Performance of ASTar at running AST for BCFs that have been stored for 16 hours at room temperature culture cabinet[26].

| Condition compared to reference | MIC values ±1 from mode value in reference / Total number of MIC values | Percent success rate |
|---|---|---|
| 16 h room temperature | 154/160 | 96.3% |
| 16 h blood culture cabinet | 178/183 | 97.3% |

## Conclusion

The future of AST relies upon devices that can provide accurate diagnostic information to physicians quickly. Rapid AST devices can enable more effective implementation of antimicrobial stewardship programs, but only if they provide accurate MIC results. The controlled inoculum that the ASTar System produces allows it to consistently provide inoculum concentrations within the CLSI and EUCAST recommended guidelines. In comparison to the standard practice method of fixed dilution, controlled inoculum reduces the risk of the inoculum effect causing erroneous MIC values. With rising antimicrobial resistance, the inoculum effect is likely to become a greater issue for accurate AST results in the future. The current literature already indicates an association between treatment success and the presence of the inoculum effect. Microbiologists and physicians will increasingly seek ways to reduce this impact by providing robust and consistent inoculum preparation for AST. The consistent and controlled inoculum preparation of the ASTar System provides accurate MIC determination, which should ultimately help physicians to select correct antimicrobial, and dosage when appropriate.

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