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 deviating (high or low) 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 automatically prepares 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. 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 bacteria 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 standardise MIC determination and facilitate laboratory result reproducibility.

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].

One approach to creating an inoculum from positive blood cultures 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

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 standardise inoculum concentrations are time-consuming, which has prevented the widespread adoption of this procedure.

Most microbiology laboratories 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.

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.

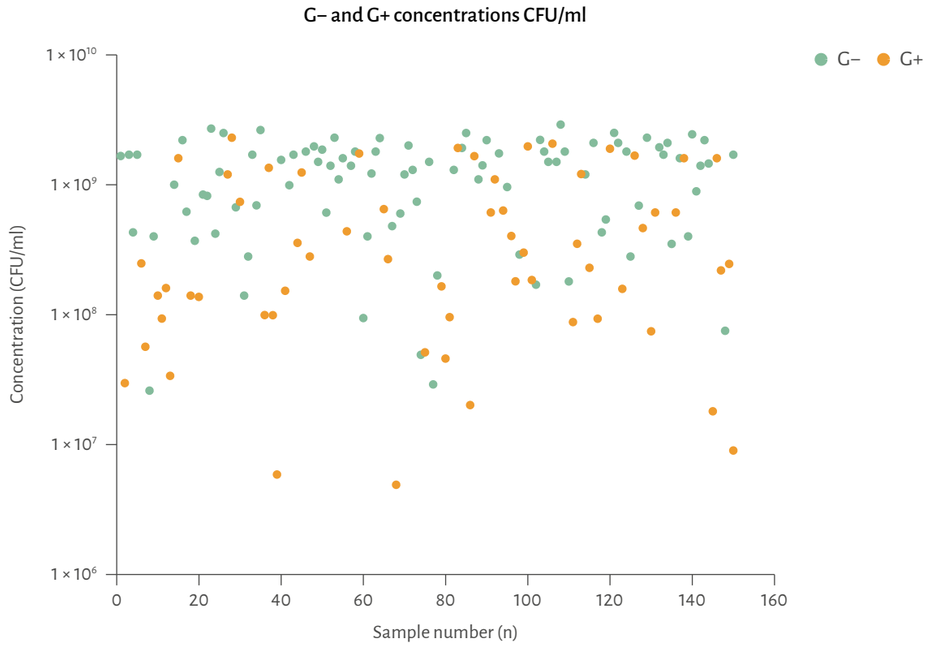

Figure 1. Viable count concentrations of Gram-positive and Gram-negative species present in positive BCFs. Species present: *A. baumannii, E. cloacae, E. coli, E. faecalis, H. influenzae, K. pneumoniae, L. grayi, L. monocyogenes, P. aeruginosa, P. mirabilis, S. aureus, S. capitis, S. epidermidis, S. 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 laboratories work, as determining appropriate inoculum concentration is time-consuming. A summary of the automated method is shown in Figure 2.

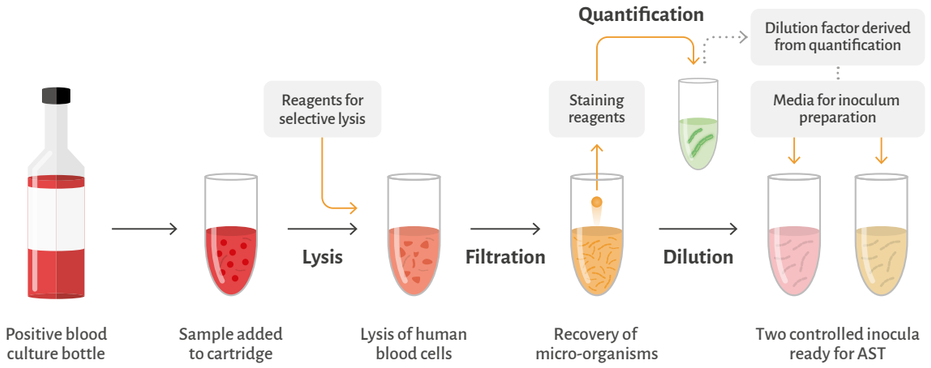

Figure 2. An overview of the sample preparation method used in the ASTar System.

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, only 73% of Gram-negative samples would have been in range. The strategy to prepare a controlled inoculum minimises 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 demonstrated 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 reproducible MIC results. When comparing samples from blood culture flasks run on ASTar just after positivity and at 16 hours after positivity, the MIC results were within +/-1 dilution in >98% of the cases[26]. Consequently, blood culture flasks signalling positive after the work shift the previous day can safely be run on ASTar the following morning.

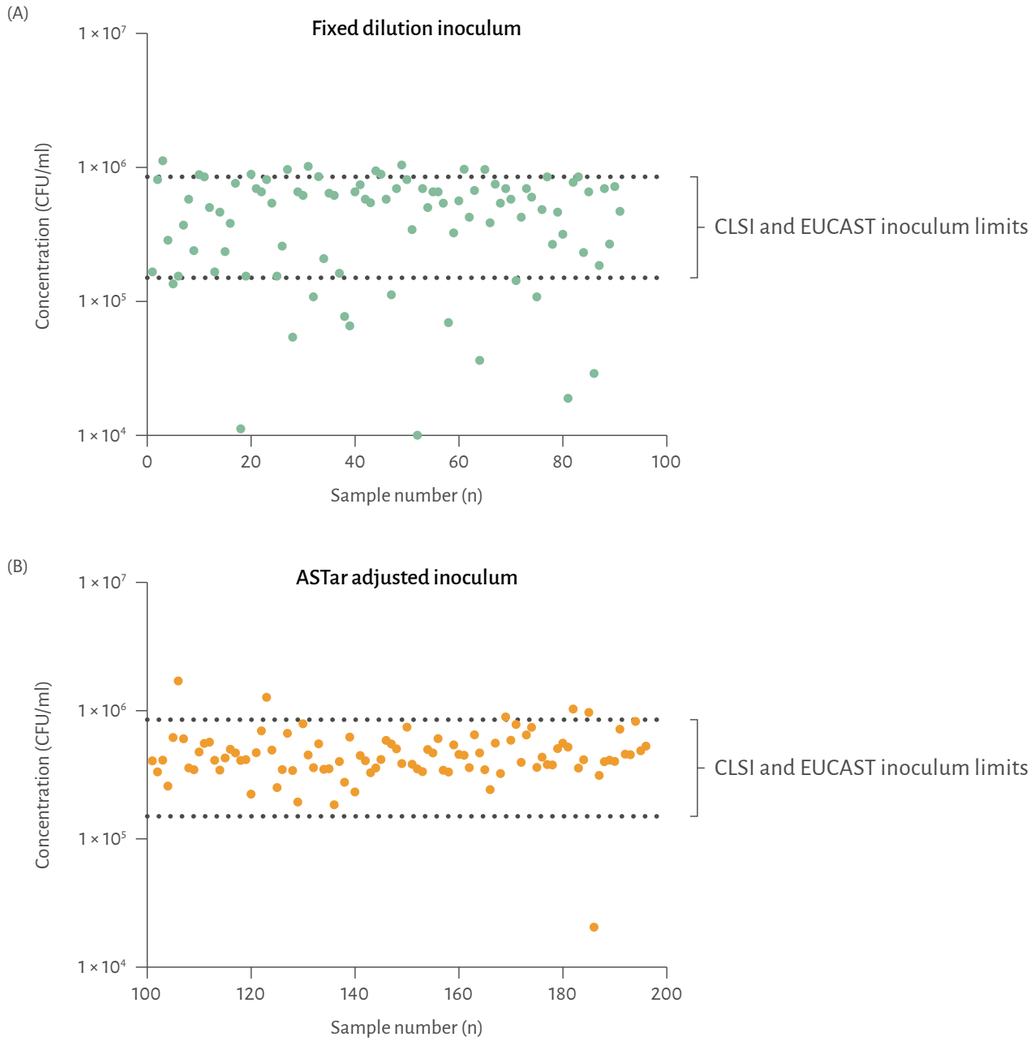

Figure 3. (A) Inoculum concentration of 100 samples following fixed dilution method. (B) Inoculum concentration of 100 samples following ASTar automated controlled sample preparation. Species present: *A. baumannii*, *C. freundii*, *C. koseri*, *E. asburiae*, *E.aerogenes*, *E.cloacae* complex, *K. aerogenes*, *K. oxytoca*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *S. marcescens*. Data from ECCMID poster 2019 (ref 27).

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 antimicrobials and appropriate dosages.

References

1. Laupland KB, Church DL. Population-based epidemiology and microbiology of community-onset bloodstream infections. *Clin Microbiol Rev*. 2014;27(4):647–664. doi:10.1128/CMR.00002-14
2. Singer M, Deutschman CS, Seymour C, et al. The third international consensus definition for sepsis and septic shock (sepsis-3). *JAMA - J Am Med Assoc*. 2016;315(8):801–810. doi:10.1001/jama.2016.0287
3. Schoenberg MH, Weiss M, Radermacher P. Outcome of patients with sepsis and septic shock after ICU treatment. *Langenbeck's Arch Surg*. 1998;383(1):44–48. doi:10.1007/s004230050090
4. Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. *Lancet*. 2020;395(10219):200–211. doi:10.1016/S0140-6736(19)32989-7
5. Fleischmann C, Scherag A, Adhikari NKJ, et al. Assessment of global incidence and mortality of hospital-treated sepsis current estimates and limitations. *Am J Respir Crit Care Med*. 2016;193(3):259–272. doi:10.1164/rccm.201504-0781OC
6. Ferrer R, Martin-Loeches I, Phillips G, et al. Empiric antibiotic treatment reduces mortality in severe sepsis and septic shock from the first hour: Results from a guideline-based performance improvement program. *Crit Care Med*. 2014;42(8):1749–1755. doi:10.1097/CCM.0000000000000330
7. *June 2006 - Volume 34 - Issue 6 : Critical Care Medicine*.; 2006. Accessed September 22, 2021. https://journals.lww.com/ccmjournal/toc/2006/06000
8. Bassetti M, Peghin M, Timsit JF. The current treatment landscape: Candidiasis. *J Antimicrob Chemother*. 2016;71:ii13–ii22. doi:10.1093/jac/dkw392
9. Marquet K, Liesenborgs A, Bergs J, Vleugels A, Claes N. Incidence and outcome of inappropriate in-hospital empiric antibiotics for severe infection: A systematic review and meta-analysis. *Crit Care*. 2015;19(1). doi:10.1186/s13054-015-0795-y
10. Retamar P, Portillo MM, López-Prieto MD, et al. Impact of inadequate empirical therapy on the mortality of patients with bloodstream infections: A propensity score-based analysis. *Antimicrob Agents Chemother*. 2012;56(1):472–478. doi:10.1128/AAC.00462-11
11. Schumacher A, Vranken T, Malhotra A, Arts J, Habibovic P. In vitro antimicrobial susceptibility testing methods: agar dilution to 3D tissue-engineered models. *Eur J Clin Microbiol Infect Dis*. 2018;37(2):187–208. https://pubmed.ncbi.nlm.nih.gov/28871407/
12. Idelevich EA, Seifert H, Sundqvist M, et al. Microbiological diagnostics of bloodstream infections in Europe—an ESGBIES survey. *Clin Microbiol Infect*. 2019;25(11):1399–1407. doi:10.1016/j.cmi.2019.03.024
13. Puttaswamy S, Gupta SK, Regunath H, Smith LP, Sengupta S. A Comprehensive Review of the Present and Future Antibiotic Susceptibility Testing (AST) Systems. *Arch Clin Microbiol*. 2018;09(03):1–9. doi:10.4172/1989-8436.100083
14. Sood S, Singhal R, Bhat S, Kumar A. Inoculum Preparation. In: *Comprehensive Biotechnology, Second Edition*. Vol 2. Academic Press; 2011:152–164. doi:10.1016/B978-0-08-088504-9.00090-8
15. Hwang JH, Lee SY, Choi J. Microscopic analysis of bacterial inoculum effect using micropatterned biochip. *Antibiotics*. 2021;10(3):1–12. doi:10.3390/antibiotics10030300
16. Smith KP, Kirby JE. The inoculum effect in the era of multidrug resistance: Minor differences in inoculum have dramatic effect on MIC Determination. *Antimicrob Agents Chemother*. 2018;62(8). doi:10.1128/AAC.00433-18
17. Hobson CA, Cointe A, Jacquier H, et al. Cross-resistance to cefiderocol and ceftazidime–avibactam in KPC β-lactamase mutants and the inoculum effect. *Clin Microbiol Infect*. 2021;27(8):1172.e7-1172.e10. doi:10.1016/j.cmi.2021.04.016
18. Laure NN, Dawan J, Ahn J. Effects of incubation time and inoculation level on the stabilities of bacteriostatic and bactericidal antibiotics against salmonella typhimurium. *Antibiotics*. 2021;10(8). doi:10.3390/antibiotics10081019
19. Lenhard JR, Bulman ZP. Inoculum effect of β-lactam antibiotics. *J Antimicrob Chemother*. 2019;74(10):2825–2843. doi:10.1093/jac/dkz226
20. Miller WR, Seas C, Carvajal LP, et al. The cefazolin inoculum effect is associated with increased mortality in methicillin-susceptible staphylococcus aureus bacteremia. *Open Forum Infect Dis*. 2018;5(6):1–9. doi:10.1093/ofid/ofy123
21. Lee S, Song KH, Jung SI, et al. Comparative outcomes of cefazolin versus nafcillin for methicillin-susceptible Staphylococcus aureus bacteraemia: a prospective multicentre cohort study in Korea. *Clin Microbiol Infect*. 2018;24(2):152–158. doi:10.1016/j.cmi.2017.07.001
22. Chapin KC, Musgnug MC. Validation of the automated reading and incubation system with sensititre plates for antimicrobial susceptibility testing. *J Clin Microbiol*. 2003;41(5):1951–1956. doi:10.1128/JCM.41.5.1951–1956.2003
23. Chandrasekaran S, Abbott A, Campeua S, et al. Direct-from-Blood-Culture Disk Diffusion To Determine Antimicrobial Susceptibility of Gram-Negative Bacteria : Preliminary Report from the Clinical and Laboratory Standards Group. 2018;56(3):1–10.
24. Fiori B, D'Inzeo T, Di Florio V, et al. Performance of two resin-containing blood culture media in detection of bloodstream infections and in direct matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) broth assays for isolate identification: Clinical comp. *J Clin Microbiol*. 2014;52(10):3558–3567. doi:10.1128/JCM.01171-14
25. Chapin KC, Musgnug MC. Direct susceptibility testing of positive blood cultures by using sensititre broth microdilution plates. *J Clin Microbiol*. 2003;41(10):4751–4754. doi:10.1128/JCM.41.10.4751–4754.2003
26. ASTar BC G- Kit Instruction for Use, D28727 and D49165
27. Klintstedt M, Osman H, Russell C, et al. Automated inoculum preparation for AST from crude samples. Q-linea. Published 2019. https://qlinea.com/0986741_wp-uploads/2021/04/Poster-ECCMID-2019-P1759.pdf

Q-LINEA

www.qlinea.com

D60218 V5 10-2024