Automated inoculum preparation for AST from crude samples

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Introduction

Accurate broth microdilution (BMD)-based antimicrobial susceptibility testing (AST) requires correct inoculum (1, 2). Turbidity measurement is used for isolates, but for inoculum preparation direct from clinical samples, fewer alternatives exist. We present a rapid, automated process for preparing viable bacteria directly from positive blood cultures, giving a pre-defined inoculum for AST in a single sample preparation cartridge.

Key functions of the sample preparation process (Figure 1) are:

- Pathogen isolation
- Concentration determination
- Adjustment to selected inoculum
- Addition of fastidious supplement
- Distribution of the sample to the disc for culturing

This study investigated the performance of the first three process steps.

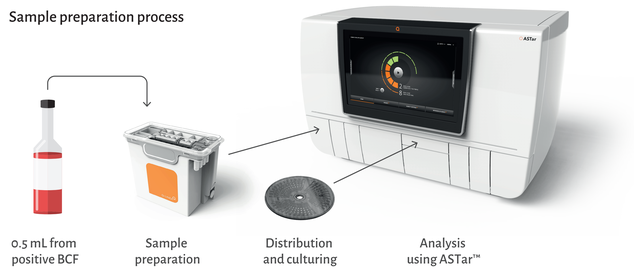

Fig 1. ASTar, an automated system for rapid antimicrobial susceptibility testing (AST) based on broth microdilution and time-lapse microscopy.

Materials and methods

Blood culture flasks (BCF) were spiked with a gram-positive or gram-negative pathogen together with 9 mL of blood from healthy donors and cultured until signaling positive. Bacterial concentration measured as colony forming units (CFU) in the positive BCF was assessed using viable count technique (VC). Subsequently, 0.5 mL of the BCF content was added to an ASTar™ (Q-linea AB, Sweden) sample preparation cartridge for selective lysis of non-microbial content and bacteria isolation using filtration. The bacterial resuspension was diluted in cation-adjusted Mueller-Hinton broth and its concentration measured and adjusted to a pre-selected value suitable for AST. This process was evaluated with a second viable count. Figure 2 shows an overview of the study design. For fixed dilution calculations, the dilution ratio was set using a learning set of positive BCF, from which a dilution ratio was calculated to maximize the likelihood of achieving EUCAST inoculum (5×10^5 CFU/mL ± 60%).

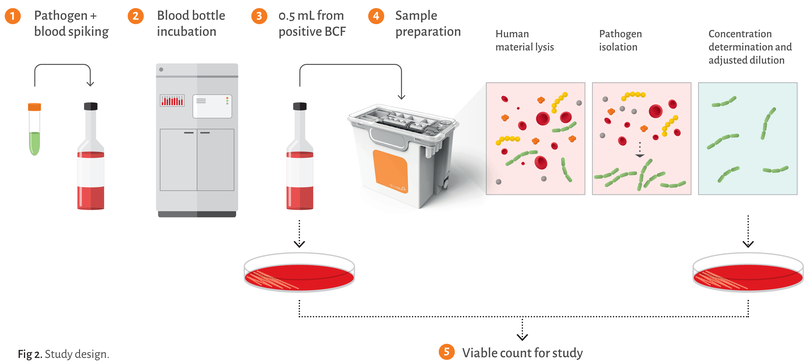

Fig 2. Study design.

Results

In a test set of gram-negative and gram-positive organisms, the spread of pathogen concentration in positive BCFs spanned approximately two orders of magnitude for gram-negative (G-) organisms and over three for gram-positive (G+) organisms (Figure 3). For the fixed-dilution experiment, the G-concentration distribution was tighter than the G+ distribution, but not more than 82% (G-) and 39% (G+) of the samples achieved the EUCAST (ISO) inoculum. In contrast, the inoculum delivered by the automated sample preparation placed 96% of G- and 86% of G+ samples within the EUCAST inoculum. Figure 4 gives the input and output concentrations of the automated concentration adjustment for a large and diverse set of G+ and G- organisms. Despite wide variation in input concentration, output inoculum is kept within EUCAST limits with only a few exceptions of relatively small magnitude.

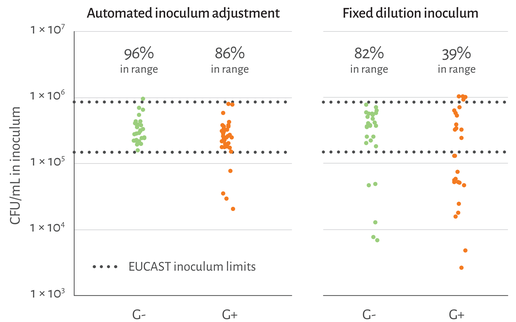

Fig 3. Comparison between inoculum concentrations achieved using ASTar automated sample processing compared with what would have been achieved using fixed dilution for gram-negative (G-) and gram-positive (G+) organisms.

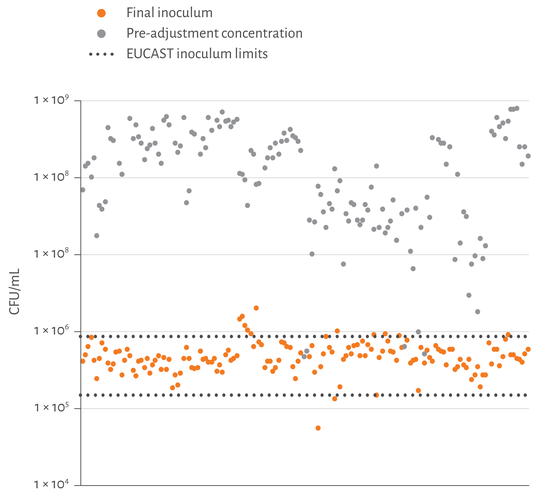

Fig 4. CFU counts of recovered and resuspended pathogens from a large set of positive BCF spiked with gram-negative and gram-positive organisms sampled approx. 0–8 h after positivity compared with automated prepared inoculum from the same samples. Dashed lines denote limits of EUCAST recommended inoculum. Organisms (160 samples): *A. baumannii, C. koseri, E. aerogenes, E. cloacae, E. coli, H. influenzae, K. oxytoca, K. pneumoniae, P. aeruginosa, P. fluorescens, P. mirabilis, P. stutzeri, S. aureus, S. marcescens, S. pneumoniae*. Fifteen species and 47 strains in total.

Conclusions

- Even in a relatively homogenous set of G- samples, CFU counts in positive BCF vary by more than two orders of magnitude, and variation in G+ samples is even greater.
- For a large heterogenous set of gram-negative and gram-positive samples, the CFU concentration spanned three orders of magnitude.
- In both cases, the automated sample processing performed by the ASTar system provides robust and consistent inoculum preparation for AST.

References

1. ISO 20776–1
2. CLSI M07–A10

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