Accurate inoculum preparation for AST from various samples

Eva Hell, Lotta Levén, Ida Niklasson, Harer Osman, Camilla Russell, Jenny Göransson, Jan Grawé, Charlotta Göransson, Markus Klintstedt, and Mats Gullberg
Q-linea, Uppsala, Sweden

Q-linea
+46 18 444 36 10
contact@qlinea.com

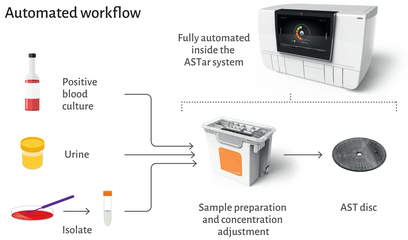
Introduction

Accurate broth microdilution (BMD)-based antimicrobial susceptibility testing (AST) requires correct inoculum (1). Today manual turbidity measurement is used for isolates, but for inoculum preparation direct from clinical samples, fewer alternatives exist. Here we present a rapid, automated process for preparing viable bacteria directly not only from positive blood cultures (as shown earlier [2]) but also urine or isolates. We also present performance metrics for automatic inoculum preparations for AST in a single sample preparation cartridge.

Key functions of the automated 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
- Time lapse microscopy of bacteria in broth for MIC determination

Automated workflow



Semi-automated workflow

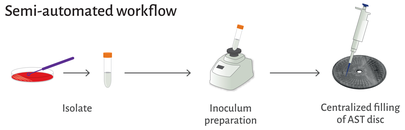


Fig 1. Simple and rapid automated or semi-automated workflows for AST from different specimen types.

Results

In a test set of gram-negative (G-) and -positive (G+) organisms, the spread of pathogen concentration in positive BCFs spanned between about two (G- organisms) and over three (G+ organisms) orders of magnitude. Not more than 82% (G-) and 39% (G+) of the samples achieved the EUCAST (ISO) inoculum with a fixed dilution strategy, while automated sample preparation delivered an inoculum placing 96% of G- and 86% of G+ samples within the EUCAST inoculum (Figure 2). The ASTar™ performance of a larger and diverse set of G+ and G- organisms using a single standard curve for both G+ and G- are shown in Figure 3.

The AST system reliably measures biomass down to an inoculum of 5×10^3 CFU/mL, relevant for processed clinical urine samples. In the example of Figure 4, at five hours the same MIC was obtained regardless of inoculum.

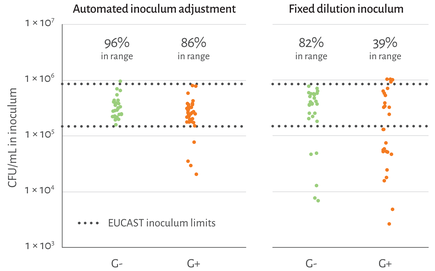


Fig 2. Comparison between inoculum concentrations achieved using ASTar automated sample processing compared with what would have been achieved using fixed dilution for gram-negative (G-) and gram-positive (G+) organisms.

However, depending on the combinations of bacteria and antimicrobials the inoculum will affect the MIC (4). A set of G+ and G- bacteria in blood culture, urine or isolates were tested and a subset of the data is shown in Figure 5. For all three tested matrices, the resulting five-hour MIC compared well to reference Sensititre™ BMD.

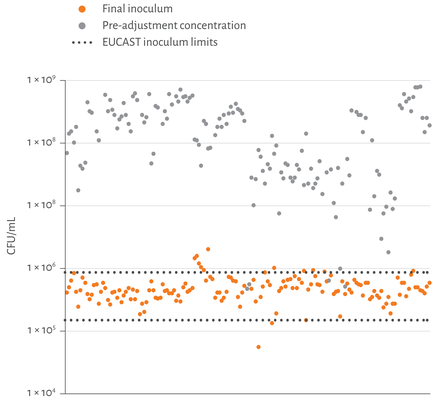


Fig 3. Concentration (CFU/mL) of recovered and resuspended pathogens from a large set of positive BCF inoculated with gram-negative and gram-positive organisms sampled approx. 0–8 h after positivity compared with automated prepared inoculum from the same samples. Dashed lines denote limits of EUCAST 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.

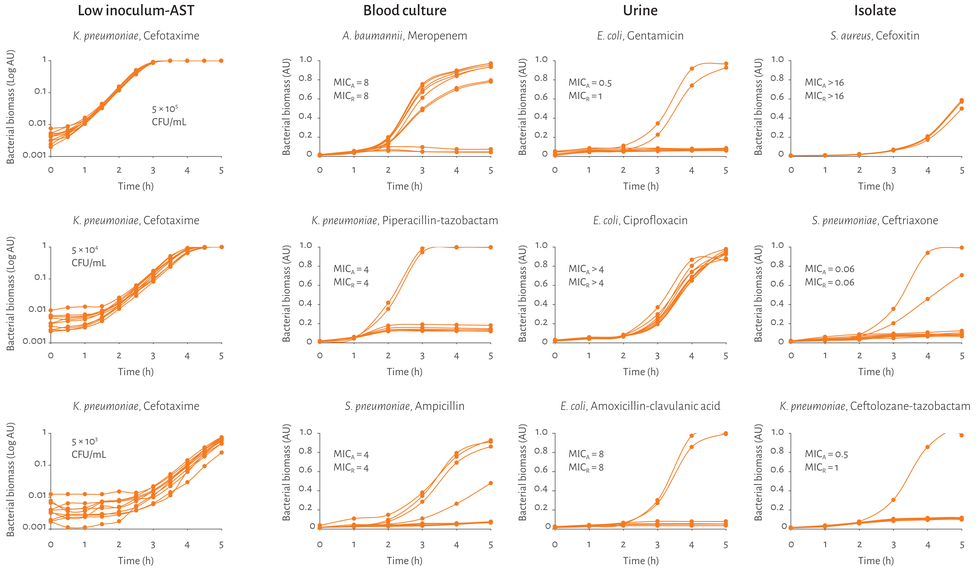


Fig 4. Inoculum between 5×10^3 – 5×10^5 CFU/mL can be measured.

Fig 5. Biomass plotted over time for different specimen types and for a selected number of bacteria and antimicrobials combinations, each curve representing one concentration (mg/L). For each AST, ASTar-MIC (MICA) and reference BMD MIC (MICR) are noted.

Materials and methods

Clinical isolates were inoculated into blood culture flasks with 9 mL blood and grown until signaled positive, or into urine. A 0.5 mL aliquot of positive blood culture or 10 mL of spiked urine (10^5 CFU/mL) was directly processed in the ASTar system, with inoculum adjustment and dilution in cation-adjusted Mueller-Hinton broth. The inoculum target is $2–8 \times 10^5$ CFU/mL for blood samples and $2 \times 10^4 – 2 \times 10^5$ CFU/mL for urine samples. Viable count (VC) was performed on both sample types; directly from unprocessed raw samples as well as after concentration adjustment. For comparison with performance of fixed dilution, a learning set of positive BCF was used to calculate a single dilution ratio maximizing the number of samples achieving EUCAST inoculum (5×10^5 CFU/mL ± 60%).

For the AST step, biomass was measured on 5–10 two-fold dilutions of each antimicrobial using time-lapse microscopy and subsequently translated into minimum inhibitory concentration (MIC) values by proprietary algorithms. Reference MIC was obtained with Sensititre™ BMD. In addition, system performance was investigated at inoculum relevant for clinical urine samples (3).

References

1. ISO 20776–1, CLSI M07–A10
2. Posters P1801 and O0400, ECCMID 2018, Posters 206 and 218 ASM 2018
3. Bonkat, G. and Pickard, R. *et al.* EAU Guidelines on Urological Infections. 2018, ISBN 978-94-92671-01-1
4. Smith, K.P. and Kirby J.E. Antimicrob Agents Chemother. 2018, 62 (8), e00433-18.

Conclusions

- The automated AST workflow generates five-hour MIC results from blood culture, urine and isolates that correlate well with Sensititre BMD reference.
- 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.
- In both cases, the automated sample processing performed by the ASTar system provides robust and consistent inoculum preparation for AST.

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