# Antimicrobial Susceptibility Test System Delivering Phenotypic MICs in Hours from Positive Blood Cultures for Fastidious- and Non-Fastidious Pathogens

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## Introduction

Rapid identification (ID) and antimicrobial susceptibility testing (AST) direct from positive blood cultures (BCF) are important in helping provide septic patients with earlier and better optimized antimicrobial treatment. The great variety of different pathogens isolated from positive BCFs puts high demands on the identification technology, and around 10% of all pathogens isolated from BCFs are so-called fastidious bacteria (require growth supplements). Currently, no 'direct AST from positive blood culture' system for fastidious bacteria is available.

We have evaluated the ability to perform AST directly on a positive BCF for both fastidious and non-fastidious pathogens and report the MIC after just six-hour's growth. Our method comprised automated sample preparation and image-based antimicrobial susceptibility testing in both standard CAMHB as well as CAMHB with additives to support the growth of fastidious pathogens.

## Materials and methods

The AST results presented are obtained using a prototype system. Blood culture flasks spiked with blood from healthy individuals and clinical isolates were cultured in a Bactec 9050 cabinet until signaled positive. A 500 µl aliquot from each positive flask was subjected to automated sample preparation followed by inoculation into discrete concentrations of different antimicrobials at several dilution ranges. Time-lapse images were taken over 18 hours. MIC is determined as the lowest concentration of antibiotic inhibiting growth (Fig 1). The MIC determined by a proprietary automated algorithm from time-lapse images after six hours growth was compared to the MIC determined manually via visual observation after 18 hours growth. If the same isolate was used in several independent spike experiments, the median value of the 18-hour MICs from that strain was used as reference MIC.

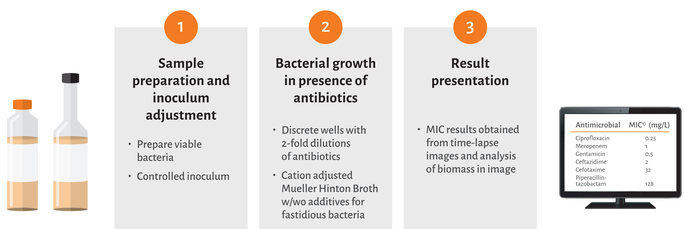


**Fig 1.** An aliquot from a positive blood culture is subjected to automated sample preparation and adjustment, transferred to a consumable with dried antibiotics for broth microdilution AST, and imaged with time-lapse imaging. The MIC is calculated from the images based on the lowest concentration of antibiotics inhibiting growth measured as total biomass in the image.

## Results

Both fastidious and non-fastidious bacteria can be analyzed using time-lapse microscopy (Fig 2). The MIC values obtained after six hours growth achieved an Overall Essential Agreement of more than 95% compared with MICs determined after 18-hour's growth (Table 1 and 2). Approximately 30% of the different bacteria-antibiotic combinations tested had a resistant or intermediate phenotype. To challenge the system, around 20% of all bacteria-antibiotic combinations had an MIC for that antibiotic on a clinical breakpoint. This explains why the Overall Categorical Agreement is lower than the Overall Essential Agreement obtained in the overall data set.

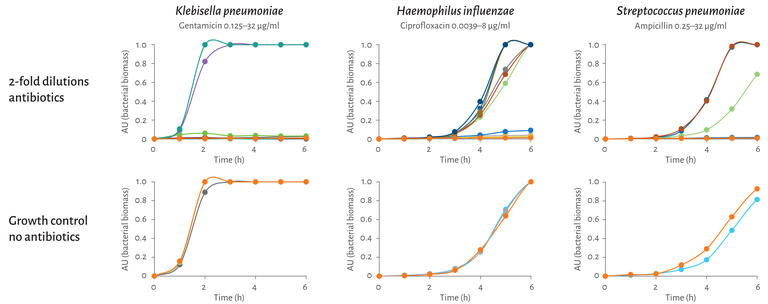


**Fig 2.** Biomass plotted over time for a selected number of bacteria and antibiotics. A growth / no growth assessment is made per concentration of each antibiotic to determine the MIC.

**Table 1.** Summary of Overall Essential Agreement (EA) and Overall Categorical Agreement (CA) for six-hour image-based AST data of fastidious pathogens isolated from blood cultures spiked with blood from healthy individuals and clinical isolates and grown to positivity. (total N = 124) mE = minor error, ME = major error, VME = very major error.

| | | | | No. of tests | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ***Streptococcus pneuomniae*** | **Total no. of tests** | **EA (%)** | **CA (%)** | **S** | **I** | **R** | **mE** | **ME** | **VME** |
| Ampicillin | 20 | 100% | 80% | 6 | | 14 | 4 | | |
| Vancomycin | 17 | 100% | 100% | 17 | | | | | |
| Cefotaxime | 19 | 100% | 89% | 6 | 7 | 6 | 2 | | |
| ***Streptococcus mitis*** | | | | | | | | | |
| Ampicillin | 11 | 90% | 100% | 11 | | | | | |
| Vancomycin | 12 | 100% | 100% | 12 | | | | | |
| Benzylpenicillin | 12 | 100% | 73% | 6 | 6 | | 2 | | |
| ***Haemophilus influenzae*** | | | | | | | | | |
| Cefotaxime | 11 | 91% | 91% | 1 | | 10 | | | 1 |
| Ciprofloxacin | 11 | 100% | 100% | 8 | | 3 | | | |
| Meropenem | 11 | 82% | 100% | 11 | | | | | |

**Table 2.** Summary of Overall Essential Agreement (EA) and Overall Categorical Agreement (CA) for six-hour image-based AST data of pathogens isolated from blood cultures spiked with blood from healthy individuals and clinical isolates and grown to positivity (total N = 672) mE = minor error, ME = major error, VME = very major error.

| | | | | No. of tests | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Antimicrobial agent** | **Total no. of tests** | **EA (%)** | **CA (%)** | **S** | **I** | **R** | **mE** | **ME** | **VME** |
| Amoxicillin-clavulanic acid | 27 | 96% | 100% | 9 | | 18 | | | |
| Ampicillin | 30 | 97% | 100% | 27 | | 3 | | | |
| Benzylpenicillin | 6 | 100% | 100% | | | 6 | | | |
| Cefotaxime | 48 | 100% | 81% | 22 | 3 | 23 | 9 | | |
| Cefoxitin | 10 | 90% | 80% | 6 | | 4 | | | 2 |
| Ceftazidime | 66 | 95% | 86% | 43 | 6 | 17 | 9 | | |
| Ceftolozane-tazobactam | 16 | 75% | 100% | 12 | | 4 | | | |
| Ciprofloxacin | 81 | 99% | 96% | 43 | 3 | 35 | 3 | | |
| Colistin | 38 | 92% | 100% | 26 | | 12 | | | |
| Daptomycin | 11 | 100% | 100% | 8 | | 3 | | | |
| Gentamicin | 64 | 98% | 95% | 39 | 3 | 22 | 3 | | |
| Levofloxacin | 14 | 100% | 100% | 3 | | 11 | | | |
| Meropenem | 81 | 91% | 100% | 78 | 3 | | | | |
| Piperacillin-tazobactam | 67 | 94% | 84% | 61 | 6 | | 6 | 5 | |
| Tetracycline | 14 | 100% | 93% | 4 | 3 | 7 | 1 | | |
| Tobramycin | 41 | 93% | 88% | 35 | | 6 | 5 | | |
| Trimethoprim/sulfamethoxazole | 11 | 100% | 100% | 8 | | 3 | | | |
| Vancomycin | 47 | 100% | 94% | 41 | | 6 | | | 3 |
| **Total** | **672** | **95.7%** | **93.1%** | **465** | **27** | **180** | **36** | **5** | **5** |

**Gram negative:** *P. aeruginosa, E. coli, A. baumanni, P. mirabilis, K. pneumoniae, E. cloacae*
**Gram positive:** *S. aureus, E. faecalis, S. epidermidis*

Overall EA: 95.7%
CA: 93.1%

## Conclusions

This study shows that it is possible to determine MICs for fastidious pathogens isolated directly from a positive BCF using image-based AST. Time-lapse images show that a common algorithm can detect differential growth within six hours for a large variety of bacteria / antibiotic combinations. MIC was determined by extracting biomass information in each concentration of antibiotic. The imaging is compatible with the additives that support the growth of fastidious organisms and the variable effects on bacterial morphology caused by a broad range of different antibiotics.

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