# P 165 Proposed breakpoints for rapid antimicrobial susceptibility testing with disk diffusion tests direct from positive blood cultures for *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pneumoniae*


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## Introduction

With increasing antimicrobial resistance, rapid antibiotic susceptibility testing (RAST) becomes important, especially in patients with blood stream infections. We have previously shown good results with early reading after 6 and 8 hours incubation (Poster 850, ECCMID 2016) with a standard McFarland 0.5 inoculum from an overnight culture.

## Objectives

The objectives of this study was to i) evaluate RAST with disk diffusion directly from positive blood culture bottles and ii) to shorten the time even further compared to using a McFarland 0.5 inoculum.

## Methods

Blood culture bottles, BACTEC™ Plus Aerobic (BD), were inoculated with pure cultures of *E. coli* (n=61), *K. pneumoniae* (n=52), *S. aureus* (n=54) and *S. pneumoniae* (n=56) together with 5 mL defibrinated horse blood and incubated in the BACTEC FX (BD). A high proportion of isolates with varying levels of non susceptibility were intentionally included. Disk diffusion was performed with EUCAST methodology, but with modified inoculum and incubation time. Approximately 150 µL (3 drops from a syringe) from the positive bottle was added to each agar plate and evenly distributed. Clinically relevant antibiotics for sepsis treatment were included (**Table 1**). Inhibition zones were read after 4, 6 and 8 hours incubation, on the same re-incubated plate. Mueller-Hinton (MH) agar from two manufacturers (BBL/BD and Oxoid/Thermo Fisher Scientific) was used. Broth microdilution (BMD) according to ISO 20776-1 (EUCAST MH-F broth for *S. pneumoniae*) was used as a reference and MICs were interpreted according to EUCAST breakpoints v 7.1, 2017.


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## Results

The time to result (defined as when a zone can be accurately read) was shortened with RAST directly from positive blood culture bottles compared with using a McFarland 0.5 inoculum. It was possible to read the majority of the zones after 6 and 8 hours, but after 4 hours the numbers varied between species (see **Table 1**).The correlation with BMD was good for most organism-agent combinations and improved with longer incubation time. Technical difficulties were found for *E. coli* and *K. pneumoniae* vs. piperacillin-tazobactam and *S. aureus* and *S. pneumoniae* vs. clindamycin. Tentative breakpoints were set for 25 organism-agent combination (**Table 1**). It was necessary to set separate breakpoints for 4, 6 and 8 hours incubation, due to improved separation with longer incubation. Zone diameter breakpoints were set to avoid false susceptibility, whereas occasional false resistance was accepted (example in **Table 2**). The area between the S and R breakpoints was defined as the "Area of technical uncertainty" (ATU, **Figure 1**) and no AST report was given for these results.

**Table 1. a) Proposed breakpoints for disk diffusion from positive blood cultures with reading after 4, 6 and/or 8 hours incubation b) compared with EUCAST breakpoints for standard incubation time from the EUCAST Clinical Breakpoint Tables v. 7.1.**
MIC-zone diameter correlation graphs for *E. coli* vs. cefotaxime (red box below) are shown in Figure 1.

**a)**

| *Escherichia coli* Antimicrobial agent and disk content | Proposed breakpoints (mm) 4h S≥ | 4h ATU* | 4h R< | 6h S≥ | 6h ATU* | 6h R< | 8h S≥ | 8h ATU* | 8h R< | Zones not possible to read (%) 4h | 6h | 8h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Piperacillin-tazobactam 30-6 µg | - | ≥12 | 12 | - | ≥12 | 12 | - | ≥12 | 12 | 15 | 0 | 0 |
| Cefotaxime 5 µg | 15 | 13-14 | 13 | 18 | 14-17 | 14 | 18 | 14-17 | 14 | 12 | 0 | 0 |
| Ceftazidime 10 µg | 15 | 12-14 | 12 | 16 | 14-15 | 14 | 17 | 14-16 | 14 | 14 | 0 | 0 |
| Meropenem 10 µg | 17 | 12-16 | 12 | 17 | 14-16 | 14 | 17 | 14-16 | 14 | 5 | 0 | 0 |
| Ciprofloxacin 5 µg | 16 | 13-15 | 13 | 19 | 15-18 | 15 | 20 | 15-19 | 15 | 8 | 0 | 0 |
| Gentamicin 10 µg | 14 | 12-13 | 12 | 14 | 12-13 | 12 | 14 | 12-13 | 12 | 2 | 0 | 0 |
| Tobramycin 10 µg | 14 | 12-13 | 12 | 15 | 12-14 | 12 | 15 | 12-14 | 12 | 2 | 0 | 0 |
| Amikacin 30 µg | 15 | 13-14 | 13 | 15 | 13-14 | 13 | 15 | 13-14 | 13 | 2 | 0 | 0 |

| *Klebsiella pneumoniae* Antimicrobial agent and disk content | Proposed breakpoints (mm) 4h S≥ | 4h ATU* | 4h R< | 6h S≥ | 6h ATU* | 6h R< | 8h S≥ | 8h ATU* | 8h R< | Zones not possible to read (%) 4h | 6h | 8h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Piperacillin-tazobactam 30-6 µg | - | ≥12 | 12 | - | ≥12 | 12 | - | ≥12 | 12 | 1 | 0 | 0 |
| Cefotaxime 5 µg | 15 | 12-14 | 12 | 18 | 14-17 | 14 | 18 | 14-17 | 14 | 2 | 0 | 0 |
| Ceftazidime 10 µg | 15 | 11-14 | 11 | 16 | 12-15 | 12 | 17 | 11-16 | 11 | 2 | 0 | 0 |
| Meropenem 10 µg | 15 | 12-14 | 12 | 17 | 13-16 | 13 | 17 | 13-16 | 13 | 4 | 0 | 0 |
| Ciprofloxacin 5 µg | 18 | 15-17 | 15 | 18 | 15-17 | 15 | 19 | 16-18 | 16 | 3 | 0 | 0 |
| Gentamicin 10 µg | 14 | 12-13 | 12 | 14 | 12-13 | 12 | 13 | 12 | 12 | 0 | 0 | 0 |
| Tobramycin 10 µg | 14 | 12-13 | 12 | 13 | 11-12 | 11 | 13 | 11-12 | 11 | 0 | 0 | 0 |
| Amikacin 30 µg | 15 | 13-14 | 13 | 14 | 11-13 | 11 | 13 | 10-12 | 12 | 0 | 0 | 0 |

| *Staphylococcus aureus* Antimicrobial agent and disk content | Proposed breakpoints (mm) 4h S≥ | 4h ATU* | 4h R< | 6h S≥ | 6h ATU* | 6h R< | 8h S≥ | 8h ATU* | 8h R< | Zones not possible to read (%) 4h | 6h | 8h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cefoxitin 30 µg screen for beta-lactam resistance | 17 | 15-16 | 15 | 19 | 17-18 | 17 | 20 | 18-19 | 18 | 33 | 9 | 9 |
| Norfloxacin 10 µg screen for fluoroquinolone resistance | 12 | <12 | - | 13 | <13 | - | 14 | <14 | - | 45 | 11 | 9 |
| Gentamicin 10 µg | 15 | 13-14 | 13 | 16 | 14-15 | 15 | 16 | 14-15 | 14 | 46 | 9 | 9 |
| Erythromycin 15 µg | 18 | 16-17 | 16 | 18 | 16-17 | 16 | 18 | 16-17 | 16 | 56 | 14 | 10 |
| Clindamycin 2 µg | - | - | - | - | - | - | - | - | - | 57 | 15 | 11 |

| *Streptococcus pneumoniae* Antimicrobial agent and disk content | Proposed breakpoints (mm) 4h S≥ | 4h ATU* | 4h R< | 6h S≥ | 6h ATU* | 6h R< | 8h S≥ | 8h ATU* | 8h R< | Zones not possible to read (%) 4h | 6h | 8h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Oxacillin 1 µg screen for beta-lactam resistance | 14 | <14 | - | 17 | <17 | - | 18 | <18 | - | 7 | 2 | 0 |
| Norfloxacin 10 µg screen for fluoroquinolone resistance | 10 | <10 | - | 11 | <11 | - | 11 | <11 | - | 21 | 2 | 0 |
| Erythromycin 15 µg | 19 | 17-18 | 17 | 19 | 17-18 | 17 | 19 | 17-18 | 17 | 19 | 2 | 0 |
| Clindamycin 2 µg | - | ≥14 | 14 | - | ≥14 | 14 | 18 | 14-17 | 14 | 18 | 2 | 1 |
| Trimethoprim-sulfamethoxazole 25 µg | 14 | 10-13 | 10 | 14 | 10-13 | 10 | 14 | 10-13 | 10 | 15 | 2 | 0 |

*ATU, Area of technical uncertainty

**b)**

| Enterobacteriaceae 16-20h S≥ | R< |
|---|---|
| | 20 | 17 |
| | 20 | 17 |
| | 22 | 19 |
| | 22 | 16 |
| | 26 | 24 |
| | 17 | 14 |
| | 17 | 14 |
| | 18 | 15 |

| Enterobacteriaceae 16-20h S≥ | R< |
|---|---|
| | 20 | 17 |
| | 20 | 17 |
| | 22 | 19 |
| | 22 | 16 |
| | 26 | 24 |
| | 17 | 14 |
| | 17 | 14 |
| | 18 | 15 |

| *S. aureus* 16-20h S≥ | R< |
|---|---|
| | 22 | 22 |
| | 17 | - |
| | 18 | 18 |
| | 21 | 18 |
| | 22 | 19 |

| *S. pneumoniae* 16-20h S≥ | R< |
|---|---|
| | 20 | - |
| | 11 | - |
| | 22 | 19 |
| | 19 | 19 |
| | 18 | 15 |

## Conclusions

Disk diffusion following direct inoculation of susceptibility plates from positive blood cultures with reading after 4, 6 and 8 hours incubation is possible if an "Area of Technical Uncertainty" (ATU) is introduced. Isolates with results within ATU after 4 or 6 hours incubation should be reincubated up to a total of 8 hours. Isolates with results within the ATU also after 8 hours incubation must be retested with standard methodology.

The method has also been evaluated for *Enterococcus faecalis*, *Enterococcus faecium*, *Pseudomonas aeruginosa* and *Haemophilus influenzae,* with promising results. Evaluation of the proposed method and breakpoints for clinical isolates at additional laboratories is in progress.


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**Table 2. Number of tests per incubation time with incorrect categorisation compared with reference BMD for *E. coli* vs. cefotaxime.**

| Isolate | MH Agar Manuf. | Incubation time 4h SIR | 4h zone (mm) | 6h SIR | 6h zone (mm) | 8h SIR | 8h zone (mm) | 16-20h SIR | 16-20h zone (mm) | Reference MIC |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Oxoid | ATU | 13 | R | 13 | R | 12 | I | 17 | 2 |
| 2 | Oxoid | ATU | 13 | R | 13 | U | 15 | I | 17 | 2 |
| 3 | Oxoid | R | 6 | S | 20 | S | 19 | S | 27 | 0,12 |
| 3 | BD/BBL | R | 6 | S | 19 | S | 21 | S | 27 | 0,12 |
| 4 | Oxoid | R | 6 | S | 19 | S | 20 | S | 29 | 0,25 |
| 4 | BD/BBL | R | 6 | S | 20 | S | 19 | S | 31 | 0,25 |
| 5 | BD/BBL | R | 10 | S | 19 | S | 21 | S | 34 | 0,12 |

**Figure 1. Inhibition zone distributions for *E. coli* vs. cefotaxime per incubation time, with corresponding MICs as coloured bars.**
The proposed ATU is shaded in grey and standard breakpoints are shown as dotted lines.