

# Disk diffusion, using EUCAST methodology, can be interpreted after 6 or 8 hours incubation.

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on behalf of the Eurostar Rapid Disk consortium\*

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## Objectives

The outcome of patients with septic shock is dependent on the rapid administration of appropriate therapy. In an era of increasing antimicrobial resistance rapid susceptibility testing is imperative. The Eurostar Rapid Disk project aims at developing a standardised rapid method for susceptibility testing using EUCAST clinical breakpoints.

## Materials and Methods

Well defined strains of *Escherichia coli* (n=45), *Klebsiella pneumoniae* (n=45), *Pseudomonas aeruginosa* (n=30) *Staphylococcus aureus* (n=55) and enterococci (n=28) were tested against up to 10 antibiotics by three laboratories on Mueller Hinton agar (Oxoid) according to the EUCAST disk diffusion methodology, with the exception of a shorter incubation time. Zone measurements were performed at 6 and/or 8h and at 18h incubation with different techniques. Växjö used manual reading with a slide gauge, Hvidovre a combination of manual reading and the benchtop Imaga (BD/Kiestra) and AMC used the fully automated reading system (BD/Kiestra).

The results of 6/8h reading was compared to the 18h results both quantitative and categorical ategorical agreement was evaluated :  
VME: Very Major Error (S→R),  
ME: Major error (R→S)  
mE: Minor Errors where all possible combinations including I category was included.

**Figure 1-3.** Three examples of the correlation between rapid AST (6 or 8h incubation) and EUCAST methodology (18h). The data from all three laboratories are included. The categorical agreement are presented per laboratory (VX=Växjö, HV=Hvidovre, AMC=Academic Medical Center). Note that for *K.pneumoniae* and piperacillin-tazobactam a substantial number of strains were ESBL-producing isolates.

\*The EuroStar Rapid Disk Project is a collaboration between Depts of Clinical Microbiology in Växjö (Sweden), Hvidovre (Denmark), Nijmegen and Amsterdam (The Netherlands), BD/Kiestra Lab automation and Thermo Fischer Scientidic (Oxoid).

The Aim of the project is to reduce the time to AST results using disk diffusion. The project is financed through a EuroStar grant.

## Conclusions

A method for rapid susceptibility testing (6 or 8h) was calibrated to EUCAST disk diffusion methodology and EUCAST clinical breakpoints.

MRSA (8h) and resistance to 3<sup>rd</sup> generation cephalosporins due to ESBLs in *E.coli* and *K.pneumoniae* (6h) were readily detected by all three laboratories.

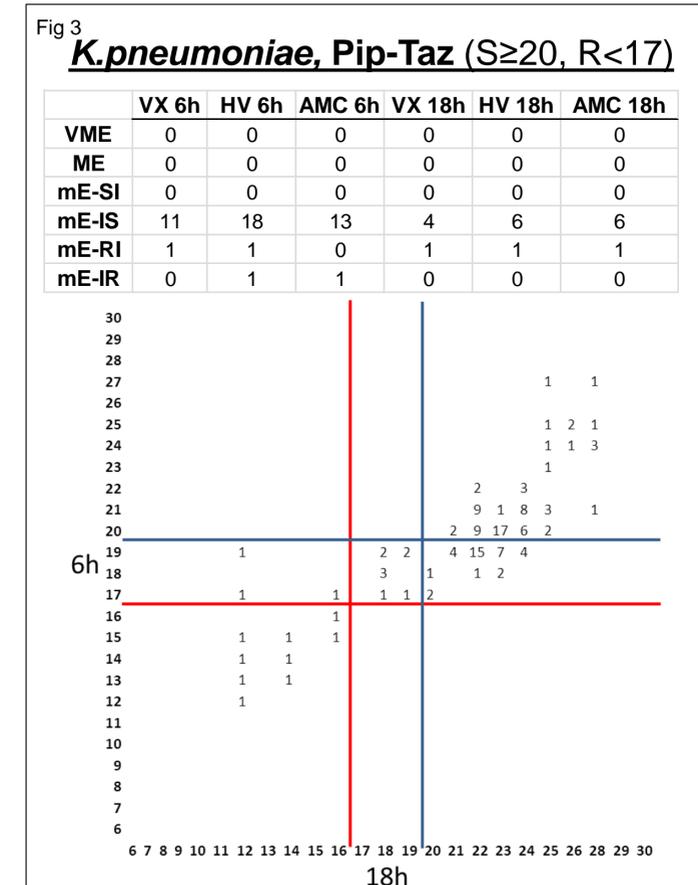
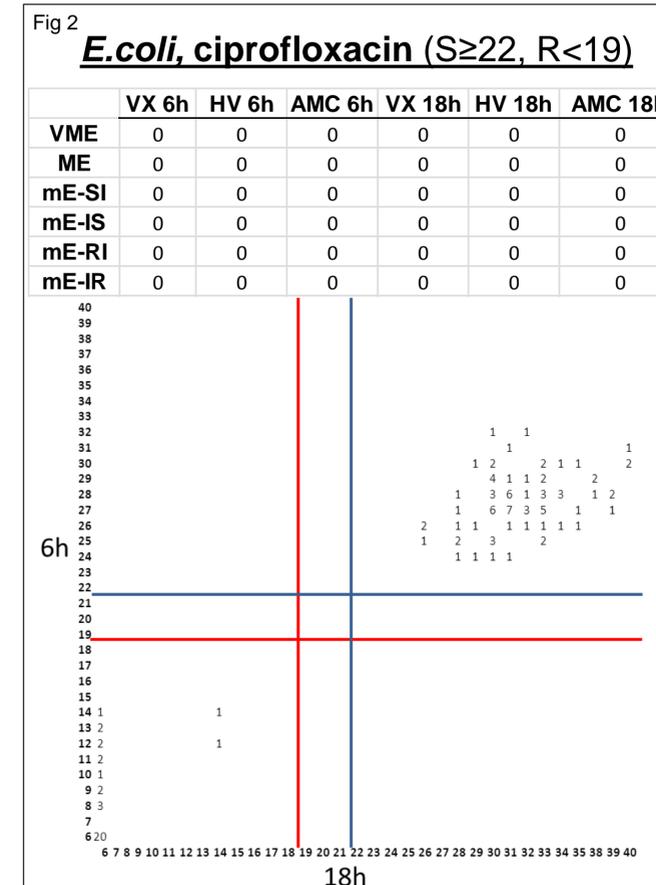
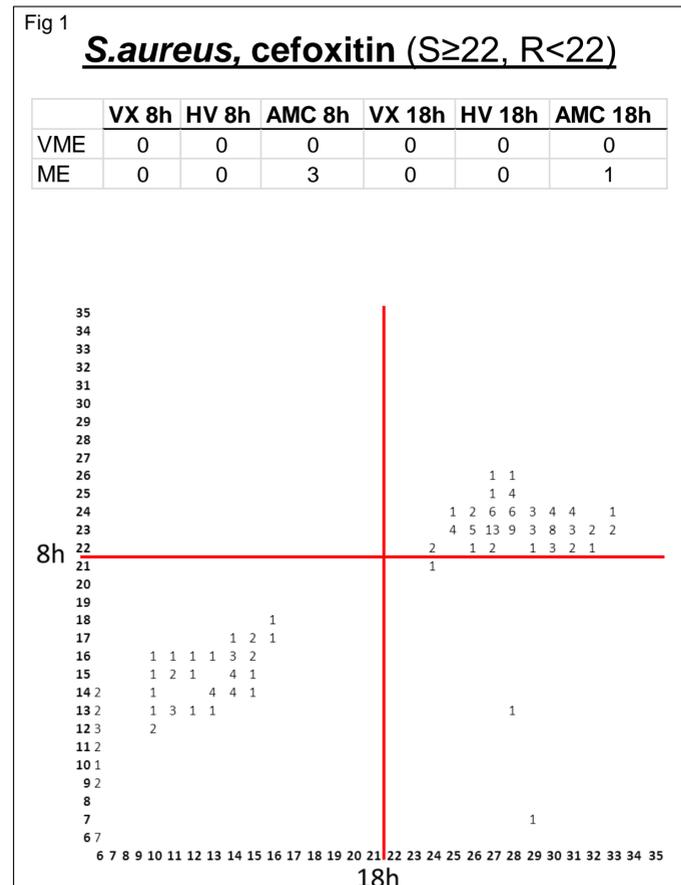
A panel of ciprofloxacin, cefotaxime, ceftazidime, meropenem and gentamicin in Enterobacteriaceae and ceftoxitin, erythromycin, clindamycin in *S.aureus* can be used for rapid susceptibility testing with disk diffusion.

## Results

Zones could be measured at 6 and 8h for >93% of the isolates. A longer incubation (18h) produced a bigger difference in zone size between wild type and non-wild type isolates than the short incubation. (Figure 1)

The rapid method allowed the use of EUCAST breakpoints for a reproducible SIR classification for all 32 drug/agent combinations except for the classification of piperacillin-tazobactam in *K.pneumoniae* and *P.aeruginosa*, ceftazidime in *P.aeruginosa* and the detection of vancomycin resistance and High Level Aminoglycoside Resistance (HLAR) detection in enterococci.

The three laboratories reported very similar data but the fully automated reading at 8h yielded a higher number of errors in *P.aeruginosa* and *S.aureus* (Figure 1). This was due to technical difficulties related to the camera when reading weak growth.



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