

**P1797 EUCAST rapid antimicrobial susceptibility testing directly from blood cultures: validating results using blood culture bottles from three different manufacturers.**

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**Background:** EUCAST is preparing to publish a standardised method for rapid antimicrobial susceptibility testing (RAST) using disk diffusion directly from positive blood culture (BC) bottles with reading after 4, 6 and 8 hours incubation. The proposed breakpoints are based on data from BC bottles from the Becton Dickinson (BD) BACTEC™. The objective of this study was to compare results for RAST using BC bottles from BD, bioMérieux and ThermoScientific.

**Materials/methods:** Five clinical isolates each of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Enterococcus faecium* and six relevant quality control strains (in triplicate) were tested. Four BC bottles from three manufacturers (Table 1) were inoculated with equal volumes of the same bacterial suspension. Five millilitres of defibrinated horse blood was added to each bottle followed by incubation in air at 35°C while tilting for 15-21h. After incubation, viable counts and disk diffusion according to EUCAST RAST methodology were performed from each bottle. Inhibition zones were read after 4, 6 and 8 hours incubation and interpreted using specific RAST breakpoints. Broth microdilution (BMD) according to ISO 20776-1 (EUCAST MH-F broth for *S. pneumoniae* and *H. influenzae*) was used as reference and MICs were interpreted according to EUCAST breakpoints v. 7.1 (2017).

**Results:** Only small differences in viable counts and zone diameters were observed between the BC bottles. In general, higher viable counts were related to smaller inhibition zones, with the smallest zones observed for BD bottles. *S. pneumoniae* generally grew poorer in bioMérieux SA and to some extent also in ThermoScientific bottles. The agreement with reference BMD for each bottle type is shown in Table 1. Three false susceptible results occurred for the ThermoScientific bottle (*S. pneumoniae* vs. erythromycin and clindamycin).

**Conclusions:** The results from this study imply that there are only minor differences in viable counts and zone sizes between BC bottles from different manufacturers. However, this was when bottles were spiked with equal amounts of bacteria. Each BC system needs to be individual investigated to confirm that EUCAST suggested RAST breakpoints based on the BD BACTEC system can be used also in other systems.

Table 1: Agreement of RAST methodology with reference BMD. For each bottle type and incubation time, results are presented as: the number of minor errors<sup>A</sup>/false resistant/false susceptible/correct categorised results. The number of results in the area of technical uncertainty (ATU)<sup>B</sup> is shown in parenthesis.

Incubation time Bottle type	Clinical isolates			Quality control strains		
	4h 5 species <sup>C</sup> , 145 readings	6h 8 species <sup>D</sup> , 225 readings	8h 8 species <sup>D</sup> , 225 readings	4h 4 species <sup>E</sup> , 66 reading	6h 6 species <sup>F</sup> , 102 readings	8h 6 species <sup>F</sup> , 102 readings
BioMérieux BACTEC™ Plus Aerobic	1/3/0/106 (31)	3/0/0/149 (37)	4/0/0/178 (40)	0/0/0/45 (16)	0/0/0/80 (13)	0/0/0/91 (11)
BioMérieux BacT/ALERT® FA Plus	4/2/0/100 (28)	4/0/0/136 (40)	5/1/0/180 (33)	0/0/0/46 (10)	0/0/0/75 (13)	0/0/0/90 (11)
BioMérieux BacT/ALERT® SA	1/3/0/97 (24)	3/0/0/133 (33)	3/0/0/174 (31)	0/0/0/45 (6)	0/0/0/81 (11)	0/0/0/82 (10)
ThermoScientific VersaTREK™ REDOX™	0/3/0/93 (27)	4/0/1/129 (35)	4/0/2/179 (38)	0/0/0/43 (8)	0/0/0/73 (10)	0/0/0/88 (10)

A: minor errors = intermediate isolates categorised as susceptible or resistant with RAST methodology.

B: Used to avoid reporting false susceptible or false resistant results. These results should be read after further incubation or retested with standard method.

C: *E. coli*, *K. pneumoniae*, *S. aureus*, *S. pneumoniae* and *E. faecalis*

D: *E. coli*, *K. pneumoniae*, *Ps. aeruginosa*, *H. influenzae*, *S. aureus*, *S. pneumoniae*, *E. faecalis* and *E. faecium*

E: *E. coli*, *S. aureus*, *S. pneumoniae* and *E. faecalis*

F: *E. coli*, *Ps. aeruginosa*, *H. influenzae*, *S. aureus*, *S. pneumoniae* and *E. faecalis*