Guidance document on broth microdilution testing of cefiderocol

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Introduction
Cefiderocol is a siderophore cephalosporin with activity against aerobic Gram-negative bacteria. It is approved for the treatment of aerobic Gram-negative infections in adults with limited therapeutic options. EUCAST has determined clinical breakpoints (MIC and zone diameter) for *Enterobacterales* and *Pseudomonas aeruginosa*. Cefiderocol exhibits pronounced activity against *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* but there was insufficient clinical evidence to determine breakpoints.

Cefiderocol shows activity against many isolates with high MIC values for and resistance to other beta-lactam agents, both cephalosporins and carbapenems. However, resistance to cefiderocol exists and these organisms can be detected with antimicrobial susceptibility testing (AST) performed with MIC or disk diffusion AST.

Disk diffusion is performed with a cefiderocol 30 µg disk according to EUCAST standard recommendations for non-fastidious organisms, see EUCAST Disk Diffusion Manual.

Broth microdilution testing methodology for cefiderocol
Cefiderocol requires low iron levels for optimal activity. Broth microdilution MIC determination must be performed in iron-depleted Mueller-Hinton broth. The iron concentration of liquid medium affects reproducibility and therefore should be strictly defined. Chelation is used for the iron depletion, and this procedure also removes other cations (i.e. calcium, magnesium and zinc). Following this process, cations are added back to concentrations of calcium 20–25 mg/L, magnesium 10–12.5 mg/L, and zinc 0.5–1.0 mg/L. The procedure necessary to produce the broth is described in detail by Hackel *et al.* [1].

The MIC of cefiderocol is read as the first well in which the reduction of growth corresponds to a button of <1 mm or is replaced by the presence of light haze/faint turbidity. The positive control should show strong growth in the form of a button of >2 mm or heavy turbidity. **See pictures with reading examples on the next page.**

References
1. Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF.
Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth.
Diagn Microbiol Infect Dis. 2019 Aug;94(4):321-325.

Examples of reading cefiderocol MIC endpoints

The MIC of cefiderocol is read as the first well in which a reduction of growth corresponding to a button of <1 mm or the presence of light haze/faint turbidity is observed (red ring). The positive control should show strong growth (button of >2 mm or heavy turbidity). Cefiderocol concentrations increase two-fold serially from column 1 (X mg/L) to column 11 (Y mg/L) wells.