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EUCAST susceptibility testing of APX001A: impact of choice of microtitre plate type, dilution method and plate storage

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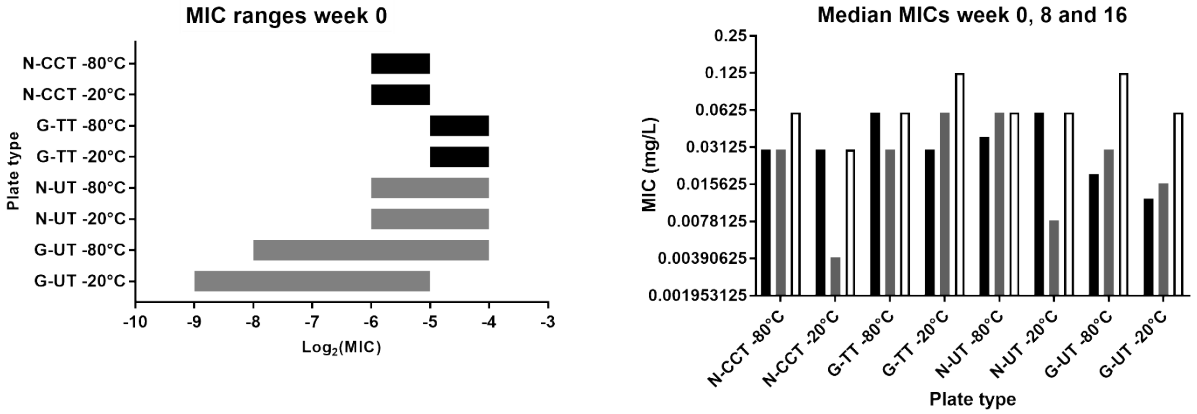
Background: MIC variation has been linked to choice of microtitre plate in the case of caspofungin suggesting that standardisation of plate type may be important for reducing interlaboratory variability. Before generating multicentre EUCAST MIC data for the new antifungal compound APX001A for ECOFF and breakpoint setting, we wished to examine if this is also the case for APX001A. Also, we wished to determine if plate storage time and freezing temperature affect MICs.

Material/methods: The APX001A MICs against four *Cryptococcus neoformans* isolates was determined using untreated and cell-culture/tissue-treated plates from Nunc and Greiner. Plates were prepared using serial dilutions (concentration range 1-0.001 mg/L) in double concentrated EUCAST medium with 1% DMSO. Half the plates were stored at –20°C and the other half at –80°C. MICs were determined after 48h's incubation and repeated after 8 and 16 weeks for three of the isolates giving 80 MICs in total.

Results: In the initial testing (<7 days storage), a larger variation in MIC was observed for the untreated plates compared to the cell-culture/tissue-treated plates (figure 1a). For the Nunc untreated and cell-culture treated plates, the MIC ranges spanned three (0.016-0.06 mg/L) and two (0.016-0.03 mg/L) dilutions, respectively, with no difference between short-term freezing temperatures. Similarly, for the Greiner plates, plate type affected performance as the MICs spanned five dilutions using untreated plates (0.002-0.03 mg/L and 0.004-0.06 mg/L frozen at –20°C and –80°C, respectively), but a two dilution MIC range (0.03-0.06 mg/L) for the tissue-treated plates (–20°C and –80°C). Repeating the MIC determinations for three of the isolates after 8 and 16 weeks of freezing showed the most stable MICs for the treated plates and when frozen at –80°C (figure 1b). At week 8, lower MICs were

seen for the Nunc plates frozen at −20°C compared to the main study but at week 16, all MICs were at level with the main study or over.

Conclusions: Although preliminary, our results suggest that type of plastic and microtitre plate storage time do affect antifungal susceptibility testing and that use of cell-culture or tissue-treated plates and storage of the plates at −80°C provide more reliable and consistent MICs. This should be confirmed in a multicentre study involving several species.



(a) (b)

Figure 1. MICs for the four tested microtitre plates and two freezing temperatures. **(a)** Log_2 transformation of the MICs at week 0 for each plate type (black: treated, grey: untreated plates) and **(b)** median MICs for week 0, 8 and 16 for each plate type and freezing temperature (Black: 0 weeks, grey: 8 weeks, white: 16 weeks). N-CCT: Nunc cell-culture treated, G-TT: Greiner tissue-treated, N-UT: Nunc untreated, G-UT: Greiner untreated plates.