EUCAST Susceptibility Testing of APX001A: Impact of Choice of Microtitre Plate Type and Plate Storage Temperature and Duration

Karin Meinike Jørgensen[1] and Maiken Cavling Arendrup[1,2,3]

[1]Unit of Mycology, Statens Serum Institut, Copenhagen, Denmark, [2]Dept. Clinical microbiology, Rigshospitalet, Copenhagen, Denmark, [3]Dept. of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

Background

MIC variation has been linked to choice of microtitre plate in the case of caspofungin (1,2) 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 was also the case for APX001A. Also, we wished to determine if plate storage time and freezing temperature affected MICs.

Material/methods

The APX001A MICs against four *Cryptococcus neoformans* isolates were determined using

1. Non-treated and cell-culture/tissue-treated plates from Nunc and Greiner.
2. EUCAST E.Def 7.3
3. APX001A concentration range 1-0.001 mg/L
4. Plate storage temperature: –20°C vs. –80°C
5. Plate storage time: 0, 8 and 16 weeks

Generating 80 MICs in total.

Results

Short term storage

Cell-culture/tissue-treated plates led to the most narrow MIC ranges for both plate manufacturer Nunc and Greiner. The temperature did not affect performance after short term freezing. MICs were in agreement within 3 dilutions across plate brand and short term freezing temperature (week 0, Figure 1a).

Non-treated plates were associated with wider MIC ranges and a greater MIC difference across freezing temperatures and plate manufacturer. The MICs covered a 6 dilution span across brand and short term freezing temperature.

Eight-16 weeks storage

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.

This confirms that more reproducible MICs are obtained using treated microtitre plates rather than non-treated.

Figures

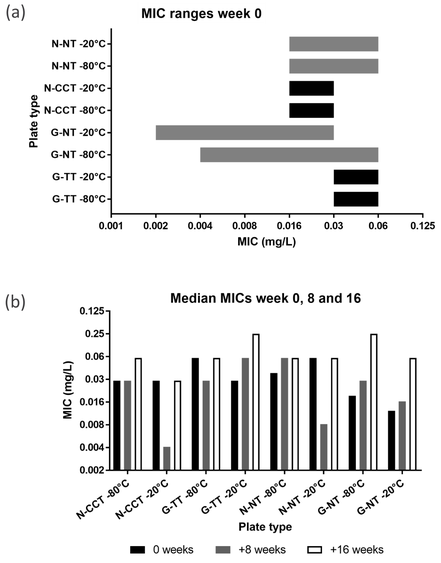

Figure 1. MICs for the four tested microtitre plates and two freezing temperatures. **(a)** MICs at week 0 for each plate type (black: treated, grey: non-treated plates) and **(b)** median MICs for week 0, 8 and 16 for each plate type and freezing temperature (Black: 0 weeks, grey: 8 weeks, white: 16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

Specific issues for non-treated plates

Air bubbles were observed in all non-treated plates, particularly for plates stored at –80°C, figure 2. This may influence MIC values determined by spectrophotometric reading as the air bubbles caused a false increase in OD (Figure 3).

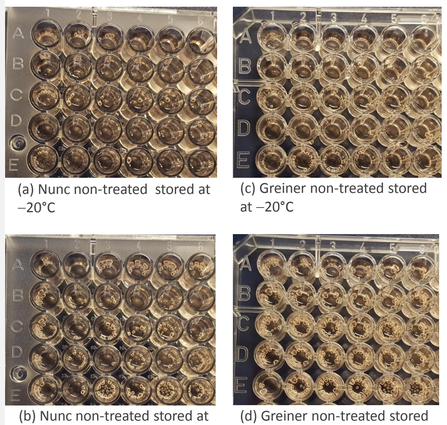

Figure 2. The number of air bubbles in the non-treated plates is higher in the plates frozen at –80°C compared to –20°C. Pictures from week 16 are shown. **(a)** Nunc non-treated stored at –20°C, **(b)** Nunc non-treated stored at –80°C, **(c)** Greiner non-treated stored at –20°C and **(d)** Greiner non-treated stored at –80°C.

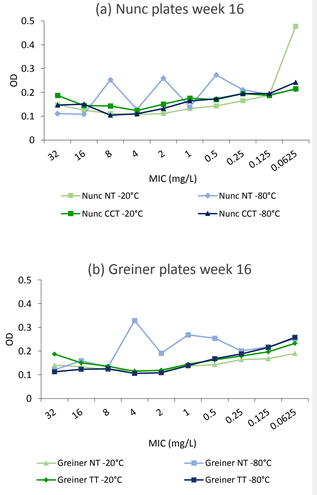

Figure 3. Growth curves of plates with air bubbles. The number of air bubbles in the non-treated plates is higher in the plates frozen at –80°C compared to –20°C. Pictures from week 16 are shown. **(a)** Nunc microtitre plates and **(b)** Greiner microtitre plates.

Conclusion

Although preliminary, our results suggest

- that type of plastic plate and storage time affect antifungal susceptibility testing results
- that more reliable and consistent MICs are obtained by the use of
  - ✓ cell-culture or tissue-treated plates
  - ✓ storage of the plates at –80°C

This should be confirmed in a multicentre study involving several species.

References

1. Espinel-Ingroff *et al.*, Antimicrob Agents Chemother. 2013 Dec; 57(12): 5836–5842.
2. Fothergill *et al.*, J Clin Microbiol. 2016 Mar; 54(3): 734–738

Acknowledgements

The study was financially supported by Amplyx Pharmaceuticals