# Comparative Evaluation of Micronaut-AM and CLSI broth micro-dilution method for antifungal susceptibility testing of *Aspergillus species* against four commonly used antifungals

**Ali Nuh, Newara Ramadan, Darius James-Armstrong and Silke Schelenz - Department of Microbiology, Laboratory Medicine, Royal Brompton and Harefield NHS foundation trust**

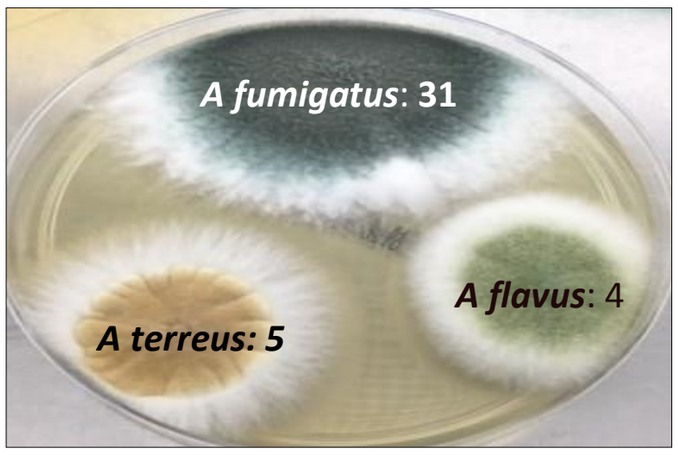
## Introduction

Both European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) recommend broth dilution method for filamentous fungi susceptibility testing.[1, 2] This method is labour-intensive and requires extra expertise and hence in the UK most clinical microbiology laboratories send filamentous fungi sensitivity to reference laboratories, which in turn increases turn-around-time and may have negative impact on the clinical management of the patients with invasive fungal diseases. This highlights the need for easily performed and reproducible susceptibility testing method for filamentous fungi that can be performed at routine microbiology laboratories.

In light of this, we evaluated Micronaut-AM plate (MN) (Merlin Diagnostika GmbH) for Aspergillus species, the most commonly isolated filamentous fungi. MN is a EUCAST-based commercial colorimetric micro-dilution method. It is relatively easy to perform and the use of metabolic dye, methylene blue, facilitates reading MIC end points. We validated it against CLSI micro-dilution method. It has been shown that MICs generated by EUCAST method correlate well with those of CLSI method.[3]

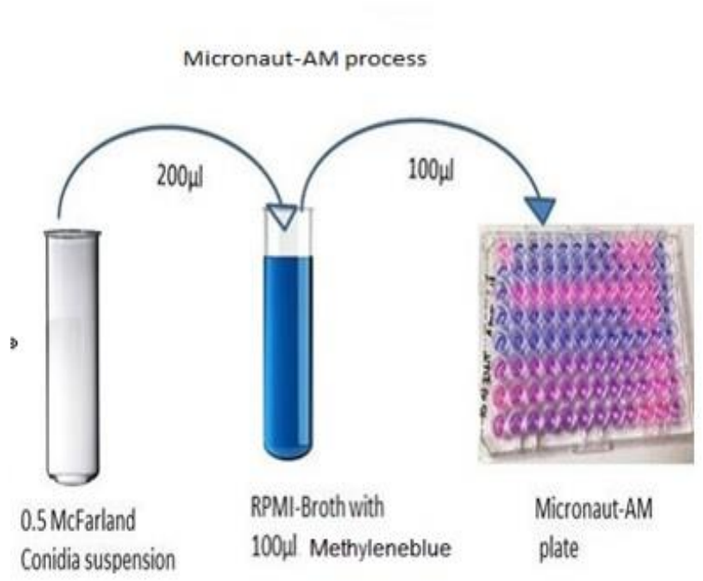
## Isolates tested

40 clinical isolates of Aspergillus species (31 *Aspergillus fumigatus*; 5 *Aspergillus terreus* & 4 *Aspergillus flavus*), 9 of them azole-resistant , from private collection of the Department of Medical Microbiology of Royal Brompton and Harefield NHS, foundation trust were tested.




## Methods

- Test was performed in accordance with the instruction of the manufacturer, except that a final conidial inoculum of 0.5-2 x$10^4$ was used.
- A fumigatus ATCC 204305 was used as a reference strain.
- MICs for Echinocandins were determined as first purple well after 24 hours incubation whereas amphotericin, voriconazole and itraconazole MICs were determined as first blue well with no growth after 48 hours incubation. The collection of Aspergillus species was referred to Bristol Mycology reference laboratory and tested by CLSI broth microdilution method. MICs generated by Micronaut were compared to CLSI results.




## Results

Micronaut-AM correlated well with CLSI broth method both in categorical results and in MIC values and there were very few discrepancies (table 1).
The level of agreement between Micronaut-AM (MN) generated MICs and the reference laboratory MICs results were very good. Overall, the level of agreement (within 2 log2 dilution) between MN and CLSI reference laboratory method was 100% for anidulafungin; 97% for amphotericin; 90% for voriconazole and 94% for itraconazole. In addition, Micronaut-AM reliably detected azole resistance.

| Antifungal | % categorical agreement | Discrepancy | | % agreement within 2log2 dilution |
|---|---|---|---|---|
| | | MD[1] | mD[2] | |
| Anidulafungin | 100 | | | 100 |
| Amphotericin | 98 | 1 [a] | | 98 |
| Voriconazole | 95 | 2 [b] | | 90 |
| Itraconazole | 98 | | 1 [c] | 94 |

Table1: % agreement between MN and CLSI

1. Major discrepancy (MD)
2.Minor discrepancy (mD)
a) An isolate of *A terreus* had MIC of 1 mg/L compared to MIC of 2mg/L in CLSI
b) 2 isolates of *A fumigatus* had MIC of 0.5mg/L and 1mg/L whereas CLSI MICs were 2mg/L and 4mg/L respectively.
c) An isolate of *A fumigatus* had MIC of 0.25mg/L compared to CLSI result of 1.5mg/L; when inoculated in VIP-check media, the isolate was negative for azole resistance.

## Conclusion

Micronaut-AM showed good agreement with reference broth micro-dilution method results for all antifungal agents tested and was able to detect azole resistance. This colorimetric method is very promising and with further evaluation appears to be a suitable alternative susceptibility testing method for *Aspergillus species*.

## References

1. *Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. CLSI document M38 Vol 37 No 15, Wayne PA: CLSI, 2017.*
2. *"EUCAST," 2017. Susceptibility testing of moulds[Online]. Available: http://www.eucast.org. [Accessed 01 March 2018].*
3. *Pfaller, M. et al. 2011. Comparison of the broth microdilution methods of the European Committee on Antimicrobial Susceptibility Testing and the Clinical and Laboratory Standards Institute for Testing Itraconazole, Posaconazole and Voriconazole against Aspergillus Isolates. Journal Of Clinical Microbiology, vol. 49, no. 3, pp. 1110-1112.*

Acknowledgments: Bioconnections for providing the kits and Peter Darby for helping with the poster design.