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# Evaluation and standardization of a screening method for the detection of azole resistant Aspergillus fumigatus

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**Background:** Azole resistance is emerging in clinical and environmental *A. fumigatus* isolates and increasing number of azole-resistant infections in azole exposed as well as azole-naïve patients are reported. Susceptibility testing of molds is difficult and not easily adopted in routine laboratories of clinical microbiology, yet it is of outmost importance to identify resistant isolates as early as possible. Delayed appropriate antifungal treatment is associated with increased morbidity and mortality. This will ensure patients to receive appropriate treatment while potentially resistant isolates undergo further investigation. We therefore evaluated the performance of an azole screening agar method for the rapid identification of potentially resistant *A. fumigatus* isolates.

**Material/methods:** A total of 79 *A. fumigatus* isolates, 40 wild-type and 39 azole-resistant with $TR_{34}$/L98H (n=9), $TR_{46}$/Y121F/T289A (n=10), G54 (n=10) and M220 (n=10) mutations, was tested blindly at three centres using 4-well screening plates, each well containing either voriconazole, posaconazole, itraconazole or a drug free control. Two batches of plates prepared commercially and in house, respectively were evaluated. 25 µl of 0.5 McFarland was added to each well and incubated for

48 h at 37°C. Growth was read by two independent observers giving a numerical score 0 (no visible growth), 1 (weak growth), 2 (growth but less than drug-free control) and 3 (growth as the drug free control). Absolute (no difference in scores) inter-observer and inter–plate agreement was calculated. The sensitivity and specificity in detecting isolates harboring *CYP51A* mutations was calculated for each reader, plate and centre for each azole individually and for the three azole wells in combination.

**Results:** The median (range among centres) inter-observer agreement was 88(80-88)% for itraconazole, 94(91-96)% for voriconazole, 94(88-95)% for posaconazole for commercially available plates and was 85(80-88)% for itraconazole, 94(93-96)% for voriconazole, 91(90-95)% for posaconazole for the in house plates. The median (range among centers) inter-plate agreement was 91(81-95)% for itraconazole, 95(91-99)% for voriconazole, 94(85-95)% for posaconazole. For all readers and plates, the sensitivity and the specificity of the 4-well plates with regard to identifying isolates with *CYP51A* mutant isolates was 95-100% across readers, plates, centres and *CYP51A* mutations.

**Conclusions:** The 4-well plate agar screening method for azole-resistant *A. fumigatus* isolates was highly reproducible and demonstrated high sensitivity and specificity in detecting isolates harboring *CYP51A* mutations among different observers, batches of plates, and centers. Importantly, performance was independent of resistance mechanism when at least one agar being positive was the criteria for resistance. The screening plate is well suited to be used in clinical laboratories to guide treatment of *A. fumigatus* infections.