



## VIPcheck™ validation

### Performance of VIPcheck™ to detect azole resistance in *A. fumigatus*

Selection of isolates. A total of ninety-six *A. fumigatus* isolates collected between 1994 and 2014 was used for the validation of the VIPcheck™ plates. Isolates were selected from the fungal culture collection of the Radboudumc, Nijmegen, the Netherlands. An additional four isolates did not sporulate at the time inoculations were performed and were therefore excluded. All but three isolates were cultured from patient specimens. Forty-five isolates were azole-sensitive based on EUCAST microbroth dilution reference method and clinical breakpoints. Fifty-five isolates showed an azole-resistant phenotype to one or more of the mold-active azoles itraconazole, posaconazole and/or voriconazole. For all isolates the full CYP51a gene and promoter region had been sequenced. As the primers for CYP51a are species specific, this confirms the *A. fumigatus* species identification.

Thirty-three isolates harbored a known resistance mechanism: TR<sub>34</sub>/L98H (11 isolates); TR<sub>46</sub>/Y121F/T289A (6 isolates); TR<sub>53</sub> (2 isolates); a substitution at codon G54 (G54W (3 isolates), G54E (2), G54R (1); M220 (M220V (1 isolate) M220R (1), M220I (1), M220K (1)); G448 (G448S (2 isolates)); P216 (P216L (1 isolate)) and G138 (G138C (1 isolate))[18, 20-27]. For eighteen isolates no Cyp51A-mediated azole resistance mechanism was found, indicating a yet unknown mechanism causing resistance.

VIPcheck™. For validation of the VIP check™ the 96 isolates were tested in duplicate. The first series was inoculated by an inexperienced researcher (observer 5) who had not used the VIPcheck™ before. The second series was inoculated by an experienced mycology technician (observer 1). Briefly, a wet sterile swab was used to collect conidia from an *A. fumigatus* colony to make a 0.5 McFarland suspension in 1 ml sterile water. A disposable pipette was used to add one drop of the suspension (25 µl) to each of the four wells. The lid was put on the VIPcheck™ and the plates were incubated for 48 h at 37 °C. The presence of growth was determined after 1 day and 2 days of incubation.

The plates were read by six observers. Observer 1 and 2 were experienced mycological technicians. Observer 3 and 4 were experienced bacteriological technicians without experience in mycology. Observer 5 and 6 were not trained in microbiology: observer 5 was a medical doctor and observer 6 was a first year intern. The VIPcheck™ plates were numbered 1 to 192. Observers were instructed to read the plates and document whether there was “distinct growth”, “minimal growth” or “no growth” for each individual well at both 24 h and 48 h.





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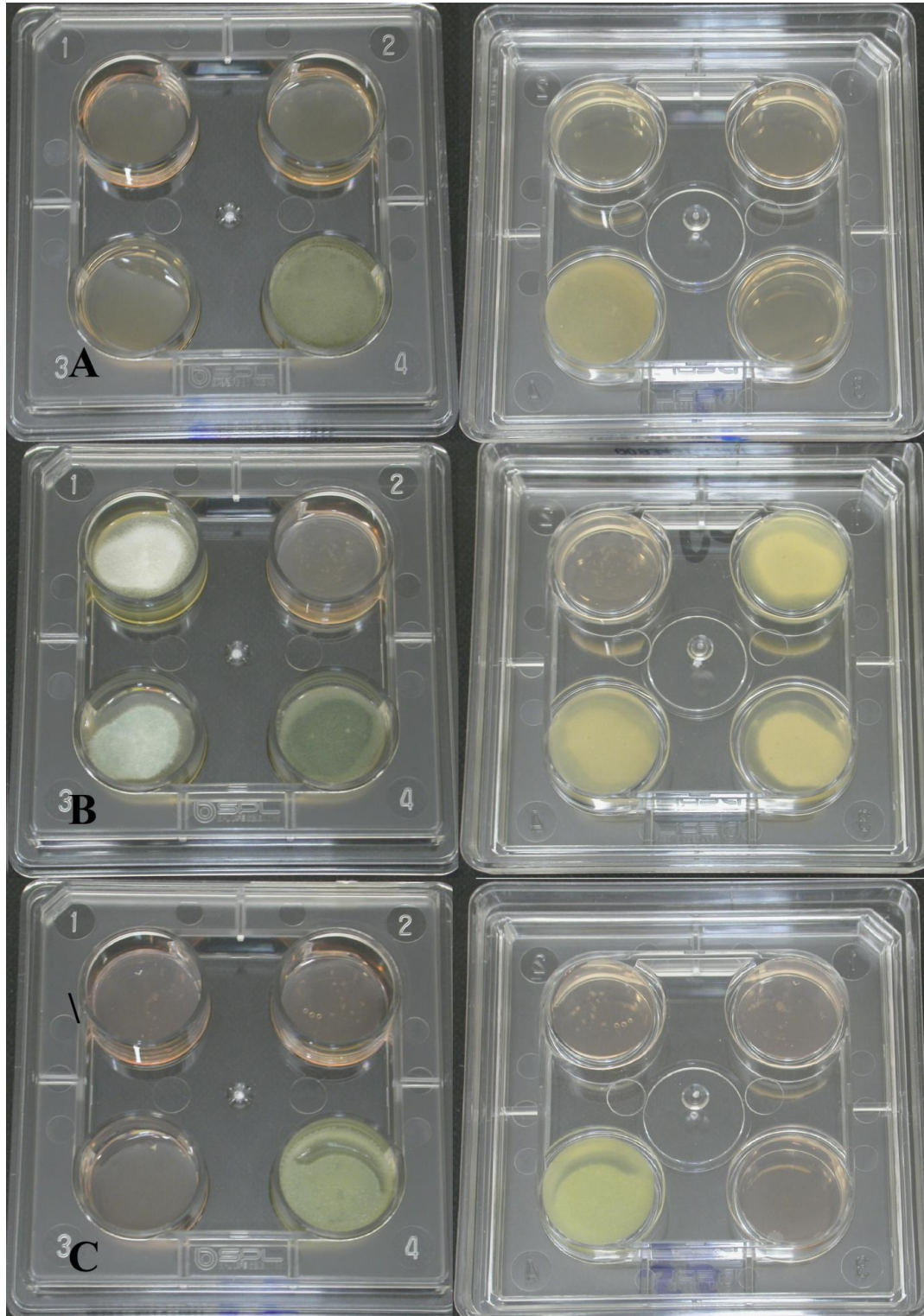
The quality control routine for a batch VIPcheck™ plates consist of the inoculation of five isolates with known phenotype: two susceptible isolates, one isolate resistant for ITC and POS and intermediate for voriconazole resulting in at most minimal growth in the well supplemented with voriconazole, one isolate resistant for both itraconazole, posaconazole and voriconazole, and one isolate with intermediate susceptibility for voriconazole resulting to minimal growth at most.

	Series	Sensitivity (95% CI)	Specificity (95% CI)	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Observer 1	1	0.94 (0.84-0.99)	1.00 (0.92-1.00)	1.00 (0.93-1.00)	0.94 (0.83-0.99)
	2	0.98 (0.90-1.00)	1.00 (0.92-1.00)	1.00 (0.93-1.00)	0.98 (0.88-1.00)
Observer 2	1	0.92 (0.81-0.98)	1.00 (0.92-1.00)	1.00 (0.92-1.00)	0.92 (0.80-0.98)
	2	0.96 (0.87-1.00)	1.00 (0.92-1.00)	1.00 (0.93-1.00)	0.96 (0.85-0.99)
Observer 3	1	1.00 (0.93-1.00)	0.96 (0.85-0.99)	0.96 (0.87-1.00)	1.00 (0.92-1.00)
	2	0.98 (0.90-1.00)	0.98 (0.88-1.00)	0.98 (0.90-1.00)	0.98 (0.88-1.00)
Observer 4	1	1.00 (0.93-1.00)	0.91 (0.79-0.98)	0.93 (0.82-0.98)	1.00 (0.91-1.00)
	2	1.00 (0.93-1.00)	0.98 (0.96-0.99)	0.84 (0.72-0.92)	1.00 (0.91-1.00)
Observer 5	1	1.00 (0.93-1.00)	0.98 (0.88-1.00)	0.98 (0.90-1.00)	1.00 (0.92-1.00)
	2	0.98 (0.90-1.00)	0.98 (0.88-1.00)	0.98 (0.90-1.00)	0.98 (0.88-1.00)
Observer 6	1	1.00 (0.93-1.00)	0.76 (0.60-0.87)	0.82 (0.70-0.91)	1.00 (0.90-1.00)
	2	1.00 (0.93-1.00)	0.67 (0.51-0.80)	0.77 (0.65-0.87)	1.00 (0.88-1.00)
Mean	all	0.98	0.93	0.95	0.98
Mean	technicians	0.97	0.98	0.98	0.97

**Table** - Performance of the VIPcheck™ after 48h incubations for 96 *Aspergillus fumigatus* isolates: 45 azole susceptible, 51 azole resistant in two replicates. Performance is reported for each observer and series individually. Mean all is the mean of all sensitivities, specificities and predictive values. Mean technicians is the mean performance for Observer 1 to 4.



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**VIPcheck™ plates** - A: front and back of a susceptible isolates after 48h incubation. B: front and back of a resistant isolates with distinct growth after 48h incubation. C: front and back of resistant isolates with minimal growth after 48h incubation.