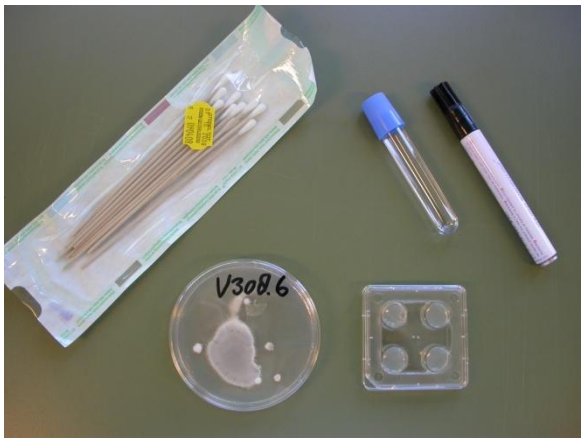
PROTOCOL VIPCHECK™

Aim: To screen *Aspergillus fumigatus* isolates for a resistant phenotype for itraconazole, voriconazole or posaconazole.

Procedure

Inoculation of agar-plates

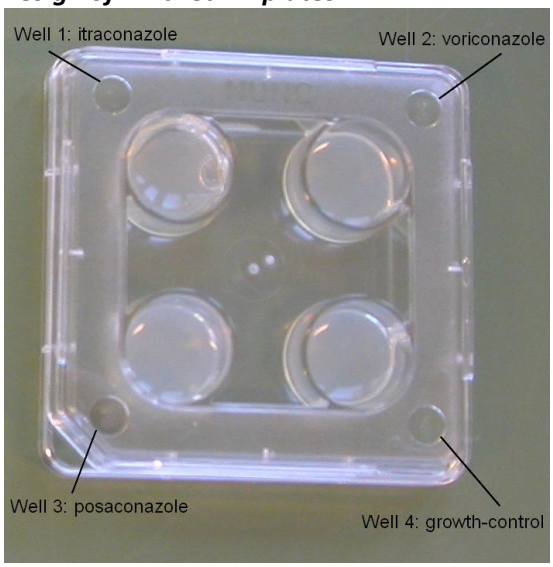
An *Aspergillus fumigatus* colony that is cultured from any clinical specimen can be screened. The colony needs to be sporulating. In most of the cases a sporulating culture is obtained after about 2 or 3 days of incubation.



Materials

- VIPcheck™ plate
- A sterile cotton swab stick
- Sterile water or AquaDest
- Sterile plastic pipette

Design of VIPcheck™ plates:



Well 1 contains itraconazole
Well 2 contains voriconazole
Well 3 contains posaconazole
Well 4 is the growth control

Step 1
Moisturise the swab stick with sterile water or Aqua Dest. Please do not moisturise too much, otherwise the Aspergillus on the agar-surface will be diluted.

Step 2
Brush past the Aspergilluscolony with the wet swab. Make sure some conidia stick to it, but do not push too hard.

Step 3
Make suspension of 0.5 McFarland (by eye) in the AquaDest or sterile water, and inoculate all wells with a single drop (about 25µl) of the suspension after removing the lid. Replace the lid after inoculation.

Step 4
Incubate the VIPcheck™ plate at 37 °C, ± 2 °C.

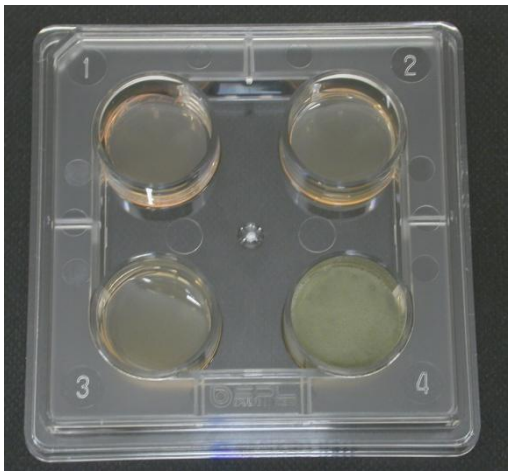
Step 5
Assess the ability to grow after 24 and 48 hours, in all four wells separately.

Any growth on wells 1, 2 or 3 indicates the possibility of an azole-resistant isolate and should prompt MIC testing of the isolate.

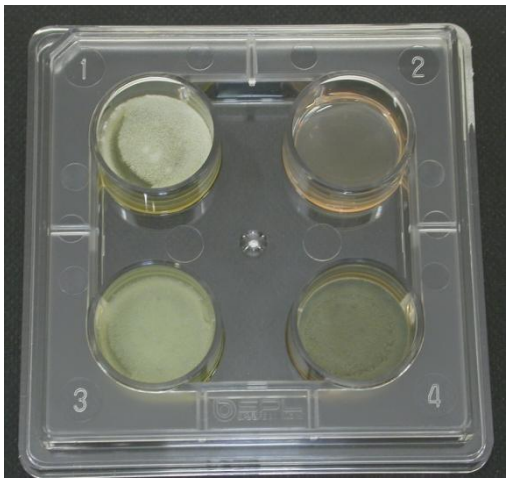
Using this method single colonies or multiple colonies can be tested on a single plate. If multiple colonies are tested, various *A. fumigatus* colonies can be sampled, thus creating a mixed inoculum. If there is a resistant colony present it will grow on one or more azole-containing wells.

Examples:

Azole susceptible isolate: Growth in well 4 (growth control)



Azole-resistant isolate: growth on well 1, 3 and 4 (growth control)



Azole-resistant isolate: growth in well 1 and 4 (growth control)