

# PROTOCOL VIPCHECK™



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

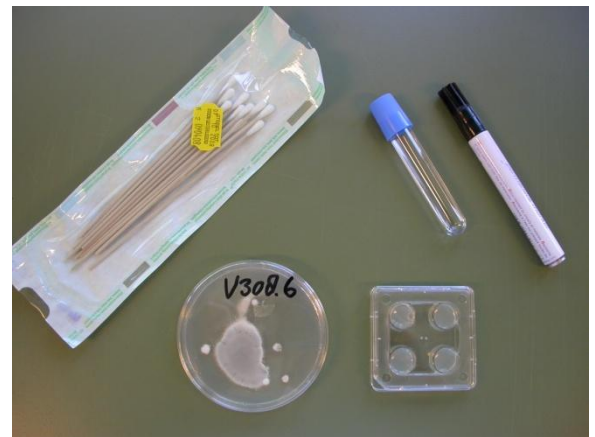
## Procedure

### Inoculation of agar-plates

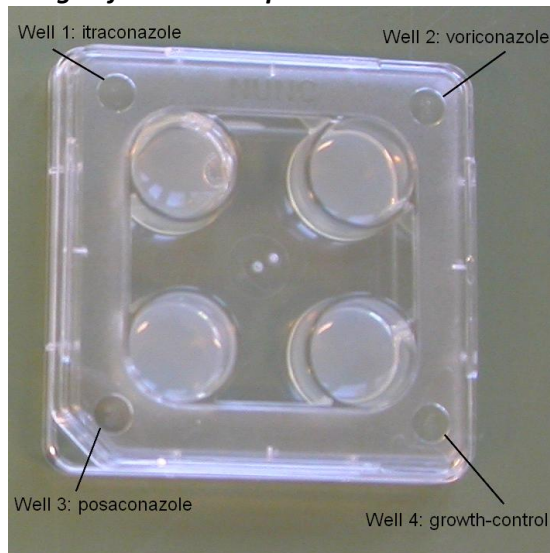
An *Aspergillus fumigatus* colony that is cultured from any clinical specimen can be screened. The colony needs to be sporulating. In most of the cases a sporulating culture is obtained after about 2 or 3 days of incubation.

### 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 *Aspergillus* colony with the wet swab. Make sure some conidia stick to it, but do not push too hard.



**Step 3**

Make suspension of 0.5-2 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.**

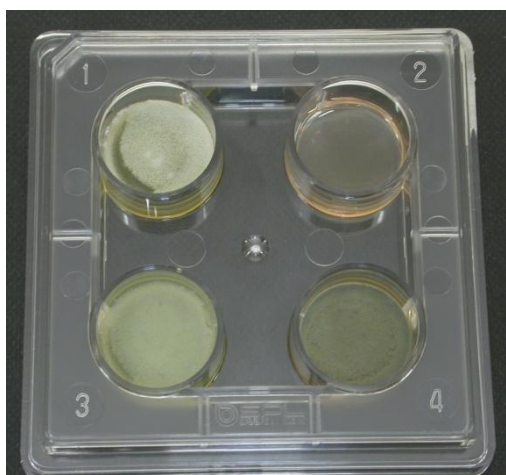
Using this method single colonies or multiple colonies can be tested on a single plate. If multiple colonies are tested, various *A. fumigatus* colonies can be sampled, thus creating a mixed inoculum. If there is a resistant colony present it will grow on one or more azole-containing wells.

**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)**

