Direct detection of resistance in clinical specimens

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Background

- Fungal infections are an increasing burden to society
  - Increased numbers of patients
- Resistance to antifungals is increasing
  - Increased use of antifungals
- Rapid detection of resistance could be of benefit to the patient
  - obvious
- Conventional or molecular detection?

Conventional versus molecular testing:

- Conventional testing detects any resistance mechanism
  - When performed properly
- AFST testing requires specific expertise …
- Molecular detection requires specific expertise …
- Molecular detection is potentially faster than conventional testing
- Sometimes conventional testing is not an option …

Conventional versus molecular testing:

- Conventional testing detects all resistance mechanisms
- AFST testing requires specific expertise …
- Molecular detection requires specific expertise …
- Molecular detection is potentially faster than conventional testing

- No culture available!

Triazoles

(voriconazole, itraconazole, posaconazol etc.)

Main resistance mechanisms:

1. point mutations in target gene (*cyp51A / erg11*)
2. increased expression levels of target gene
3. increased expression levels of efflux pumps
4. species specific intrinsic resistance

Echinocandins

(caspofungin, micafungin, anidulafungin)

Main resistance mechanism:

1. point mutations in *fks1* gene
2. species specific intrinsic resistance

Amphotericin B

(different formulations)

No acquired resistance, species specific intrinsic resistance

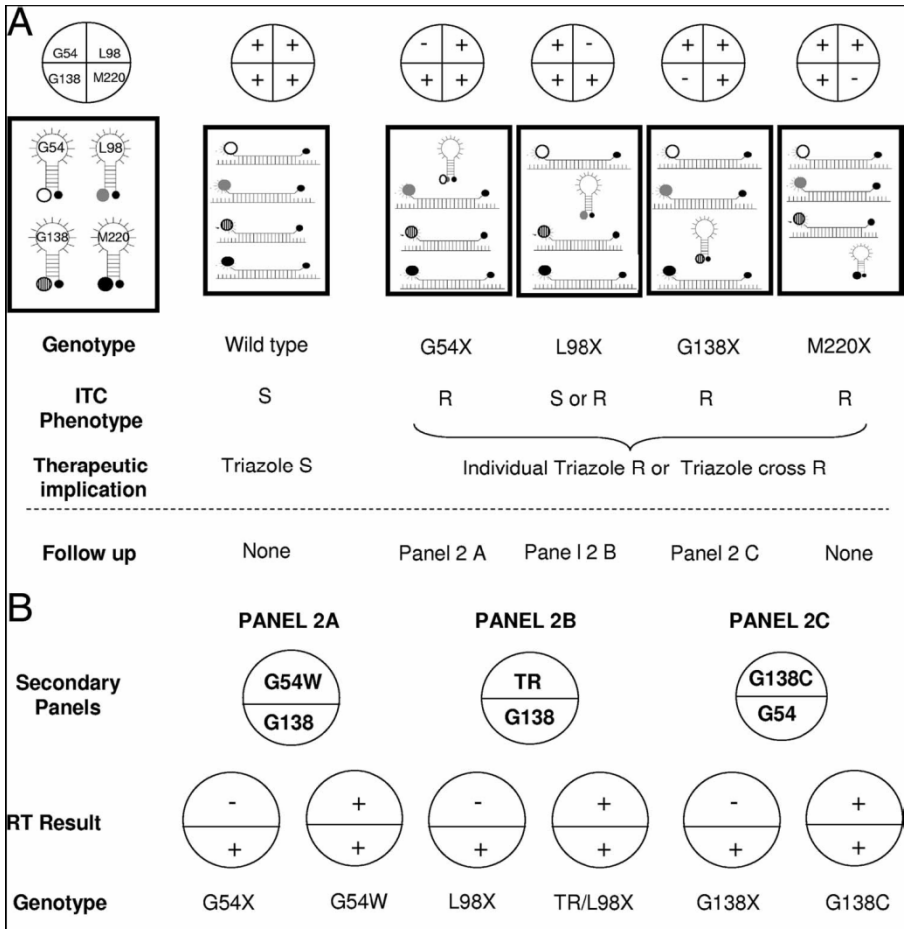
Current widespread options for molecular testing

Conventional PCR followed by DNA sequencing:

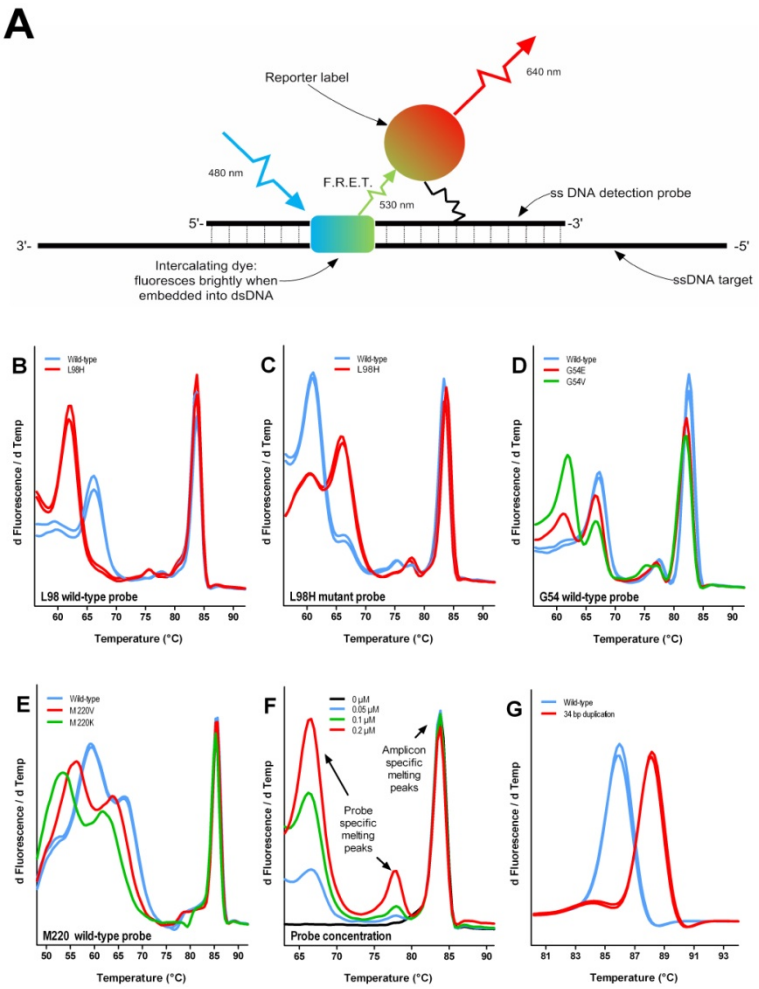
- loss of speed
- contamination risk
- does not detect all forms of resistance

Real-time PCR to detect mutations at specific positions:

- does not detect all forms of resistance



Garcia-Effron et al. JCM 2008



Klaassen et al. JAC 2010

Limitations of molecular detection

- Different fungi
- Different target genes
- Different mutations described, relation to resistance not always established
- Not all resistance mechanisms are known/detectable
- Different samples

Limitations of molecular detection

- Different fungi
- Different target genes
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- **Low DNA load !!**
  - Detection of fungal DNA already challenging using multicopy target
  - Even more challenging to target single copy genes …

A clear need to focus!

> Species

> Specific antifungals / targets / mutations

> Samples

> Patients

Focus on species

- *Aspergillus fumigatus* ☺
  - Resistance well studied
- *Candida spp.*☺
  - Resistance well studied
- Zygomycetes ☹
  - Limited information about acquired resistance
- Others …?

Focus on specific antifungals / targets / mutations

- *de novo* acquisition of mutations by a susceptible isolate
  - Many different point mutations in the *cyp51A / erg11* gene
  - Hotspot positions in *A. fumigatus*: Gly54 and Met220 in *cyp51A*
  - Hotspot region in *fks1*

- colonization/infection by an already resistant isolate ("environmental mutations")
  - TR/L98H, TR/Y121F/T289A
  - Internationally spreading

Snelders et al. AEM 2009
Verweij et al. Lancet ID 2009
Lockhart et al. AAC 2011
Chowdhary et al. JAC 2012
Klaassen et al. Mol Ecol 2012
Snelders et al. PLoS One 2012
Terpstra et al. unpublished

Focus on samples

- Not all samples are equally informative
- Respiratory samples
  - multiple genotypes present …
  - any clinical sample that is in contact with the environment may contain multiple different genotypes / antifungal susceptibility profiles
- Blood (-derivatives) / Tissues / Biopsies
  - samples from sterile sites are focussing on the invasive isolate instead of on possible co-colonizing genotypes
- Should we test any sample?
  - role for prescreening for fungal DNA load?

De Valk et al. JCM 2007
Denning et al. CID 2011

Focus on patients

- prolonged use of antifungals > acquired mutations
- azole naive patient > environmental mutations

Conclusions

Direct detection of resistance in clinical specimens using molecular techniques is technically very challenging

… but not impossible …

… and would benefit from using a clear strategy to select/deselect specific species / samples / targets / patients to be tested

... in combination with highly optimized technical procedures

Thank you for your attention