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Establishing a Mycology Service

Improving the detection of invasive fungal infections in immunocompromised patients

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Invasive fungal infection (IFI)

- Morbidity and mortality rates high (50 - 90%) in immunocompromised
- Most frequently implicated: *Aspergillus* spp. and *Candida* spp.
- Diagnosis: clinical suspicion, culture and non-specific radiology findings … IFI often diagnosed late
- Delayed diagnosis & treatment: worse outcome
- Uncertain diagnosis: empirical antifungal use (£££)
- Delayed / uncertain diagnosis: increased admission duration (£££)
- Inappropriate use of antifungals can lead to resistance

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Cost of Antifungal Agents

Antifungal	Cost per day (inc. VAT)	Cost per 14 days (inc. VAT)
Ambisome	£527.40	£7,383
Anidulafungin	200mg = £503 100mg = £251	£3,779
Caspofungin	70mg = £447 50mg = £351	£5,022
Caspofungin	70mg = £447	£6,266
Fluconazole 200 mg Oral	£0.09	£1.26
Fluconazole 400 mg IV	£1.16	£16.24
Micafungin	£409	£5728
Posaconazole	£112	£1571

Patients at risk of IFI

Immunocompromised:

- Bone marrow and haematopoietic stem cell transplant patients
- HIV patients
- Neutropenic patients
- Solid organ transplant patients, and
- Others receiving immunosuppressive therapy (e.g. rheumatology)
- Premature neonates

Need for improved IFI diagnostics

Rapid, more informative diagnostic tools can influence patient management by:

1. Initiating earlier intervention & reducing mortality
2. Narrowing differential diagnosis in complex septic patients
3. Reducing empiric antifungal agents
4. Permitting appropriate choice of antifungal (susceptibility testing)
5. Improving epidemiological data

Establishing a Mycology service

- Evaluation of a serological assay for detecting fungal infection.
- Implementing serological and molecular assays.
- Susceptibility testing.
- Designing RT-PCRs for different targets indicative of infection by the pathogen and infection in the host.
- Potential for the design of a more sensitive and specific serological assay.

Establishing a Mycology Service

- **Serological assays:**

Galactomannan - *Aspergillus* spp.

(1→3)-B-D-Glucan - pan-fungal

- Molecular assays:

Host target

Pathogen targets –*Aspergillus* spp. and

-*Candida* spp. (5 most frequent species)

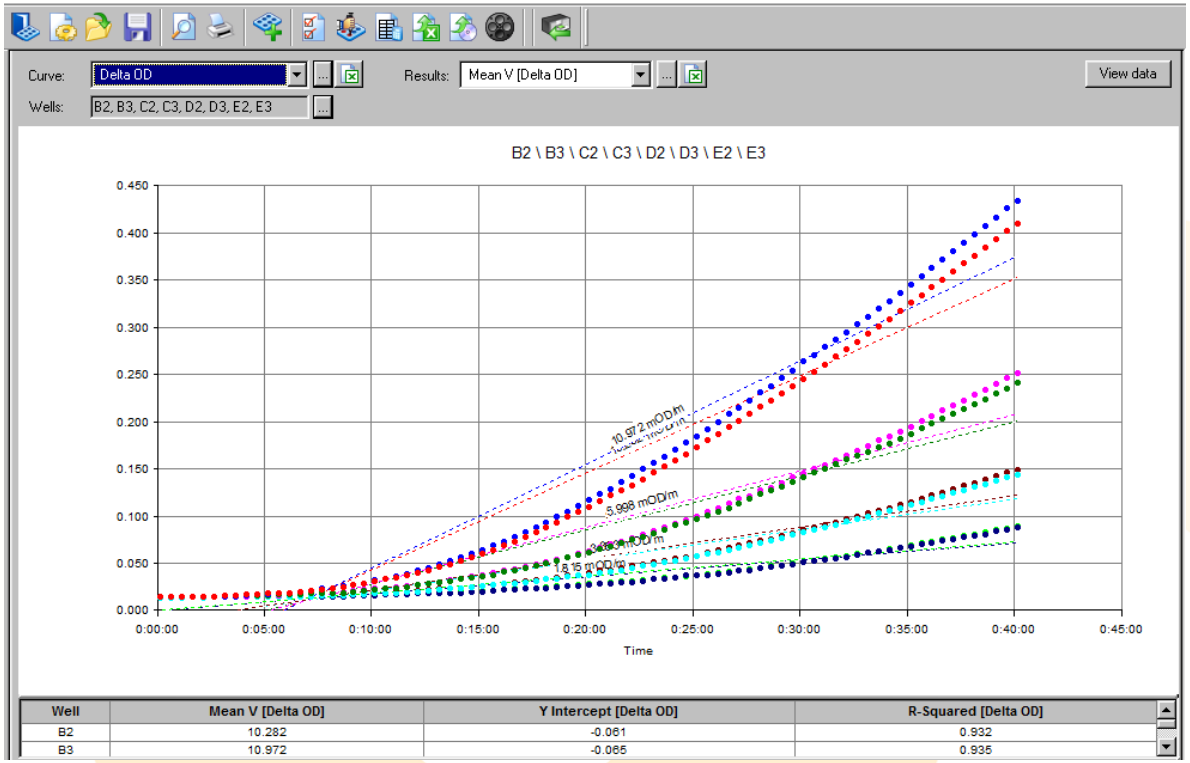
Serological assay- galactomannan (GM)

- Available for diagnosing *Aspergillus* spp.
- Evaluated using serum and Broncho-alveolar (BAL) samples (100 ul).
- Bio-Rad Platelia™ -One-stage immunoenzymatic sandwich EIA using a rat monoclonal antibody (EB-A2).
- Semi-automated.
- False-positives reported with tazocin and other beta-lactam agents.

Serological Assay- (1→3)-BDG

- (1→3)-Beta-D-glucan (BDG), a cell wall polysaccharide found almost exclusively in fungi.
- Fungitell –(Associates of Cape Cod): pan-fungal serological, qualitative, colorimetric assay.
- Evaluated using serum samples (5 ul).

Delta OD Curve 405 of standard curves



- BDG and GM concordant positive 15/41 GM positives (37%)
- BDG and GM concordant negative 89/96 GM negatives (93%)

(1→3)-BDG assay

- Provides a rapid diagnosis for fungal infection.
- Low sample volume required.
- High specificity was observed and BDG has a high PPV for *Candida*, *Aspergillus* or *Fusarium* species.
- Useful for treatment monitoring.
- An automated platform under evaluation.
- Contaminants possible: dialysis membranes and filters made from cellulose. Cotton gauze and sponges and some drugs.
- Our findings, the assay had a low PPV compared with GM and is sensitive to environmental contaminants.

Establishing a Mycology Service

- Serological assays:

Galactomannan - *Aspergillus* spp.

(1→3)-B-D-Glucan - pan-fungal

- **Molecular assays:**

Host target

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-*Candida* spp. (5 most frequent species)

Molecular detection options

- Consensus *Aspergillus* spp. PCR (Evaluated and approved across multiple sites).
- *Aspergillus* spp. PCR design and development real-time reverse-transcription PCRs (RT-PCRs) using different gene targets (serine proteinase and velvet gene).
- *Candida* spp. PCR for speciating the 5 predominant species: (*C.albicans, C.glabrata, C.krusei, C.parapsilosis, C.tropicalis*).

'Consensus' *Aspergillus* PCR

- Readily available as a rapid, qualitative, real-time PCR for *Aspergillus* spp., relative to an internal control.
- Consensus *Aspergillus* spp. PCR targets a region of the 18S ribosomal gene.
- Sequence-specific probe to confirm the presence of *Aspergillus* spp.
- Risk of false positives: reagents and environmental *Aspergillus* spp. contamination (ubiquitous).

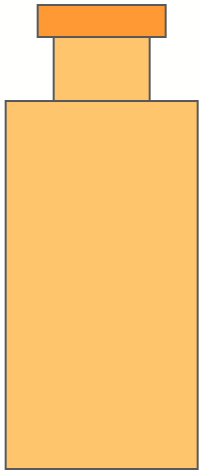
Alternative PCRs

- RT-PCR is used to monitor levels of mRNA (expressed gene) present, which is suggestive of increased amount of protein expression thus active growth.
- Reduced risk of false positive due to detection of *Aspergillus* spp. contaminants.
- A human host marker of IFI will further support diagnosis, as a second identifier.

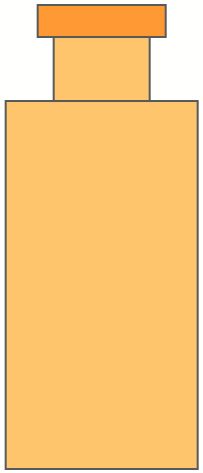
Aspergillus spp. RT-PCR

- Two targets under investigation are the serine proteinase (SP) and published velvet genes.
- SP - a protein expressed in high quantity during the protein investigation work carried out 5 years ago at King's College Hospital.

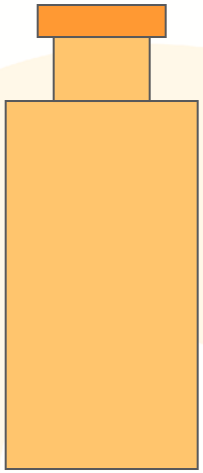
Developed a model System to ‘mimic’ the conditions for the invasive growth of *A. fumigatus* in human epithelial lung cells



A. fumigatus



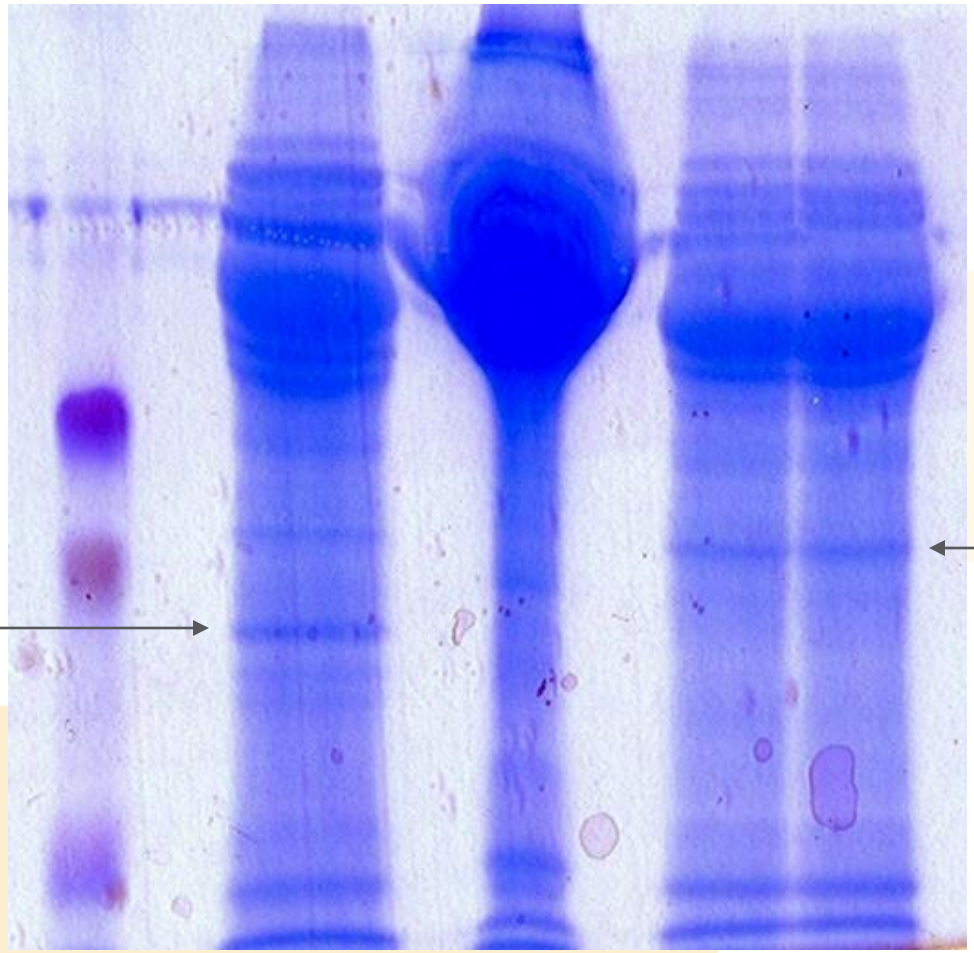
HEL cells



A. fumigatus + HEL cells

Grown in Eagle’s Minimal Essential Medium for 1 and 5 days at 37^{o}C
Harvested mycelium/cells for RNA extraction
Collected culture filtrate for extracellular proteins

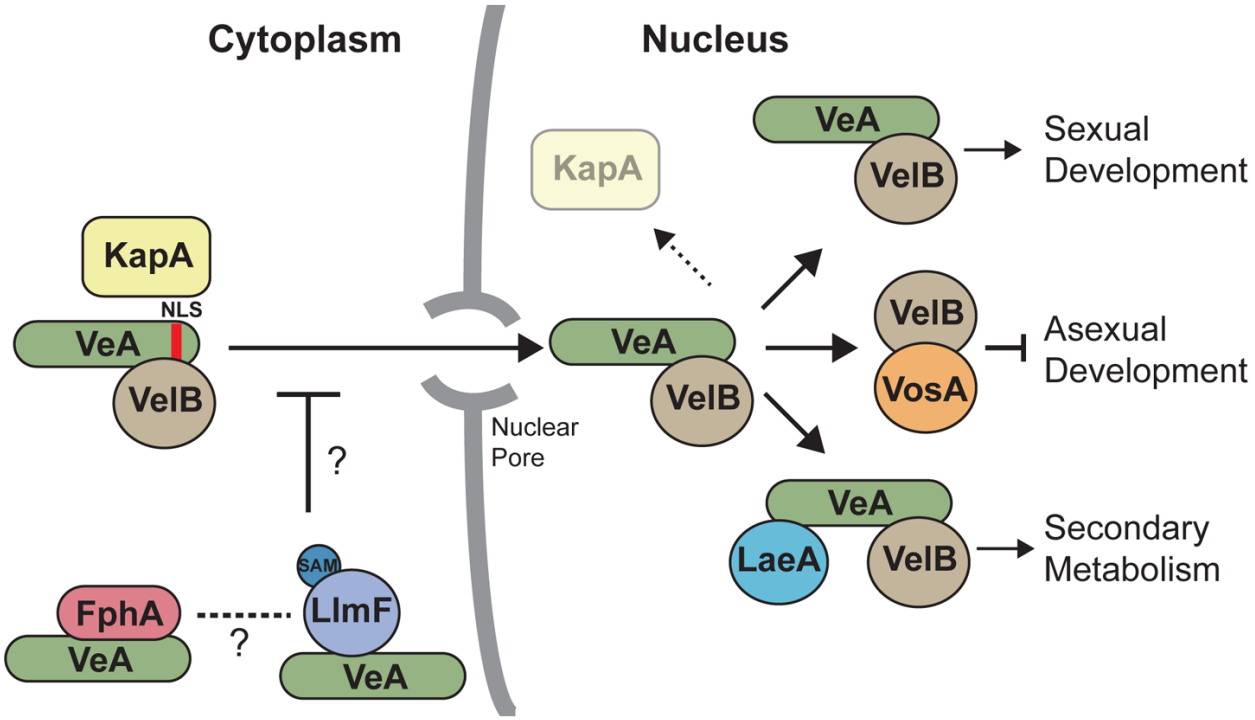
Day 5 Polyacrylamide gel of model system *Aspergillus*



Donated by
Dr M. Smith

Velvet complex proposal

- The velvet genes are part of a velvet complex that is present in development of hyphae during the sexual stage of *Aspergillus* spp.



Palmer J et al Jan 2013 PLOSGenetics Secondary Metabolism and Development Is Mediated by LlmF Control of VeA Subcellular Localization in *Aspergillus nidulans*

- The presence of an increase in mRNA expression would indicate increased protein expression, active growth.

Invasive *Aspergillus* infection detection

- Rapidly sequence PCR amplicons from multiple primer sets to identify suitable sequence-specific probes for detection of invasive *aspergillus* infection using next-generation sequencing (NGS).
- The NGS tool will provide more sequence data for the development of an informative molecular assay.
- Test in a model system to confirm protein expression data.
- Provides opportunity to develop rapid, affordable protein-based assays. Potentially with the aim of producing a lateral flow device.

Candida spp. PCR

- Speciation of 5 most frequent *Candida* spp. pathogens can influence patient management.
- Plan: a universal primer set targeting the 28S rRNA and the design of sequence-specific probes for the 5 species using a real-time PCR platform is planned.
- Obtain rapid and conclusive results.
- Thus, determine the most appropriate antifungal agent(s) for treating *Candida* spp.

Future/Summary

- Implement the mycology service at King's College Hospital and the other Viapath partners, using the current serological assays and PCR.
- Potential to provide the service to other transplant centres and burns centres.
- Develop an improved, standardised, informative molecular assay, using RT-PCR for a conclusive detection of IFI.
- Research 'Pathogen – Host' combination molecular assay.
- Identify and characterise proteins that may be suitable for preparing antibody clones for an antigen/antibody serology assay.
- Introduce in-house susceptibility assays.

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Thank you