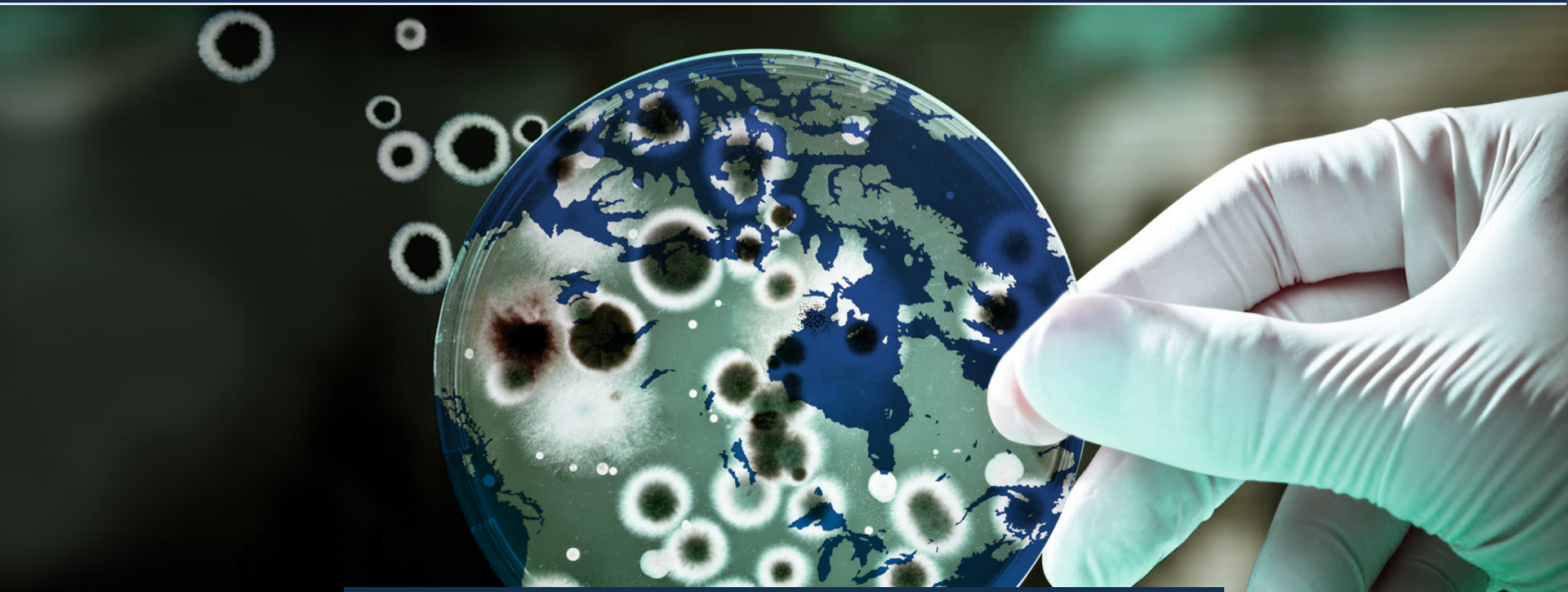
IFI DIAGNOSTICS:

Something Old, Something New: Beyond Lactophenol Cotton Blue



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Disclosures

Advisory Board Member/Consultant:

- CSL Behring Canada, Pfizer Canada

Speaker:

- CSL Behring Canada & Sunovion

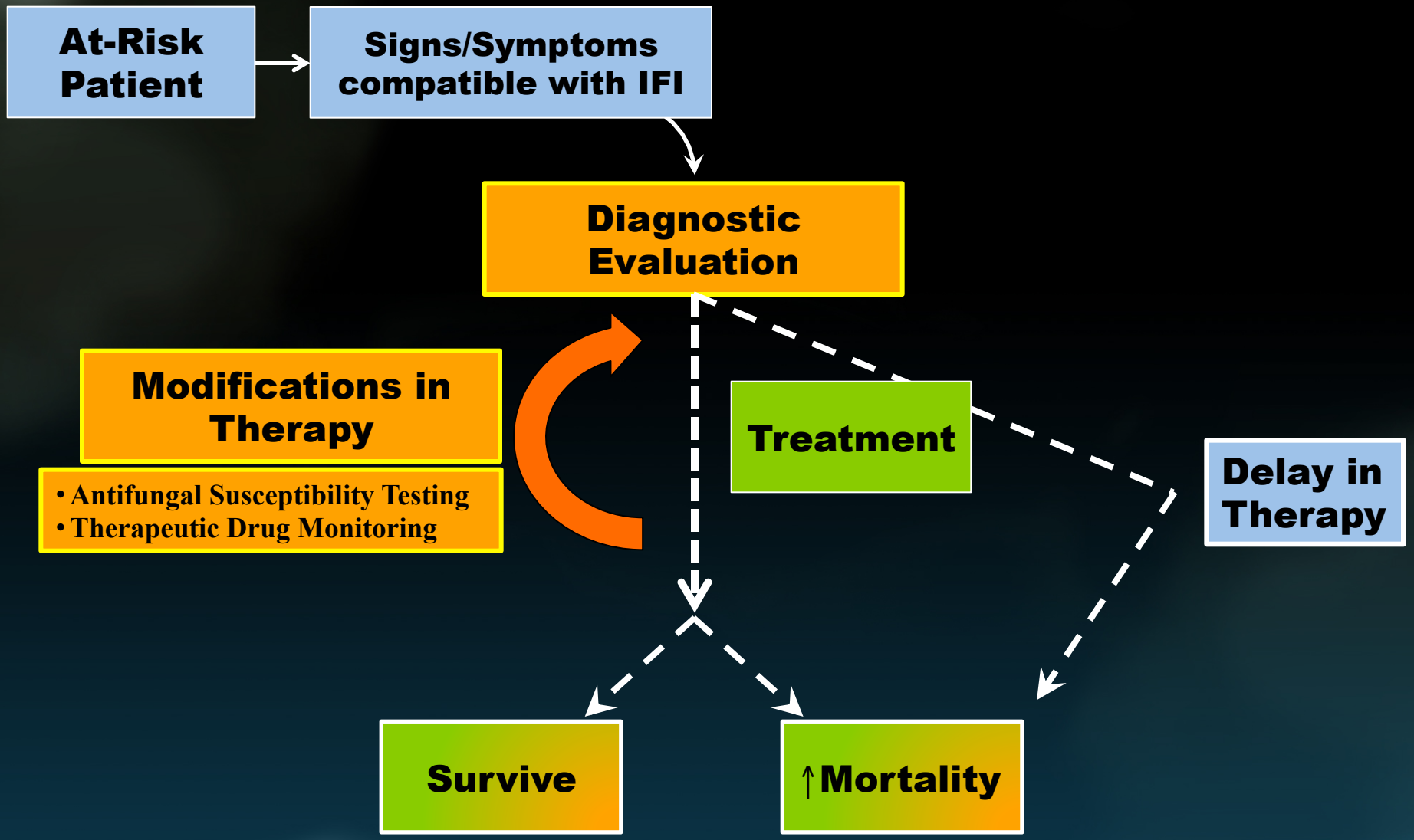
Grant & Honorarium:

- CSL Behring Canada, Astellas Canada

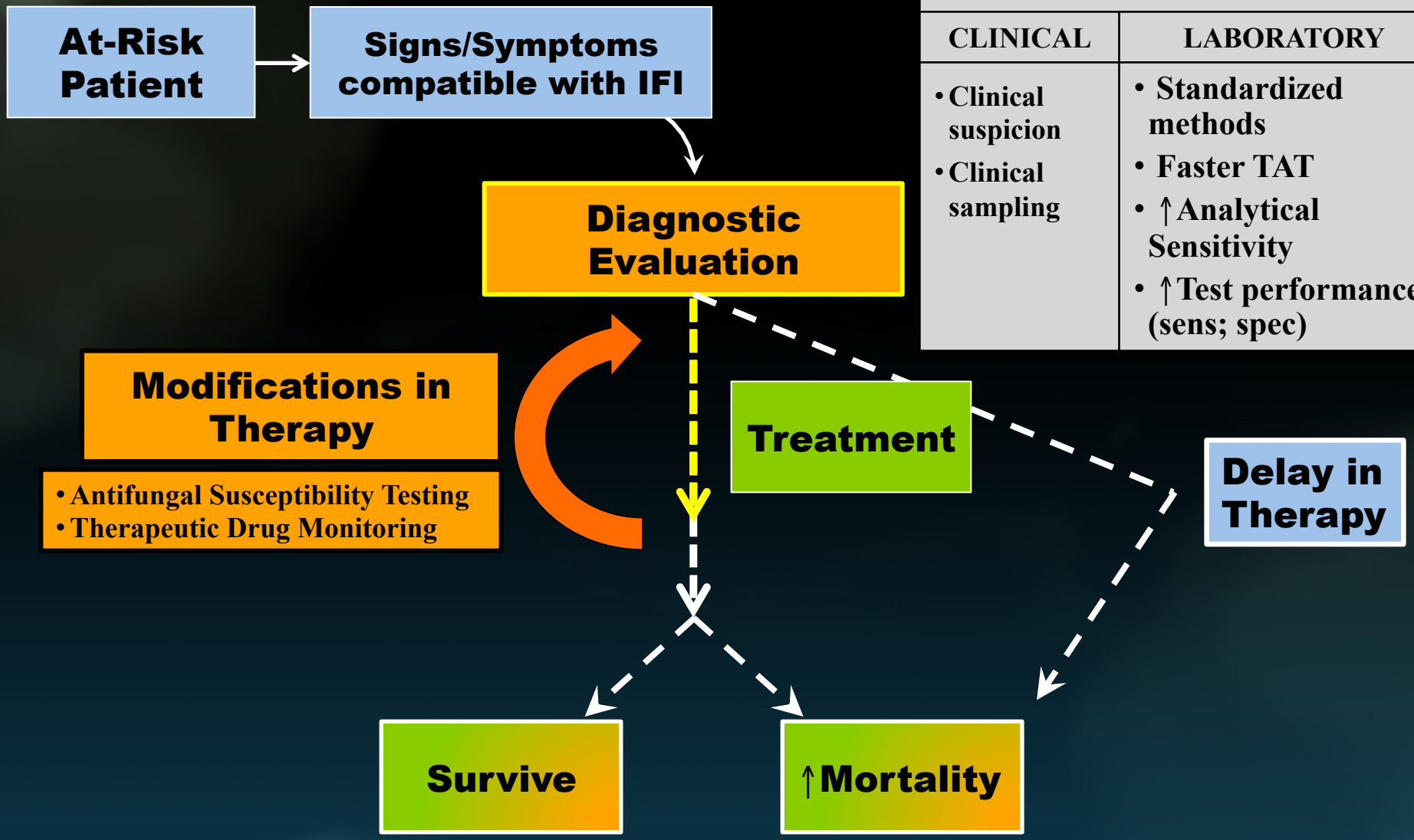
Objectives:

- Overview of diagnostic strategies for IFI in haematological patients
- Discuss advantages and disadvantages of different diagnostic modalities
- State emerging diagnostic tools

The Problem:

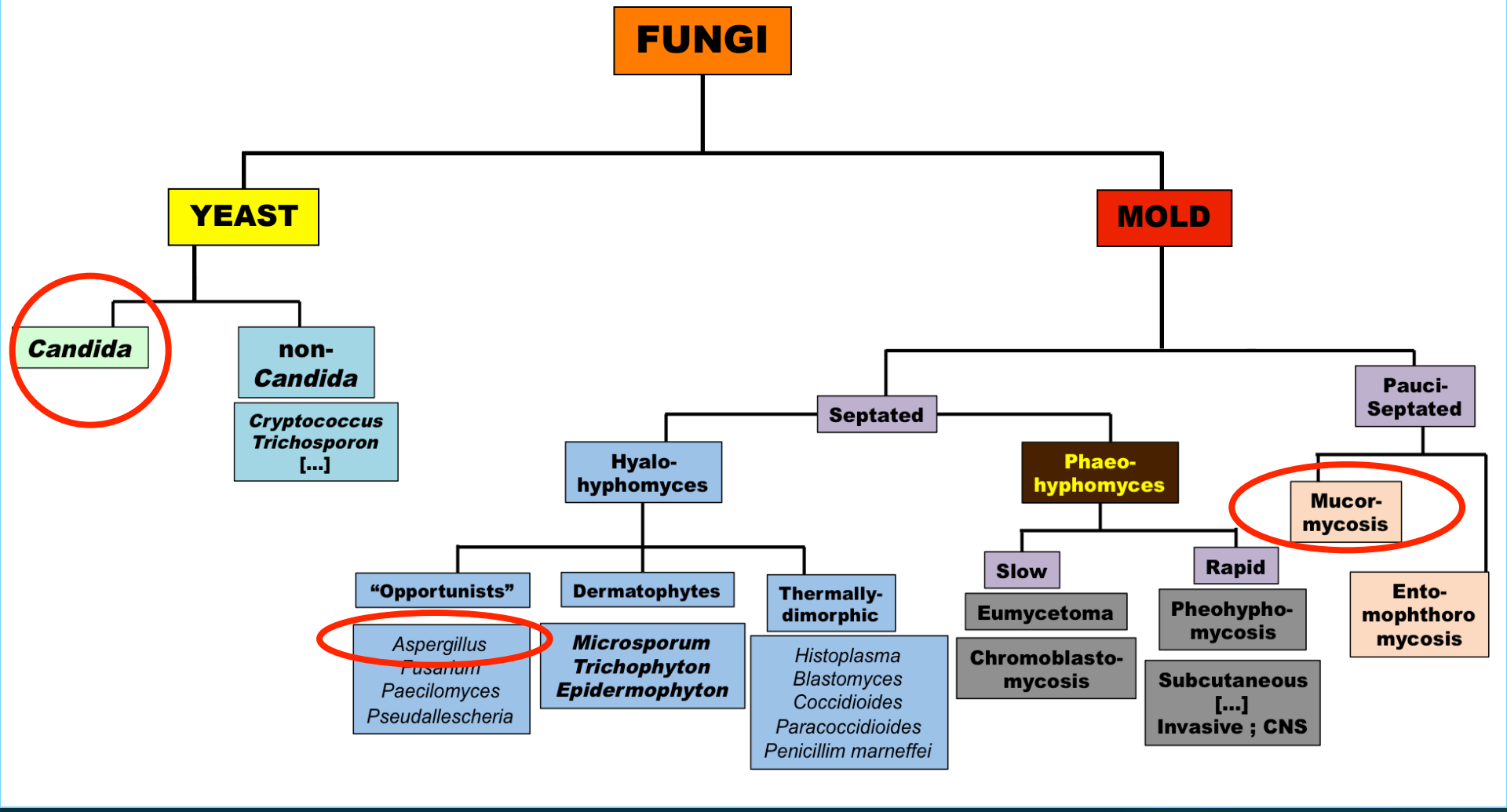

At-Risk Patient
Signs/Symptoms compatible with IFI
Diagnostic Evaluation
Modifications in Therapy
• Antifungal Susceptibility Testing
• Therapeutic Drug Monitoring
Treatment
Delay in Therapy
Survive
↑Mortality

The Problem:

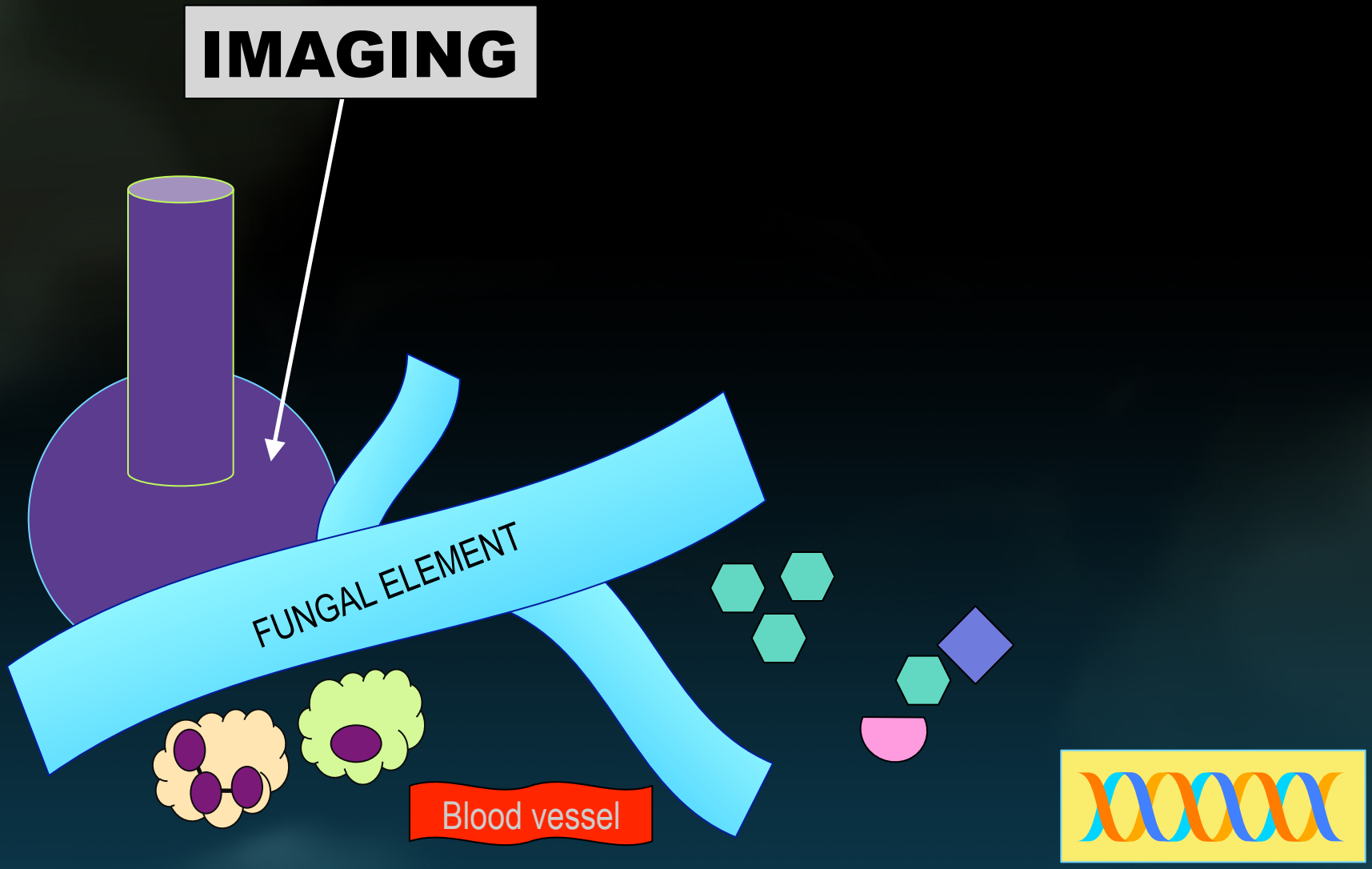


EARLIER DIAGNOSIS	
CLINICAL	LABORATORY
• Clinical suspicion • Clinical sampling	• Standardized methods • Faster TAT • ↑Analytical Sensitivity • ↑Test performance (sens; spec)

Clinical Mycology:



IFI DIAGNOSTICS:



IMAGING:

Chest CT: GOLD STANDARD

- 1896: CXR… ? Time to move forward ?

Mod-High sensitivity: 80-85% (cf. autopsy)*

Earlier detection: 5 d (median)

"Characteristic signs":

- EARLY: Halo sign; Fluffy nodules; Pleural-based wedges
- LATE: Air-crescent sign; Cavitation
- Non-specific findings… adjunctive Dx tests

Typical radiological evolution:

- On appropriate antifungal Tx, ↑ volume of infiltrates (4x) during 1st week (d7)
- Stable (x few days)
- ↓ thereafter
- F/U scan no sooner than 2 weeks (if clinically stable)

*Kami et al., Mycoses 2002

CT IMAGING:

ADVANTAGES:

- **Non-invasive**
- **Availability**
- **Speed of the test**
- **Systematic use: associated with improved outcomes**

DISADVANTAGES:

- **You have to know where to look: Lungs, Sinuses… elsewhere (?GI, GU?)**
- **Non-specific signs**
  - incl. Non-specific radiology report: "… could be compatible with fungal or mycobacterial disease, or parasites or alien plant life"
- **Limited distinction capability**
  - Fungus vs. Non-fungus
  - Which fungus?

Emerging Imaging Modalities:

FDG PET/CT

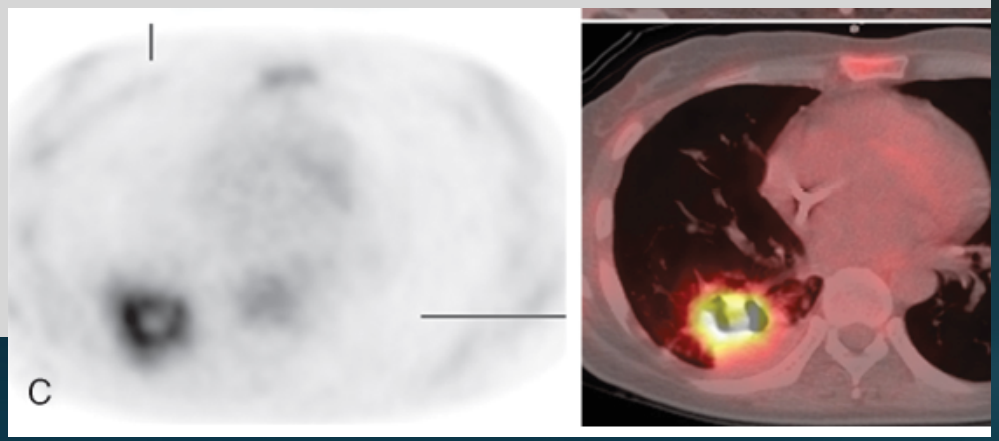
- FDG uptake in infl cells ; glc metabolism (“respiratory burst”)
- Rationale: non-specific findings on CT

Kim et al.*:

- IA (imm compr): hypermetabolic nodule, NO halo
- NIA (imm compt): isometabolic noule, (+) halo

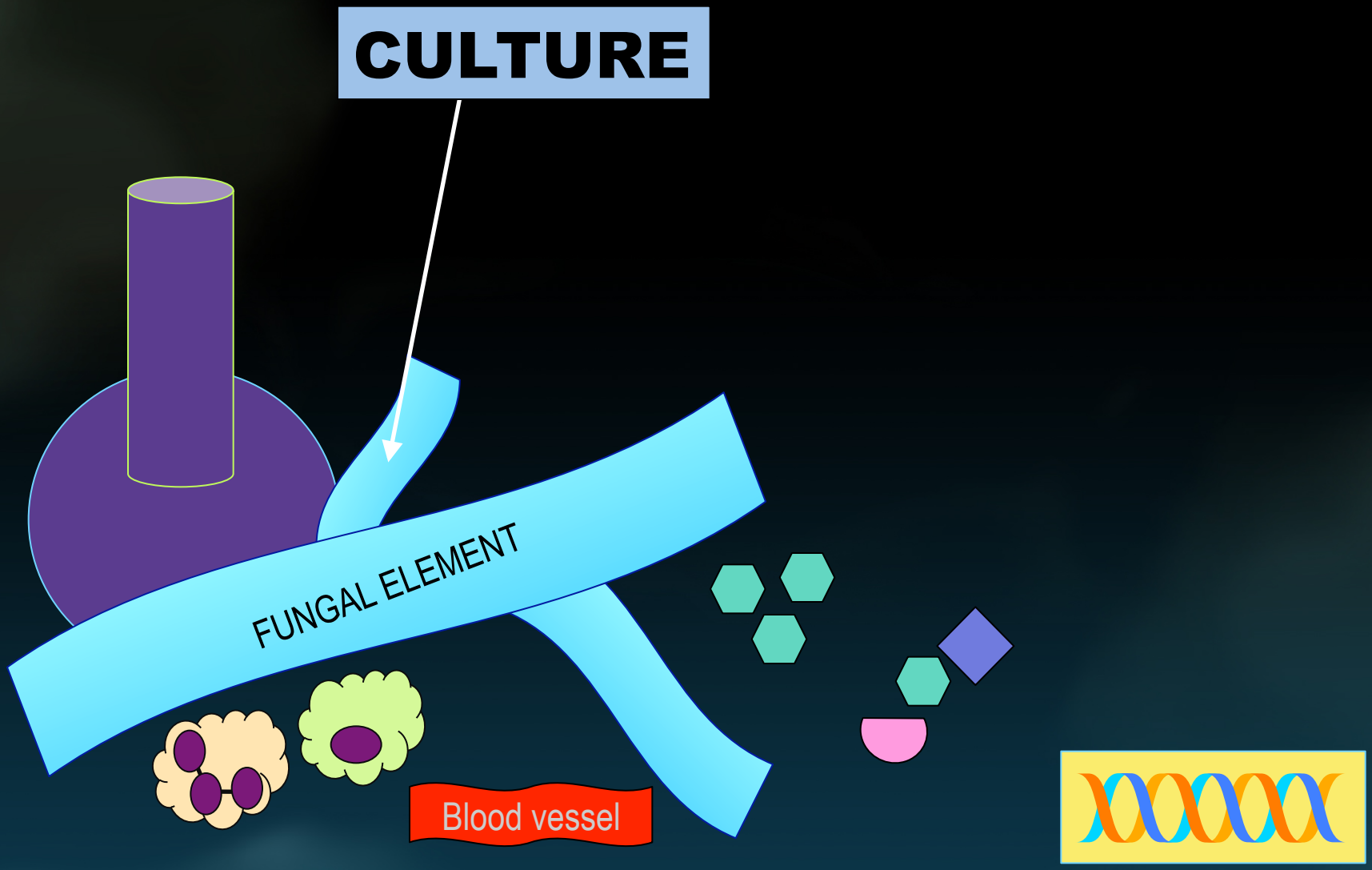
More studies needed

Work done on fungal labeling…

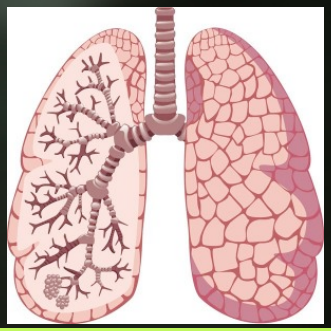


*J Comput Assist Tomogr 2013

IFI DIAGNOSTICS:



CULTURE:

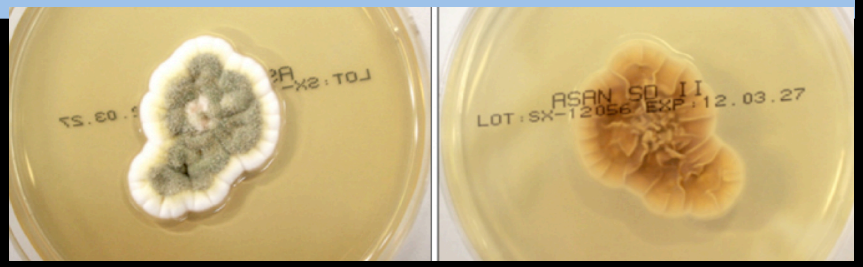


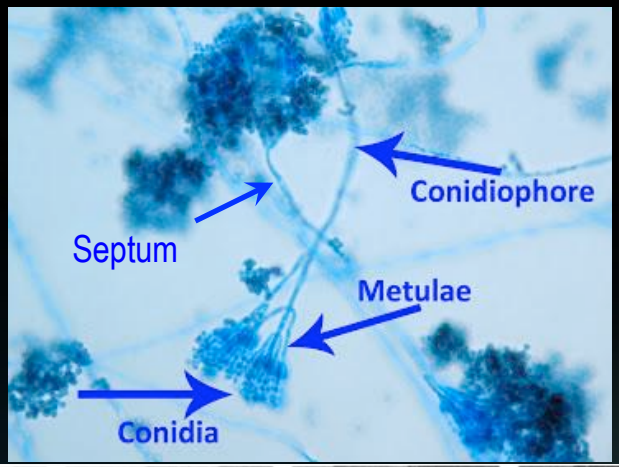
RIGHT Clinical sample

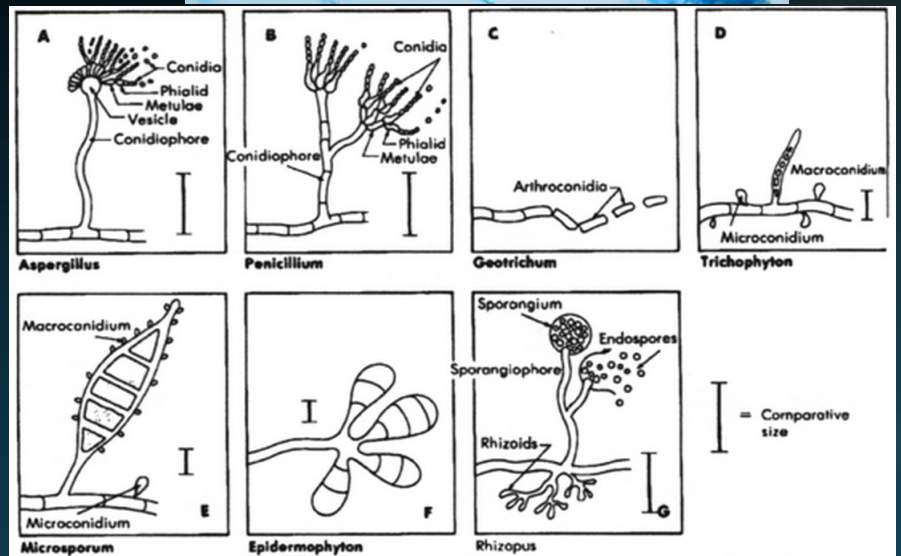
RIGHT Culture media

(Some) Different types of fungal media:

- BHI (Brain-Heart-Infusion) agar
- Czapek's agar
- Inhibitory Mould Agar
- Mycosel agar
- Potato Dextrose Agar
- Sabouraud-BHI
- Sabouraud's Dextrose Agar
- Potato flake agar







CULTURE:

ADVANTAGES:

- **HIGH specificity for IFI (70-95% cf. autopsy)***
- **Provides an isolate for further testing, including**
- **Antifungal Susceptibility testing**

DISADVANTAGES:

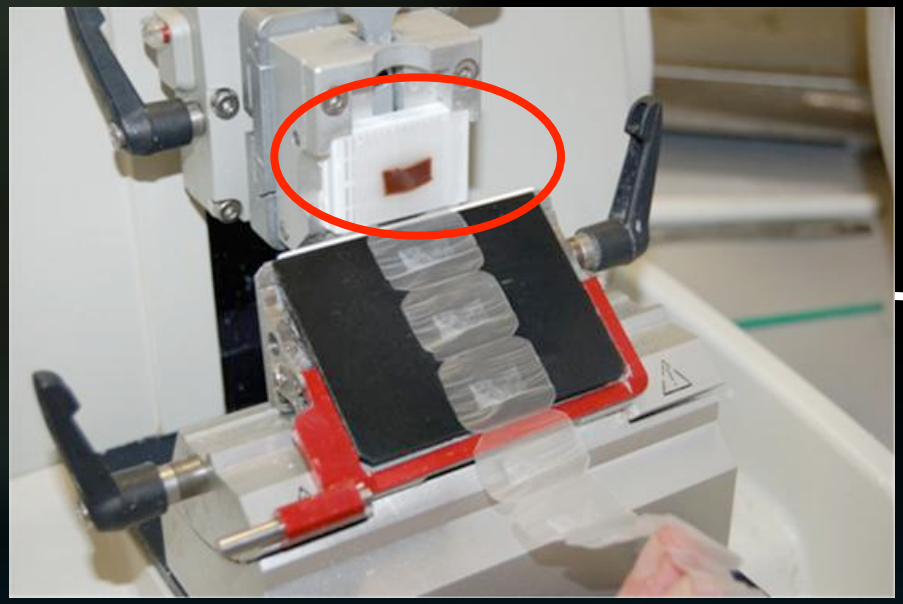
- **Usually requires invasive procedure for sampling**
- **LOW sensitivity: 0 – 88% (cf. histology)***
- **Precarious sampling / sample processing**
  - Pan-Micro lab testing + Path
- **Requires expertise**
- **May be slow**
  - weeks to complete

*Guarner et al. CMR 2011

IFI DIAGNOSTICS:

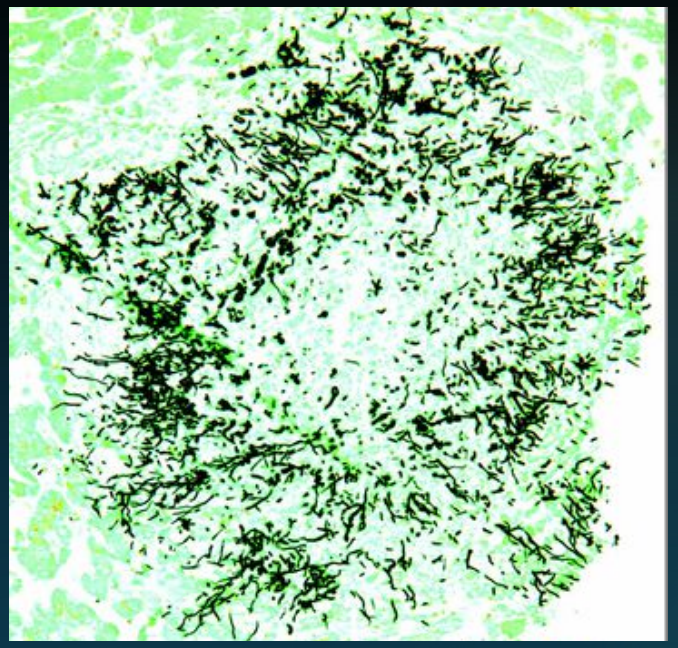
HISTOPATHOLOGY

Histopathology:



Pathology Stains:

- **H&E**
- **PAS**
- **GMS**



Histopathology:

ADVANTAGES:

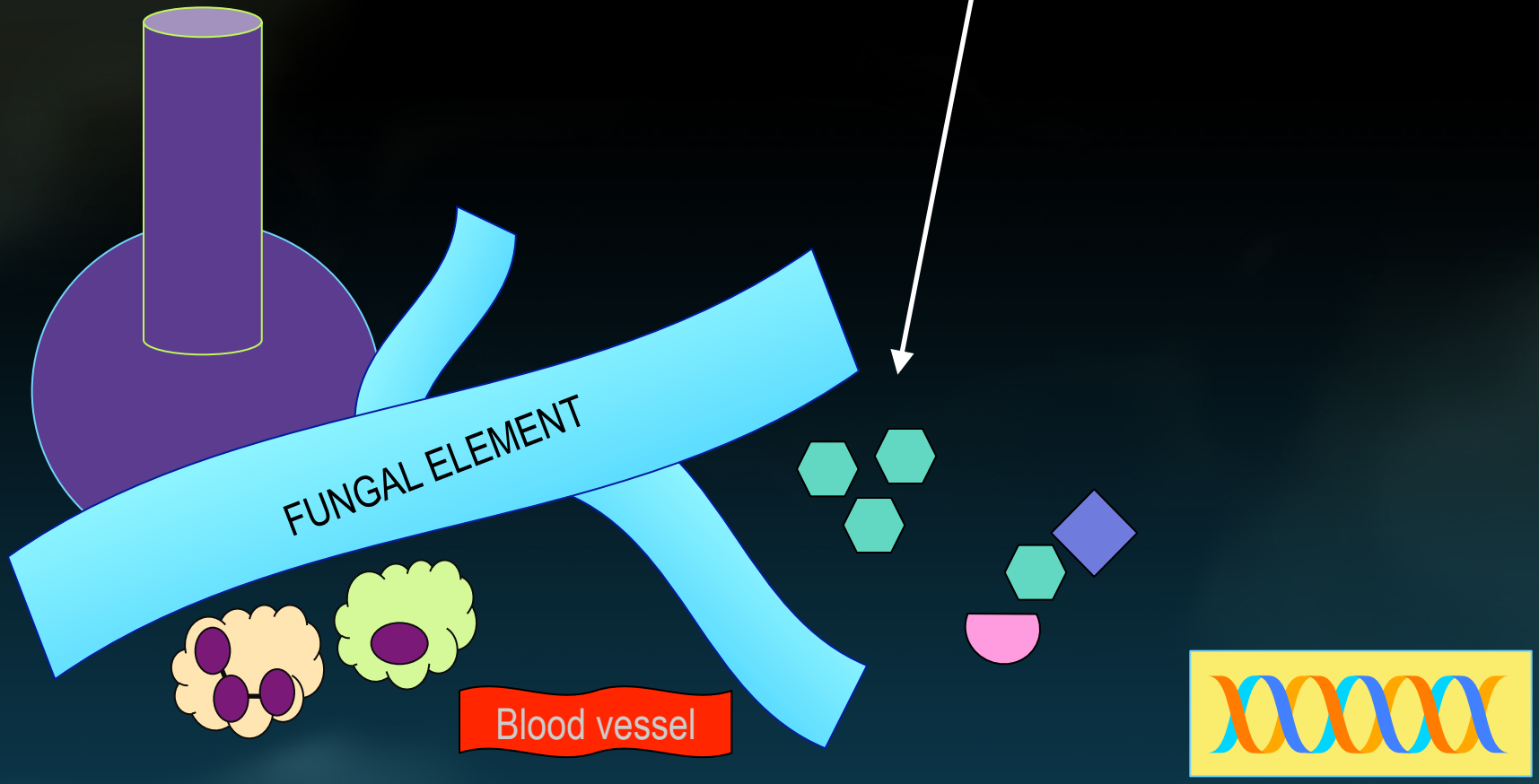
- **Pretty**
- **Distinguishes: contamination vs. colonization vs. infection**
  - Micro stains: H&E, PAS, GMS, Fontana-Masson, Calcofluor
  - Host response: Inflammation, necrosis, hemorrhage
- **Provides presumptive Dx pending cultures (if they do grow)**

DISADVANTAGES:

- **Usually requires invasive procedure for sampling**
- **Overall accuracy: 20 – 80% (cf. culture)**
- **Morphological diagnosis (i.e. "they all look the same")**
  - polymorphic fungal elements
- **No speciation (or even genusiation)**
- **Dual infection**
- **Requires interaction with a Pathologist**

IFI DIAGNOSTICS:

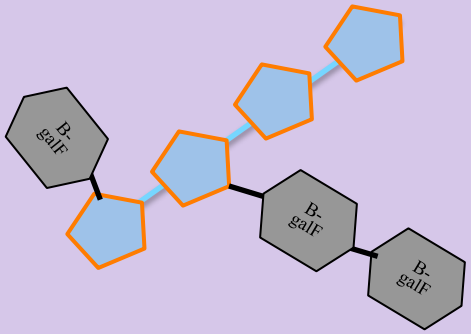
ANTIGEN DETECTION



ANTIGEN DETECTION:

GALACTOMANNAN (GM)

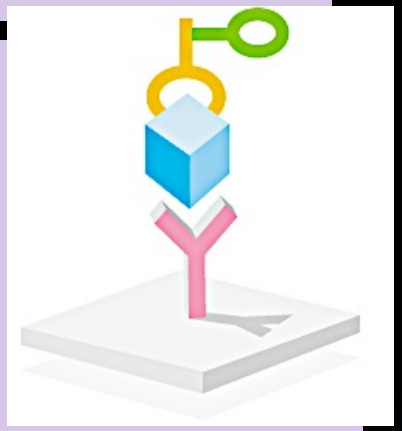
- **Polysaccharide found in cell wall of most *Aspergillus* sp.**
- **Released from growing hyphae**
- **Commercial kit**



β-D-GLUCAN (BDG)

- **Polysaccharide found in cell wall of MANY FUNGI**
- **Commercial kit**
  - Manufacturer recommendations made on 2 studies (mainly, non-neutropenic patients)
- **Meta-analysis: (+)=2++ samples**
  - LOW Sens: 50%
  - HIGH Specificity: 99%

GALACTOMANNAN:

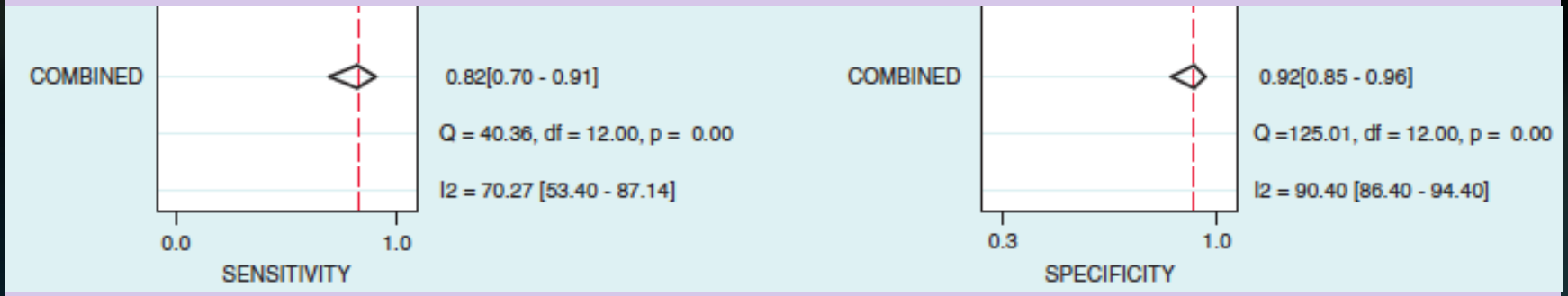


- Standard test: EIA
- Extensively studied in Heme population (Allo-SCT, AML/chemoTx)
- **<u>Serum</u>*: Sens: 58–65%; Spec:65–95%**
  - Heterogeneity in : design/patients/OD cut-off
  - May precede radiologic/microbiologic Dx by **5-8 d** (median)
  - Useful for **<u>PRE-EMPTIVE</u>** strategy in at-risk (non-prophylaxed patients)
  - False (+):
    - Pip/Tazo (previously… ?generic)
    - Other fungi (e.g. *Fusarium*)
    - Prophylaxed patients (low pre-test prob; ∴Dx-driven)

*Pfeiffer et al., CID 2006

GALACTOMANNAN:

- **BAL:**
  - Diagnostic-driven, NOT screening
  - In meta-analysis restricted to hematology patients*, using GAL-GM cut-off of 1.5:



SENS: 82%

SPEC: 92%

- Combining BAL-GM to S-GM or BAL-PCR: ↑Sens by 5-9%

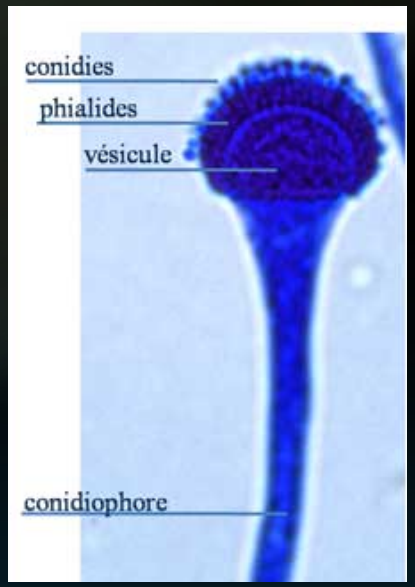
*Heng et al., Crit Rev Microbiol. 2015

GALACTOMANNAN:

ADVANTAGES:	DISADVANTAGES:
• Not technically demanding • Costs can be reduced by sample batching • Fast TAT: 4 h • Serial serum GM: monitor response to therapy	• (+) results need confirmation (repeat testing) • Limited microbiological spectrum: • *Aspergillus* vs. NON-*Aspergillus* • WHICH *Aspergillus* ??

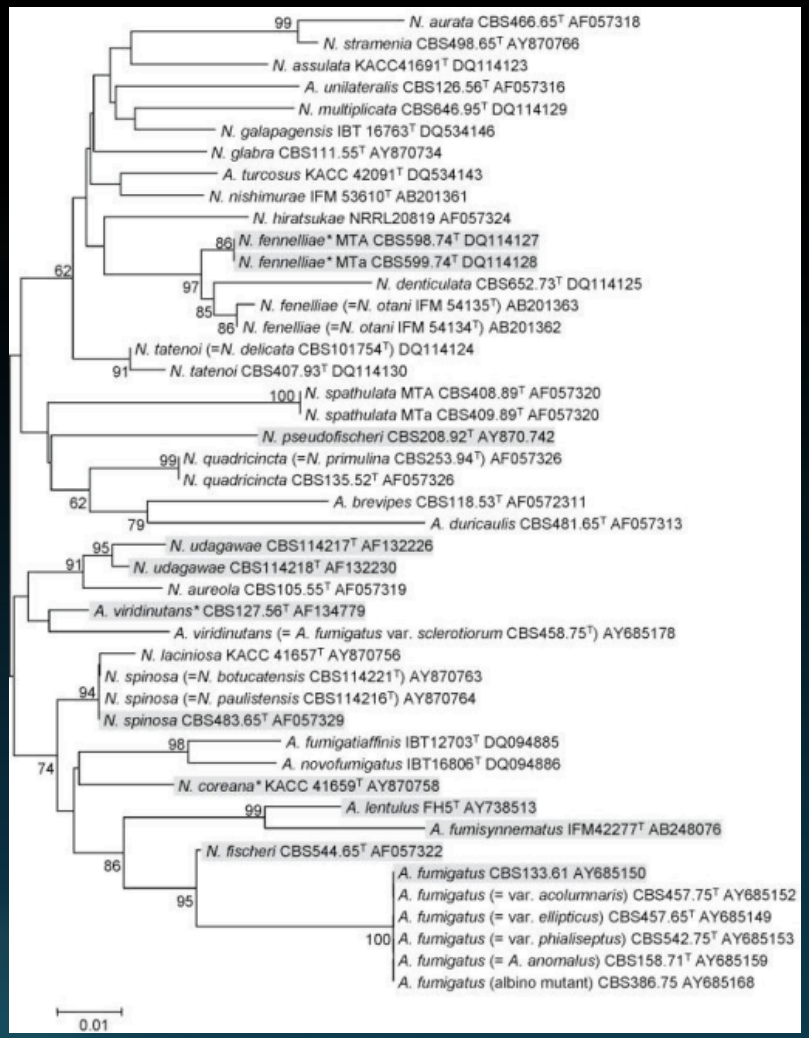
Will the real *A. fumigatus* please stand up?

PHENOTYPE (MORPHOLOGY)



GENOTYPE

Aspergillus species complex (section *Fumigati*)

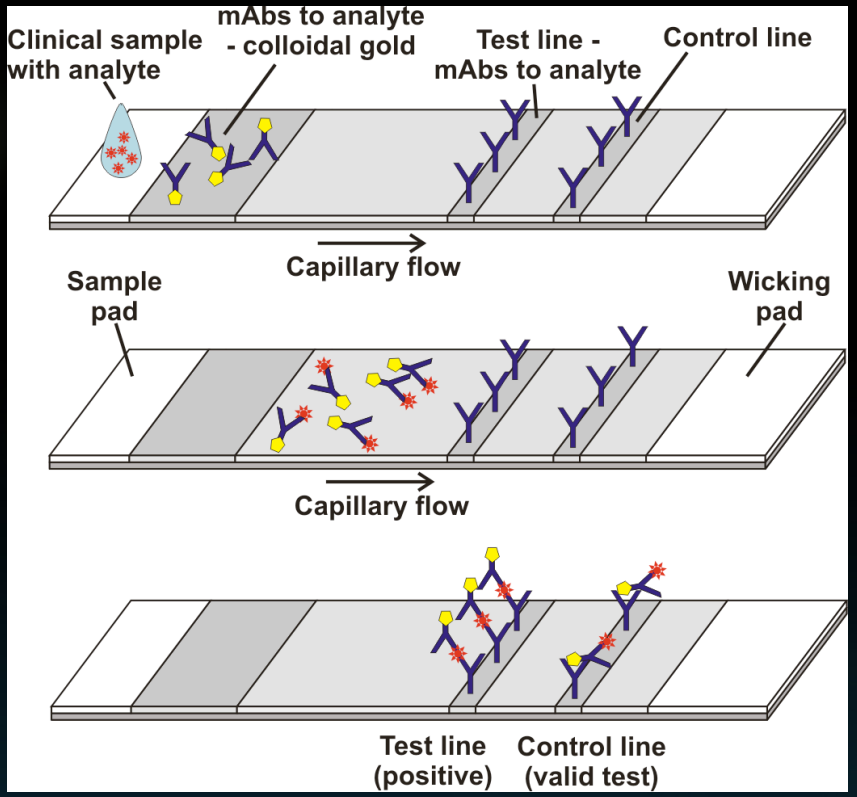


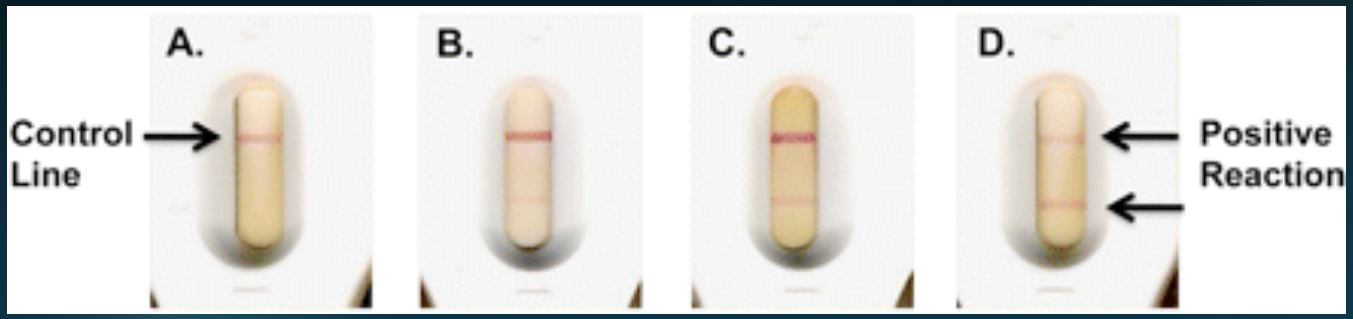
ANTI-FUNGAL SUSCEPTIBILITY PROFILE

	A.fumi (ss)	pheno-copies
AmB	0.25-1	>1
Vori	0.25 – 1	>1
Terb	2 – 8	< 1.66

(+)GM ≠ Vori(S)

GM by Lateral Flow Assay:





*Wiederhold et al., Clin Vaccine Immunol 2009

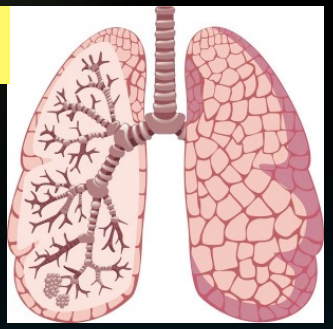
IFI DIAGNOSTICS:

NUCLEIC ACID-based Testing

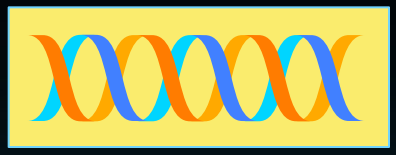
NUCLEIC ACID-based TESTING (NAT):

- PCR: Polymerase Chain Reaction
- "Why not just PCR it out ?"

Clinical Dx



- Screen vs. Diagnose
- Sample? Blood (which component)? BAL?



Specimen type

Specimen processing

DNA extraction

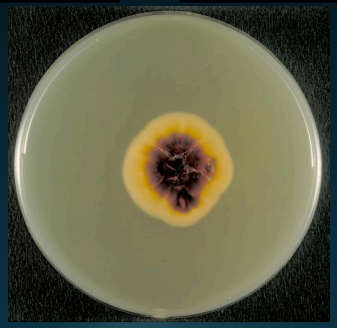
PCR assay design:

- Target?
- Primers?
- Reagents?

Downstream analysis

- (+)/(-) ? 2++?
- Sequencing
- Database ?

Micro Dx

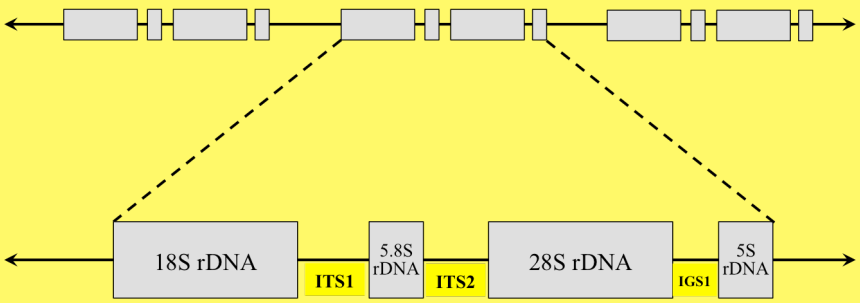


QUALITY (QC / QA)

PCR:

- ***Aspergillus*-specific PCR**
  - Serum: SENS: 72%; SPEC: 96.5%
  - Lack of standardization of methods
  - Commercial kit (*Candida* 7-plex; Mould 11-plex)
  - European Aspergillus PCR Initiative (EAPCRI)
- **Real-time PCR for detection of *Candida***

- **Pan-Fungal PCR:** detect unknown fungi in clinical specimens
  - Path(+), Culture(-)
  - Blood



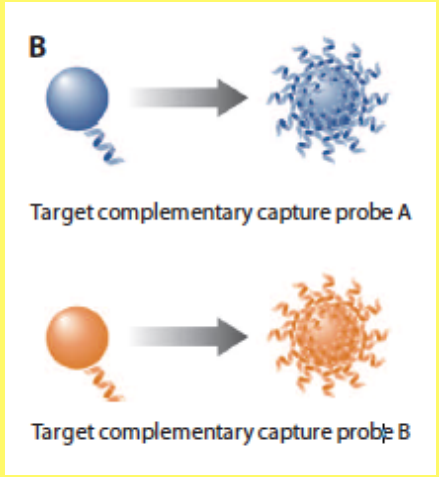
- May be esp. useful for Mucormycosis

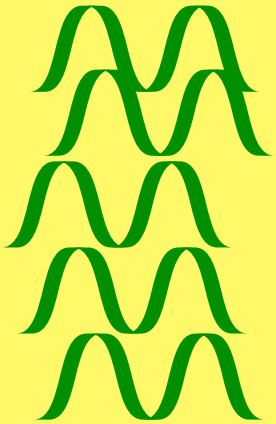
Emerging NAT: T2Candida assay

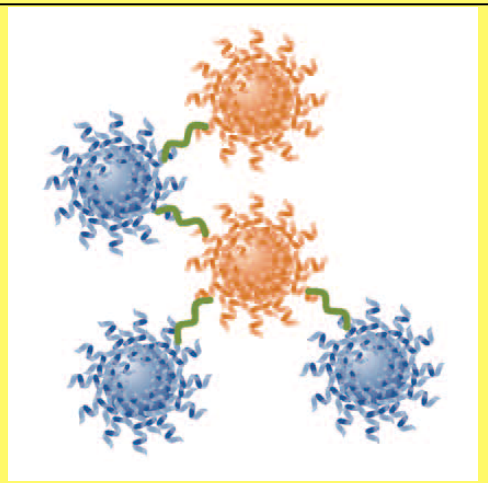
- Detection of *Candida* from whole blood

Whole blood (2mL) → **Lyse blood cells** → **Lyse *Candida*** → **PCR lysate**

Amplified DNA detecting using magnetic resonance technology



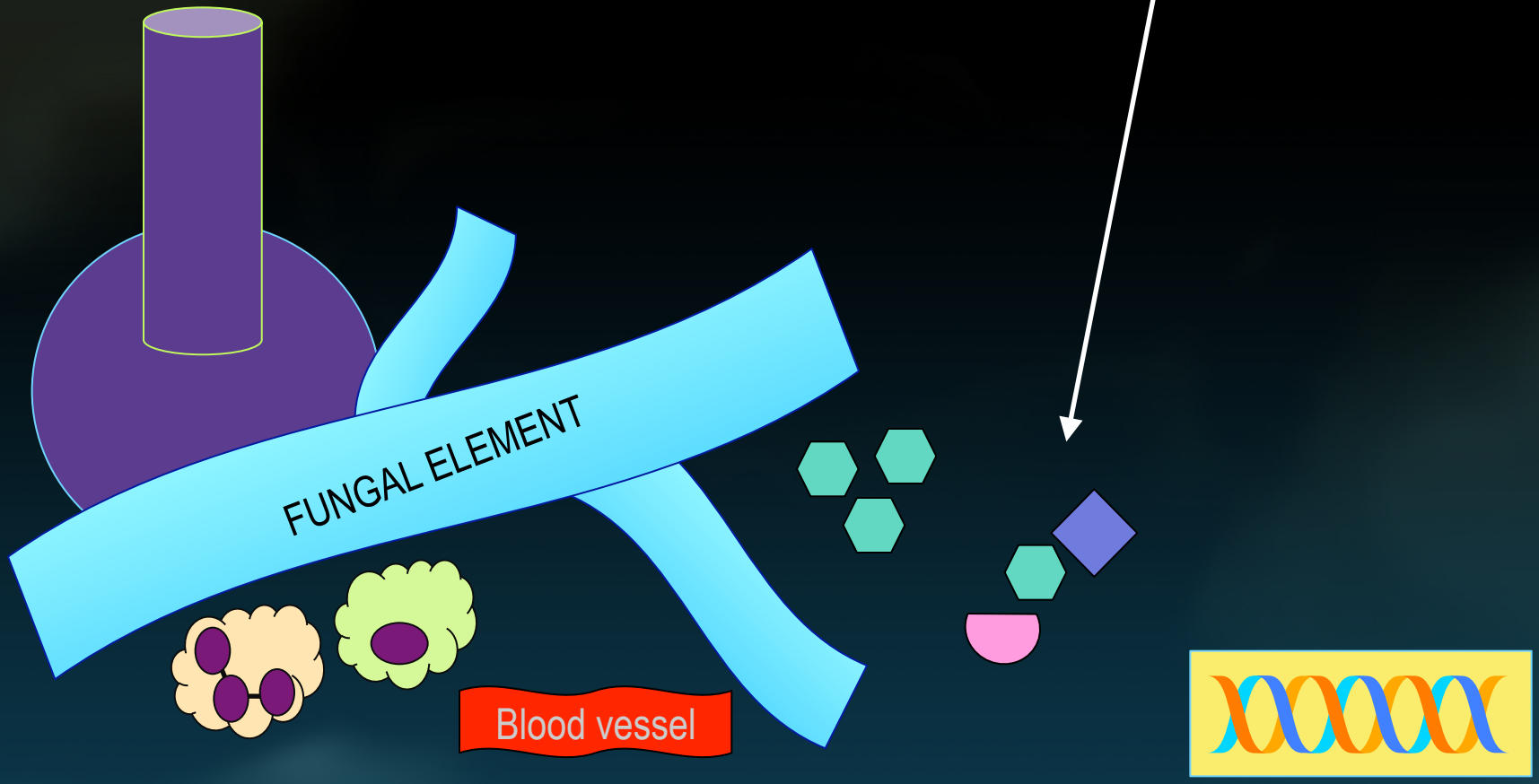




- **Time to detection: 3 hours**
- **Detects: *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis***
- **Analytical senstivity: 1 CFU / mL**

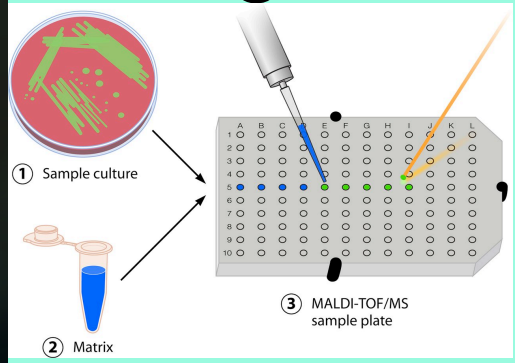
IFI DIAGNOSTICS:

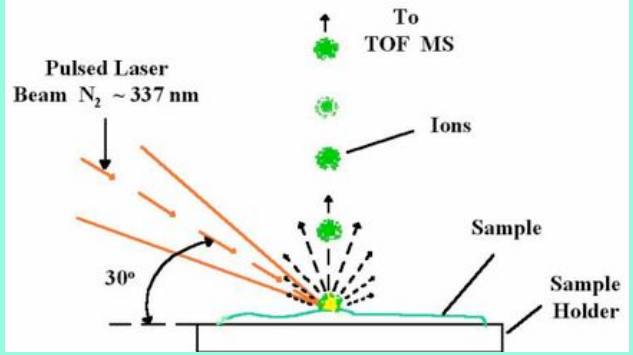
PROTEOMIC Analysis

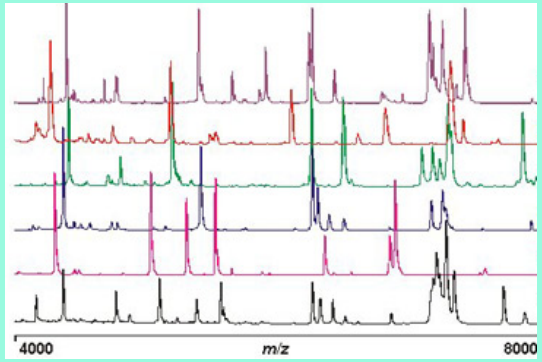


Proteomic Analysis: MALDI-TOF MS:

- Identifies protein fingerprints of unknown fungi based on spectral patterns







- >90-95% concordance with conventional identification

Advantages	Restrictions
↓ Time to identification	Standardize fungal growth
Identify FF	Standardize protein extraction
Has been applied to clinical specimens (e.g. Blood/*Candida*)	Database curation

SUMMARY:

- **EARLIER DIAGNOSIS: Key to Improved outcomes**
- **CLASSICAL micro / MOLECULAR micro**
- **Ongoing performance optimization & validation**
- **Incorporation into clinical trials**
  → Integration into clinical practice

Summary & Closing Remarks

Dr. Shariq Haider

Thank you!