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Rapid Diagnostics: Bacteriology & Mycology

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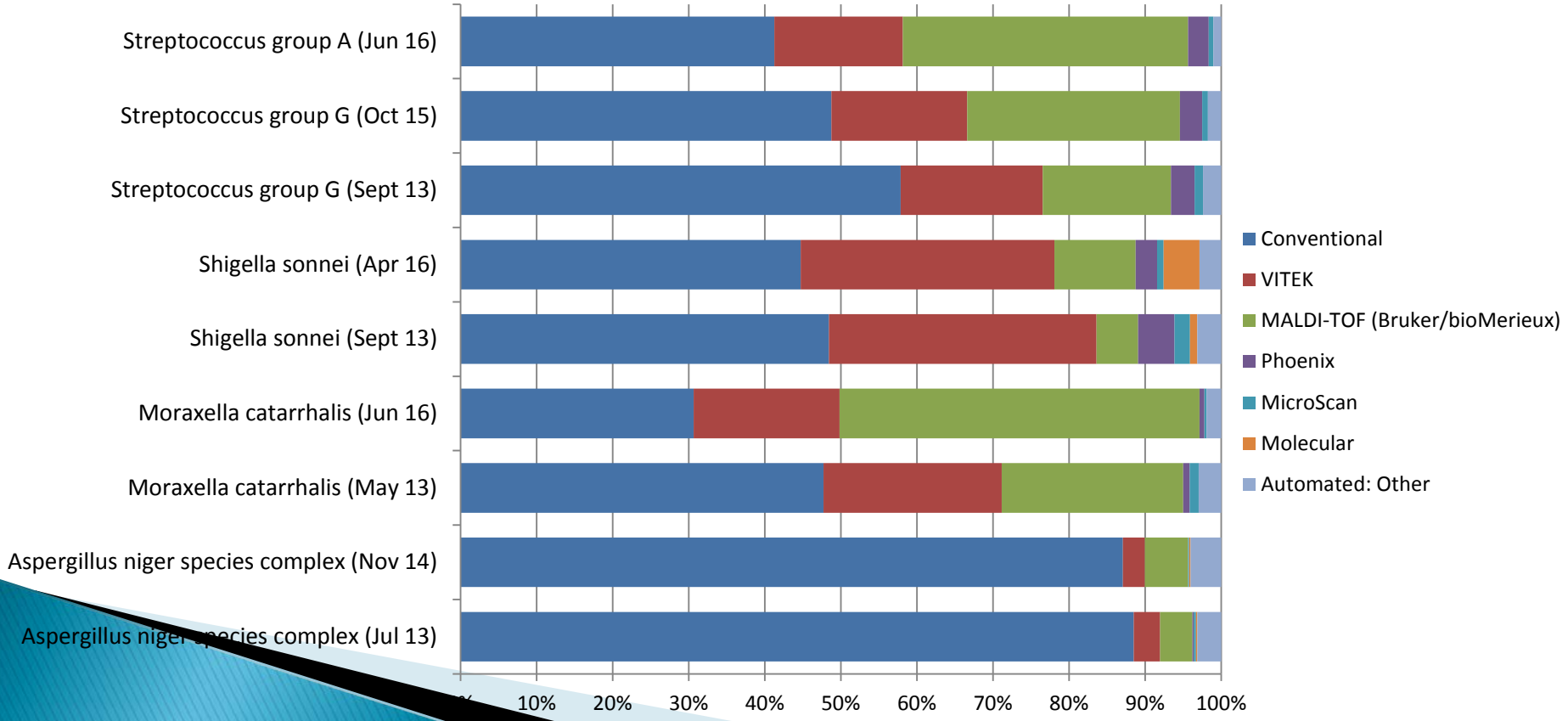
UK NEQAS Microbiology Division Scientific Meeting, 02.12.2016

General Bacteriology

- Trends in methodology
  - Gradual move from conventional methods to MALDI-ToF

Methods used by participants to identify pathogens sent out in UK NEQAS General bacteriology scheme



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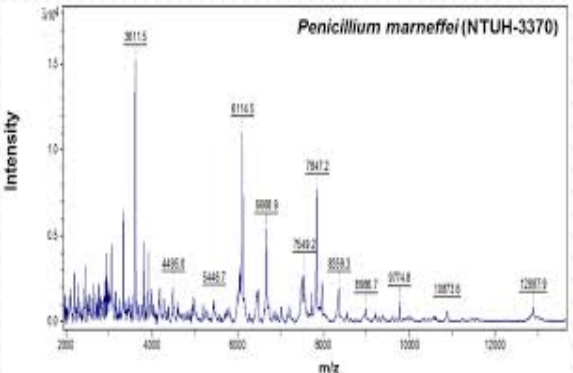
Proteomics

Advantages

- Power to identify the increasing numbers of micro-organisms
- Speed (~ 5mins)
- No downstream manipulation
- Minimal training
- ID good as the database

Limitations

- Need an existing database
- Initial outlay
- Immobile
- Skill required to analyse spectra



Non culture

- EntericBio RT PCR
  - TAT 3 hours
  - Easy to use – no previous molecular experience required
  - Negative screening reduces culturing 90-95%
  - Positive PCR results allow lab to perform targeted culturing specific to pathogen detected
  - Throughput of 48 samples per run
  - Test panels all use same setup procedure on a single platform

Urinary antigens

- Legionella:
  - Introduced in 2011
    - 5 EIA assays (including 1 in-house)
    - 5 rapid assays
  - 5 years later
    - 4 EIA assays
    - 11 rapid assays

	Distribution (Year)	
Legionella Assay	**2854 (Jun 2011)**	**3977 (Sept 2016)**
Bartels LUA EIA	6	11
Binax LUA EIA	5	18
BioRad Legionella EIA	3	2
Biotest LUA EIA	4	3
In house EIA	1	-
ACCUSAY Legionella	3	-
BinaxNOW Legionella	182	244
bioMerieux: bioNexia	-	1
Legionella Monlab Test	-	1
Legionella K-SeT	-	4
Legionella V-Test	1	6
Meridian: TRU	-	14
Pro-Lab: Proflow	-	4
Quidel: Sofia Legionella FIA	-	17
Remel: Xpect	10	-
SAS Legionella	1	-
Servibio: UriSign	-	1
SSI Diag: ImmuView	-	9
Trinity: Uni-Gold	-	3
EIA total	19 (8.8%)	34 (10.1%)
Rapid total	197 (91.2%)	304 (89.9%)

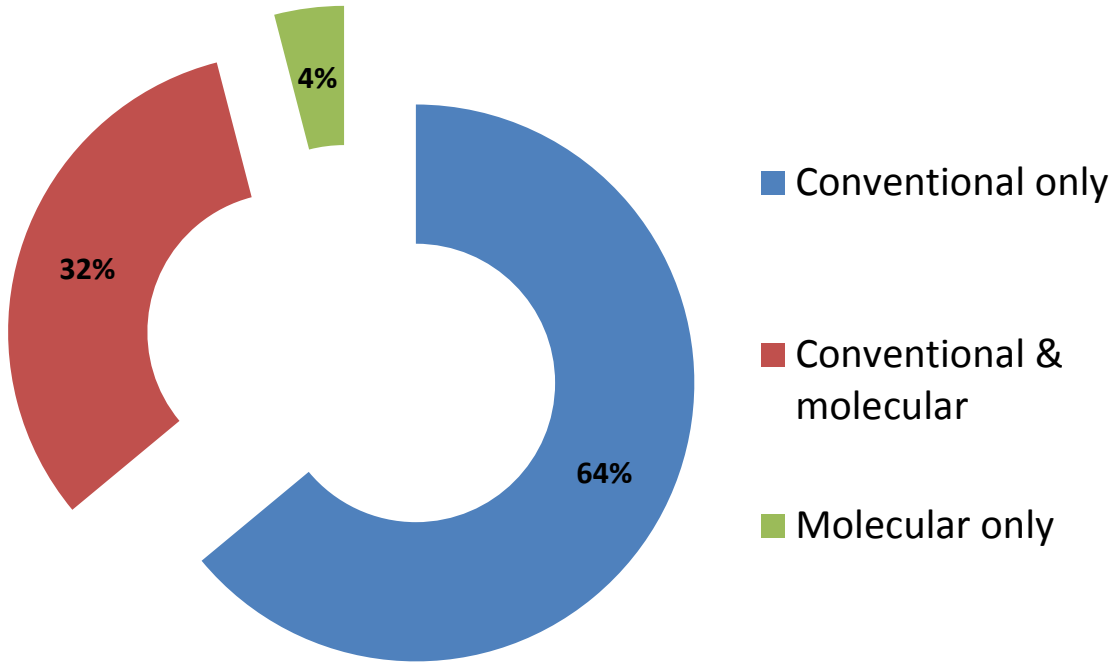
Urinary antigens

- Pneumococcal
  - Piloted in 2013
  - Introduced in 2014
    - 2 rapid assays
  - 2 years later
    - 6 rapid assays

	Distribution (Year)	
Pneumococcal assay	**3474 (May 2014)**	**3977 (Sept 2016)**
BinaxNOW S. pneumoniae	197	232
Meridian: TRU	–	5
Quidel: Sofia	–	11
Servibio: UriSign	–	1
SSI Diag: ImmuView	–	9
Trinity: Uni-Gold	3	6

MRSA screening

- Majority use conventional methods (309/322)
  - Chromogenic agar
- One third use molecular methods (116/322)
  - Cepheid Xpert



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Which is the most useful diagnostic test for fungal diagnosis?

- Direct microscopy (Gram, calcoflour, lactophenol)
- Culture (macro/microscopic)
- Histopathology (staining, immunofluorescence)
- Antibody detection (CFT, ID)
- Antigen detection (1-3 beta D glucan, galactomannan, mannan, histoplasma GM)
- Molecular techniques (18S, ITS 28S)
- Proteomics
- WGS/NGS

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Conventional /Traditional

Advantages	Limitations
Microscopy –direct investigations	Loss of experienced mycologists
Culture Gold standard—allows susceptibility testing	Increase in the diversity of fungal pathogens
Histopathology tissue biopsies	Time consuming
	Biopsies invasive and may not contain fungal elements

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Serology (Antibody)

Advantages	Limitations
Endemic mycoses	Sensitivity dependant on type of disease
Immunodiffusion (*Aspergillus*/*Candida* precipitans)	Negative result ≠ no fungus
Complement fixation for *Histoplasma* antibodies M and H antigens in urine	Time consuming
EIA for Coccidioidomycosis (patients unable to produce a sputum)	Experienced trained staff
	Patients have low immune response

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Antigen detection Galactomannan

Advantages	Limitations
Sample readily available Positive result before culture Ability for screening and or diagnostic monitoring of IA Excellent NPV, good PPV	Variable positive index for ELISA Variable storage conditions Manual (automation) False positives GM transient EQA

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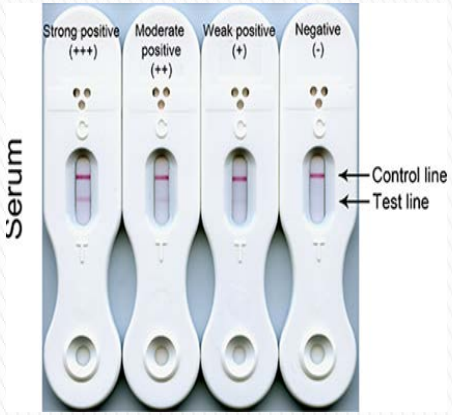
Antigen detection- POCT

Cryptococcal/Aspergillus

- Rapid
- Serum
- Qualitative and semi-quantitative detection of cryptococcal antigen.
- Quantative for Aspergillus
- Bedside



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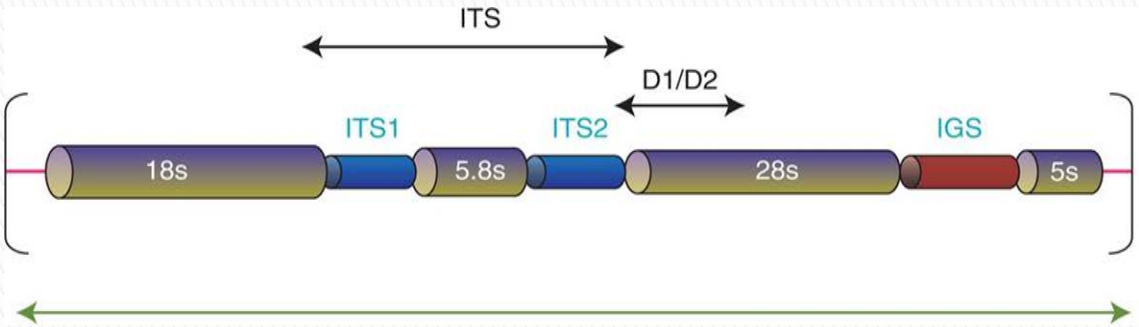
Molecular diagnostics–culture/ non culture

Advantages

- Power to identify the increasing numbers of fungi
- Taxonomy and phylogeny
- Con/RT/qPCR
- No need for viable cells
- No need for culture
- High sensitivity
- & specificity

Limitations

- Great variability of PCR methods and confirmatory nature of the technology
- Variable in PPV NPV
- Skill mix required
- Costly
- Genebank



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Proteomics

Advantages

- Power to identify the increasing numbers of fungi
- Speed (~ 5mins)
- No downstream manipulation
- Minimal training
- ID good as the database

Limitations

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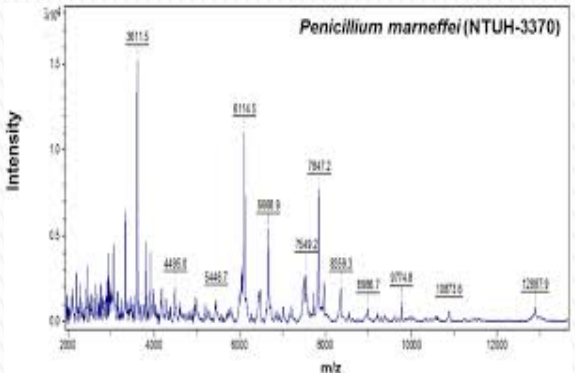
- Variability of spectra if tested at various growth conditions
- Initial outlay
- Immobile
- Skill required to analyse spectra



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Fungal diagnostics –IA

Diagnostic tests

- Conventional: Culture

Microscopy

- 1–3BetaDGlucan assay
- Galactomannan EIA
- Lateral flow device
- PCR
- Radiological findings
- Clinician assessment
- ?MALDI–ToF

Combination testing

- 78 BALS
- GM, beta-d-glucan, ALF, Conventional and PCR
- Sensitivity all 4 70–88%
- GM and ALF 94%
- GM and PCR 100% (spec for prob/prov 95–98%)

Hoeniq *et al* 2014 J. Clin Micro

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Summary

- Results from our schemes show the trend towards the use of rapid diagnostic test to compliment or replace conventional methods
- All diagnostic tests have their advantages and limitations.
- There is no stand alone test to provide a definitive diagnosis for fungi