Mycobacteriology & Mycology

APHL ID Lab Con 2023

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Comparison of three tests for latent tuberculosis infection in high-risk people in the USA: an observational cohort study

Observational Cohort Study comprised of people at high risk for latent tuberculosis infection (LTBI) or progression to TB disease

- Participants: \( n = 22,131 \) enrolled beginning July 2012
  - Enrolled at 18 TBESC-affiliated clinics at 10 sites across the U.S.
  - Tested for LTBI at study entry
  - Assessed for progression to TB disease for 2 years

- Three tests for LTBI: \( n = 20,149 \) with valid results for each
  1. Tuberculin Skin Test (TST)
  2. QuantiFERON (interferon gamma (IFN-\(\gamma\)) release assay, IGRA)
  3. T-SPOT.\(\text{TB}\) (IGRA)
Data analysis to:

• Determine agreement between TST and the 2 FDA-approved IGRA assays
• Determine the ability of each test to accurately predict progression to TB disease
• Assess concordance/discordance between IGRAs and TST to guide appropriate test utilization

Data stratified by important subgroups including:

• Age group (<5 years, 5–9 years, 10–14 years, 15–24 years, 25–44 years, 45–64 years, and ≥65 years)
• U.S. born/non-U.S. born
• Other risk groups: close contacts, homeless people, people living with HIV
Results
Proportion of positive test results for participants with valid results for all 3 test types, n=20,149

– Increase in age associated with increase in positive results.
– Non-US born participants had a higher proportion of positive results than US-born for any test, overall and by risk group.
– Ratio of positive TST to positive IGRA was inversely associated with age.
– Discordance between TST and IFN-γ release assay results varied by age among non-US-born participants and was greatest among the 848 non-US-born children younger than 5 years.

→204 (87.2%) of 234 non-US-born children younger than 5 years with at least one positive test were TST-positive and IGRA-negative.
Outcomes/Conclusion

• Test agreement was highest between the two IGRAs

• IGRAs should be used preferentially for the diagnosis of LTBI in high-risk populations, especially in children <5 years of age and older people born outside the U.S.
Whole Genome Sequencing Prediction of Antimicrobial Susceptibilities in Non-tuberculous Mycobacteria

Solanki et al. Front Microbiol 13:1044515
Mini review discussing methods for antimycobacterial susceptibility testing (AST) in non-tuberculous mycobacteria (NTM)

• Phenotypic AST challenges
  – 160 species – variations in treatment options for specific organisms (that requires specific AST)
    • Some species have intrinsic resistance
    • There is variant to variant specific AST profiles (e.g., inducible macrolide resistance in *M. abscessus* complex)
  – Limited standardized AST methods available for NTM
  – Guidelines for AST are limited to a few drugs, depending on NTM identification

• Can the model of WGS for TB be used for NTM?
<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Further improvements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypic DST</td>
<td>• Long TAT</td>
<td>• Research demonstrating the clinical importance of conflicting MICs</td>
</tr>
<tr>
<td>• Established method</td>
<td>• Varying incubation requirements</td>
<td>• Evaluating novel drugs with regard to plate media</td>
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<tr>
<td>• Reproducibility</td>
<td>• Complicated inoculum calibration</td>
<td></td>
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<tr>
<td>• Direct measurement of susceptibilities</td>
<td>• Inconsistent results</td>
<td></td>
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<tr>
<td>• Widely understood interpretation</td>
<td>• Subject to variation depending on technical experience</td>
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<tr>
<td></td>
<td>• True MIC could be between any two concentrations</td>
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<tr>
<td></td>
<td>• Poor correlation between <em>in vitro</em> drug sensitivity results and clinical outcomes</td>
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<tr>
<td>WGS</td>
<td>• Inferred susceptibility results</td>
<td>• Advancements in the accurate prediction of resistance in NTM via databases</td>
</tr>
<tr>
<td>• Identifies additional mutations compared to genotypic tests</td>
<td>• Lack of databases available which can accurately predict resistance</td>
<td>• Develop machine learning models for use with NTM</td>
</tr>
<tr>
<td>• Reduction in TAT</td>
<td>• Requires high value equipment and software</td>
<td>• Identify all mutations associated with novel drugs</td>
</tr>
<tr>
<td>• Concordance between phenotypic and WGS results</td>
<td>• Requires a skilled bioinformatician</td>
<td>• The need for clinical studies describing the value WGS adds on front line decision making</td>
</tr>
<tr>
<td>• Skill set widely available</td>
<td>• DNA must be extracted from positive cultures and cannot be performed directly from primary samples</td>
<td>• Investigate the cost-effectiveness</td>
</tr>
<tr>
<td>• Accessible in low and middle-income countries</td>
<td>• Impact of WGS on NTM treatment outcomes are unknown</td>
<td></td>
</tr>
<tr>
<td>• Can be used in conjunction with machine learning without the need for databases of known resistance genes</td>
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</tbody>
</table>
Next Steps

• Need for a database for NTM that has WGS data matched with AST data (like CRyPTIC For TB).
  – Comprehensive Antibiotic Resistance Database (CARD) is a collection of well-characterized, peer-reviewed resistance determinants and their associated antimicrobials (NTM data is included).
  – There are newer drugs for treatment and gene mutations associated with resistance are yet to be elucidated, as well as guidelines and standards for phenotypic AST

• New CLSI M24S published Feb 27, 2023. Few changes including addition of species to *M. fortuitum* group, testing for different colony types of MAC and addition of QC ranges for fobrepodacin and omadacycline.
Recognition of Diagnostic Gaps for Laboratory Diagnosis of Fungal Diseases: Expert Opinion from the Fungal Diagnostics Laboratories Consortium

The recently created Fungal Diagnostics Laboratory Consortium (FDLC) comprises 26 diagnostic laboratories in North America and has the following goals:

• Survey the fungal diagnostic gaps that exist (this paper)
• Provide diagnostic mycology services to immunocompromised patient populations
• Improve availability and access to fungal diagnostics
• Facilitate and enhance applied research and collaboration
• Work with industry to support new assay development, commercialization and implementation
Survey gaps:

→ 5 fungal diagnostic priorities

→ 6 disease-specific gaps

→ 6 method/approach specific gaps
Priority 1): Development of best practice guidelines for fungal diagnostics

Priority 2): Development/validation of standardized NAAT for molecular detection of fungi directly in clinical specimens

Priority 3): Development and multi-center validation of new diagnostic tests

Priority 4): Workforce development – overcome laboratory shortage of staff with knowledge and skillsets in performing fungal diagnostic tests

Priority 5): Laboratory capacity building – enhance laboratory diagnostic tests for rapid and accurate diagnosis of fungal diseases

Disease-specific gaps
I. Pneumocystis pneumonia
II. Mucormycosis
III. Aspergillosis
IV. Candidemia
V. Endemic mycoses
VI. Emerging and rare mycoses

Method/approach-specific gaps
I. Direct identification of fungal pathogens in FFPE tissues
II. Mold identification by MALDI-TOF MS
III. Mold blood culture
IV. Fungal point-of-care test
V. Cystic fibrosis fungal culture
VI. Antifungal susceptibility testing
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Emerging and Rare Mycoses

• Example: Multi drug resistant *Candida auris*

• Current methods for ID:
  – MALDI-ToF (commercial biochemical systems e.g., Vitek2, MicroScan may misidentify)
  – LDT NAAT (some methods directly on the specimen), but no FDA-cleared rapid molecular methods

• Diagnostic gap: an easy-to-use, cost-effective, rapid, direct-on-specimen, standardized, NAAT

• Solution: Multicenter development of a NAAT
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Antifungal Susceptibility Testing

• Current methods for AST:
  – CLSI reference methods: broth microdilution, disk diffusion
  – Established breakpoints: most-common pathogenic yeasts; one for molds, *Aspergillus* and voriconazole

• Diagnostic gap: Few established breakpoints (low frequency of fungal disease makes determination challenging), ECVs may be of use but are institution-specific

• Solution: Perform longitudinal study with clinical outcome data, accurate species ID and MIC data to establish clinical breakpoints, and for less-common bug/drug combinations, novel drug classes and emerging pathogens e.g., *C. auris*. 
<table>
<thead>
<tr>
<th>Approach</th>
<th>Microscopy/histology</th>
<th>Culture</th>
<th>MALDI-TOFMS</th>
<th>Sequencing ID</th>
<th>BDG</th>
<th>GM</th>
<th>Antigen antibody</th>
<th>NAAT</th>
<th>Diagnostic gap</th>
<th>Proposal</th>
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<tbody>
<tr>
<td>Disease specific</td>
<td></td>
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<td></td>
<td>NAAT cannot differentiate colonization and mal infection; BDG has low specificity</td>
<td>Standardize NAAT; NAAT (particularly qNAAT) coupled with BDG for accurate diagnosis</td>
</tr>
<tr>
<td>Pneumocystis pneumonia</td>
<td>+/+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−/−</td>
<td>+</td>
<td>NAAT cannot differentiate colonization and mal infection; BDG has low specificity</td>
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<tr>
<td>Mucomycosis</td>
<td>+/+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−/−</td>
<td>+</td>
<td>Culture/histology lacks sensitivity and speed for diagnosis</td>
<td>Define optimal tissue process for culture; standardize NAAT for screening and easy diagnosis</td>
</tr>
<tr>
<td>Aspergillosis</td>
<td>+/+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>Lack of NAAT standardization; insufficient data to understand the utility of NAAT in conjunction with GM to optimize diagnosis</td>
<td>Standardize NAAT; multi-center clinical trials to evaluate NAAT in conjunction with GM to optimize diagnosis</td>
</tr>
<tr>
<td>Candidemia</td>
<td>+/+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>Naive culture based detection of Candida species in blood</td>
<td>Clinical trials are needed to address the feasibility of implementing non-culture-based rapid detection platforms and determine their clinical impact and outcomes</td>
</tr>
<tr>
<td>Endemic mycoses</td>
<td>+/+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+/+</td>
<td>+</td>
<td>Lack of availability and ability to perform antigen and antibody assays in clinical laboratories</td>
<td>Development of low-complexity antigen/serology assays may increase clinical laboratores’ capabilities in timely diagnosis of disease</td>
</tr>
<tr>
<td>Emerging and rare fungal infections</td>
<td>+/+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−/−</td>
<td>+</td>
<td>Lack of knowledge and capabilities to detect and recognize these emerging, rare or uncommon fungal pathogens</td>
<td>Multicenter efforts to understand the prevalence, characteristics, and clinical features of these rare fungal infections</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Method/Approach specific</th>
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<tbody>
<tr>
<td>Fungal ID in FFPE</td>
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<td></td>
<td>Difficulty with identification to species of fungal pathogens present in FFPE tissues</td>
<td>Standardize molecular approach to directly identify fungal pathogens present in FFPE tissues</td>
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<td>Mold ID by</td>
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<td></td>
<td>Limited diagnostic spectra for molds in MALDI databases</td>
<td>Standardize and expand MALDI mold database</td>
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<td>MALDI-TOF MS</td>
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<td>Suboptimal recovery of mold from blood culture</td>
<td>Multicenter efforts to optimize and standardize mold blood culture</td>
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<td></td>
<td>Lack of POC for rapid diagnosis of fungal diseases</td>
<td>Implement low-complexity and affordable LSA assays to increase clinical laboratory capacity for fungal diagnosis</td>
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<td>Fungal POCT</td>
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<td>Fungal culture for CF</td>
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<td>Lack of standardized laboratory approach for fungal culture in CF</td>
<td>Standardize CF fungal culture protocol</td>
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<td>AST</td>
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<td>Increasing clinical need for antifungal drug susceptibility testing, particularly for molds</td>
<td>Multicenter efforts to develop a feasible and affordable approach for mold susceptibility testing in clinical laboratories</td>
</tr>
</tbody>
</table>

*AST, antifungal susceptibility testing; BDG, 1,3-β-D-glucan; CF, cystic fibrosis; FFPE, formalin-fixed and paraffin embedded; GM, galactomannan; ID, identification; NAAT, nucleic acid amplification test; qNAAT, quantitative NAAT; POC, point-of-care test; +, available; −, not available.
In summary, this information is tabulated according to the Approach, Methods, Diagnostic Gap and Proposed action going forward.

- Example, *Pneumocystis* pneumonia

**TABLE 2** Summary of fungal disease-specific and method/approach-specific diagnostic gaps and proposals to fill the gaps

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