NEW YORK STATE Department of Health | Wadsworth Center

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

Ho *et al*. Lancet Infect Dis. 2022 January ; 22(1): 85–96.

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-γ) release assay, IGRA)
  3. T-SPOT.*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?

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

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

Zhang *et al*. J Clin Microbiol 59:e01784-20.

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

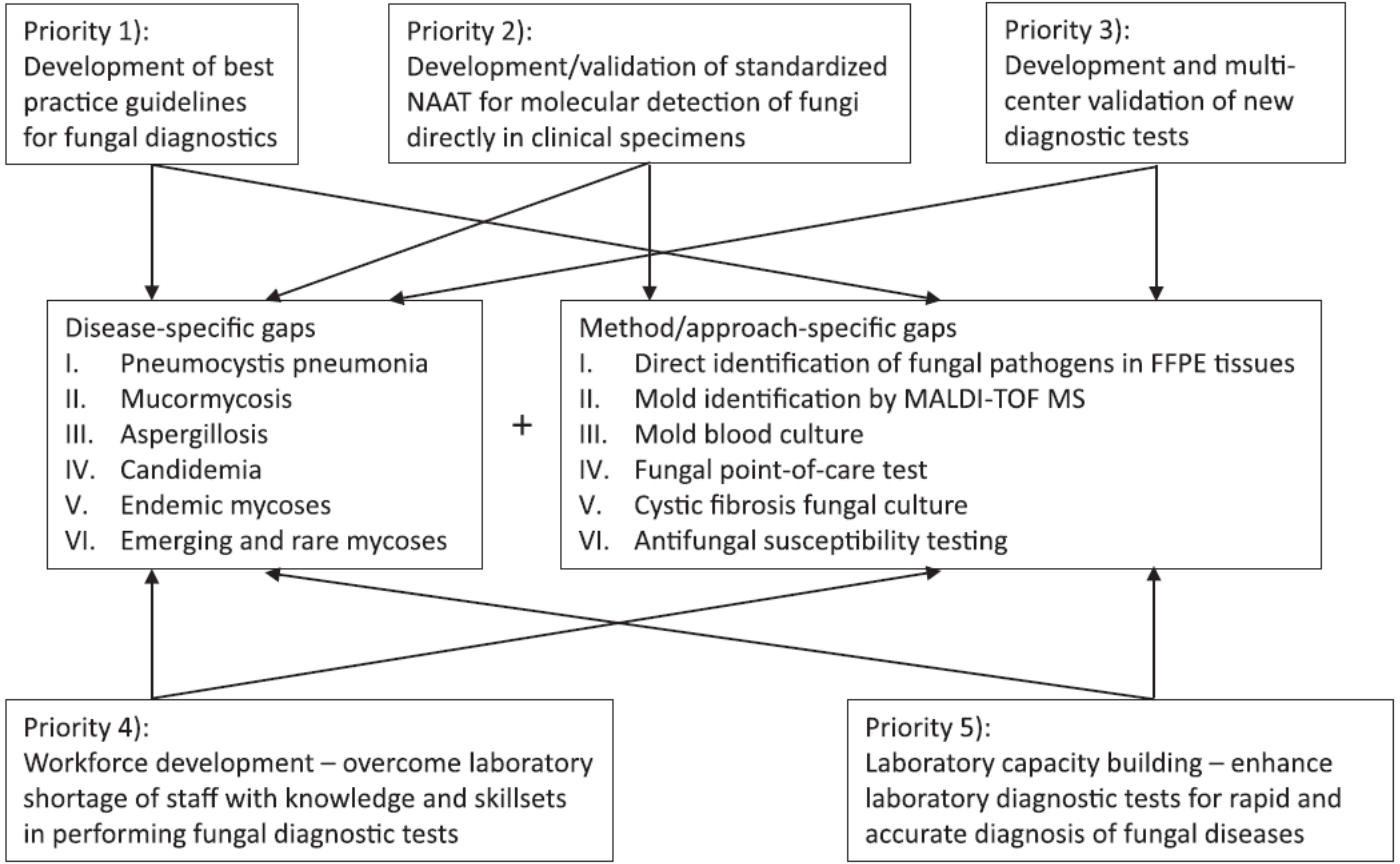
- Survey the fungal diagnostic gaps that exist (this paper)
- Provide diagnostic mycology services to immunocompromised patient populations
- Improve availability and access to fungal diagnostics
- Facilitate and enhance applied research and collaboration
- Work with industry to support new assay development,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

Survey gaps:

→ 5 fungal diagnostic priorities

→ 6 disease-specific gaps

→ 6 method/approach specific gaps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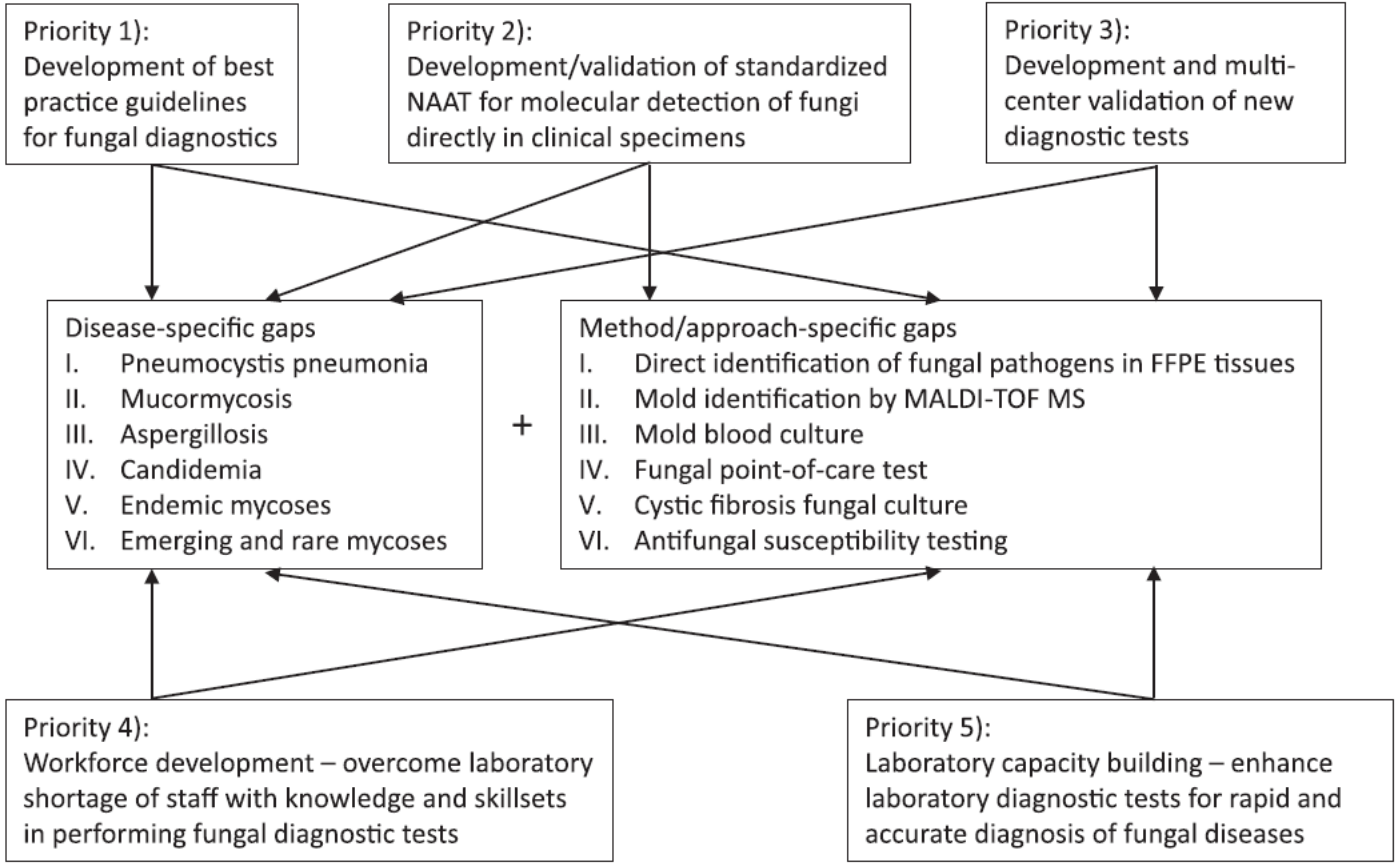
Development of best practice guidelines for fungal diagnostics
Priority 2):
Development/validation of standardized NAAT for molecular detection of fungi directly in clinical specimens
Priority 3):
Development and multi-center validation of new diagnostic tests
Disease-specific gaps
I. Pneumocystis pneumonia
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
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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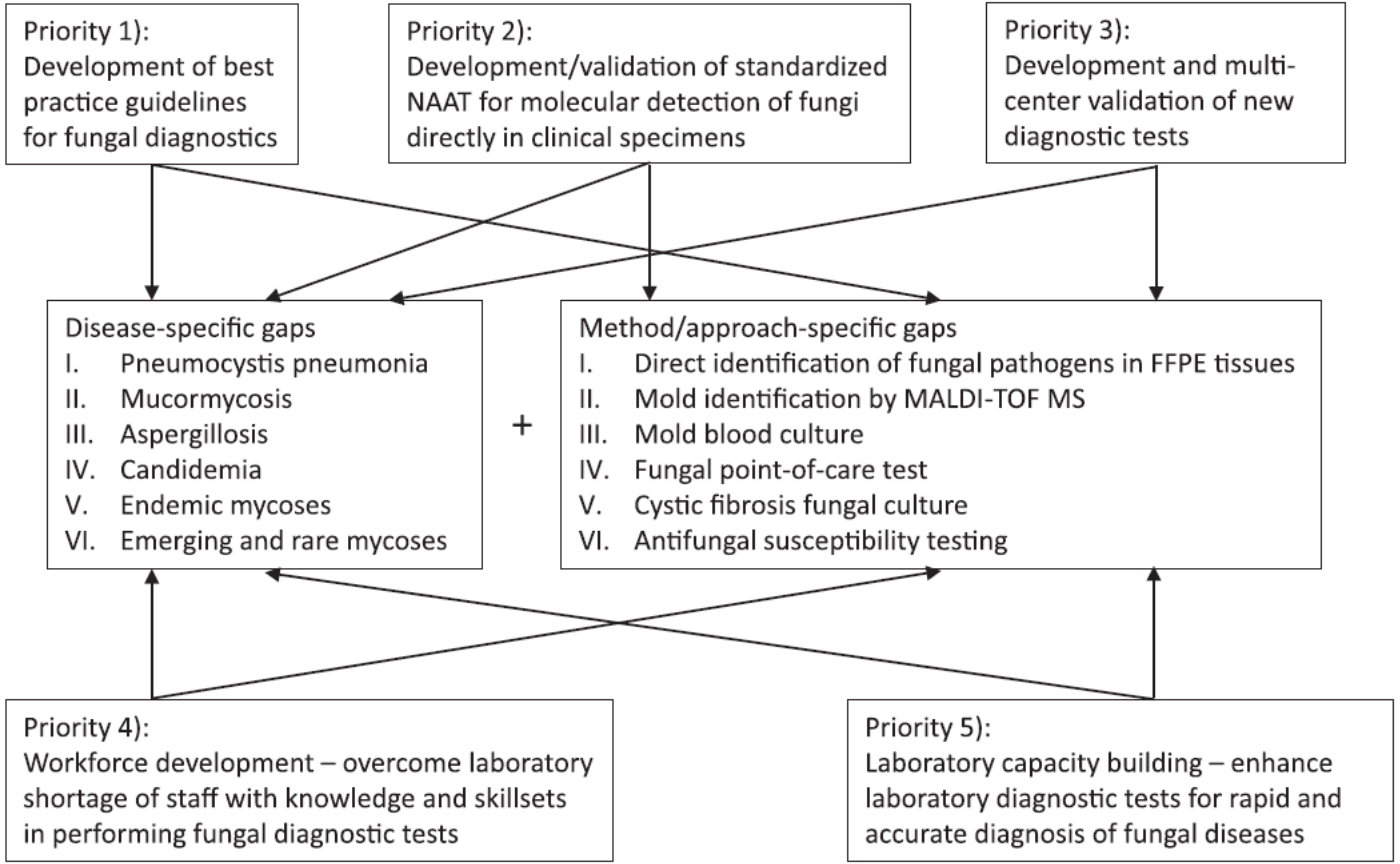
Emerging and Rare Mycoses

- 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 2 Summary of fungal disease-specific and method/approach-specific diagnostic gaps and proposals to fill the gaps[a]

Approach	Method: Microscopy/ histology	Culture	MALDI-TOF MS	Sequencing ID	BDG	GM	Antigen/ antibody	NAAT	Diagnostic gap	Proposal
Disease specific										
Pneumocystis pneumonia	+/+	−	−	+	+	−	−/+	+	NAAT cannot differentiate colonization and real infection; BDG has low specificity	Standardize NAAT; NAAT (particularly qNAAT) coupled with BDG for accurate diagnosis
Mucormycosis	+/+	+	+	+	−	−	−/−	+	Culture/histology lacks sensitivity and speed for diagnosis	Define optimal tissue process for culture; standardize NAAT for screening and early diagnosis
Aspergillosis	+/+	+	+	+	+	+	+/−	+	Lack of NAAT standardization; insufficient data to understand the utility of NAAT in conjunction with GM	Standardize NAAT; multicenter clinical trials to evaluate NAAT in conjunction with GM to optimize diagnosis
Candidemia	+/+	+	+	+	+	−	+/−	+	Non-culture-based direct detection of *Candida* species in blood	Clinical trials are needed to address the feasibility of implementing non-culture-based rapid detection platforms and determine their clinical impact and outcomes
Endemic mycoses	+/+	+	+	+	+	−	+/+	+	Lack of availability and ability to perform antigen and antibody assays in clinical laboratories	Development of low-complexity antigen/ serology assays may increase clinical laboratories' capabilities in timely diagnosis of disease
Emerging and rare fungal infections	+/+	+	+	+	+	−	−/−	+	Lack of knowledge and capabilities to detect and recognize these emerging, rare or uncommon fungal pathogens	Multicenter efforts to understand the prevalence, characteristics, and clinical features of these rare fungal infections
Method/approach specific										
Fungal ID in FFPE									Difficulty with identification to species of fungal pathogens present in FFPE tissues	Standardize molecular approach to directly identify fungal pathogens present in FFPE tissues
Mold ID by MALDI-TOF MS									Limited diagnostic spectra for molds in MALDI databases	Standardize and expand MALDI mold database
Mold blood culture									Suboptimal recovery of mold from blood culture	Multicenter efforts to optimize and standardize mold blood culture
Fungal POCT									Lack of POCT for rapid diagnosis of fungal diseases	Implement low-complexity and affordable LFA assays to increase clinical laboratory capacity for fungal diagnosis
Fungal culture for CF									Lack of standardize laboratory approach for fungal culture in CF	Standardize CF fungal culture protocol
AST									Increasing clinical need for antifungal drug susceptibility testing, particularly for molds	Multicenter efforts to develop a feasible and affordable approach for mold susceptibility testing in clinical laboratories

[a]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; POCT, 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

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