What updated ID guidelines are said and done

Best practices for collection and handling of blood cultures

American Journal of Infection Control 2015;43:1222-37

Nontuberculous Mycobacterial Pulmonary Disease

Clinical Infectious Diseases 2020;71:e1–e36

Microbiological Laboratory Testing in the Diagnosis of Invasive Fungal Infections

Am J Respir Crit Care Med 2019;200:535–550.

Bacteremia and fungemia

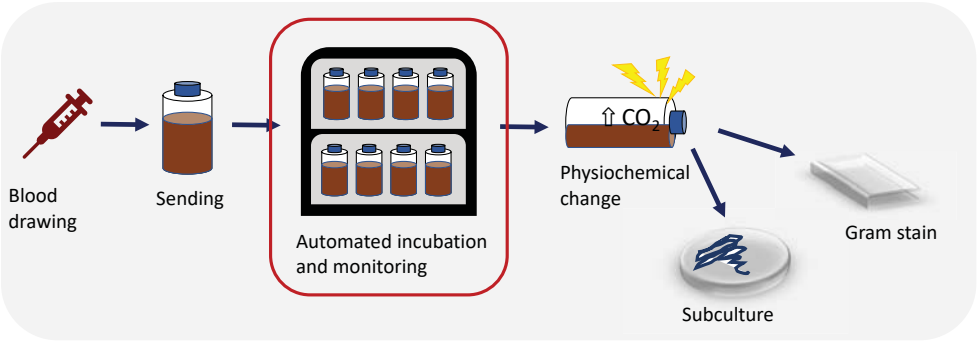
Antimicrobial effects of blood peptides and/or antibiotics › **Microorganism load is low and intermittent**

Clinical Infectious Diseases 2016;63:1332–9

Best practices for collection and handling of blood culture

- Appropriate volume of blood
- Number of sets
- Timing
- Proper handling and transport
- BC bottles with antibiotic binding agents

American Journal of Infection Control 2015;43:1222-37



Automated blood culture detection system

Workflow of blood culture collection and handling

How it can optimize microorganism detection

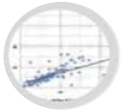
BacT/Alert Virtuo BC system

Automatic robotic loading

Directly measure blood volume and immediate notification

Automatic scanning for full traceability

New algorithm to faster time of detection



Automated unloading of negative bottles

J Clin Microbiol 2017;55:2413–2421.
www.biomerieux-usa.com

1

BacT/Alert Virtuo vs. BacT/Alert 3D blood culture systems

(Aerobic and anarobic bottle set)

Comparison of positive rate and time to detection (TTD)

Jacobs *et al*, 2017

Total 5,709 sets (POSITIVE 430 sets)

Positivity rates – comparable

Mean TTD (h)

Virtuo **15.9** h vs. BTA3D **17** h (p = 0.001), particularly shorter for enteric GNR and enterococci

J Clin Microbiol 2017;55:2413–2421.

2

BacT/Alert Virtuo vs. BacT/Alert 3D blood culture systems

(Aerobic and anarobic bottle set)

Comparison of positive rate and time to detection (TTD)

Kim *et al*, 2019

Total 1,904 sets (POSITIVE 623 sets)

Positivity rates – comparable

Median TTD (h)
Virtuo **11.5** h vs. BTA3D **11.8** h ($p < 0.001$), particularly shorter for *E. coli* and *S. aureus*

Ann Lab Med 2019;39:278-283.

Up-to-date trend

Automated blood culture detection system with
- Full automation
- Shorten time to detection

Non-tuberculous mycobacterial (NTM) pulmonary disease

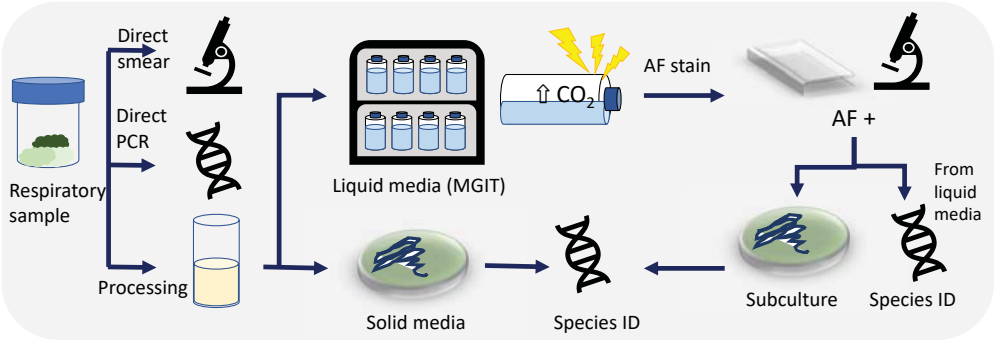
NTM pulmonary disease

An official ATS/ERS/ESCMID/IDSA clinical practice guideline 2020

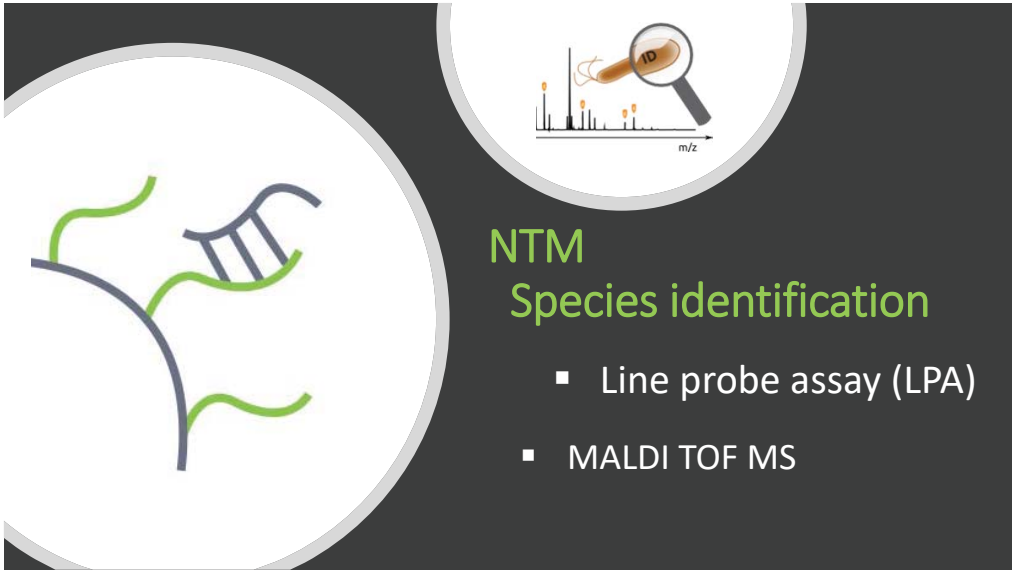
Laboratory diagnosis of respiratory sample

- Collect at least 3 samples over an interval of at least a week
- Culture on both liquid and solid media
- Correct species identification
  (*for clinical significance and treatment regimen*)

Clinical Infectious Diseases 2020;71:e1–e36

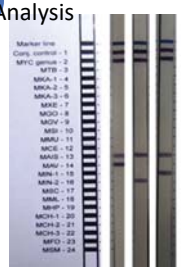


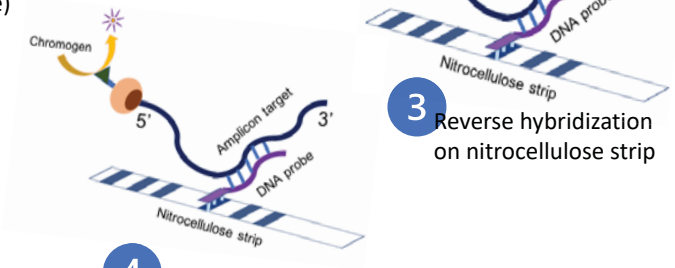
Workflow of respiratory sample for mycobacterial culture



Line probe assay key steps

1. DNA extraction from culture isolates (liquid or solid culture)
2. Amplification
3. Reverse hybridization on nitrocellulose strip
4. Chromogenic detection
5. Analysis





LPA vs. Conventional testing or standard PCR and gene sequencings for species ID

No. of isolates	Accuracy
INNO-LiPA Mycobacteria	
157 (reference and clinical)	99.4%
GenoType Mycobacterium CM/AS	
131 (clinical)	90.8 %
317 (clinical)	91.2%

Limitations

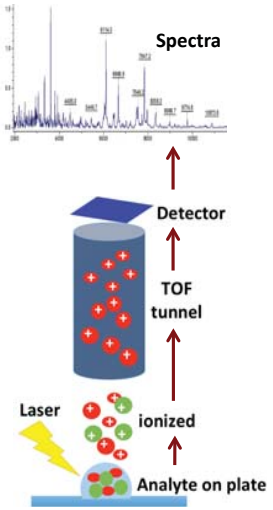
M. avium-M. intracellulare-M. scrofulaceum or *M. malmoense*; *M. fortuitum* complex; *M. abscessus-chelonae*; *M. szulgai*; *M. simiae*

M. lentiflavum; *M. abscessus*
Uncommon species cross reactivity with *M. intracellulare, M. fortuitum*

J Clin Microbiol 2001;39:4477–4482., Journal of Medical Microbiology 2009;58:900–904., J Clin Microbiol 2010;48:307–310.

Matrix-assisted laser desorption ionization time-of-flight mass spectometry (MALDI-TOF MS)

1. Isolate mixed with matrix or analyte is placed on the plate
2. Laser desorbs the analyte into small-ionized molecules
3. The molecules are funneled through an TOF tunnel
4. Molecules of different masses/charges fly at different speeds
5. The molecules pass through an ion detector
6. Production of mass spectra unique to specific genera or species



Clin Microbiol Infect 2010;16:1604-13., Clin Infect Dis 2013;57:564-72.

MALTI TOF: A developing tool for mycobacteria ID

- New extraction protocols enhance the amount of proteins available for ID
- Updating mycobacterial spectra in commercial databases

Clinical Microbiology and Infection 2018;24:599-603

Vitek MS
(Biomerieux, France)

Saramis v4.12: 1,286 spectra from 45 *Mycobacterium* spp.

Biotyper
(Bruker, USA)

Bruker v5.0: 912 spectra from 159 *Mycobacterium* spp.

Study timelines of MALDI TOF for mycobacteria ID

No. of isolates[a]	Year	Test (version)	Reference assay[b]	Accuracy
178	2013	Bruker (v3.0)	Gene sequencing	93.8% (species level), 98.3% (genus level) (score ≥ 2)
199	2014	Bruker (v3.1) vs. Vitek MS (Saramis 4.12)	-	Overall 94.9% (species level)[c] Vitek MS 87.4%, Bruker 79.3%[c]
125	2015	Bruker (v3.1)	Gene sequencing	Overall 51.2% (score ≥ 2), and 68.8% (score ≥ 1.9)
244	2019	Bruker (v5.0) vs. Vitek MS (v3.0)	Gene sequencing	**In complex/group level:** Overall, Bruker 92% vs. Vitek MS 95% **In species/subspecies level** Overall, Bruker 62% vs. Vitek MS 57%

[a] Clinical and/or reference strains
[b] 16S rRNA, rpoB, and/or hsp65 gene sequencing
[c] Combined U of Washington/Bruker database with UW extraction protocol

J Clin Microbiol 2013;51:2875–2879., J Clin Microbiol 2014;52:130–138., J Clin Microbiol 2015;53:2737–2740., Am J Clin Pathol 2019;152:527-536.

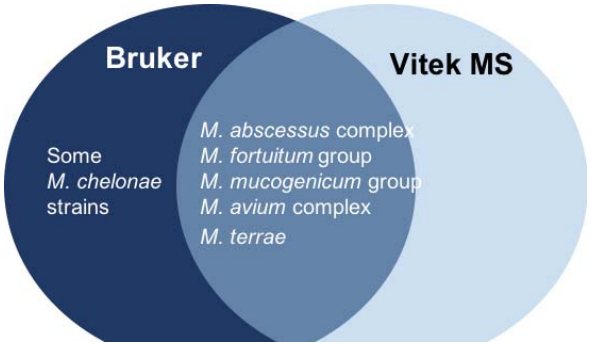
MALDI TOF ID from automated liquid media with smear POS

- Decontaminate and digest smear POS sputum sample
- Inoculate into automated liquid media bottles
- Once positive, process POS bottle for Vitek MS v3.0

Total **73** isolates	Overall (%)
Correct ID	88%
No ID	9%
Mis ID	3%

J Clin Microbiol 2018;56:e00219-18.

Limitations of MALDI TOF for mycobacteria ID



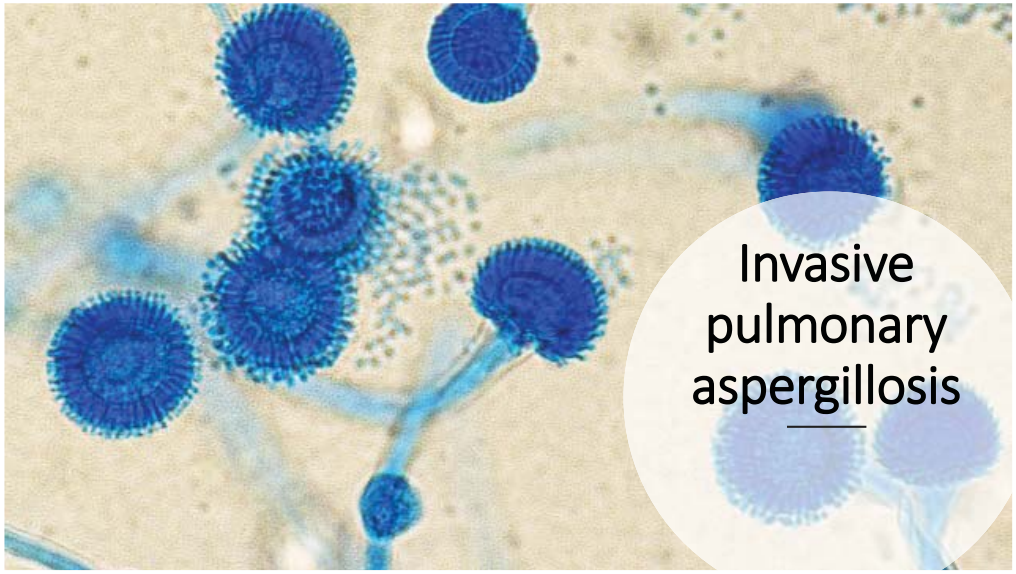
- Solid vs liquid media
- Extraction protocol
- Database version
- **Close related genera and complex**

Am J Clin Pathol 2019;152:527-536.
Clinical Microbiology and Infection 2018;24:599-603.

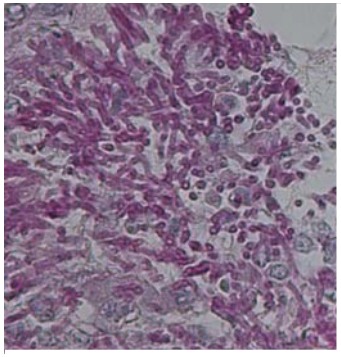
Up-to-date trend

Correct species ID for NTM isolates by using molecular assays

- Judgement of clinically significant isolate
- Decision on treatment regimen



Invasive pulmonary aspergillosis



Sterile material (lung tissue biopsy)

POS histopathology (with evidence of tissue damage) | POS culture (with clinical features of infection) | POS Aspergillus PCR from tissue patho.(when fungal hyphae seen)

Proven invasive pulmonary aspergillosis (IPA)

Clinical Infectious Diseases 2020;71:1367–76.

Host factors

- Prolonged neutropenia
- Hematologic malignancy
- Receipt of HSCT or SOT
- Prolonged Rx of corticosteroids
- Rx with T-cell immunosuppressants
- Rx with TKI
- Inherited severe ID

Radiological findings

- Dense, well-circumscribed lesion(s) with or without a halo sign
- Air crescent sign
- Cavity
- Wedge-shaped and segmental or lobar consolidation

Mycological evidence

- POS Aspergillus culture or POS fungal elements from non-sterile samples
- POS galactomannan (GM) from blood or BAL sample
- POS Aspergillus PCR from blood and/or BAL sample

Probable IPA

Clinical Infectious Diseases 2020;71:1367–76.

29 studies (2000 - 2018)

Cochrane Database of Systematic Reviews 2019, DOI: 10.1002/14651858.CD009551.pub4.

Blood Aspergillus PCR: A meta-analysis

- Populations: Hematologic malignancy or HSCT
- Several PCR protocols and gene targets
- Serum or whole blood 200 µL – 10 mL
- Reference standard: IA EORTC/MSG criteria
- Mean prevalence (proven/probable IA) **16.3** %

Blood Aspergillus PCR: A meta-analysis

PCR testing	Results	
1 single POS PCR test	Sensitivity 79%,	20% **false NEG** and 20% **false POS**
	Specificity 80%,	
≥ 2 POS PCR tests	Sensitivity 60%,	40% **false NEG** and 5% **false POS**
	Specificity 95%,	

" PCR shows moderate diagnostic accuracy in high-risk patient groups "

- The subgroup analyses show antifungal prophylaxis might impair test performance

Cochrane Database of Systematic Reviews 2019, DOI: 10.1002/14651858.CD009551.pub4.

Limitations of blood Aspergillus PCR

- Non-neutropenic patients
- On mold-active antifungal prophylaxis
- No standardization protocol
- Standardized interpretative criteria

J Fungi 2020;6:18, doi:10.3390/jof6010018

Galactomannan

- Widely used testing
- Cross reactivity
- False positivity

Aspergillus PCR

- Limited availability
- High specificity
- Cost

Galactomannan **vs.** Aspergillus PCR

When combined blood Aspergillus PCR + serum GM

- Increased diagnostic test accuracy (**60** → **90**%)
- Give an early diagnosis of IPA
- Increased proven or probable IA–free survival

Br J Haematol 2013;161:517–524.
Clin Infect Dis 2015;60:405–414.

Up-to-date trend

Diagnosis of IPA

- Using more Aspergillus PCR testing
- A combination of GM and PCR testing

Clinical Microbiology: Up to date
Microbiological Diagnosis and Identification

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