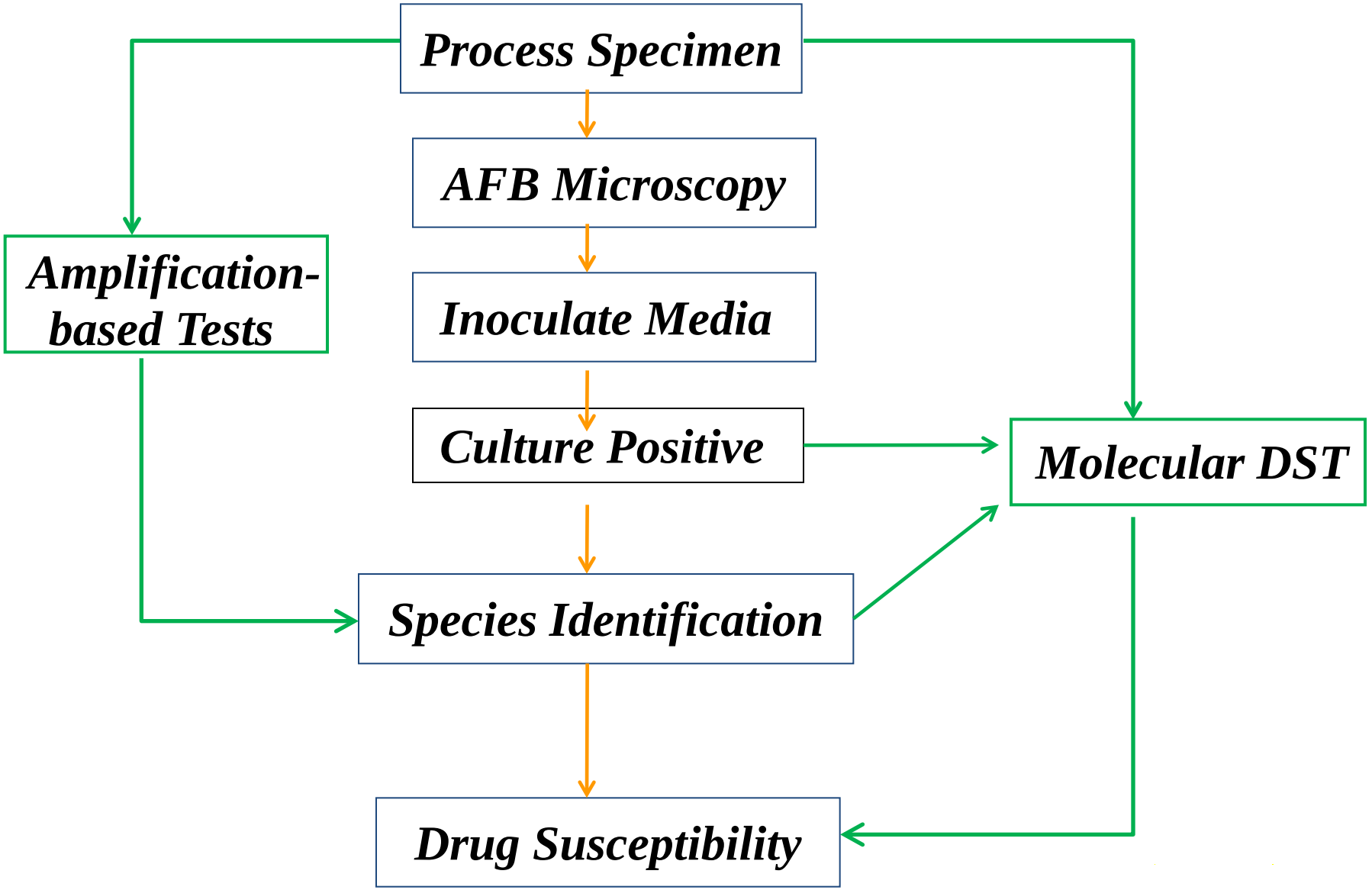
Overview: Mycobacterial Culture, Identification, and Drug Susceptibility Testing

Mycobacterial Testing Algorithm



Overview, Purpose, and Methods

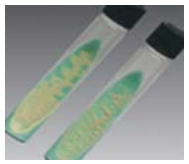
MYCOBACTERIAL CULTURE

Mycobacterial Culture

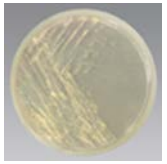
- Gold standard for detection of *Mycobacterium tuberculosis* complex (MTBC)
- Use of culture increases the number of tuberculosis (TB) cases found over smear alone
  - For MTBC, fewer organisms needed for positive culture than for positive AFB smear
- Culture used for species identification, drug susceptibility testing (DST), and genotyping
- Culture also used to monitor patient response to treatment

Culture Media

- Two major categories of media
  - Solid: egg-based and agar-based
  - Liquid: also often referred to as broth media
    - Used with automated systems
    - 3 are FDA cleared in US:



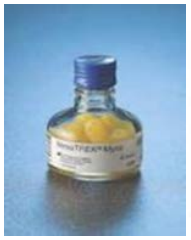
LJ media



7- H 11 media

Biomerieux
BacT/ALERT® 3D

Becton Dickinson
BACTEC MGIT™



Thermo Scientific
VersaTREK™

- Most labs use liquid and one type of solid

Reporting

- Negative report issued at 6–8 weeks
  - Automated systems incubate liquid media for 6 weeks, solid for 6-8 weeks before negative
- Positive report as soon as media turns positive and AFB are observed
  - Update report when identification made
  - Minimally, report of identification should indicate either MTBC or non-tuberculous mycobacteria (NTM)

Contamination

- Most specimens for AFB testing come from non-sterile sites
  - Despite decontamination, some contamination of culture media is to be expected
  - Common contaminants include molds, yeast, bacteria, and some NTM
- Acceptable contamination rate for liquid media is 5–8% and 3–5% for solid media

Biosafety Recommendations for Manipulations of Mycobacterial Cultures

- All procedures for isolation of MTBC including culture propagation and manipulation of the cultures are performed in BSL-3 facilities
- Essential practices for manipulation of MTBC cultures:
  - use of containment equipment (e.g., biosafety cabinet, centrifuge safety cups)
  - Minimization of aerosol production
  - use of respiratory protection

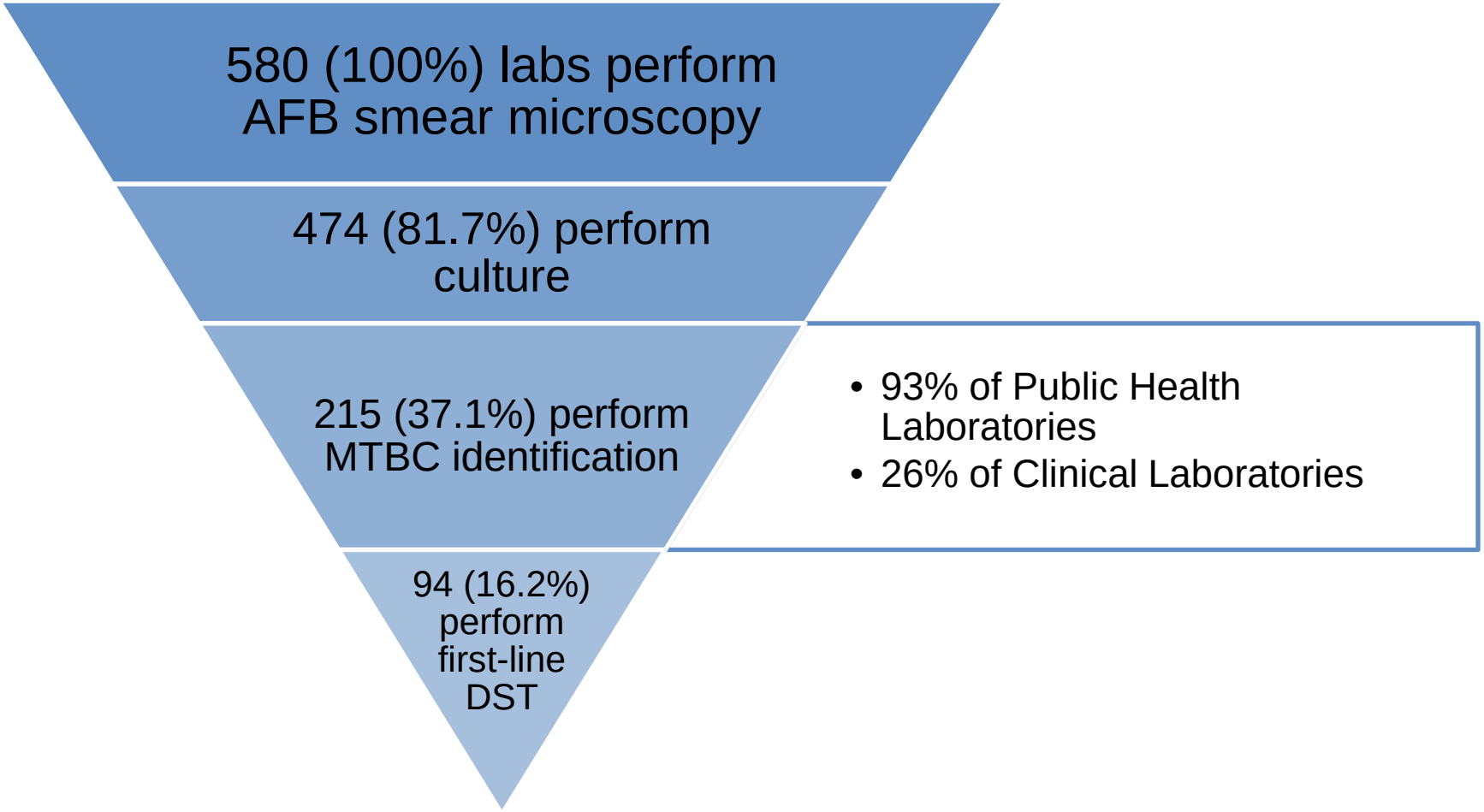
Overview, Purpose, and Methods

MYCOBACTERIAL IDENTIFICATION

Identification of Mycobacteria

- Accurate and prompt identification is important for patient management and public health response
- Identification results are used for
  - Diagnosis of clinically significant disease
  - Respiratory isolation decisions
  - Initiating or discontinuing contact investigations

National TB Laboratory Services Survey



APHL. National TB Laboratory Services Survey Report. 2012

Clinical Significance of MTBC and NTM

- Identification of MTBC is the most important finding in the laboratory and has serious clinical and public health consequences
- While some NTM can cause disease, not all NTM isolation is clinically significant
- Accurate and timely identification of mycobacteria is crucial
  - Use a multi-faceted approach that includes a rapid identification and phenotypic assessment before issuing a final report

Identification Methods

- Classical methods
  - Growth characteristics and conventional biochemical reactions
- Rapid methods:

Method	Advantages	Limitations
GenProbe® Accuprobe®	Identifies four common mycobacteria; Most common method used; FDA-cleared	No nucleic acid amplification occurs during this assay; sufficient culture growth is necessary for identification
High Performance Liquid Chromatography (HPLC)	Can identify MTBC and NTM from broth culture and directly from clinical specimens	High equipment costs; FDA-cleared system requires mature solid medium growth; Problems with identification of rapidly-growing mycobacteria
Line Probe assays	Increased sensitivity; Some assays detect mutations for MTBC drug resistance	Can be difficult to differentiate bands; Not FDA-cleared
MALDI-TOF	Rapid identification; Used for many bacteria and fungi in the laboratory	Database limitations; Initial cost investment high; Not FDA-cleared
DNA Sequencing	Quicker turnaround time (TAT); Ability to recognize new strains	High cost; Specialized equipment, expertise and training; Not FDA-cleared

Recommended Turnaround Time (TAT)

- Identification of MTBC ≤ 21 days from specimen receipt
  - Molecular methodologies have dramatically decreased the TAT for identification
  - Laboratory workflow and testing practices affect TAT
  - Referral of testing can lead to increased TAT
    - Submitting laboratories should routinely monitor TAT of the referral laboratory

Tenover, et. Al, 1993; Healthy People 2010 goals

Referral of Isolates for Identification

- Reference facilities should be used by laboratories that lack appropriate technologies and resources
  - Healthcare providers and TB Control Programs should be consulted to determine the level of TB laboratory services provided in your jurisdiction
- Any AFB isolate not identified in-house should be sent within one working day to reference laboratory

Transport

- Isolates of MTBC (including broths known to be positive for MTBC) are considered Category A (Infectious Substances)
- Patient specimens (e.g., sputum) are considered Category B (Biological Substances)
- Transport of both isolates and patient specimens is regulated by the Department of Transportation (DOT) and the International Air Transport Association (IATA) rules
- Persons involved in shipping must be trained and certified since the process is complex and all regulations* must be followed completely

**For details regarding these regulations, please see the information provided in the Reference section*

Overview, Purpose, and Methods

GROWTH-BASED DST OF MTBC

DST of MTBC

- Guides choice of chemotherapy—provides the best chance of cure
- Detects drug resistance or confirms the emergence of drug resistance
- Offers insight into appropriate treatment for contacts of patients with active TB
- Used to estimate the prevalence of primary and acquired drug resistance in a community

Recommended Panel for DST

- Initial MTBC isolates from ALL patients should be tested for susceptibility against four primary drugs
  - INH, RMP, EMB, and PZA*
- Isolates resistant to RMP or any two primary drugs should be tested against second-line drugs
  - Minimally, second-line panel should include amikacin, kanamycin, capreomycin and at least one fluoroquinolone
- DST should be repeated after 3 months if patient remains culture positive

***INH = Isoniazid, RMP = Rifampin, EMB = Ethambutol, PZA = Pyrazinamide**

Clinical and Laboratory Standards Institute [CLSI] M24-A2
American Thoracic Society, CDC, IDSA, MMWR 2003, TB Treatment Guidelines

DST Performed From Culture

- Indirect DST is performed after growth is identified as MTBC
- MTBC cultures must be pure; contaminating bacteria can potentially cause false-resistant results
- Broths should be sub-cultured to 7H10/7H11 and blood agar to assess purity and colony morphology
- If a culture is mixed with NTM or other bacteria, laboratories should attempt to re-isolate the MTBC

Growth-based Methods for DST

	MGIT 320 or 960	VersaTREK	Indirect Agar Proportion	Sensititre
Company	Becton Dickinson	Thermoscientific	N/A	Thermoscientific
Media	Liquid broth	Liquid broth	Solid	Liquid broth
Format	Tube	Tube	Petri plate	96-well microtitre plate
FDA approved	Yes (cleared)	Yes (cleared)	No (laboratory developed test)	No (research use only)

Considerations for DST Referral

- If possible, laboratories should refer liquid cultures for DST rather than waiting for growth on solid media
  - Submitting and referral laboratories should be familiar with shipping guidelines for infectious substances
- Consider the panel of drugs that the referral laboratory tests
- Submitting laboratories should monitor TAT of the referral laboratory

RESOURCES AND REFERENCES

Packing and Shipping Guidance

- ASM website-Guidance: http://www.asm.org/images/pdf/Clinical/pack-ship-7-15-2011.pdf
- DOT Guidance: https://hazmatonline.phmsa.dot.gov/services/publication_documents/PHH50-0079-0706%20Transporting%20Infectious%20Substances%20Safely.pdf
- More DOT guidance: http://www.phmsa.dot.gov/staticfiles/PHMSA/Hazmat/digipak/pdfs/presentation/Infectious_Substances(04_07).pdf
- IATA Infectious Substances website: http://www.iata.org/whatwedo/cargo/dgr/Pages/infectious_substances.aspx

References

- Manual of Clinical Microbiology, 10th Edition.Chapter 28
- http://www.ecdc.europa.eu/en/publications/Publications/1105_TER_Basics_TB_control.pdf
- Bauer, J., et al. (1997). "False-positive results from cultures of Mycobacterium tuberculosis due to laboratory cross-contamination confirmed by restriction fragment length polymorphism." J Clin Microbiol 35(4): 988-991.
- Review of False-Positive Cultures for Mycobacterium tuberculosis and Recommendations for Avoiding Unnecessary Treatment William J. Burman and Randall R. Reves
- Clinical and Laboratory Standards Institute [CLSI] M24-A2, M48,
- American Thoracic Society, CDC, IDSA, MMWR 2003, TB Treatment Guidelines
- Centers for Disease Control and Prevention, Tuberculosis website. http://www.cdc.gov/tb/
- Centers for Disease Control and Prevention/National Institute of Health. Biosafety in microbiological and biomedical laboratories (BMBL). 5th Edition. 2009. HHS Publication No. (CDC)21-1112. http://www.cdc.gov/biosafety/publications/bmbl5/index.htm

References

- Centers for Disease Control and Prevention. “Guidelines for safe work practices in human and animal medical diagnostic laboratories; Recommendations of a CDC-convened biosafety blue ribbon panel”. MMWR 2012;61 (Suppl): 1-102.http://www.cdc.gov/mmwr/preview/mmwrhtml/su6101a1.htm
- European CDC: http://www.ecdc.europa.eu/en/publications/Publications/1105_TER_Basics_TB_control.pdf
- ACILT African Centre for Integrated Laboratory Training http://www.cdc.gov/globalaids/resources/laboratory/Lab-Training-Center.html
- “Monitoring the performance of mycobacteriology laboratories: a proposal for standardized indicators,” KD McCarthy et al., INT J TUBERC LUNG DIS 12(9):1015–1020.
- APHL. Assessing Your Laboratory, TB Self-Assessment Tool http://www.aphl.org/aphlprograms/infectious/tuberculosis/Pages/TB-Self-Assessment-Tool.aspx
- Kent and Kubica, Public Health Mycobacteriology; A Guide for the Level III Laboratory. U.S. Department of Health and Human Services, 1985