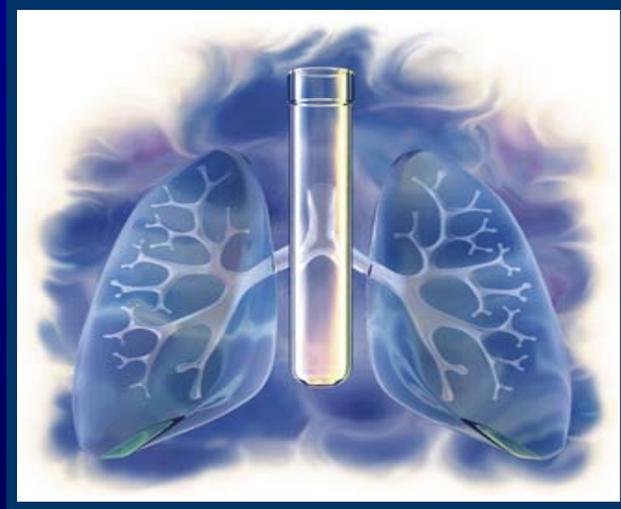


TB Detection and Characterization: The past, the present and the future



Ellen Basinger and Denise Toney, Ph.D.

Commonwealth of Virginia

Division of Consolidated Laboratory Services

Microbial Identification and Characterization

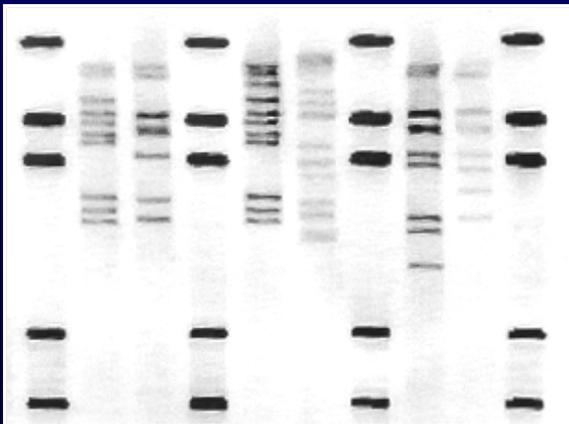


Phenotypic

- Biological & biochemical
- DNA Culture Probes
- Antibiotic & drug susceptibility testing

Genotypic

- DNA Fingerprinting
 - Spoligotyping
 - RFLP
 - VNTR analysis
- Probe hybridization
- Nucleic Acid Amplification (NAA)
 - GenProbe MTD



2008 Mycobacteriology Testing

Annual Workload

Primary Isolation

- 8956 patient specimens
 - » 394 Mycobacterium spp. (Non-tuberculosis)
 - » 632 *M. tuberculosis*
- 4144 Number of individual patients
- 102 number of individual patients for whom at least one culture was positive for *M. tuberculosis* complex.

Reference Culture Identification

- 636 cultures submitted
- 105 number of individual patients that had at least one reference isolate identified as *M. tuberculosis* complex.

2008 Mycobacteriology Testing

Drug Susceptibility

First Line

- 194 Number of first line drugs on all initial *M. tuberculosis* isolates

Second Line

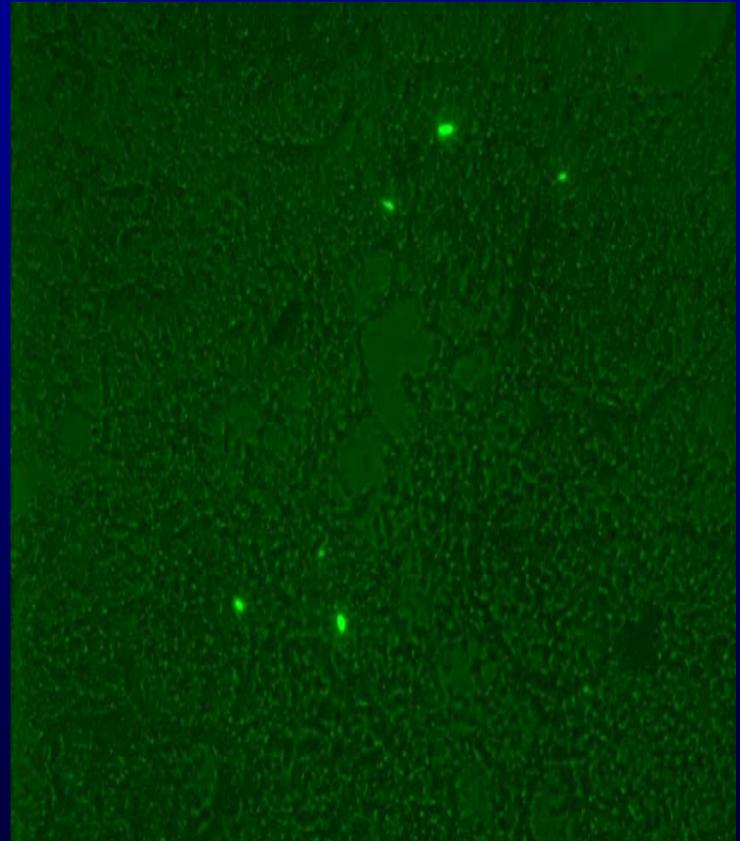
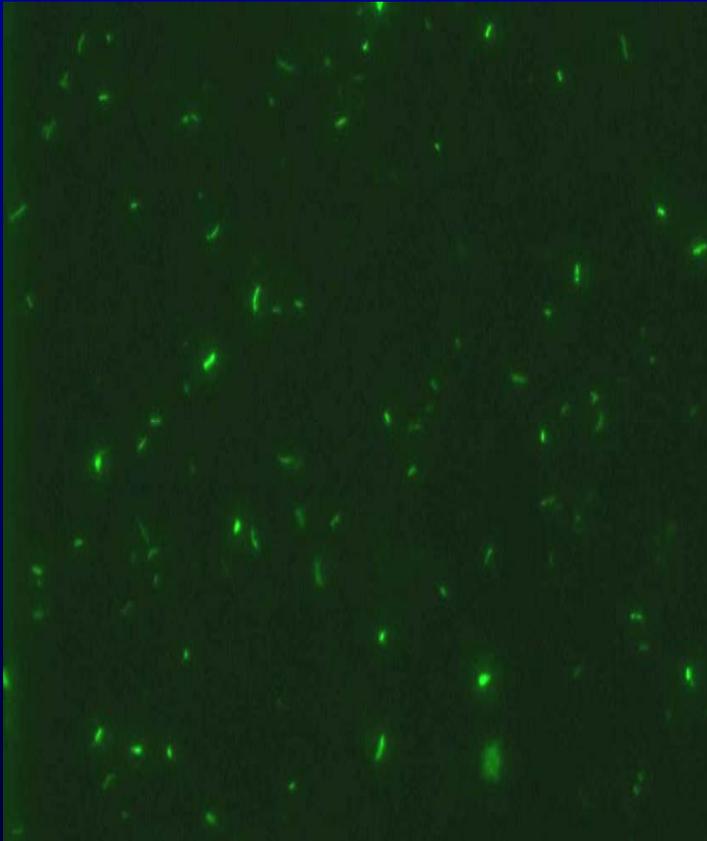
- 52 Second line drugs on all resistant *M. tuberculosis* isolates.

Drugs for most other *Mycobacteria* spp.
available through CDC and National Jewish
Hospital upon request.

Specimens

- Respiratory, body fluids, and tissues
- Processed for primary isolation within 24 hrs of receipt in the lab (excluding weekends)
- Digested and decontaminated with NaCl/NaOH
- Concentrated smears prepared
 - Stained with Auramine O for presence of acid fast bacilli (AFB) using fluorescent microscopy
 - Positive smear results on new patients phoned to the submitter the same day
 - Reports mailed the same day

Acid Fast Bacilli in Specimens Stained with Auramine O



AFB appear as yellow fluorescing rods

Primary Isolation

Solid media

- Lowenstein-Jensen (LJ) egg based tubed media

Broth based media

- Mycobacteria Growth Indicator Tube (MGIT)
- Middlebrook 7H9 with OADC enrichment
- PANTA: antibiotics to reduce growth of contaminants
- Oxygen quenching fluorescent technology
- Rapid detection of mycobacterial growth



MGIT 960 Instrument

- Continuous incubation at 37°C
- Continuous monitoring for fluorescence
- Growth of any organism is detected
 - Mycobacteria, yeast, other bacteria
- Smears prepared from broth
 - Growth determined to be acid-fast
- Tubes monitored for 6 weeks
- Tubes with no growth discarded as “negative”

MGIT 960 Tubes



Positive tubes fluoresce

LJ Slants

- Incubated aerobically at 37°C
- Macroscopically examined weekly for the presence of growth
- Slants are examined for 8 weeks
- Slants with no growth discarded as negative
- Slants with growth have smears made; Kinyoun stain performed
- No growth detected on media: broth or solid
- Negative Final Culture Report mailed



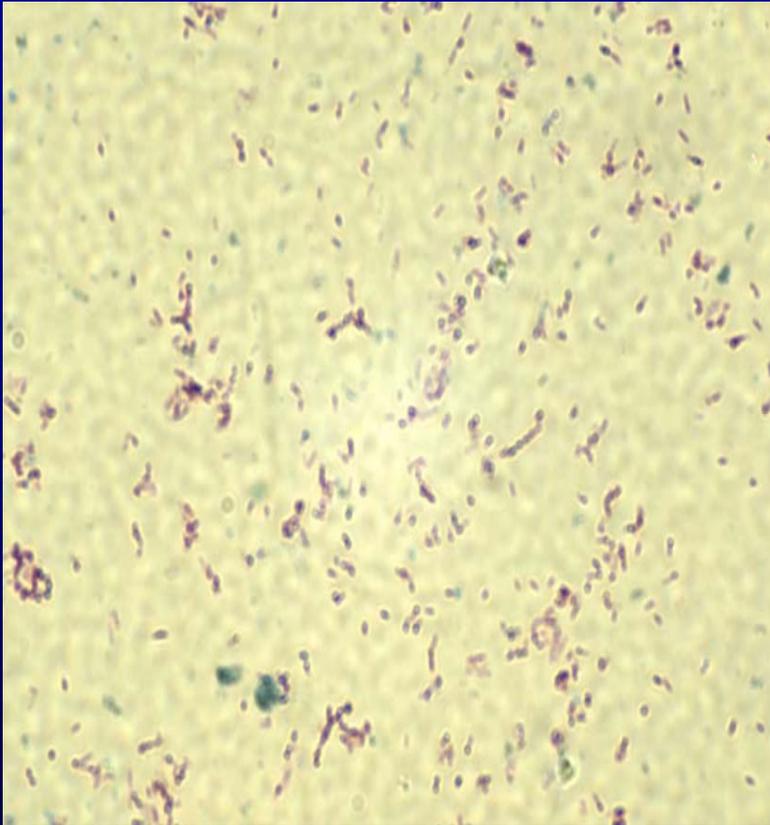
Identification for Acid Fast Organisms (MGIT or LJ Culture)

Smear morphology: TB Kinyoun stain

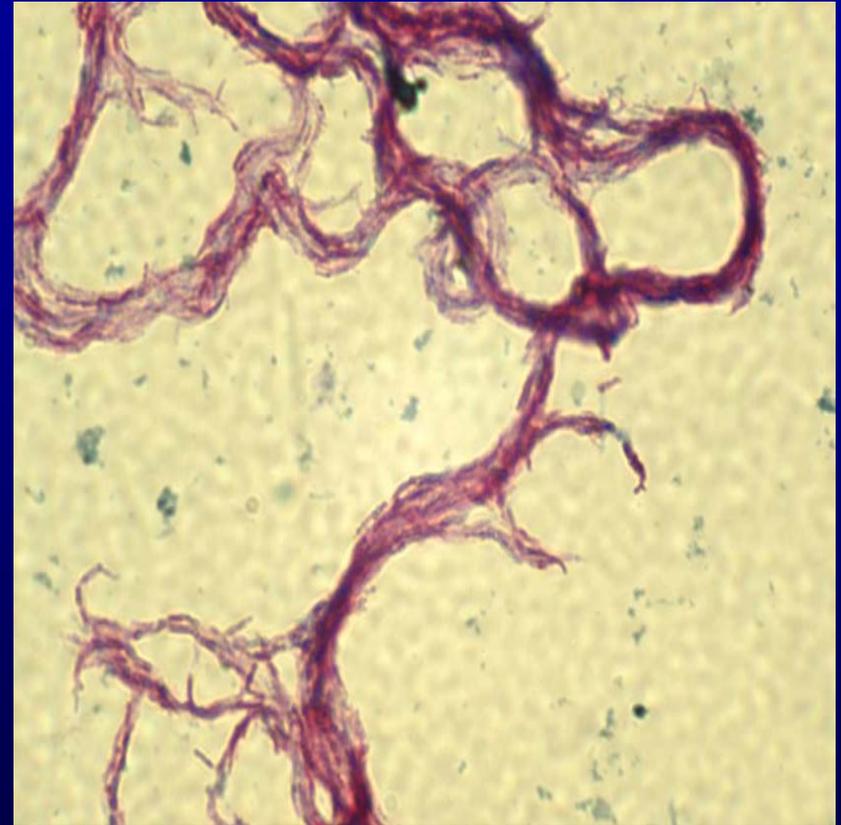
- “Cording” typical of *M. tuberculosis*
 - DNA probe for M.tb complex
 - Performed directly from culture tube; Average time for isolation is 7-21 days
- Pleomorphism and branching
 - DNA probe for *M. avium* performed directly from culture tube
- Other *Mycobacterium* spp. suspected
 - Conventional biochemicals
 - Additional probe testing – *M. kansasii* and *M. goodii*

Acid Fast Bacilli in Culture

Stained with Kinyoun (Carbol-fuchsin)



Pleomorphism and branching
often seen in *M. avium* complex



“Cording” typical of
M. tuberculosis

Growth on LJ Slant



M. tuberculosis



Non photochromagen
M. avium

Growth on LJ Slant

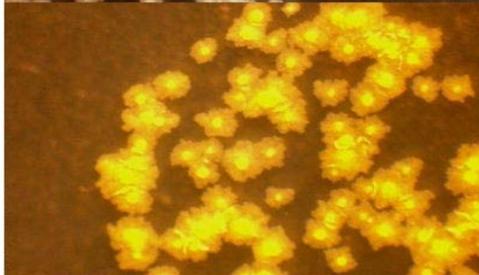


Scotochromagen
M. scrofulaceum

M. kansasii colonies
before exposure



M. kansasii colonies
after exposure to light
and re-incubation



Photochromagen
M. kansasii

Positive DNA Culture Probe for *M. tuberculosis* complex

- *M. tuberculosis* complex results phoned for all new patients
 - Diagnostic of the clinical syndrome, tuberculosis
- Preliminary Culture Report mailed
- Conventional biochemicals for speciation of *M.tb* complex by request or when testing indicates possible *M.bovis*.
 - Average of 3 weeks
- Final Culture Report mailed
 - *M. tuberculosis* complex

Drug Susceptibility Testing

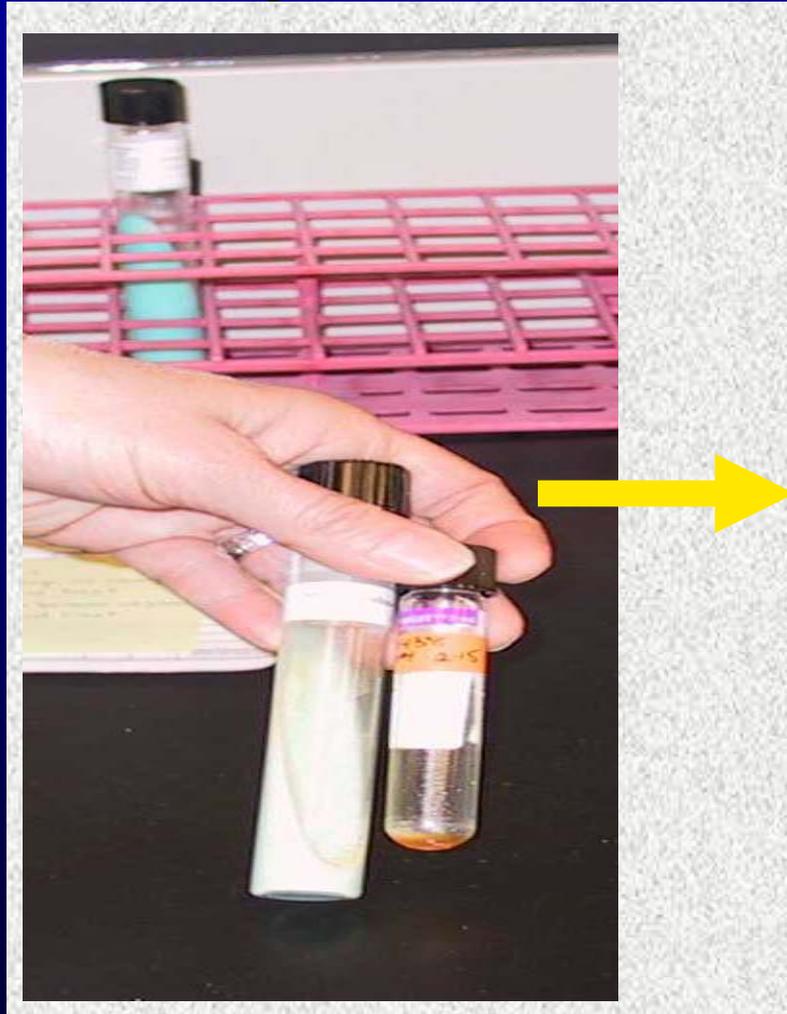
Mycobacterium tuberculosis



- First line drug testing
 - Isoniazid (INH), Rifampin, Ethambutol Streptomycin, Pyrazinamide (PZA)
- Results available within 7-12 days after speciation
- Resistant strains - results phoned to submitter
- Second line drug testing
 - Ethionamide, Capreomycin, Ofloxacin, INH at a higher concentration
 - Sent to CDC for additional drug susceptibility testing

Genotyping

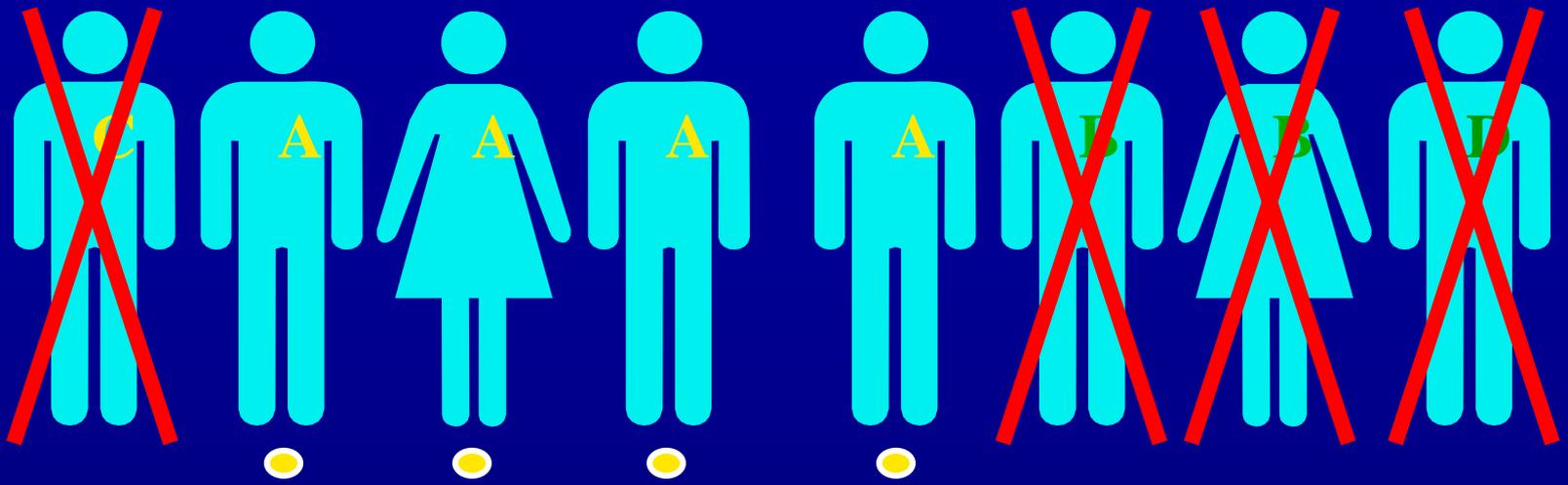
Mycobacterium tuberculosis



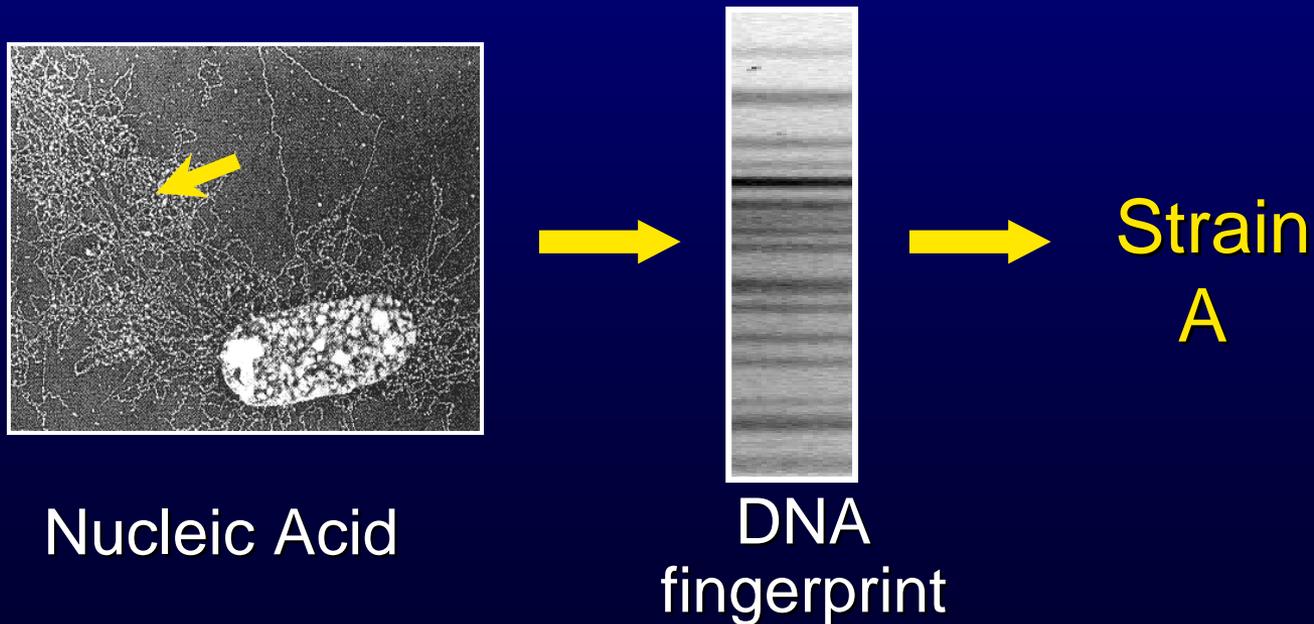
Different Strains

M. tuberculosis Strain Typing

- Commonly Used Methods
 - IS6110 Restriction Fragment Length Polymorphism (RFLP)
 - Spoligotyping
 - Mycobacterial Interspersed Repetitive Units – Variable Number Tandem Repeat (MIRU – VNTR) typing
- Testing performed by Regional Lab (MI) or the CDC



Genotyping (DNA fingerprinting) allows for the tracking of TB strains circulating in a population

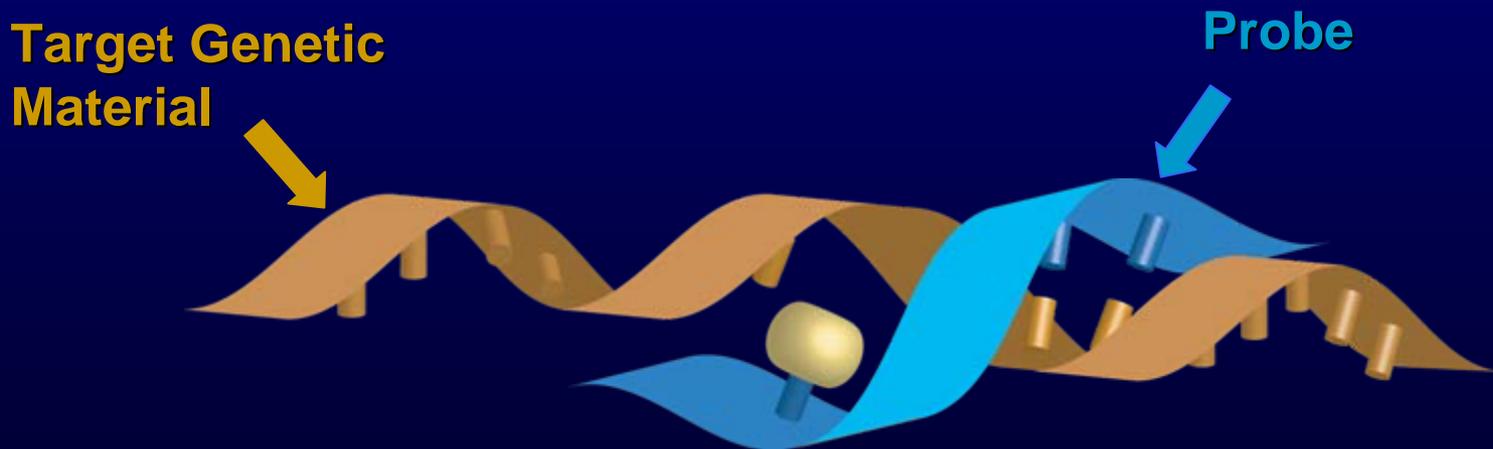


Advantages of TB Genotyping

- Increases the understanding of TB transmission within VA and nationally
- Can help to uncover information that may not be obtained with traditional epidemiologic methodologies and investigations
 - Contact investigations can often be limited to individuals directly exposed.
 - Often more precise as compared to phenotypic methods
- Can often discriminate between strains and determine whether infections are due to:
 - Recent transmission
 - Reactivation of a previous infection
 - Infection with a new strain

Nucleic Acid Amplification Tests (NAATs)

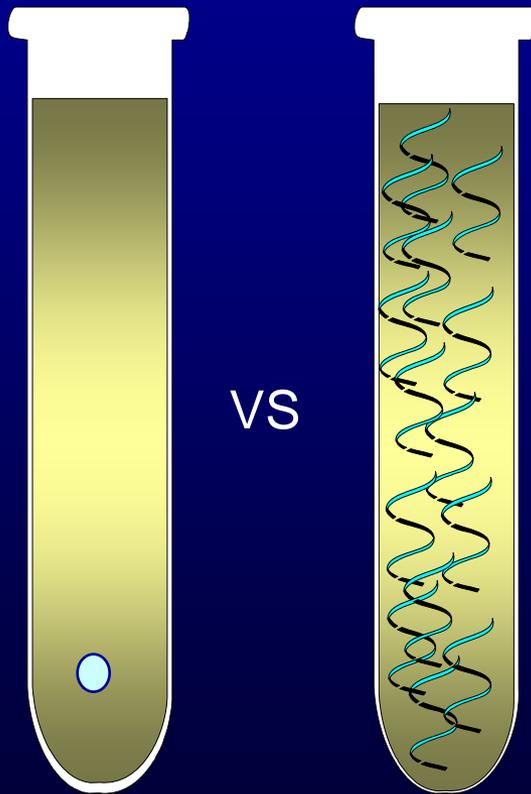
- Identify a region of genetic material unique to a particular organism (ie. *M. tuberculosis*)
- FDA approved the GenProbe Amplified *M. tuberculosis* Direct Test for AFB smear (+) respiratory specimens in 1995 and for smear (-) respiratory specimens in 1999



Why is an amplification test for TB needed?

- Direct detection with probes is not possible
- Microscopic AFB smears are rapid, but insensitive and non-specific
- Culture is sensitive and specific, but too slow (2-8 weeks).
- Clinical Significance
 - Isolate patients to prevent spread of disease
 - Treatment decisions: Is it *M.tb* or MOTT?
 - Reduce morbidity and mortality
 - Reduce health care costs for unnecessary isolation/treatment

AMPLIFIED *Mycobacterium Tuberculosis* (MTD) Assay



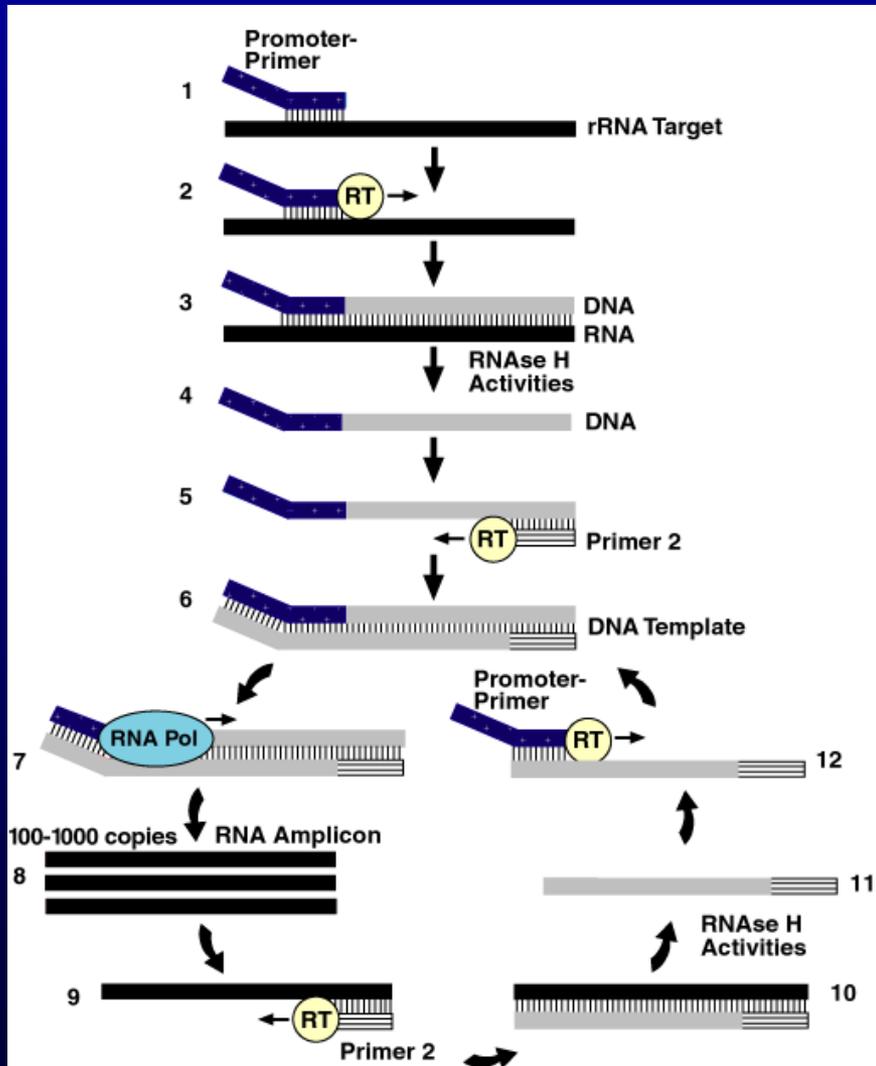
- Amplified molecular assay detects *M. tuberculosis* directly from sputum samples in less than 3.5 hours
- Utilizes a Transcription-Mediated Amplification system (TMA) to detect rRNA
- One *M. tuberculosis* organism can contain up to 10,000 copies of rRNA (biological amplification)

AMPLIFIED MTD Test Detects All Members of *M. tuberculosis* Complex

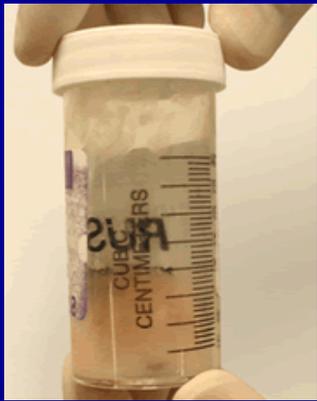


Mycobacterium africanum
Mycobacterium bovis
Mycobacterium microti
Mycobacterium tuberculosis
Mycobacterium canetti

TMA Technology



- Primer hybridizes to target and initiates the amplification reaction
- Enzymes drive the reaction:
 - T7 RNA polymerase - Transcribes RNA from DNA
 - Reverse transcriptase (MMLV) - Synthesizes DNA from RNA or DNA and has RNase H activity to degrade RNA after it has been copied into DNA



How will this work for Local HDs?

- Collect sputum and ship to DCLS to rule out TB
- DCLS will AUTOMATICALLY test the first smear (+) sputum with NAAT
- If the first sputum is smear (-) but a subsequent sputum is smear (+) the second specimen will be tested
- Testing of smear (-) specimens or priority cases is by special request ONLY and requires VDH or DCLS approval



How will this work for private providers?

- NAAT will be performed on all first time smear positive specimens received from hospitals participating in DCLS' fee for service testing at NO additional cost
- Testing for private providers will ONLY be done in special situations. Providers must contact VDH or DCLS to get approval PRIOR to submission of specimens.

What is the MTD test FDA approved and not approved for?

Approved for:

- Testing smear (+) and (-) specimens (NOTE: Smear (-) specimens NOT tested at DCLS)
- Testing patients who have taken TB medications for LESS than 7 days
- Patients with high clinical suspicion of TB

NOT Approved for:

- Specimens from patients receiving TB medications in the past 12 months
 - NOT a test of cure; MTD can detect nucleic acids from dead and live organisms, so may remain positive long after treatment is completed and the culture is negative
- Testing children or patients unable to produce sputum

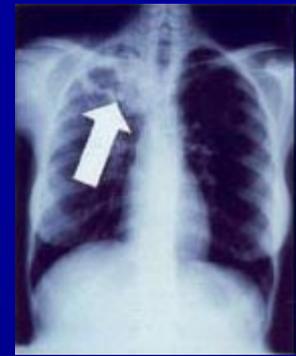


How good is this test?

- Respiratory smear (+) specimens from untreated patients with high suspicion for TB.
 - Sensitivity = 95%
 - Specificity = 98%
- Respiratory smear (-) specimens from untreated patients with high suspicion for TB.
 - Sensitivity = 66%
 - Specificity = 98%



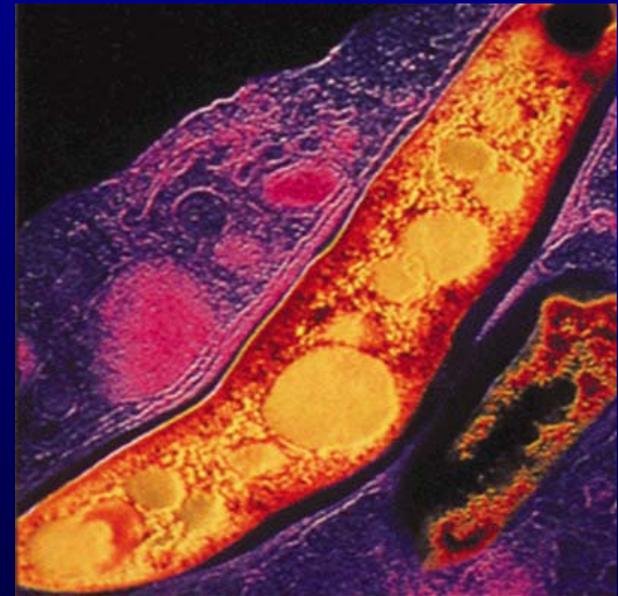
Interpretation of Results



- Not a perfect test – false positive and false negatives can occur
 - Poor specimen quality
 - Contamination
 - Low numbers of Mycobacterium
 - Inhibited due to a naturally occurring inhibitor in the specimen or processing reagent (ex. blood)
 - Cross-reactivity (rare!!)
- Does not replace culture results which are the “gold standard”.
- Interpret within the context of the patient’s symptoms, chest x-ray, smear and culture

Smear (+), NAAT (+)

- Presume active TB disease
- Start contact investigation
- Start TB medication
- Keep in isolation until cleared
- Confirm by culture result



Smear (+), NAAT (-)



- Suspect non-tuberculous mycobacterium (NTM).
- **Does not rule out TB**
- Consider delaying treatment, contact investigation and removing from isolation.
- BUT.....if highly suspected of TB or lives in congregate setting or with high risk individuals request a second NAAT and/or consult TB control.
- Confirm findings with culture result

Smear (-)** , NAAT (+)



- Likely has active TB disease
- Consider submitting another specimen for NAAT to verify
- Presumed to have TB if two or more specimens are NAAT positive
- Use clinical judgment to determine whether to start treatment, start contact investigation and place on isolation.
- Confirm by culture result

** MUST BE PRE-APPROVED

Smear (-)** , NAAT (-)

- For smear (-) specimens, sensitivity is low
- Diagnosis of TB cannot be excluded
- **MUST rely on clinical judgment**
- Consult VDH TB Control to determine if patient can be considered non-infectious if two sputum specimens test smear (-) and NAAT results are negative
- Confirm by culture result

** MUST BE PRE-APPROVED

Conclusion

- NAAT will provide LHDs with additional laboratory results to base decisions upon
- LHDs will not need to do anything different when collecting sputum to rule out TB
- Do not request or expect NAAT testing on a patient that has been on TB medications for more than 7 days or been treated within the last year
- DCLS and TB Control is available to assist with patient consultations and interpretation of results!

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Contact Information:

Ellen M. Basinger
Group Manager, Microbial Reference
Division of Consolidated Laboratory Services (DCLS)
600 North 5th Street
Richmond, Virginia 23219
(804) 648-4480 ext. 210

Denise M. Toney, Ph.D.
Lead Scientist
DCLS
600 North 5th Street
Richmond, Virginia 23219
(804) 648-4480 ext. 282

