

TB Detection using Molecular Methods

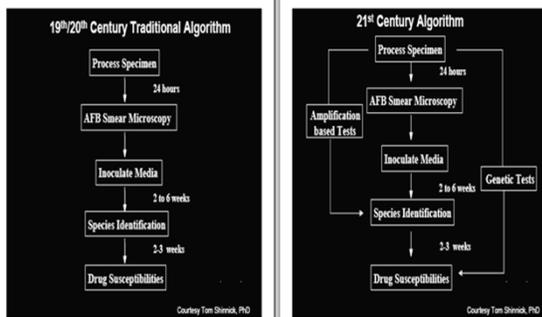


Denise Toney, Ph.D.

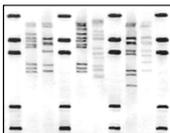
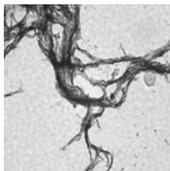
Commonwealth of Virginia

Division of Consolidated Laboratory Services

Introduction



Microbial Identification and Characterization



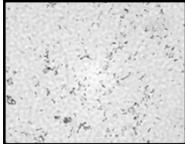
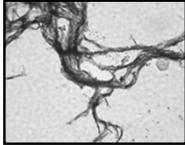
Phenotypic

- Microscopy
- Biological & biochemical
- Antibiotic & drug susceptibility testing

Genotypic

- Probe hybridization
- Nucleic Acid Amplification (NAA)
 - GenProbe MTD
- DNA Fingerprinting
 - Spoligotyping
 - IS6110 RFLP
 - VNTR analysis

Identification for Acid Fast Organisms (MGIT or LJ Culture)



Smear morphology: TB Kinyoun stain

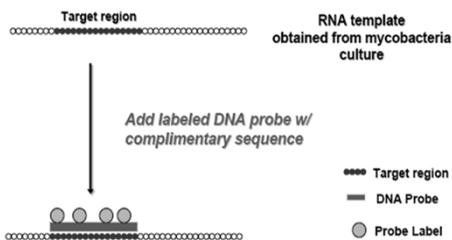
- "Cording" typical of *M. tuberculosis*
 - DNA probe for *M.tb* complex performed directly from culture tube
 - Average time for isolation is 7-21d
- Pleomorphism and branching
 - DNA probe for *M. avium* performed directly from culture tube
- Other *Mycobacterium* spp. suspected
 - Conventional biochemical
 - Additional probe testing - *M. kansasii* and *M. goodii*

Identification of *Mycobacteria* from Culture using Hybridization Probes (AccuProbe®)



- From Culture ONLY
 - No amplification step
 - Needs lots of target nucleic acid
- Gen-Probe AccuProbes® available for:
 - *Mycobacterium tuberculosis* complex
 - *Mycobacterium tuberculosis*, *M. bovis* (including attenuated BCG), *M. africanum*, *M. microti*, *M. canetti*
 - *M. avium* complex
 - *M. goodii*
 - *M. kansasii*

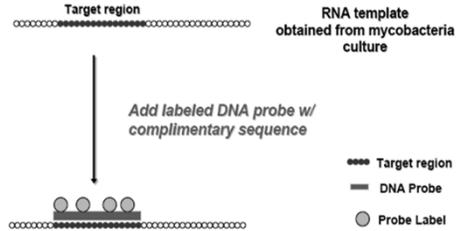
Nucleic Acid Hybridization Probes



- Acridinium ester-labeled DNA probes hybridize to *Mycobacterium* specific 16S rRNA target (AccuProbe®)

Modified from Wolk DM et al., 2001 *Infect. Dis. Clin. N. Amer.*, 15:1157-1204

Nucleic Acid Hybridization Probes



- Acridinium ester on the DNA probe is chemiluminescent
- DNA probe-rRNA hybrids emit light following addition of the detection reagent (hydrogen peroxide/sodium hydroxide)

Nucleic Acid Hybridization Probes



- Chemiluminescence measured in a luminometer and the amount of light emitted proportional to amount of DNA-RNA hybrids formed
- Total time for AccuProbe test is ~2 hours

Sensitivity and Specificity *M. tuberculosis* Complex

AccuPROBE / CULTURE IDENTIFICATION						
AccuPROBE	Pos	Pos	Neg	Neg	Sensitivity/ Specificity	Percent Agreement
Culture	Pos	Neg	Pos	Neg		
SITE 1	422	1	1	541	99.8%/99.1%	99.8%
SITE 2	185	0	4	213	98.9%/100%	99.0%
Total	607	1	5	754	99.2%/99.9%	99.6%

When the discordant samples were retested, the correct results were obtained with the exception of one isolate from Site 2 which was nonviable.

MAYO CLINIC

Nucleic Acid Hybridization Probes

Mycobacteria	Sensitivity	Specificity
<i>M. avium</i>	99.3%	100%
<i>M. intracellulare</i>	100%	100%
<i>M. avium</i> complex	99.9%	100%
<i>M. goodnae</i>	98.8%	99.7%
<i>M. kansasii</i>	92.8%	100%
<i>M. tuberculosis</i> complex	99.2%	99.0%

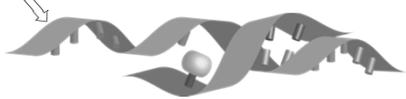
Courtesy of Nancy Wengenack, Ph.D. (Mayo Clinic)

- ### Limitations
- False Negatives
 - AccuProbe® test can be negative for *Mtb* complex and still contain TB and eventually test culture positive
 - Why--Number of organisms is below the detectable limit of the test
 - Accuprobe testing is FDA-cleared for testing with cultures ONLY

Nucleic Acid Amplification Tests (NAATs)

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

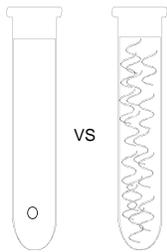
Target Genetic Material



Benefits of a Nucleic Acid Amplification Test (NAAT) for TB

- 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 *Mtb* or MOTT?
 - Reduce morbidity and mortality
 - Reduce health care costs for unnecessary isolation/treatment

AMPLIFIED *Mycobacterium Tuberculosis* (GenProbe MTD) Assay



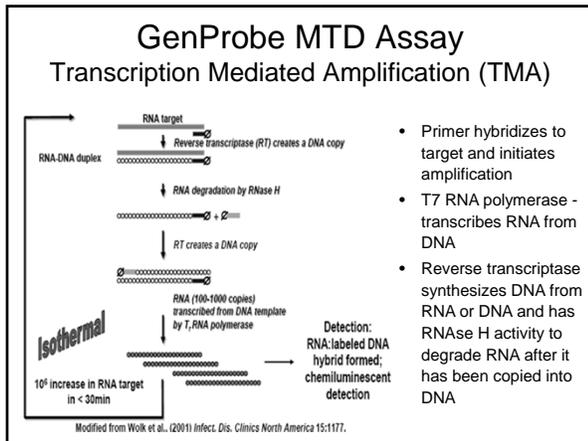
One *Mtb* organism can contain up to 10,000 copies of rRNA (biological amplification)

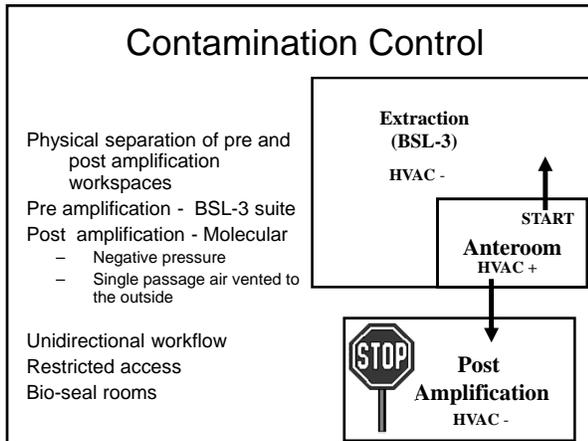
- Amplified molecular assay detects *M. tuberculosis* directly from sputum samples in less than 3.5 hours
- Utilizes a Transcription-Mediated Amplification system (TMA) to detect rRNA directly from respiratory specimens
- Limitations:
 - Only detects *Mtb* complex
 - Negative does not rule out a positive; still need to culture
 - Cross reactions can occur with other rare *Mycobacteria*

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



- Mycobacterium africanum*
- Mycobacterium bovis*
- Mycobacterium microti*
- Mycobacterium tuberculosis*
- Mycobacterium canettii*





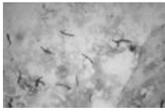
GenProbe MTD test FDA-cleared

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



Sensitivity and Specificity

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



Limitations



- 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 (+) Interpretation

NAAT (+)

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



NAAT (-)

- Suspect non-tuberculous mycobacterium (NTM).
- Does not rule out TB
- Consider delaying treatment, contact investigation and removing from isolation UNLESS 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

Smear (-)** Interpretation

NAAT (+)

- Likely has active TB disease
- Consider submitting another specimen for NAAT
- Presumed 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



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 2 sputum specimens test smear (-) and NAAT results are negative
- Confirm by culture result

** Testing must be pre-approved

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