

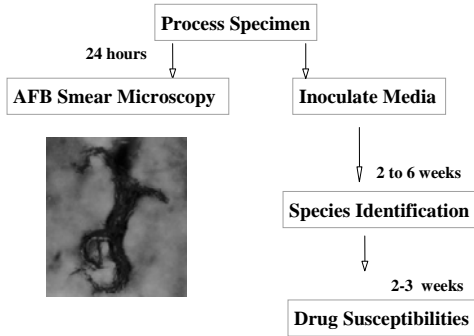


Nucleic Acid Amplification Testing for the Diagnosis of TB

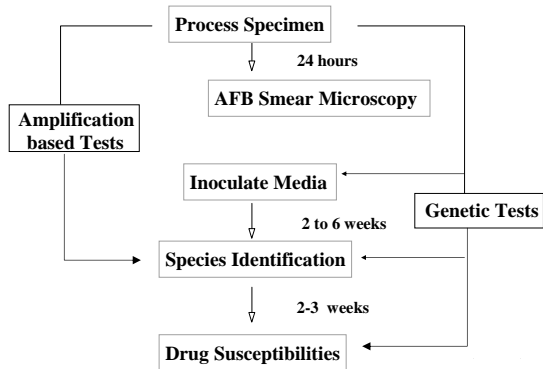
David Warshauer, PhD
Deputy Director, Communicable Diseases
Wisconsin State Laboratory of Hygiene



19th/20th Century Traditional Algorithm



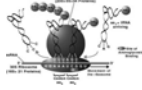
21st Century Algorithm



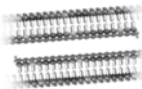
Nucleic Acid Amplification Tests

- FDA-cleared for use with respiratory specimens

- Amplified *M. tb* Direct Test® (MTD): Gen-Probe, Inc.



- Amplicor® *M. tuberculosis* (MTB): Roche Diagnostics



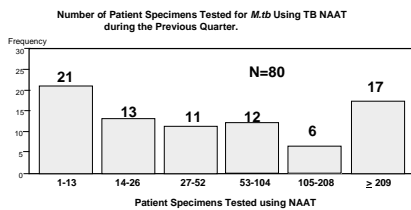
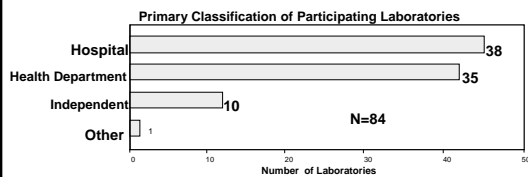
Nucleic Acid Amplification Tests

- Commercial tests available outside US

- BD ProbeTec™ MTB Direct Detection
- COBAS® Amplicor® MTB Test
- COBAS® TaqMan® MTB Test
- Hain Genotype® Mycobacteria Series
- Cepheid GeneXpert®
- Innogenetics INNO-LIPA™

- Home-brew tests
- Off-label use of FDA-cleared tests

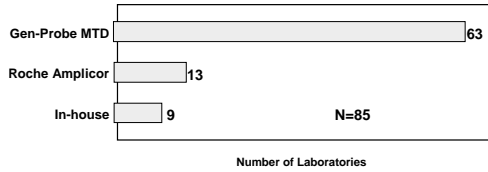
CDC *M. tb* NAAT Performance Evaluation Program



Courtesy Laurina Williams, PhD

**CDC *M.tb* NAA Testing Performance
Evaluation Program-TB MPEP**

Amplification Procedure Used for Direct Detection of *M.tb*

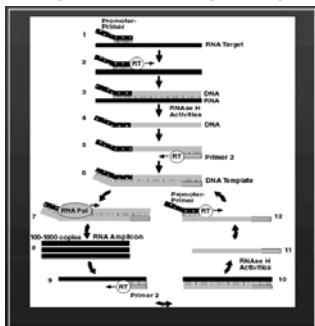


Courtesy Laurina Williams, PhD

**Amplified *M. tb* Direct Test® (MTD):
Gen-Probe, Inc.**

- Smear positive and smear negative specimens
- Transcription mediated amplification
- Ribosomal RNA target
 - Multiple copies
- Detection by a labeled Mtb complex-specific DNA probe
- Assay time 2.5-3 hours

Transcription Mediated Amplification (TMA)



**Amplicor® *M. tuberculosis* (MTB):
Roche Diagnostics**

- Smear positive specimens only
- Polymerase chain reaction assay (PCR)
- Target—584-bp region of the 16S rRNA gene
- Detection by a colorometric reaction with an Mtb complex-specific probe
- Assay time 4-6 hours

FDA-approved specimens

- Respiratory specimens-not grossly bloody
 - Sputum----induced or expectorated
 - Bronchial specimens
 - Bronchoalveolar lavages
 - Bronchial aspirates
 - Tracheal aspirates
- Test must be performed within 72 hours of decontamination
- Test must be performed in conjunction with mycobacterial culture

Patient Requirements

- Suspected of having pulmonary TB based on clinical evaluation
- Have not received anti-tuberculous therapy
 - Less than 7 days of therapy
 - Or have not received such therapy in the last 12 months

Nucleic Acid Amplification Tests

- Turnaround time of 24 to 48 hours
- Detect *M. tuberculosis* complex NA
- Do not distinguish live and dead bacilli
- Sensitivity
 - >95% for AFB smear-positive TB patients
 - 55-75% of AFB smear-negative, culture-positive TB patients
- Performance improves with increased clinical suspicion of TB

NAAT Performance – Respiratory Specimens

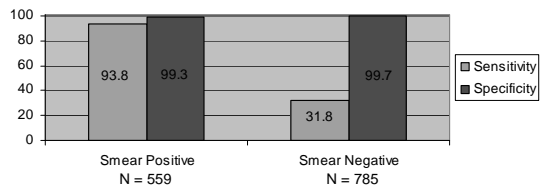
Table 1 Pooled values* [95% confidence intervals] of diagnostic odds ratio (DOR), sensitivity, and specificity of five commercial nucleic acid amplification tests (NAATs)

Test	NAA method	AFB+			AFB-		
		DOR	Sensitivity	Specificity	DOR	Sensitivity	Specificity
Amplacor	PCR	117 (56 to 246)	0.96 (0.94 to 0.97)	0.82 (0.8 to 0.86)	77 (51 to 115)	0.61 (0.57 to 0.65)	0.97 (0.968 to 0.974)
Cobas AmpliCor	PCR	99 (56 to 173)	0.96 (0.95 to 0.97)	0.74 (0.68 to 0.8)	220 (144 to 335)	0.64 (0.59 to 0.69)	0.992 (0.992 to 0.994)
BDP	SDA	181 (139 to 233.6)	0.98 (0.96 to 0.99)	0.89 (0.84 to 0.93)	94 (52 to 175)	0.71 (0.64 to 0.76)	0.97 (0.964 to 0.974)
E-ATD	TMA	314 (99 to 995)	0.97 (0.95 to 0.98)	0.96 (0.93 to 0.97)	157 (48 to 512)	0.76 (0.7 to 0.8)	0.97 (0.966 to 0.974)
LCx	LCR	42 (12 to 142)	0.96 (0.94 to 0.98)	0.71 (0.64 to 0.78)	71 (28 to 132)	0.57 (0.5 to 0.64)	0.98 (0.978 to 0.985)

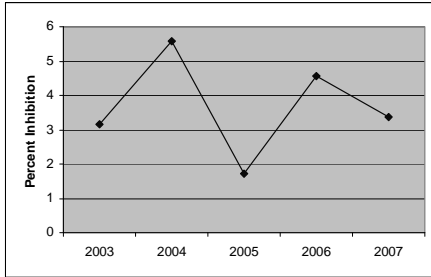
PCR, polymerase chain reaction; SDA, strand displacement amplification; TMA, transcription mediated amplification; LCR, ligase chain reaction; DOR, diagnostic odds ratio. *Random effect model.

Greco, S. et al Thorax 2006;61:783-790

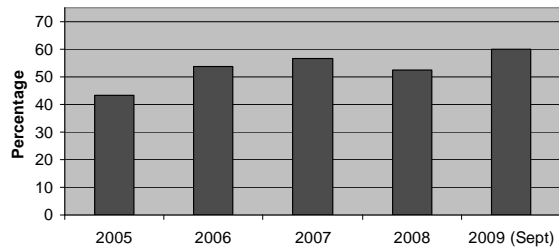
WSLH MTD Sensitivity and Specificity, July 2000 to October 2008



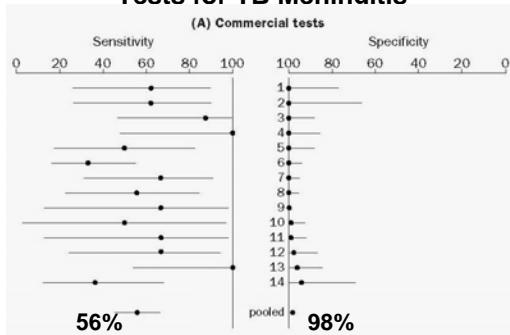
**Percentage of Inhibited Samples
2003-2007**



**Percentage of Culture-Confirmed Pulmonary TB
Cases Detected by NAA in Wisconsin, 2005-2009**



---Off-Label Testing---
**Diagnostic Accuracy of Commercial
Tests for TB Meningitis**



Pai, M. et al. The Lancet Infectious Diseases 2003, 3:633-643

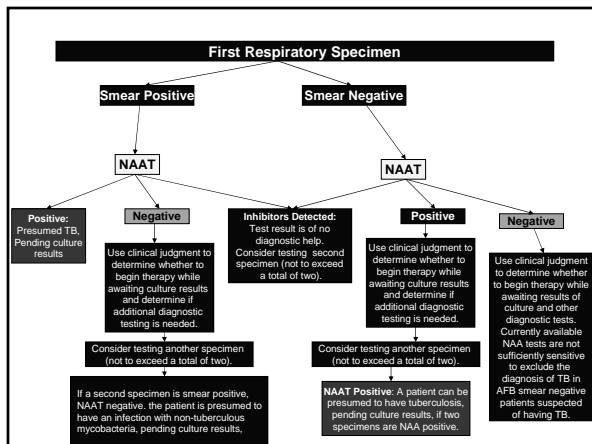
Current CDC Recommendations for NAAT ---2009

- “NAAT should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities”
- NAAT as standard practice

MMWR, 2009, 58:7-10

CDC Algorithm

- Collect at least one respiratory specimen, preferably the first, for NAAT
- Collect additional specimens for smear and culture
- Interpret NAAT results in correlation with the AFB smear results



Who should be tested?

- CDC recommends NAAT on first sputum of all patients suspected of TB for whom the test result would alter case management or TB control activities
 - NAAT should not be ordered routinely when clinical suspicion of TB is low.
- Definition of a “suspect” case can vary among clinicians?
- Clinicians, TB programs, and laboratorians must collaborate to develop criteria/definition for patients to be tested

Wisconsin criteria for NAAT

- Signs and symptoms
- Risk factors
- Patient in airborne isolation
- Reported to local health department as a suspect case

- All initial smear positive respiratory specimens automatically tested

NAAT in Smear Negative Patients

- Especially valuable in smear negative patients
 - Positive NAAT influences the clinician to start treatment
 - Avoidance of other invasive procedures
 - Avoidance of potentially toxic therapy for other diagnosis
 - Reduction of transmission

Some clinicians delay treatment decisions until laboratory results are available even though TB is considered a clinically based diagnosis

Test results must be available as soon as possible to reduce delay in initiation of therapy

Pascopella et al., 2004, J. Clin. Microbiol. 42:4209

Challenges to Implementing NAAT Guidelines

- NAAT adds significant cost to the laboratory
- Low volume test

	Amplicor®		Gen-Probe®	
Reagents	3 controls		2 controls	
	3 patients	\$180	3 patients	\$240
	3 inhib ctrl		3 inhib ctrl	
Labor*	2.5 hr	\$50	2 hr	\$40
Direct Costs per Patient Result	\$77		\$93	

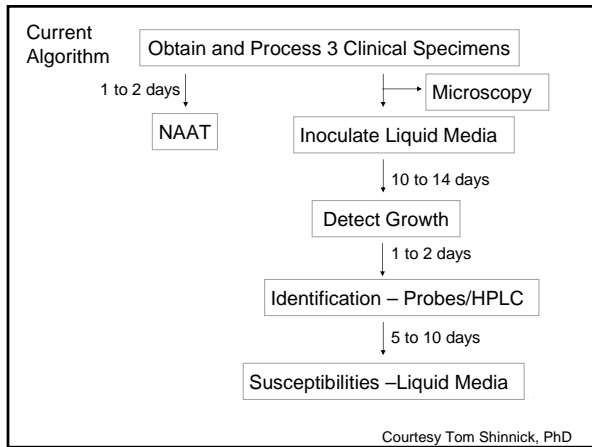
*Wage of \$20/hour

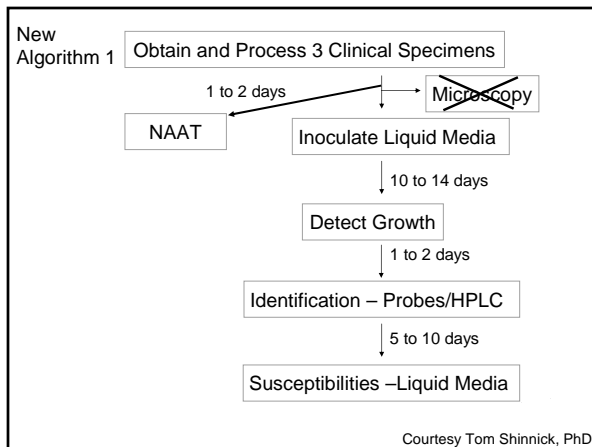
Challenges to Implementing NAAT Guidelines

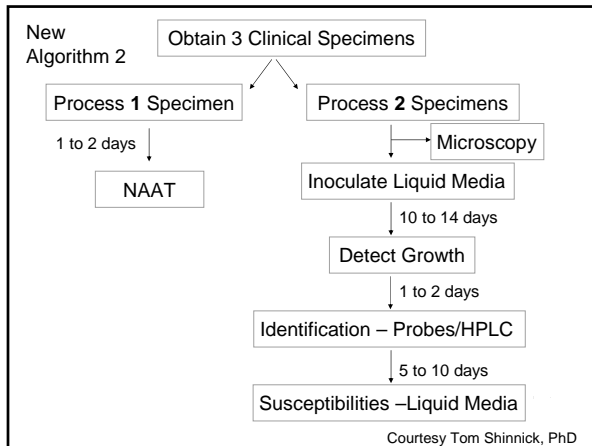
- In current algorithm, NAAT is an add-on test
- The overall costs and benefits of NAAT may vary with prevalence of TB
- Optimal, cost-effective testing regimens have not yet been developed

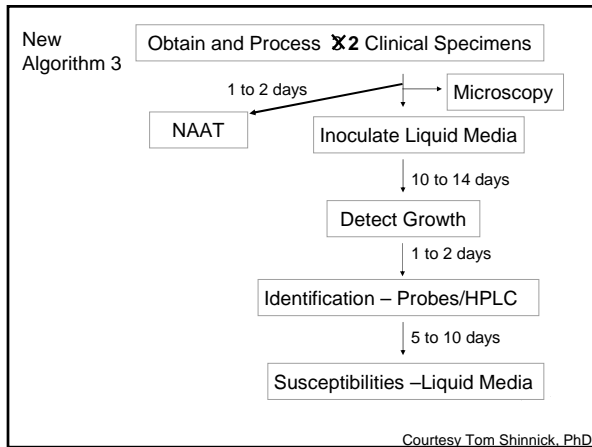
Need to establish new algorithms that include NAAT

- One specimen, preferably the first diagnostic specimen for NAAT, smear, and culture
- Can we decrease the number of sputums for smear and culture?
 - How many are necessary?
- Can we eliminate solid media to decrease costs?







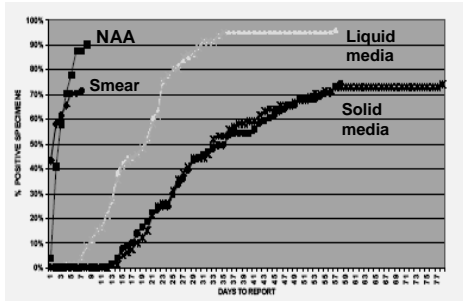


NAAT Testing Algorithm in a Public Health Laboratory

Processing: 5 days; NAAT 4 days; broth medium monitored 7 days
 NAAT (first specimen) – AFB smear and culture (3 specimens) – 797 pt [81 TB]

Assay	Sens	Spec	PPV	NPV	Mean TAT
AFB Smear	70	98	79	96.7	1
NAAT	90	100	100	98.9	2
Culture x 3	96	100	100	99.6	18

Moore et al - DMID 52:247-254(2005)



Moore et al - DMID 52:247-254(2005)

Use of NAAT for Shortening Respiratory Isolation (RI)

- RI requires single patient rooms, negative pressure and filtration, sterilization, or dispersion of exhaust air.
- Only 1 of every 10-25 isolated patients actually have TB
- Current guidelines for removal from RI
 - 3 consecutive negative smears over at least a 2 day period in addition to clinical assessment that risk is low

Use of NAAT for Shortening Respiratory Isolation

- Campos et al: AJRCCM 2008, 178(3):300-305,
 - Compared NAAT on a single first sputum to 3 sputum smears for assessing need for RI
 - 493 TB suspects (74% HIV-positive)
 - 46 (9.3%) culture confirmed TB
 - EMTD assay and an in-house NAAT

Use of NAAT for Shortening Respiratory Isolation

- First NAAT detected all 35 smear-positive TB patients
 - Included 3 who had negative smears of their first sputa
 - Performance similar in HIV seropositive and seronegative patients
- NAAT positive in 5/11 smear-negative patients

Performance of NAAT and Serial Sputum Smears in Diagnosis of TB

	NAAT	3 Smears
Sensitivity	0.87 (0.74-0.95)*	0.76 (0.61-0.87)
Specificity	1.00	0.96 (0.94-0.98)
PPV	1.00	0.73 (0.58-0.85)
NPV	0.99 (0.97-1.00)	0.98 (0.96-0.99)

* 95% CI

Conclusions: Use of NAAT for Shortening Respiratory Isolation

- A single first-sputum NAAT can rapidly and accurately identify the subset of TB suspects who require RI according to serial sputum smears.
- Replacement of serial smears with a single NAAT could permit the vast majority of patients to be discharged from RI within a single day

What's Down the Road?



Hain GenoType® Series Mycobacteria

GenoType® Mycobacterium CM/AS

GenoType® MTBC

GenoType® MTBDRplus

GenoType® Mycobacteria Direct



Molecular Basis of DR in MTBC

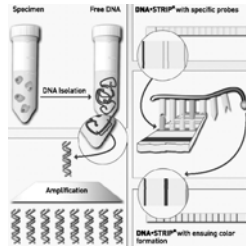
Drug	Gene Locus	Gene function	Percent of Resistance
Isoniazid	<i>katG</i>	Catalase-Peroxidase	40 - 100 %
	<i>inhA</i>	Enoyl-ACP-Reduktase	appr. 25 %
	<i>ahpC</i> -Promoter	Alkyl-Hydroxid-Peroxidase	appr. 10 %
Rifampicin	<i>rpoB</i>	β-Subunit of RNA-Polymerase	> 90 %
Pyrazinamide	<i>pncA</i>	Pyrazinamidase	appr. 95 %
Streptomycin	<i>rpsL</i>	ribosomal Protein S12	appr. 60 %
	<i>rrs</i>	16S rRNA	appr. 20 %
Ethambutol	<i>embB</i>	Arabinosyl-Transferase	appr. 60 %
Chinolone	<i>gyrA</i>	DNA-Gyrase A	appr.80-90%

GenoType® MTBDRplus

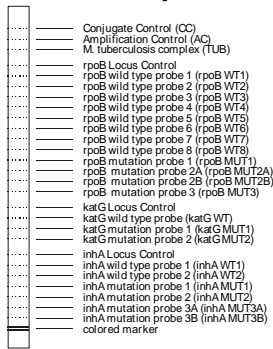
- permits the molecular identification of the *M. tuberculosis* complex
- resistance to rifampicin and isoniazid from culture growth or pulmonary smear-positive patient material
- *rpoB* gene for rifampin
- for testing of high level isoniazid resistance, the *katG* gene is examined
- for testing of low level isoniazid resistance, the promoter region of the *inhA* gene is examined.

GenoType® MTBDRplus

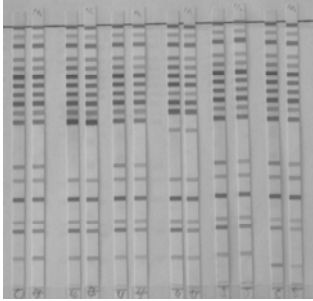
1. DNA preparation
2. PCR
3. Hybridization
4. Evaluation



Reaction zones of the GenoType® MTBDRplus



Possible Results



Research Needs for Future Advancements

- Studies to develop, evaluate, and select the most effective and efficient NAAT and culture algorithms
- Develop and evaluate tests for non-respiratory specimens
- Develop tests with improved performance and ease-of-use
- Develop tests that will enhance the diagnosis of TB in children
- Develop multiplex assays that can detect *M. avium* complex, *M. kansasii* and other NTM
- Develop tests to detect resistance to both first and second line drugs

Summary

- Advantages of NAAT
 - More rapid diagnosis
 - Initiation of earlier treatment
 - Cost savings with reduced patient isolation
 - Faster reporting to TB Programs
 - Fewer transmissions

Acknowledgements

Tom Shinnick, Ph.D CDC
Julie Tans-Kersten, WSLH

Thank You