The Clinical Impact of Rapid Nucleic Acid Amplification Tests for Detection of *M. tuberculosis*

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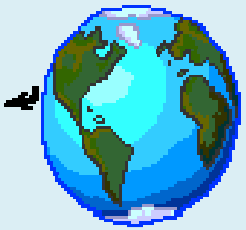
COLUMBIA UNIVERSITY MED CENTER (CUMC),
NEWYORK-PRESBYTERIAN HOSPITAL

NEW YORK, NEW YORK USA



"TOWARDS ZERO TB"

GLOBAL BURDEN IS ENORMOUS



- *GLOBAL PANDEMIC
  - ✓ 9 Million Cases Annually
  - ✓ 2 Billion People Infected



- GLOBAL MORTALITY
  - ✓ 2 Million Cases annually
- GLOBAL MDR DRUG RESISTANCE
  - ✓ Hot Spots (60% of Cases)
    - – India, China, Russian Federation
  - ✓ MDR: 1 in 20 new cases
  - ✓ XDR-TB: 84 countries
  - ✓ Totally Drug Resistant Strains
    - Mumbai 2012

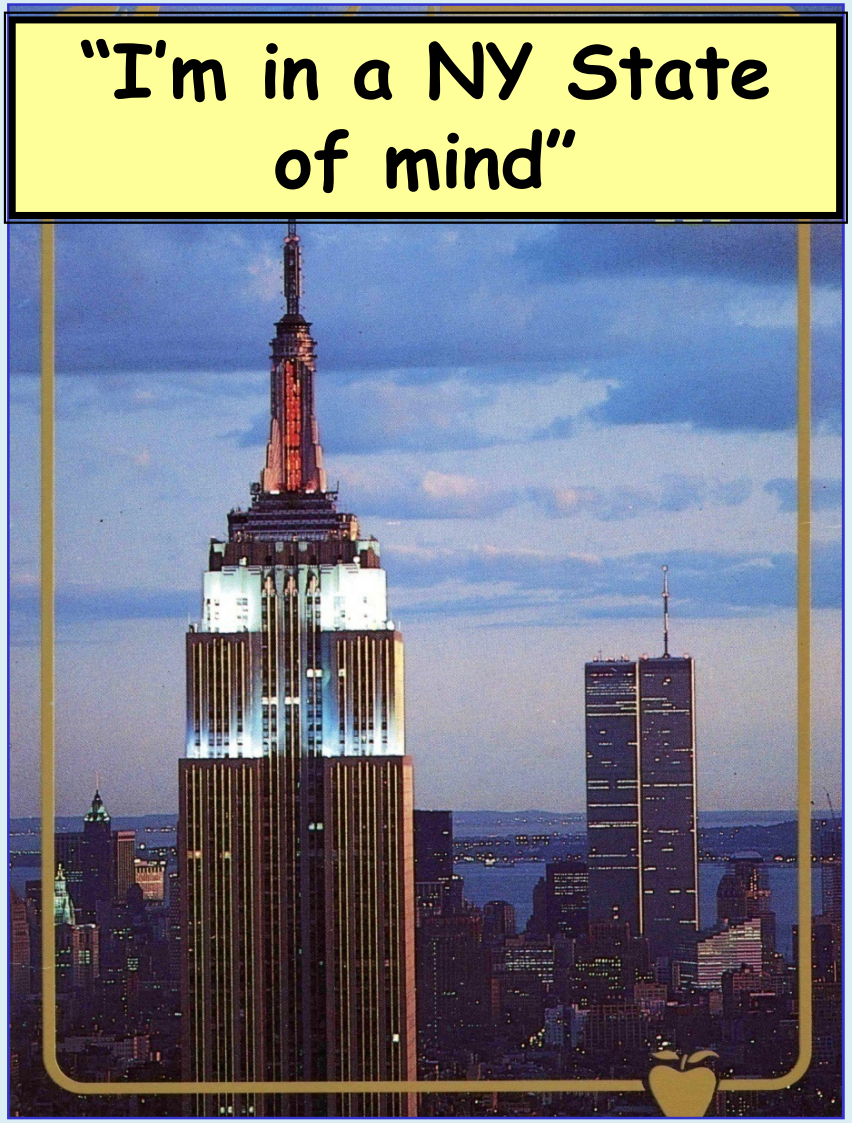


- TB ANYWHERE IS TB EVERYWHERE

*Global TB Report: World Health Organization 2012

TB IN THE BIG APPLE

"I'm in a NY State of mind"







HISTORY OF TB IN NYC
BREAKING THE CHAIN OF TRANSMISSION

1992 Peak Epidemic Yr	1997	Interventions
~4,000 cases (52/100,000)	↓ 55% fewer cases (21/100,000)	**D**irectly **O**bserved **T**herapy
1 in 5 TB cases were MDR 61% of US MDR cases	↓ 87% less MDR	**LAB MANDATES:** DAILY TB LAB RESULTS AFB SMEARS, ID & DRUG SUSCEPTIBILITY
Decline in Public health infrastructure	Downsizing large homeless shelters Increased number of TB clinics	
		DOH budget ↑ from $4 million to >$40 million
		Improved screening, isolation (e.g. prisoners)



The Emergence of Drug-Resistant TB in NYC,
T. Frieden et al NEJM 328, 1993
Tuberculosis in NYC – Turning the Tide,
T. Frieden et al NEJM 333:229, 1995

TB IN THE BIG APPLE

THINK TB

PATIENTS

- *NYC 2012
  - ✓ 8 cases/100,000
  - ✓ ~3 x US cases
- 84% Foreign Born
  - ✓ China most common followed by Mexico
- 6% TB cases were Healthcare workers
  - ✓ Occupational exposure confirmed in 2 of 39 cases

TESTS

- Culture "Gold Standard"
  - ✓ 24% case were culture neg
- AFB Smear Results
  - ✓ Of 499 cases with pulmonary disease
    - – 52% were AFB smear negative
- Drug Resistance
  - ✓ MDR (18 cases)
  - ✓ XDR (2 cases)
    - – 100% ↑ over 5 yrs

*NYC DOHMH 2012 Towards Zero TB
Bureau of Tuberculosis Control Annual Summary

FAST TRACKING TB DETECTION

*NYC DOH MANDATED REPORTING

- **AFB smear positives from any anatomic site**
  - ✓ **Do not delay AFB positive smear result pending NAATs**
- **Nucleic Acid Amplification Test positives**
- **Culture positives for MTBC**
  - ✓ **Initial culture is sent for DNA analysis NYCDOH**
- **Susceptibility test results**
- **Clinical suspicion of TB such that clinician initiates isolation or anti-TB Treatment**
- **Extrapulmonary: Biopsy, pathology tissue consistent with TB (including necrotizing granulomas)**

*Reporting:Through NYS electronic reporting system within 24 hr

NYC TB CASES BY SITE 2012 (n=651)

THINK BEYOND THE LUNG

Pulmonary Disease Only 64%	Extrapulmonary Disease Only 23%			Both Pulmonary & Extra Pulmonary 13%
	Lymphatic	n=93	39%	
	Pleural	n=51	22%	
	Bone/joint	n=26	11%	
	Meningeal	n=13	6%	
	Genitourinary	n=12	5%	
	Peritoneal	n=10	4%	
	Laryngeal	n=1	<1%	
	Other	n=30	13%	

FAST TURNAROUND TIME TO RESULTS CRITICAL TO RAPID DETECTION OF MTB



CULTURE RESULTS

> 2 to 6 wks

AFB STAIN

\+ NAAT

SAME DAY
< 1 hr

EARLY MTB DETECTION
THE ESSENTIAL LINK TO BREAKING THE CHAIN OF TB TRANSMISSION

Comparison of NAAT Tests

FEATURES	MTD Gen-Probe	Xpert® MTB/RIF Cepheid
TECHNOLOGY	Transcription Mediated Amplification	Nested real- time PCR
TARGET	16S rRNA	*rpoB* gene
AMPLICON # ESTIMATED	Relative Light Units (RLU) are measureable	No quantitative measure
SELF-CONTAINED	No	Yes
AUTOMATION		
INTERNAL CONTROL		
DRUG RESISTANCE	No	Rifampin (marker for MDR)

CEPHEID
GeneXpert MTB/RIF Assay

PROS

- VERY EASY TO USE
  - ✓ TEST DAILY, ALL SHIFTS
- SINGLE USE DISPOSABLE CARTRIDGES
  - ✓ NO AMPLICON CONTAMINATION
- SEDIMENT & DIRECT SPECIMEN
- DETECTS MTB Complex DNA
- DETECTS RIFAMPICIN RESISTANCE by *rpo* GENE MUTATIONS

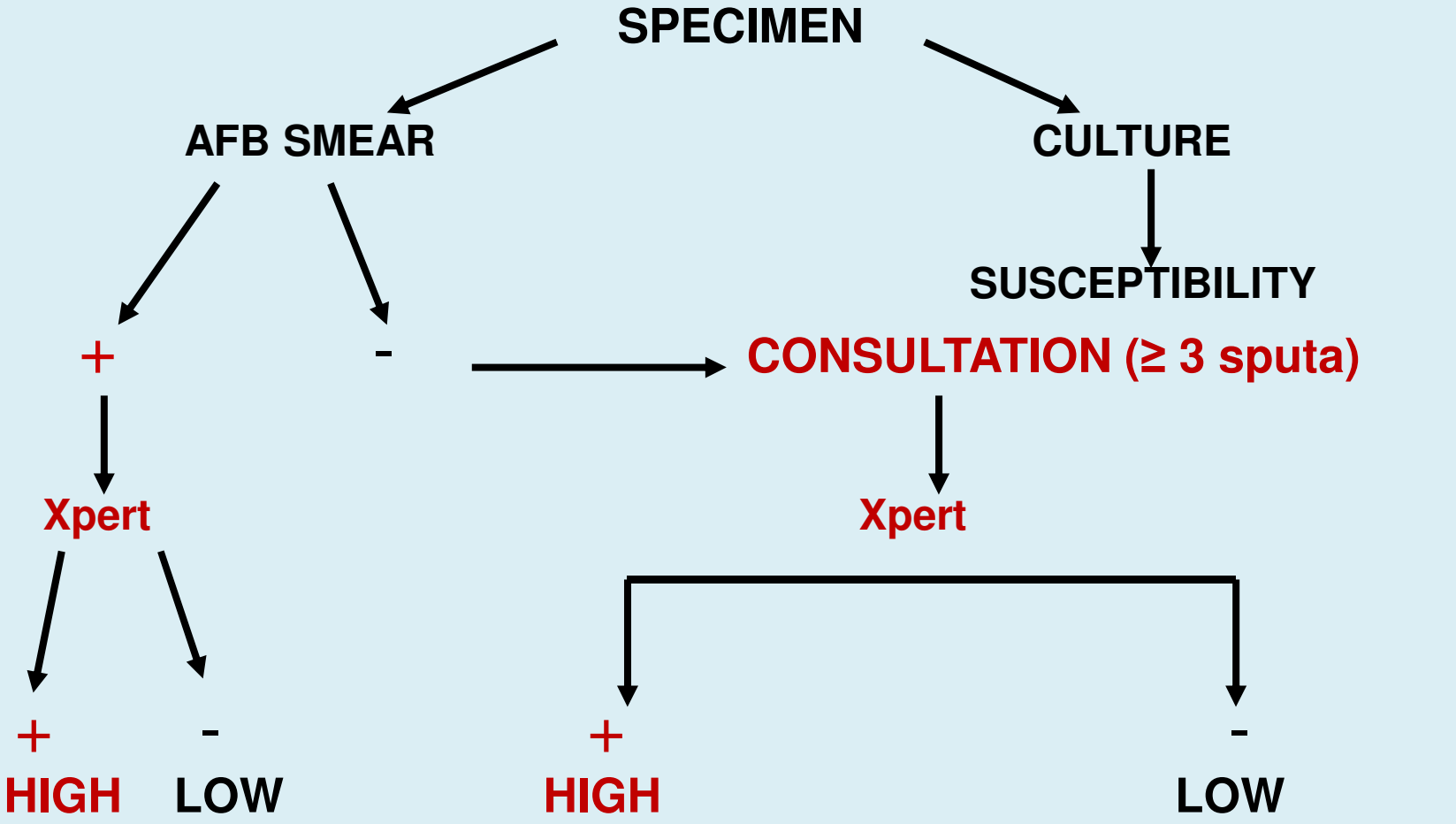


NEEDS IMPROVEMENT

- RIFAMPIN RESISTANCE ONLY
  - ✓ INH RESISTANCE SHOULD BE DETECTED
- SHOULD DIFFERENTIATE MTB FROM *M. bovis*
- ASSAY SHOULD BE QUANTITATIVE
  - ✓ ?ELIMINATE AFB SMEARS?

FAST TRACKING TB DIAGNOSIS

CUMC-NYPH ALGORITHM



INDEX OF SUSPICION FOR TB

CUMC PARADIGM

OPTIMIZING NAAT SENSITIVITY

SPECIMENS

- **Freeze all sediments from patients prospectively**
  - ✓ **When NAAT is tested, we also test previously collected samples**
    - – **Increases assay sensitivity**
    - – **Can identify concurrent pulmonary & extrapulmonary TB infections**
- **Multiple pulmonary specimens per patient**
  - ✓ **3 sputa in one day: Decreases Turnaround Time**
  - ✓ **Increases sensitivity of paucibacillary specimens**
- **MTB NAAT Tests**
  - ✓ **AFB smear-positives: Test routinely**
  - ✓ **AFB smear-negatives: Restricted test**
    - – **Consult with Micro and ID or pulmonary**
- **Pathology routinely notifies Microbiology of specimens that are AFB positive or show necrotizing granulomas**
  - ✓ **Was specimen sent to Micro?**
  - ✓ **Follow up with clinicians: Educate to THINK TB**

CUMC TB Dx Fast Track

- PATIENTS WITH AFB SMEAR +/CULTURE + SPECIMENS (2012)
  - ✓ TB 50% (18 patients)
  - ✓ MAC 15% (259 patients)
  - ✓ RAPID GROWERS 41% (33 patients)
- OPTIMIZE USE OF Xpert TB NAAT

NAAT from Specimens 2011-2012	AFB Smear	Sens %	Spec %	PPV %	NPV %
PULMONARY N= 629	POSITIVE	99	100	100	100
	NEGATIVE	90	100	100	99
EXTRA PULMONARY N=106	POSITIVE	100	100	100	100
	NEGATIVE	80	100	100	98.6

NAAT PERFORMANCE EXTRAPULMONARY SPECIMENS 2006-2014

SPECIMENS	Tested (Culture+)	Xpert TB +	Sens %	Spec %	PPV %	NPV %
LUNG TISSUE	89 (6)	5	83	100	100	98.8
TISSUE BX (e.g. Bone, Pleural)	97 (11)	9	81.8	100	100	97.7
LYMPH NODE	42 (10)	6	60	100	100	88.9
WOUNDS	44 (9)	9	100	100	100	100
CSF	127 (6)	6	100	100	100	100

*CSF specimens received = 2087

NAAT POOR SENSITVITY
PLEURAL FLUIDS vs. TISSUES

PATIENT	SPECIMEN	MTB CULTURE POSITIVE			NAA INHIB
		AFB SMEAR	MTD *RLU	Xpert	
1	PLEURAL FLUID	neg	neg	neg	pos
2	PLEURAL FLUID	neg	neg	neg	pos
3	PLEURAL FLUID	neg	*77,295	neg	neg
	PLEURAL TISSUE	neg	*>3 million	pos	neg

*Cut off for negative = 30,000 RLU
Cut off for positive = 500,000 RLU

EXTRAPULMONARY SPECIMENS

INTRINSIC HURDLES

THINK BEYOND THE LUNG

- PATIENTS HAVE ATYPICAL PRESENTATIONS
  - ✓ DIAGNOSIS & TREATMENT CAN BE MISSED OR DELAYED
  - ✓ INFECTION CONTROL PRECAUTIONS DELAYED
    - – ? Airborne Isolation Precautions?
    - – TB exposures during diagnostic procedures (e.g. draining abscesses, operating room procedures)
- PAUCIBACILLARY SPECIMENS
  - ✓ NAAT lower Sensitivity & lower Neg Predictive Values
  - ✓ Obtaining >1 extrapulmonary specimen will increase assay sensitivity but is difficult
- NO GUIDELINES FOR NAAT USE WITH EXTRAPULMONARY SPECIMENS
- NA AMPLIFICATION INHIBITORS MORE COMMON
  - ✓ Less inhibitors in tissue specimens than body fluids

NAAT EXPEDITING TB DIAGNOSIS

Patient	1	2	3
Age	5 yo	51 yo	48 yo HIV+
Specimen	Brain Bx	Bone Tissue	3 Sputa
Primary Dx	Metastatic tumor	Osteomyelitis	PCP pneumonia
Microbiology Tests	AFB Smear neg/NAAT pos (2 hours)		
Culture	MTB Culture pos (21-26 days)		
Pathology	Lymphs, AFB neg, granuloma	Necrotizing granuloma	N/A
Final Dx	Tuberculoma	Skeletal TB & lymphadenitis	Pulmonary TB

CUMC Isolation Practices

THINK TB

- High risk patients with pulmonary symptoms
  - ✓ Endemic areas, Prior Hx TB, HIV +, homeless, Immunosuppressed , IVDU, Prison, Organ Tx
- Patients with persistent cough, fever, unexplained weight loss, abnormal chest radiograph
- Tuberculin skin test or IGRA test positive coupled with Hx of exposure to TB or cough & fever
- Pediatric facilities: Generally young children are not considered infectious but transmission to healthcare workers during procedures causing aerosolization has been reported. (Int J Tuberc Lung Dis, 2005 9:589-692)

Promptly place suspect infectious TB pts on Airborne Isolation in a negative pressure room

CUMC INFECTION CONTROL PRACTICES

Disease	Isolation Category	Room Required	Protective Equipment for Room Entry
Pulmonary TB Confirmed/Suspect	Airborne	Negative Pressure	N-95 Mask
Extrapulmonary TB with draining lesion	Airborne & Contact	Negative Pressure	N-95 Mask, Gown & Gloves
Extrapulmonary TB, no drainage, no pulmonary disease	None	N/A	N/A
TB Meningitis, no pulmonary disease			
NTM			

Impact of NAAT Results
CUMC Infection Control Practices

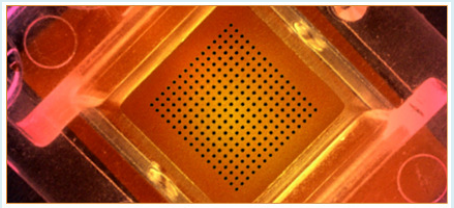
NAAT POSITIVE SPECIMENS- HIGH INDEX SUSPICION	
AFB Smear pos or AFB Smear neg	Remain on Airborne Isolation precautions until • 2 wks Tx & clinical improvement or • Discharged on DOT
NAAT NEGATIVE SPECIMENS – LOW INDEX OF SUSPICION	
AFB Smear pos	D/C Airborne Isolation if clinically cleared or has a history of NTM disease
AFB Smear neg	D/C Airborne Isolation if pt Dx with another disease (e.g. NTM) which responds to Tx or clinical history of NTM disease

WHAT'S NEW IN SUSCEPTIBILTY TESTING?

METHODS	PRINCIPLE	PROS	CONS
MOLECULAR	•Detect R mutants	•Same Day Results	•Expensive •Can Miss Strains without Target Mutation
XpertMTB/ RIF	•Nested Real Time PCR	•Single Use Cartridges with PCR •Quick & Easy	•Detects only Rif Resistance – Marker for MDR •INH needed

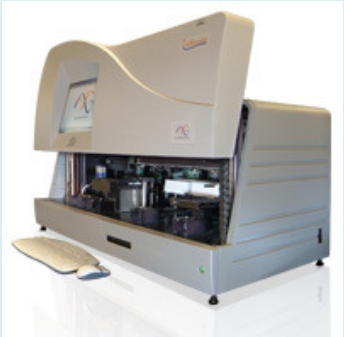
AUTOGENOMICS
INFINITI MDR – RIP ASSAY

- **FEATURES**
  - ✓ **Microarray Biochip technology for detection of MTB resistance to Rifampin & INH**
  - ✓ **Rapid TB ID & susceptibility to RIP from liquid or solid culture (3 h)**
  - ✓ **Differentiates *M. bovis* from MTBC by detecting *pncA*[57] mutation in the PZA gene**



- **INFINITI INSTRUMENT**
  - ✓ **Culture Heat Killed**
  - ✓ **PCR Amplification**
  - ✓ **Load amplified products**
  - ✓ **Load consumables & chips**
  - ✓ **Line listing – presence/absence specific mutations (R) & wild type (S) sequences**
  - ✓ **Predict S/R RIF + INH, MTB vs *M.bovis***

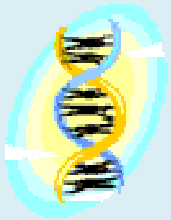




AUTOGENOMICS CUMC STUDY

STUDY STRAINS

- 103 CUMC MTBC ISOLATES
  - ✓ 92% MTB
  - ✓ 7% *M. bovis*/BCG
  - ✓ 1% *M. africanum*
- RESISTANCE
  - ✓ 3% Rifampin mono-resistant
  - ✓ 19% INH mono-resistant
  - ✓ 13% MDR (RIF/INH resistant)



THE RAPID INFINITI SYSTEM WAS COMPARED AGAINST 2 CURRENT GOLD STANDARDS

- ✓ PHENOTYPIC STANDARD BROTH SUSCEPTIBILITY TESTS (MGIT & BACTEC)
- ✓ SEQUENCING

THE RAPID INFINITI SYSTEM DETECTS RESISTANCE BY TARGETING SPECIFIC GENE MUTATIONS

- ✓ 7 RESISTANT MUTATIONS FOR RIF^R (*rpoB*)
- ✓ 3 FOR INH^R
  - 2 (*katG*) & 1 (*inhA* promoter)
- ✓ PZA^R DETECTION OF *M.BOVIS* (*pncA*)

- THE RAPID INFINITI SYSTEM ALSO PROBES FOR WILD TYPE SEQUENCES to detect presence rare SNPs

AUTOGENOMICS RESULTS

RIFAMPIN

- **INFINITI compared to SEQENCING *rpoB***
  - ✓ 95% Sensitivity
  - ✓ 100% Specificity
  - ✓ 100% PPV
  - ✓ 99% NPV
- **INFINITI compared to PHENOTYPE**
  - ✓ 94% Sensitivity
  - ✓ 98% Specificity
  - ✓ 89% PPV
  - ✓ 99% NPV

INH

- **INFINITI compared to SEQUENCING & PHENOTYPE**
  - ✓ 95% Sensitivity
  - ✓ 100% Specificity
  - ✓ 100% PPV
  - ✓ 97% NPV

COMING ATTRACTIONS

- MTBC ID & SUSCEPTIBILITY DIRECTLY FROM SPECIMEN
- NON TUBERCULOUS MYCOBACTERIA
  - ✓ IDENTIFICATION

IDENTIFICATION

100% Detection of *M.bovis*/BCG by *pncA*[57] for PZA Resistance



SAME DAY DIFFERENTIATION.... TB/NOT TB IS CRITICAL

- **CUMC:** 20% AFB SMEAR +/ NAAT NEG PULMONARY CASES ARE OFTEN MAC
  - ✓ RULE OUT TB ??
  - ✓ MAC DRUGS STARTED
- **CUMC:** 84% AFB SMEAR+/NAAT NEG IN COPD PTS MOST OFTEN INDICATE MAC DISEASE
  - ✓ IMPACTS PT TX & MANAGEMENT
  - ✓ WITH CLINICAL IMPRESSION CAN R/O TB
- RAPID DX & START OF APPROPRIATE THERAPY
  - ✓ TB, MAC OTHER NTM
- PT MANAGEMENT
  - ✓ HOSPITALIZATION? DISCHARGE? ISOLATION PRECAUTIONS?
- NO TEST IS 100%
  - ✓ "TB OR NOT TB" IS A CLINICAL CALL

NonTuberculous Mycobacteria
CLINICAL SIGNIFICANCE

- SKIN & SOFT-TISSUE INFECTIONS
  - Multiple Nodular Lesions
- PULMONARY INFECTION
  - Unilateral Noncavitary Lesion
- ENDOCARDITIS - 9% MORTALITY
- FOREIGN MATERIAL
  - Prosthetic Devices
- POSTSURGICAL SITES
  e.g. sternal wounds

- NTM ARE NOT "ATYPICAL MYCOBACTERIA"!
- DISEASE, COLONIZATION, CONTAMINATION?
- American Thoracic Society RECOMMENDATIONS FOR CLINCAL SIGNIFICANCE
  - 3 CULTURE +/AFB +/-
  - 2 CULTURE +/1 AFB SMEAR +
  - 1 BAL CULTURE +/ AFB SMEAR $\geq$ 2+
  - ISOLATION FROM STERILE BODY SITE

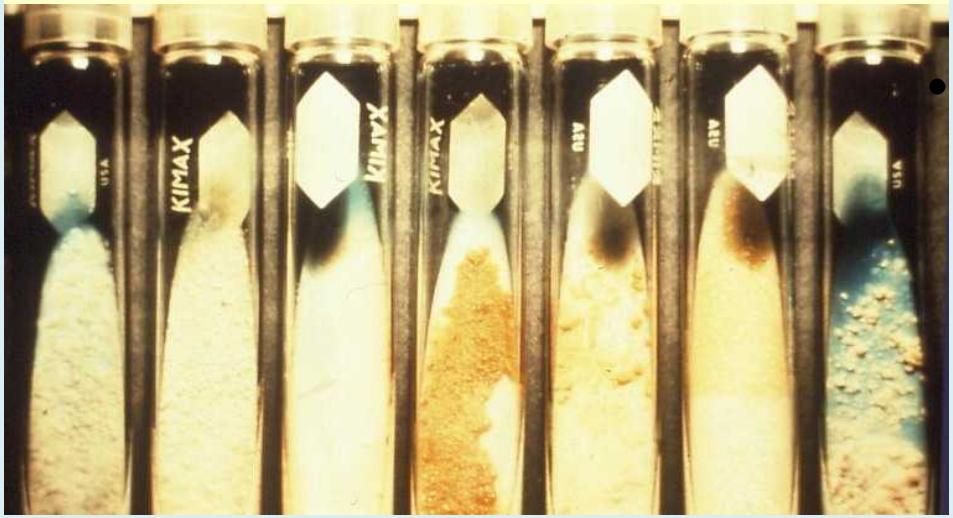
THERE ARE NO NAATs FOR NTM IDENTIFICATION

MYCOBACTERIA

MTB complex (MTBC)

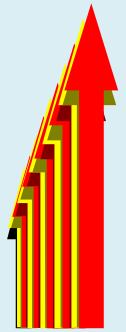
- *M. tuberculosis*
- *M. bovis, M. bovis BCG*
- *M. africanum, M. microti*
- *M. canettii*

MYCOBACTERIAL LINE-UP



Non Tuberculous Mycobacteria (NTM)

- ***M. avium* Complex (MAC)**
  - ✓ *M. avium*
  - ✓ *M. intracellulare*
- **Slow Growers**
  - ✓ *M. kansasii*
  - ✓ *M. xenopi*
- **RAPID GROWERS**
  - ✓ Grows 1 to 2 wks

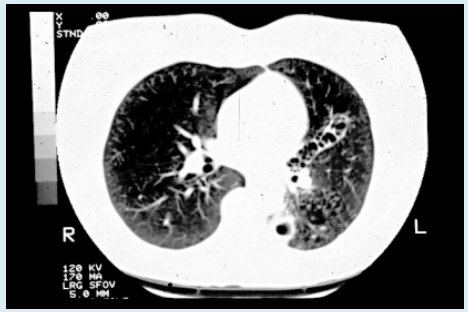


CUMC FACTS & FIGURES
PTS WITH POSITIVE CULTURES

YEARS	MTBC % (n)	MAC % (n)	RAPIDS % (n)	OTHERS % (n)
2007	11 (36)	68 (227)	10 (34)	11 (36)
2008	5 (21)	81 (314)	12 (47)	2 (6)
2009	5 (17)	78 (278)	10 (37)	7 (25)
2010	5 (19)	76 (312)	9 (36)	10 (42)
2011	6 (16)	81 (210)	6 (17)	6 (17)
2012	6 (18)	77 (259)	10 (33)	9 (32)
2013	4 (11)	80 (247)	13 (39)	4 (12)

COMMON CLINICALLY IMPORTANT NTM

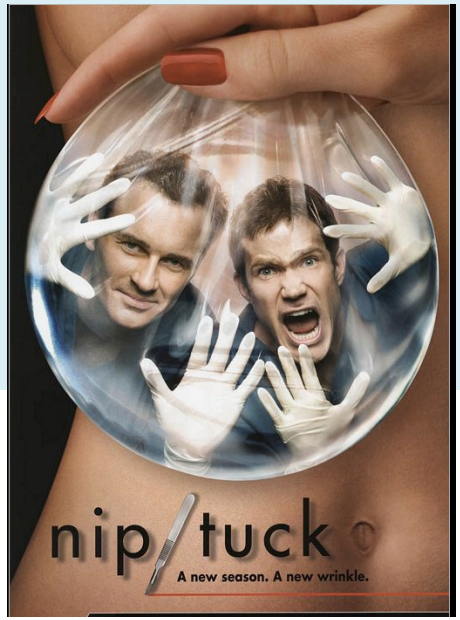
- **MAC DISEASE**
  - ✓ **HOT TUB SYNDROME (SPAS)**
  - ✓ **COPD**
  - ✓ **NODULAR BRONCHIECTASIS**
  - ✓ **DISSEMINATED DISEASE**



- **RAPID GROWING MYCOBACTERIA (RGM)**
  - ✓ ***M. abscessus, M. chelonae, M. fortuitum***
  - ✓ **SKIN & SOFT TISSUE INFECTIONS**
    - ✓ **STERNAL WOUND INFECTIONS**
  - ✓ **Cystic Fibrosis pts, PROSTHETIC VALVES**
  - ✓ **COSMETIC SURGERY (DR)**
    - **"LIPOTOURISM"**



Nip & Tuck



NEW YORK CITY DEPARTMENT OF
HEALTH AND MENTAL HYGIENE
Thomas Farley, M.D., M.P.H.
Commissioner

2013 ALERT # 38

Outbreak on East Coast of Rapidly-growing Mycobacterium Infections following Cosmetic Surgery Performed in the Dominican Republic

One Case Reported in New York City

- An outbreak of *Mycobacterium abscessus* and *chelonae* infections associated with cosmetic surgery performed in the Dominican Republic has been identified, including one case in New York City and seven in other states.
- Please report to the Health Department any suspect cases of nontuberculous *Mycobacterium* skin and soft tissue infections in patients who report recent cosmetic procedures in the Dominican Republic.

CUMC COSMETIC SURGERY CASES

YEAR	#CASES	BODY SITE	NTM SPECIES	COUNTRY
2000	2	Breast, Buttocks	*M. fortuitum*	Unknown
2002	1	Breast	**M. abscessus*	
2003	1	Buttocks		Dominican Republic (DR): Clinic in Santo Domingo
2004	8	Abdomen	1- *M. fortuitum* 7- **M. abscessus*	
2006	1		*M. fortuitum*	
2007	1			
2009	1	Buttocks	**M. abscessus*	
2010	1	Abdomen		
2011	1	Breast	*M. fortuitum*	
2013	4	Breast, buttocks	**M. abscessus*	Brazil
2014	4	Breast, Abdomen		DR

* Subspecies *bolletii*

MOLECULAR RGM IDENTIFICATION

- **Gene Sequencing**
  - ✓ **16S is no longer adequate**
    - ✓ **Cannot differentiate subspecies within *M. abscessus* complex**
  - ✓ ***rpoB* gene**
  - ✓ ***hsp65* needed**
- **Phenotypic Identification Insufficient**
  - **– Time to identification > 1 week**
  - **– Often inaccurate**



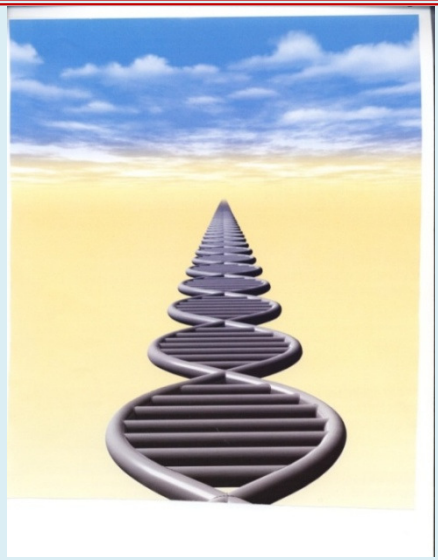
RAPID IDENTIFICATION FROM CULTURE

Critical to appropriate administration of appropriate empirical antibiotics

IDEAL TEST TO FAST TRACK Dx & DETECT MTB

- BY-PASS NALC-NaOH DIGESTION & DECONTAMINATION
- ELIMINATE AFB SMEARS
  - ✓ QUANTITATIVE ASSAY
- MOLECULAR DETECTION RESISTANCE TO RIPE
- ULTRA SENSITIVE: PAUCIBACILLARY SPECIMENS
  - ✓ DETECTION IN SALIVA OR URINE
    AVOID INVASIVE SPECIMENS
  - ✓ EXTRAPULMONARY SPECIMENS
    (body fluids, paraffin blocks)
- DISTINGUISH VIABLE FROM NON-VIABLE CELLS
  - ✓ GUIDE AIRBORNE PRECAUTIONS
  - ✓ MEASURE OF RESPONSE TO TB THERAPY

FOLLOW THE HELICAL ROAD TO THE FUTURE



High Sensitivity
Rapid Dx, Rapid Dx

NAAT (Xpert) ALGORITHMS



- DISCONTINUE AFB SMEARS…..PROBLEMS
  - ✓ Smear data improves NAAT interpretation/accuracy
  - ✓ Smear positive specimens = quality specimen
  - ✓ Smear conversion from positive to neg: Measures response to therapy
- 1-2 NEGATIVE NAAT (NO AFB SMEAR) vs 2-3 AFB SMEAR NEGATIVE SPECIMENS TO DISCONTINUE AIRBORNE ISOLATION….PROBLEMS
  - ✓ Poor specimen Quality/Quantity = false negative results & false negative cultures
  - ✓ Both NAAT and Smear optimize Infection Control decisions and diagnosis
- 1-2 AFB SMEAR POS SPECIMENS, NOT 3 SPECIMENS (WHO) RECOMMENDED

Millman et al (2013) Rapid Molecular Testing for TB to Guide Respiratory Isolation in the U.S.: A Cost Benefit Analysis. PLOS ONE 8:e79669

MUCHAS GRACIAS



Valencia



THANK YOU



New York City

