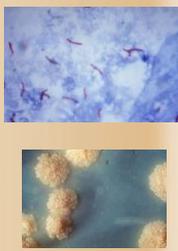


Once Upon a Time

TB Labs - Progress and Challenges

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TB/Refugee Nurse Training  
November 12, 2014



Once upon a time,  
There were AFB smears,  
And TB cultures.....



AFB smear results were available in 1-3 days

TB Cultures took 4-6 weeks to identify

And during that time.....

Patients were started on TB regimens...

And the 1<sup>st</sup> round of contact investigations were complete...

All of them!

And then the culture results arrived showing up to 1/3 were not TB.



Many with DOT

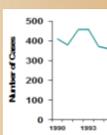


It was a lot of work.

TB suspects lived with the uncertainty of their diagnosis for a long time.



In the early 1990s there was a resurgence of TB.

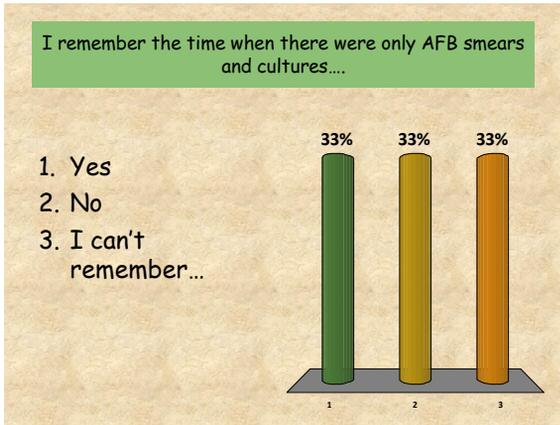


The TB genome was mapped.

New technologies were implemented more widely...

...liquid media  
...faster results



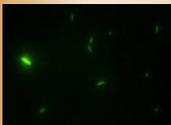


## AFB SMEARS

Fast forward...

AFB Smears

Now fluorescent; easier and faster to read.




### AFB Smear Results

| Fluorescence Microscopy (CDC Scale) |                | Report As:                                |
|-------------------------------------|----------------|---|
| 250X                                | 450X           |   |
| 0 AFB/smear                         | 0 AFB/smear    | No AFB seen                               |
| 1-2/30 fields                       | 1-2/70 fields  | Report exact count; order repeat specimen |
| 1-9/10 fields                       | 2-18/50 fields | 1+  |
| 1-9/field                           | 4-36/10 fields | 2+  |
| 10-90/field                         | 4-36/field     | 3+  |
| >90/field                           | >36/field      | 4+  |

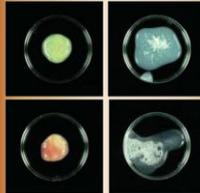
Available the afternoon on day of receipt in the state lab

In the past, 300 fields were viewed!

- Which of the following is **incorrect** regarding smear results for active TB disease?
1. Smear-positive result and grade indicates relative bacterial burden
  2. Drug resistant TB cases can be rapidly identified because they are more likely to be smear positive
  3. Changes in smear status are important for monitoring response to therapy
  4. Smear positive result and grade are useful for identifying the most infectious cases

### More About AFB Smears

- Not very sensitive
  - 50-70% for pulmonary TB
  - 5000 to 10,000 AFB/mL for identification
- Not specific for MTBC
- Value for TB
  - Inexpensive and rapid
  - Infectiousness
  - Monitor therapy
  - Determine need for more testing
- Primary method for TB diagnosis in developing countries



It all starts with the specimen!

## NUCLEIC ACID AMPLIFICATION - "RAPID TESTS"

### Nucleic Acid Amplification (rapid test) (1)

Test done on respiratory specimens for the rapid identification of genetic material unique to TB

GeneXpert now used at DCLS

GeneXpert is currently approved for:

- sputum samples from adults
- Cannot be done on bloody samples
- Can't be on TB drugs >2days at sputa collection
- Single fluoroquinolones considered a TB med
- Cannot be done on sample more than 3 days after processing
- Can be approved by TB Control for select smear negative samples

### Nucleic Acid Amplification (rapid test) (2)

Test done on respiratory specimens for the rapid identification of genetic material unique to TB

GeneXpert now used at DCLS

- DCLS runs this on the first smear + sputum
- Results are "DNA probes", but not cultures
- Does not distinguish between live and dead bacteria
- Provides RIF sensitivity information if MTB DNA detected

### Nucleic Acid Amplification (rapid test) (3)

**Diagnostic:** rapidly identifies smear + samples as MTB, **BUT** a negative NAA in smear negative samples does **not** rule out TB disease

Benefits:

- Rapid turn around time
- Provides early information on MDR TB likelihood
- Provides early information to guide contact investigation

### Nucleic Acid Amplification (rapid test) (3)

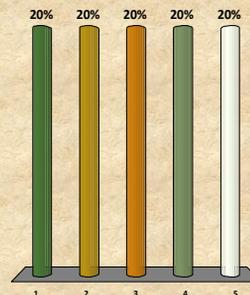
Many private labs can perform this test or a similar in house test

Other NAA Tests:

- MTD by Gen-Probe- "mycobacterium direct"; result an "RNA probe"
- PCR - RT-PCR TMA
- Line Probe Assays- HAIN Genotype INNO-LIPA
- DNA sequencing

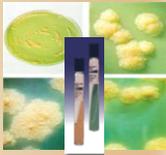
### A NAA test showing MTB "DNA detected" means:

1. The NAA culture is MTBC DNA probe "positive"
2. The client can be released from isolation
3. The culture will likely grow MTB
4. That the sample is RIF sensitive
5. The specimen will be shown to be sensitive to all first line drugs when DSTs are available



# AFB CULTURE

## AFB Culture



**The Gold Standard**

- 10 to 100 AFB/mL for culture
- Usually available in 14-21 days
- Significance of culture
  - Confirm TB
  - Obtain isolate for DST
  - Evaluate therapy
  - Determine length of therapy
  - Genotyping
- Only 85-90% of cases of pulmonary TB are culture positive

## The MTB Complex

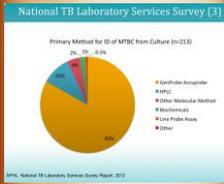
Includes several related organisms, all of which can cause TB disease and are treated similarly

**MTB Complex:**

- *M. tuberculosis*
- *M. bovis*
- *M. africanum*
- *M. microti*
- *M. Canetti*
- *M. Caprae*
- *M. pinnipedii*,
- *M. mungi*

## Identification of Mycobacteria from Growth in Culture

- Conventional biochemical tests
- DNA probes (AccuProbe®; Gen-Probe, Inc. ©)
- HPLC
- MALDI-TOF
- Lab developed tests - "in house"
  - PCR/RE
  - Genetic sequencing

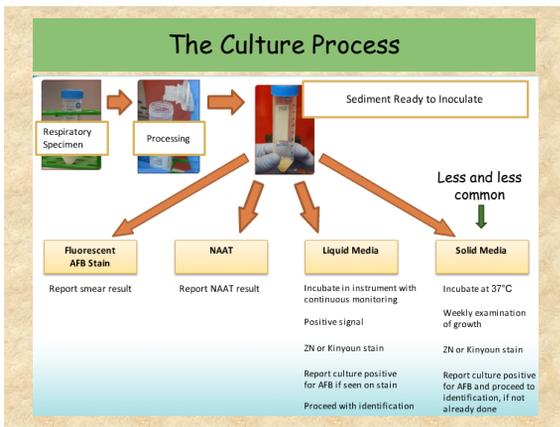


**National TB Laboratory Services Survey (3)**

Primary Method for ID of MTBC from Culture (n=233)

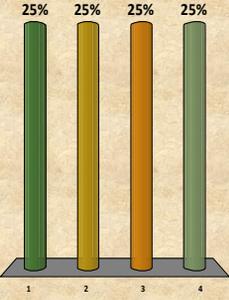
| Method                 | Percentage |
|------------------------|------------|
| AccuProbe              | 87%        |
| HPLC                   | 10%        |
| Other Molecular Method | 2%         |
| Microarray             | 1%         |
| Line Probe Assay       | 1%         |
| Other                  | 1%         |

© 2014, National TB Laboratory Services Survey Report, 2013



## Having growing MTB cultures is important because:

1. It is needed to do drug susceptibility testing
2. It is needed for genotyping
3. It is used for monitoring response to therapy
4. All of the above



| Reason  | Percentage |
|---|------------|
| 1. It is needed to do drug susceptibility testing | 25%        |
| 2. It is needed for genotyping                    | 25%        |
| 3. It is used for monitoring response to therapy  | 25%        |
| 4. All of the above                               | 25%        |

## DRUG SUSCEPTIBILITY

### Drug Susceptibility Testing (DST) of MTBC

Demonstrates which drugs will be effective against the TB organism

- Initial isolate is tested against first-line drugs
  - INH, RIF, PZA, EMB
- If RIF-resistant, or resistant to any two 1st line drugs, test 2<sup>nd</sup> line drugs
  - To include FQ, AMK, KAN, CAP

### Drug Susceptibility Testing (DST) of MTBC

#### Usefulness of DST?

- Determine an adequate regimen
- If sensitive, EMB may be stopped
- If drug resistant, impacts:
  - Contact investigation
  - Isolation decisions

- Broth based tests usually available within 14-21 days of receipt of specimen
- Second line drug testing can take 28 days from culture

### Drug Susceptibility Testing (DST) of MTBC



Agar Proportion Method



Broth Based Method

### What Delays Drug Susceptibility Results?

- Slow or no growth on culture
- Mixed culture
- Contaminated culture

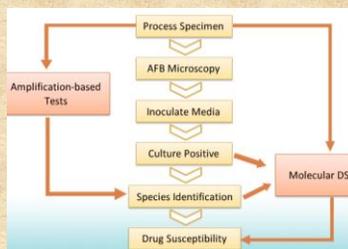


### Solutions for contaminated cultures

- Good oral hygiene generally
- No food before sample collection
- Rinse mouth well with water, but not mouthwash, when sample collected
- Mouth with no contact with collection tube
- Refrigerate sample after collection
- Transport sample on ice packs
- Transport to lab promptly

A contaminated culture doesn't "count"

## Mycobacterial Testing Algorithm



## MOLECULAR DRUG SENSITIVITY TESTS



Discordant results not uncommon

### Molecular Drug Susceptibilities

Rapid tests look for known mutations that cause drug resistance, or look for mutations to the wild strain.

#### Specimen Criteria:

- Isolate or NAA + Sediment
- High risk patient
  - RIF-R
  - From population with high MDR rates
  - Exposed to DR case
  - Failing therapy
- Cases of public health importance, with impact on
  - Public health measures
  - Public health response
- Known RIF resistance
  - Conventional resistance
  - Molecular resistance by submitter
- Mixed or non-viable cultures
- Other reasons

2012

### Molecular Drug Susceptibilities

- Sent to the CDC by DCLS
- Results available within 3 days of the sample's arrival

Molecular Detection of Drug Resistance (MDDR), looks for common genetic mutations of known drug resistant strains

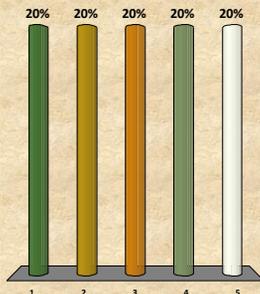
Use caution in evaluating results

Discordant results not uncommon

Virginia TB Medical Consultants should be involved

## Molecular drug sensitivity tests

1. Are used to confirm DSTs for 1<sup>st</sup> line drugs
2. May be used with contaminated cultures
3. Must be requested through TB Control
4. Can provide information on 2<sup>nd</sup> line drug DSTs
5. All of the above



## GENOTYPING

