MARYLAND DEPARTMENT OF HEALTH

# Say What? Interpreting Lab Reports for Patient Management.

**Richard Oatis**

**Supervisor, Mycobacteriology**

April 25, 2018

# AFB Smears/Microscopy

- Report should be semi quantitative.
- Fluorochrome is the preferred method – Zhiel-Neelsen/Kinyoun not as sensitive.

# Positive smear report

**TB Clinical**

**Microscopy Report** **Performed by:** Bryan Burall **Date:** 3/2/18

Fluorochrome - **Numerous AFB Found**



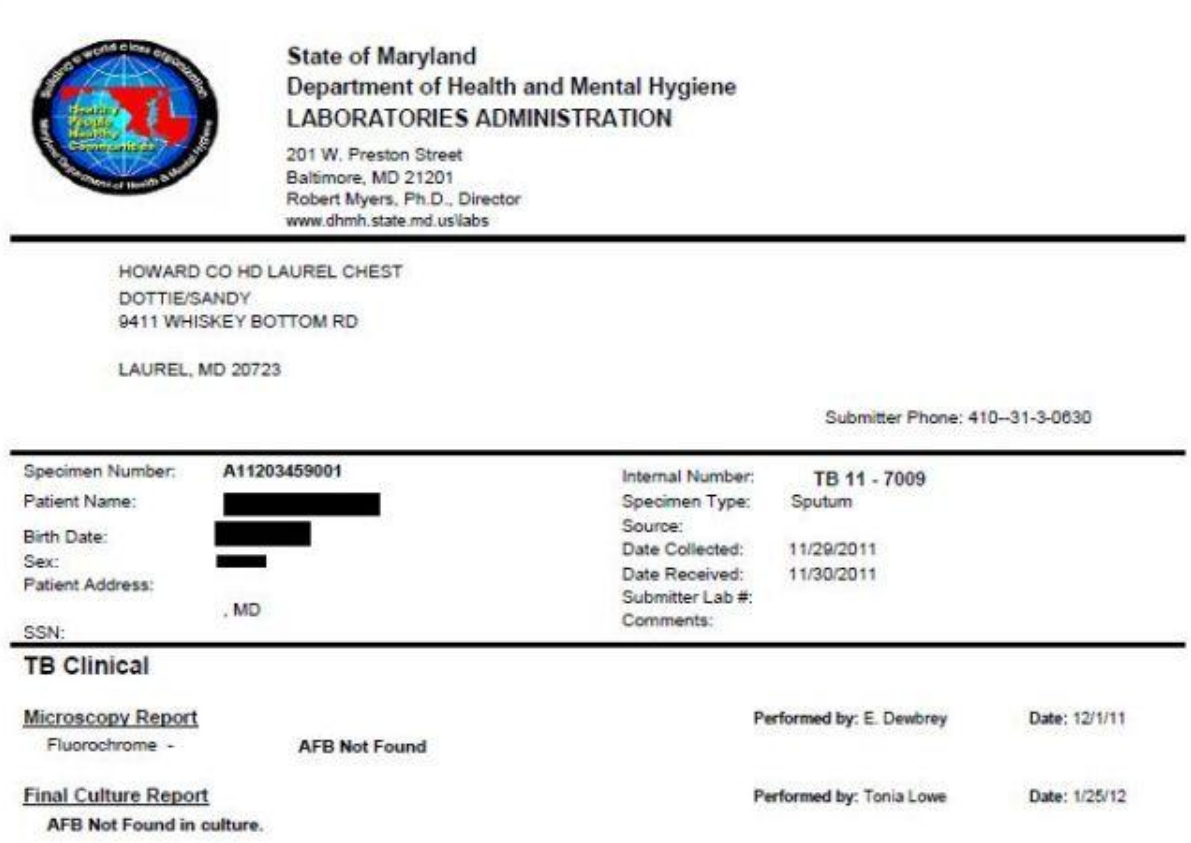
MARYLAND Department of Health

# TB Culture

- Usually takes 1 to 2 weeks for smear positive specimens to grow, but can vary.

- Media negative usually at 6 weeks.

# Negative culture report



State of Maryland
Department of Health and Mental Hygiene
LABORATORIES ADMINISTRATION

201 W. Preston Street
Baltimore, MD 21201
Robert Myers, Ph.D., Director
www.dhmh.state.md.us/labs

HOWARD CO HD LAUREL CHEST
DOTTIE/SANDY
9411 WHISKEY BOTTOM RD

LAUREL, MD 20723

Submitter Phone: 410--31-3-0630

Specimen Number: A11203459001
Patient Name: [redacted]
Birth Date: [redacted]
Sex: [redacted]
Patient Address:
, MD
SSN:

Internal Number: TB 11 - 7009
Specimen Type: Sputum
Source:
Date Collected: 11/29/2011
Date Received: 11/30/2011
Submitter Lab #:
Comments:

**TB Clinical**

Microscopy Report — Performed by: E. Dewbrey — Date: 12/1/11
Fluorochrome - AFB Not Found

Final Culture Report — Performed by: Tonia Lowe — Date: 1/25/12
AFB Not Found in culture.

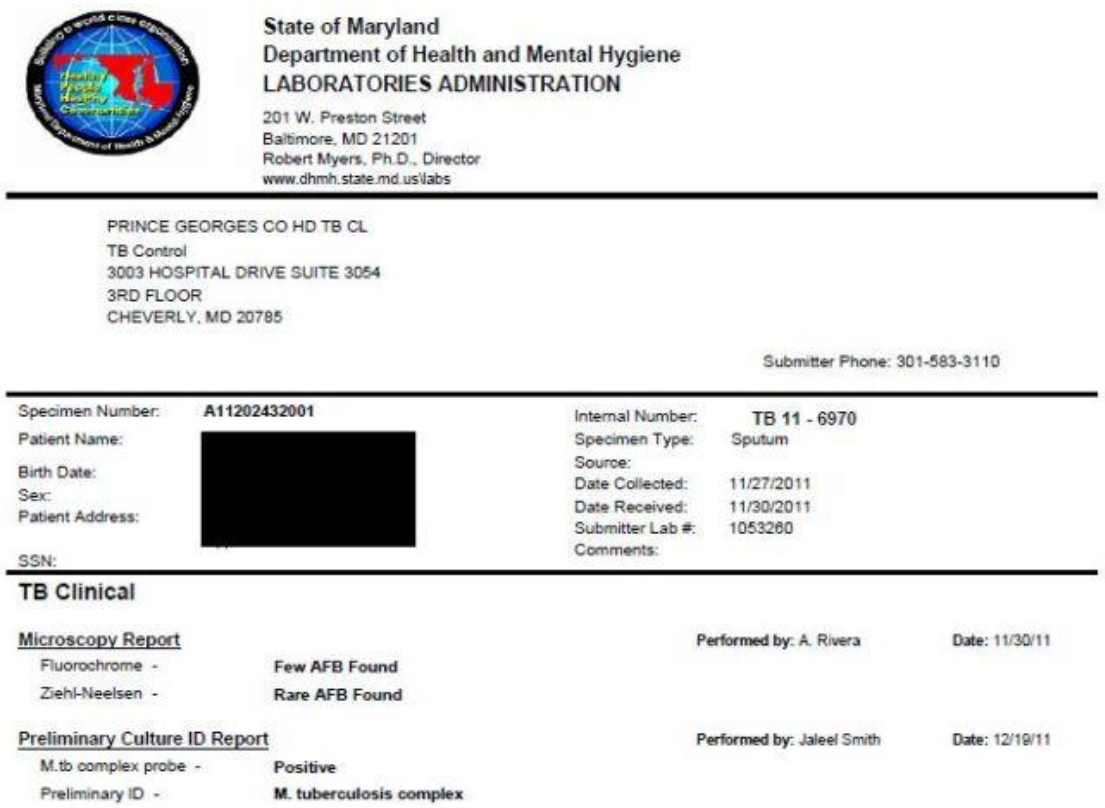


MARYLAND Department of Health

# DNA probes

- Commercially available for *M. tb* complex, *M. avium* complex, *M. kansasii* and *M. gordonae*.
- Utilizes a tagged segment of DNA which is complementary to a specific target sequence in an organism's nucleic acid.
- Can be performed same day culture becomes positive.

# Positive DNA probe report



State of Maryland
Department of Health and Mental Hygiene
LABORATORIES ADMINISTRATION

201 W. Preston Street
Baltimore, MD 21201
Robert Myers, Ph.D., Director
www.dhmh.state.md.us\labs

PRINCE GEORGES CO HD TB CL
TB Control
3003 HOSPITAL DRIVE SUITE 3054
3RD FLOOR
CHEVERLY, MD 20785

Submitter Phone: 301-583-3110

Specimen Number: A11202432001
Patient Name: [redacted]
Birth Date:
Sex:
Patient Address:
SSN:

Internal Number: TB 11 - 6970
Specimen Type: Sputum
Source:
Date Collected: 11/27/2011
Date Received: 11/30/2011
Submitter Lab #: 1053260
Comments:

**TB Clinical**

**Microscopy Report** — Performed by: A. Rivera — Date: 11/30/11

| | |
|---|---|
| Fluorochrome - | **Few AFB Found** |
| Ziehl-Neelsen - | **Rare AFB Found** |

**Preliminary Culture ID Report** — Performed by: Jaleel Smith — Date: 12/19/11

| | |
|---|---|
| M.tb complex probe - | **Positive** |
| Preliminary ID - | **M. tuberculosis complex** |



MARYLAND Department of Health

# Other culture identification techniques

- Biochemical testing
- HPLC
- Line probe assay
- Sequencing
- MALDI-TOF

# Nucleic acid amplification

- DNA within a processed specimen can be amplified and detected without culture growth.
- Usually performed within 48 hours of receipt of specimen.

# Nucleic acid amplification vs conventional DNA probes

- Both utilize a DNA segment to target a specific sequence in an organism's genome.
- Nucleic acid amplification (NAA) includes a molecular amplification process, and can be performed directly on specimens instead of positive cultures.

# NAA vs. DNA Probe

- Culture or isolate – positive for Mycobacterial growth – DNA probe. Usually >7 days from specimen collection date.
- Concentrate or sediment – processed specimen – NAA test. Usually <7 days from specimen collection.

# GeneXpert report

**TB Clinical**

**Microscopy Report** — **Performed by:** E. Dewbrey — **Date:** 8/5/13

Fluorochrome - **Numerous AFB Found**

Ziehl-Neelsen - **Numerous AFB Found**

**Nucleic Acid Amplification** — **Performed by:** Kelly Alban — **Date:** 8/5/13

MTBC - **Detected**

*Mycobacterium tuberculosis complex (MTBC) target DNA detected, which is presumptive for the presence of MTBC in the specimen.*
*It does not necessarily indicate the presence of viable organism.*
*Culture results to follow.*

Rifampicin - **Detected**

*A mutation in the rpoB gene has been detected.*
*Over 96% of clinical isolates that are resistant to Rifampicin have a mutation in the rpoB gene that affects binding of Rifampin to the target, thus confering resistance.*
*Growth based susceptibility testing to follow if a viable organism is isolated.*
*Referred to the CDC for further testing.*
*Disclaimer: The GeneXpert has not been cleared or approved by the FDA. Performance characteristics have been determined by the State of Maryland Laboratories Administration.*
*Test results are for research use only. Not for use in diagnostic procedures.*

# Drug susceptibilities

- Should generally be available within two weeks of culture ID.

- May not be performed automatically by all labs – Call lab to confirm they are performed!

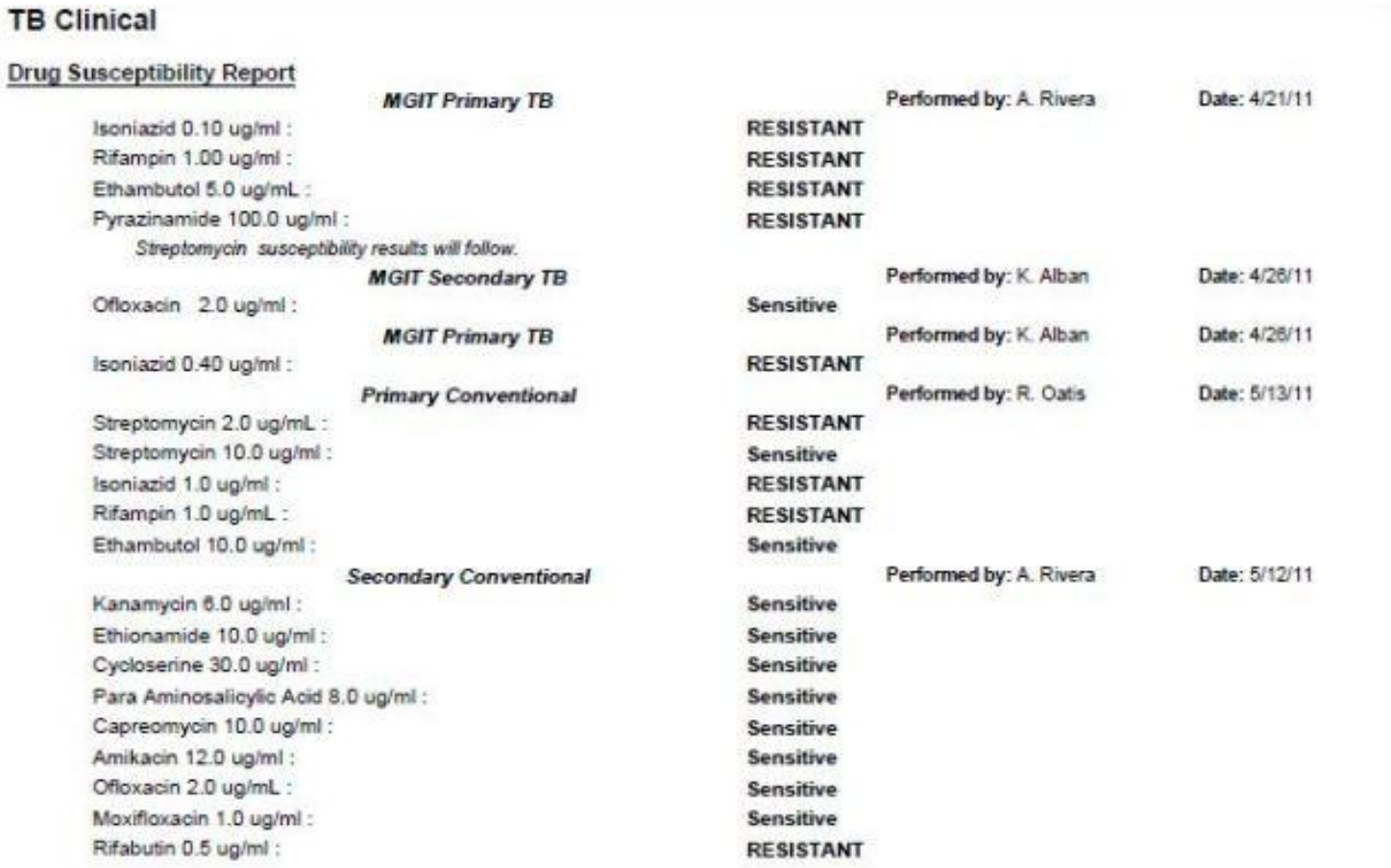
# Drug susceptibility methods

- Liquid media (MGIT)- Quick results for first line drugs: Streptomycin, INH, Rifampin, Ethambutol, PZA. Usually within 10 to 14 days of positive culture.
- Solid Media/Conventional/Agar Proportion - Allows for visual inspection of growth and wider range of drugs. Requires 3 weeks incubation to complete!

# Susceptibility methods (cont.)

- MIC (Sensititre) – No standardized sensitive/ resistant interpretation – not FDA approved.
- Sequencing/MDDR – Looks for genetic mutations associated with drug resistance – may not correlate with growth-based methods!

# Growth-based drug report



**TB Clinical**

**Drug Susceptibility Report**

| | | | |
|---|---|---|---|
| ***MGIT Primary TB*** | | Performed by: A. Rivera | Date: 4/21/11 |
| Isoniazid 0.10 ug/ml : | **RESISTANT** | | |
| Rifampin 1.00 ug/ml : | **RESISTANT** | | |
| Ethambutol 5.0 ug/mL : | **RESISTANT** | | |
| Pyrazinamide 100.0 ug/ml : | **RESISTANT** | | |
| *Streptomycin susceptibility results will follow.* | | | |
| ***MGIT Secondary TB*** | | Performed by: K. Alban | Date: 4/26/11 |
| Ofloxacin 2.0 ug/ml : | **Sensitive** | | |
| ***MGIT Primary TB*** | | Performed by: K. Alban | Date: 4/26/11 |
| Isoniazid 0.40 ug/ml : | **RESISTANT** | | |
| ***Primary Conventional*** | | Performed by: R. Oatis | Date: 5/13/11 |
| Streptomycin 2.0 ug/mL : | **RESISTANT** | | |
| Streptomycin 10.0 ug/ml : | **Sensitive** | | |
| Isoniazid 1.0 ug/ml : | **RESISTANT** | | |
| Rifampin 1.0 ug/mL : | **RESISTANT** | | |
| Ethambutol 10.0 ug/ml : | **Sensitive** | | |
| ***Secondary Conventional*** | | Performed by: A. Rivera | Date: 5/12/11 |
| Kanamycin 6.0 ug/ml : | **Sensitive** | | |
| Ethionamide 10.0 ug/ml : | **Sensitive** | | |
| Cycloserine 30.0 ug/ml : | **Sensitive** | | |
| Para Aminosalicylic Acid 8.0 ug/ml : | **Sensitive** | | |
| Capreomycin 10.0 ug/ml : | **Sensitive** | | |
| Amikacin 12.0 ug/ml : | **Sensitive** | | |
| Ofloxacin 2.0 ug/mL : | **Sensitive** | | |
| Moxifloxacin 1.0 ug/ml : | **Sensitive** | | |
| Rifabutin 0.5 ug/ml : | **RESISTANT** | | |

# Molecular drug report

**Results for Molecular Detection of Drug Resistance (Sanger Sequencing, complete panel); Conventional Drug Susceptibility Test in progress.**

| Locus (region) examined* | Result | Interpretation (based on in-house evaluation of 550 clinical isolates) |
|---|---|---|
| rpoB (RRDR) | **Mutation:** CAC>TAC; His526Tyr | Rifampin resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are RMP-R.) |
| inhA (promoter) | No mutation | Isoniazid resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are INH-R.) |
| katG (Ser315 codon) | **Mutation:** AGC>ACC; Ser315Thr | |
| embB (Met306,Gly406) | Silent mutation: CTG>CTA; Leu355Leu<br>Neutral mutation: GAG>GCG; Glu378Ala | Cannot rule out ethambutol resistance. (79% of EMB-R isolates in our in-house evaluation of 550 clinical isolates have a mutation other than the ones detected at this locus.)<br>The Leu355Leu mutation is a synonymous (silent) single-nucleotide polymorphism (SNP) and does not result in an amino acid change and is not considered clinically significant. The Glu378Ala mutation is likely a neutral mutation and is not associated with resistance |
| pncA (promoter, coding region) | No mutation | Cannot rule out PZA resistance. (86% of PZA-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.) |
| gyrA (QRDR) | No mutation | Cannot rule out fluoroquinolone resistance. (80% of FQ-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.) |
| rrs (1400 region) | No mutation | Effect of the tlyA mutation on Capreomycin resistance is unknown.<br>Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 550 clinical isolates:<br>• 91% of AMK-R isolates have a mutation in the rrs locus;<br>• 87% of KAN-R isolates have a mutation in either the rrs locus or the eis locus;<br>• 55% of CAP-R isolates have a mutation other than the one detected in either the rrs locus or the tlyA locus.) |
| eis (promoter) | No mutation | |
| tlyA (entire ORF) | **Mutation:** CGG>GGG; Arg64Gly | |

*A negative results (e.g., no mutation) does not rule out contributory mutations present elsewhere in the genome.

# IGRAs

- Blood test for latent TB infection which detects increases in interferon-gamma.
- Reports may contain the quantitative values for the assay as well as a qualitative result.

# T-spot

- Measures number of white blood cells producing interferon-gamma.
- < 5 spot = Negative
- 5-7 spots = Borderline
- >7 spots = Positive
- Invalid

# Quantiferon-Gold In-Tube

- Measures change in levels of interferon-gamma in serum (Antigen – Nil).
- 0.34 IU/mL or less = Negative
- 0.35 IU/mL or greater = Positive
- Indeterminate

# Quantiferon-Plus

- Introduces a fourth tube that targets CD8 cells.
- A positive result for either Antigen tube (TB1 or TB2) yields a positive interpretation.

# Gold In-Tube report

| Test Name | | | Date Reported |
|---|---|---|---|
| Quantiferon | | | 03/02/2018 |
| Result: | | POSITIVE | |
| Nil: | 0.08 IU/mL | | |
| Antigen: | 0.68 IU/mL | | |
| Mitogen: | > 10 | | |

# Plus report

| Quantiferon Result | TB1 | TB2 | Units | Date Reported |
|---|---|---|---|---|
| POSITIVE | 6.11 | > 10 | IU/mL | 02/02/2018 |

Release Statement

QFT-PlusR test reports include two calculated values as well as a single test interpretation. The reported values are calculated as follows: (1) TB1 Antigen (TB1 Ag) minus Nil, and (2) TB2 Ag minus Nil.

For a test to be considered positive only one of the two reported values (TB1 or TB2) must be greater than or equal to 0.35 IU/ml and the TB minus and the Nil must be greater than or equal to 25% of the NII value (as determined by the laboratory).

In some cases the laboratory will report a test as indeterminate even if one of the TB Ag values is greater than or equal to 0.35 IU/ml. An indeterminate result is considered invalid and the test must be repeated. Please submit a new specimen. For further questions or concerns, please contact the TB Laboratory at (443) 681-3942.

# Additional questions?

- TB Lab: (443) 681-3942
- Rich: (443) 681-3944
- Email: richard.oatis@maryland.gov