What's New With Your Maryland State Lab.

Richard Oatis
Supervisor, Mycobacteriology

State of Maryland
Department of Health and Mental Hygiene
Labs Administration
1770 Ashland Ave
Baltimore, MD 21205
(443)681-3944
richard.oatis@maryland.gov

In case you plan on falling asleep…

- Lab move to the new facility.
- Specimen quality assurance.
- GeneXpert testing for smear-negative specimens.
- Quantiferon testing: what we do now and what's next.

Contact info:

- Lab results (Val): (443)681-3942
- Weird questions (Rich): (443)681-3944
- Fax: (443)681-4506
- Website: http://dhmh.maryland.gov/laboratories/Pages/Tuberculosis-(TB)-Laboratory.aspx

The new building:

Main lobby:

BSL-2 space:

BSL-3 space:

Today:

Our view:

Growing pains…

- Move begins 04/16/15, but small cracks in HEPA filter housing prevent work at 1770 until 04/26/15.
- Drain pipe leak in penthouse shuts down main processing lab in September 2015. Repairs continue…

Our new BSL-3

- Separate suite of labs isolated from the rest of the lab space.
- Designed with additional features to enhance biological containment.
- Restricted access – a code is required to get in.
- Employees working in BSL-3 must sign in/out and notify security upon entrance/exit.

BSL-3 PPE

Powered Air Purifying Respirator (PAPR):


THANK YOU!

Quality specimens

- Our results can only be as good as the specimen we receive.
- We hope to assist all TB cases managers and physicians in providing the best possible TB specimens.

Sputum specimens

- First morning specimens are often best.
- 5 mL is the optimum volume.
- Watery specimens may not yield good results!

Other specimen collection considerations:

- Check that tube is closed tightly and properly as much as possible!
- Check that biohazard “ziplock” bag is sealed properly.
- Refrigerate specimens as much as possible during storage/transport.
- Submit to lab ASAP – do not batch!

Our goal for time to specimen receipt in lab is 24 hours!

- Expedite lab testing.
- Disallow for the growth of contaminating organisms.

Contaminated cultures:

TB Clinical

Microscopy Report — **Performed by:** M. Plehn — **Date:** 1/11/16

Fluorochrome - AFB Not Found

Final Culture Report — **Performed by:** A. Rivera — **Date:** 1/28/16

Contaminated. Please submit another sample.

Current turnaround times:

CY2015	CY2014	Description of turnaround times (TAT) for initial diagnostic specimens
		1. Promote rapid delivery of specimens. (TAT goal: Specimens should be received in the laboratory within 24 hours of specimen collection.) Report the percent of specimens received within 1, 2, and 3 calendar days.
14	28	% of specimens received within 1 calendar day.
39	54	% of specimens received within 2 calendar days.
59	69	% of specimens received within 3 calendar days.

How can we work together to solve this?

- Identify changes to practices or workflow to expedite specimen submission.
- Notify the lab of any ways we may be to assist – would adjustments to courier schedule help?

GeneXpert

- Nucleic acid amplification (NAA) assay for direct detection of *M. tb* complex.
- Only can be performed on respiratory (sputum, bronch wash, etc.) and tissue specimens.
- Also detects mutations associate with Rifampin resistance.

GeneXpert – when should it be done?

- All smear-positive specimens with no recent history of AFB + culture.
- Specimen should be within 3 days of start of treatment.
- Performed within 7 days of being processed in lab.
- Can be performed on smear-negative specimens upon phone request.

CDC recommends:

- "NAA testing 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."
- http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5801a3.htm

At our lab:

- 316 patients tested with GX in 2015, up from 223 in 2014.
- Of the 316 tested, 142 were requested on smear-negative specimens (45%).
- Of these 142 requested, only 4 were GeneXpert positive (3%).

Concerns of these results:

- Money/lab time spent.
- Negative results can be misleading – GX should not be used to rule out TB!

Negative GeneXpert result:

- In 2015, there were 5 patients with negative GeneXpert results but positive cultures.
- Another 5 cases were only positive upon testing a second specimen.
- These concerns increase with smear-negative specimens – too few TB bacilli to detect!

What we can do as a result:

- All GeneXpert results should be interpreted in light of patient history/clinical presentation.
- Be mindful of specimen quality.
- Call the lab and request additional tests be run if there are concerns with negative results.

GeneXpert to remove patients from respiratory isolation:

- First announced February 2015.
- “One or two” negative GeneXpert results is sufficient for removing TB suspects from isolation.
- NOT intended for patients with confirmed active TB - not a good test of cure.
- http://ir.cepheid.com/releasedetail.cfm?releaseid=896300

Take home messages:

- All TB suspects should have a GeneXpert requested.
- GeneXpert should not be used to “rule out” TB – culture results still important!
- Specimen quality, patient history, and clinical presentation all need to be considered when interpreting GeneXpert results.

Quantiferon

- An interferon-gamma release assay whereby whole blood is exposed to proteins specific to *M. tb.* complex (but not BCG) to test for latent TB infection.
- The test requires three specialized collection tubes: a blank (Nil) tube, the test (Antigen) tube, and a positive (Mitogen) tube.
- Only known to cross-react with three fairly uncommon Mycobacterial infections: *M. kansasii, M. marinum, M. szulgai.*

Quantiferon: how do we interpret results?

Nil [IU/mL]	TB Antigen minus Nil [IU/mL]	Mitogen minus Nil [IU/mL][1]	QuantiFERON®-TB [IU/mL]	Report/Interpretation
≤ 8.0	< 0.35	≥ 0.5	**Negative**	*M. tuberculosis* infection NOT likely
	≥ 0.35 and < 25% of Nil value	≥ 0.5		
	≥ 0.35 and ≥ 25% of Nil value	Any	**Positive**[2]	*M. tuberculosis* infection likely
	< 0.35	< 0.5	**Indeterminate**[3]	Results are indeterminate for TB Antigen responsiveness
	≥ 0.35 and < 25% of Nil value	< 0.5		
> 8.0[4]	Any	Any		

The controversy:

- No equivocal zone around cut point lends to “flip flopping” upon repeat testing.
- Some research suggests a higher cutoff point (.70) may yield results with better correlation to other testing methods.

Why must our lab use 0.35?

- FDA approved the test with this cut point.
- Must interpret the test as described in package insert by Federal regulation.

Why isn't the cutoff being changed?

- No gold standard for latent TB infection.
- Balancing sensitivity (rate of false negatives) vs. specificity (rate of false positives).
- $$$$

What do the numbers mean?

- Amount of CD4 cells in specimen.
- How well blood tubes were shaken during collection.
- How long blood tubes were incubated.
- Variations in preparation of reagents in lab.
- Room temperature of lab.
- Time between blood collection and testing.

What this means for you:

- Interpretation on lab report (positive, negative, indeterminate) is more important than the numbers.
- Still must be interpreted in light of patient history.

How does the lab assure the quality of QFT results?

- Positive and negative controls run daily as well as kit standards.
- All positive results repeated to demonstrate precision.
- Discordant results repeated until an consensus reached.
- Testing can be repeated on same specimen up to a week from receipt if there are concerns.

What's next for QFT?

- Quantiferon-TB Gold Plus.
- May receive FDA approval some time this year.
- Already seeing use overseas.

The good:

- Designed to stimulate CD8 as well as CD4 blood cells.
- May better capture recent exposures, active TB disease, HIV + patients, and young children.
- Mostly no change to specimen collection, processing, and testing procedure.

The bad: 4 tubes instead of 3!

- 2 separate antigen tubes: one targeting CD4 cells, one targeting CD8 cells.
- More time to collect, more discomfort for patients.
- More time to test in lab.

The ugly: $$$$!

- One would expect tubes to cost more per test since 1 additional tube required.
- Less tests will fit on each ELISA plate, which may add cost.
- But what about additional savings?

Current QFT plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1N	1A	1M	*S1*	*S1*	9N	9A	9M	17N	17A	17M	25N
B	2N	2A	2M	*S2*	*S2*	10N	10A	10M	18N	18A	18M	25A
C	3N	3A	3M	*S3*	*S3*	11N	11A	11M	19N	19A	19M	25M
D	4N	4A	4M	*S4*	*S4*	12N	12A	12M	20N	20A	20M	26N
E	5N	5A	5M	*S5*	*S5*	13N	13A	13M	21N	21A	21M	26A
F	6N	6A	6M	*S6*	*S6*	14N	14A	14M	22N	22A	22M	26M
G	7N	7A	7M	*S7*	*S7*	15N	15A	15M	23N	23A	23M	
H	8N	8A	8M	*S8*	*S8*	16N	16A	16M	24N	24A	24M	

Possible QFT-Plus plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1 N	3 N	5 N	7 N	9 N	S1	S1	13 N	15 N	17 N	19 N	21 N
B	1 TB1	3 TB1	5 TB1	7 TB1	9 TB1	S2	S2	13 TB1	15 TB1	17 TB1	19 TB1	21 TB1
C	1 TB2	3 TB2	5 TB2	7 TB2	9 TB2	S3	S3	13 TB2	15 TB2	17 TB2	19 TB2	21 TB2
D	1 M	3 M	5 M	7 M	9 M	S4	S4	13 M	15 M	17 M	19 M	21 M
E	2 N	4 N	6 N	8 N	10 N	11 N	12 N	14 N	16 N	18 N	20 N	22 N
F	2 TB2	4 TB1	6 TB1	8 TB1	10 TB1	11 TB1	12 TB1	14 TB1	16 TB1	18 TB1	20 TB1	22 TB1
G	2 TB2	4 TB2	6 TB2	8 TB2	10 TB2	11 TB2	12 TB2	14 TB2	16 TB2	18 TB2	20 TB2	22 TB2
H	2 M	4 M	6 M	8 M	10 M	11 M	12 M	14 M	16 M	18 M	20 M	22 M

What will it take to get us there?

- FDA approval…???
- Training…should be minimal.
- Lab validation of assay – may present challenges.
- Updating our LIMS to accommodate extra tube result.

Questions?