What’s New With Your Maryland State Lab.

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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:

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- 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:

<table>
<thead>
<tr>
<th>TB Clinical</th>
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<tbody>
<tr>
<td><strong>Microscopy Report</strong></td>
</tr>
<tr>
<td>Fluorochrome: AFB Not Found</td>
</tr>
<tr>
<td><strong>Final Culture Report</strong></td>
</tr>
<tr>
<td>Contaminated. Please submit another sample.</td>
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</tbody>
</table>
Current turnaround times:

<table>
<thead>
<tr>
<th>CY2015</th>
<th>CY2014</th>
<th>Description of turnaround times (TAT) for initial diagnostic specimens</th>
</tr>
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</table>
| 14     | 28     | 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. |
| 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.
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?

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<tbody>
<tr>
<td>≤ 8.0</td>
<td>&lt; 0.35</td>
<td>≥ 0.5</td>
<td>Negative</td>
<td><em>M. tuberculosis</em> infection NOT likely</td>
</tr>
<tr>
<td></td>
<td>≥ 0.35 and &lt; 25% of Nil value</td>
<td>≥ 0.5</td>
<td>Positive²</td>
<td><em>M. tuberculosis</em> infection likely</td>
</tr>
<tr>
<td>≥ 0.35 and ≥ 25% of Nil value</td>
<td>Any</td>
<td></td>
<td>Indeterminate³</td>
<td>Results are indeterminate for TB Antigen responsiveness</td>
</tr>
<tr>
<td>&gt; 8.0</td>
<td>Any</td>
<td>Any</td>
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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:

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<td>17A</td>
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Possible QFT-Plus plate layout:

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<td>22 M</td>
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</table>
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?