Evaluation of a low-cost air sampling system for the detection of *Mycobacterium tuberculosis* in coughing patients

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### Background & Objective

- In Low and Middle-Income Countries (LMICs), healthcare workers have at least three times higher incidence of Tuberculosis (TB), compared those in high-income countries.
- Aerosol droplets from coughing patients with TB pose a significant occupational risk to these workers and the people around them.
- *Mycobacterium tuberculosis* in these fine aerosols, often less than 5 µm in diameter, are still assessed for infectiousness by microscopic exam of sputum, which is neither sensitive nor specific for infectiousness.
- Prior studies in air filtration of patients with TB have suffered from issues of fungal overgrowth and difficulty growing mycobacteria.

### Hypotheses

- In patients with active pulmonary TB, aerosol particles whilst coughing can be captured and cultured using an aerosol filter.
- Decontamination with disinfectant washes can reduce fungal overgrowth and difficulty growing mycobacteria.

### Methods

#### Clinical samples

- Newly diagnosed with TB
- 2 or 3+ Auramine smear

#### Lab Validation

- Dilutions of TB & Fungus
- Inoculations on filters or media
- Direct or Decontamination with TSP or NaOH
- Drying for 24h
- GeneXpert & culture

#### Air filter on day 0 & 14

- Induced cough for 15 as much as possible
- PTFE air filter with 0.3 µm pores
- Vacuum pump, Gilian BDX-II, filters at 2L/min
- Filter brought from hospital to lab in humid box

### Results

**Initial results**: cultures on filter are positive, then die suggesting filter material kills mycobacteria

**Comparison** of TB colonies present on day 0

<table>
<thead>
<tr>
<th>Filter Type</th>
<th>TB colonies present</th>
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<tbody>
<tr>
<td>Control Culture</td>
<td>2100</td>
</tr>
<tr>
<td>l in 10</td>
<td>1000</td>
</tr>
<tr>
<td>l in 100</td>
<td>250</td>
</tr>
<tr>
<td>l in 1000</td>
<td>78</td>
</tr>
<tr>
<td>l in 10,000</td>
<td>14</td>
</tr>
<tr>
<td>l in 100,000</td>
<td>0</td>
</tr>
</tbody>
</table>

**PCR on filter**: + + + + + +

**Culture on fresh filter**: + + + - - -

**Culture on stored filter**: + - - - - -

### Discussion & Directions

- While our samples did not achieve culture positivity, there is evidence that air samples can effectively tested by qPCR for assessing microbial load.
- Other studies showed that only 5% of TB patients elicited contagious aerosol droplets, named “super-infectors.” Our study did not have sufficient power to find these patients.
- Our air sampling system can detect as few as 100 mycobacterial colony forming units using PCR
- Our patients had high microbial load on sputum smear, although *M. tuberculosis* in their cough-generated aerosols were below our threshold of detection
- Future studies will include use of liquid media filter and the use of a polycarbonate filter.

### Acknowledgments

- NIH Fogarty International Center Grant: R25TW009340
- All clinical samples were GeneXpert and Culture negative, so validation experiment was performed

### References