Improvements in Efficiency and Safety: Phenol/Alcohol Fixing of AFB Smears

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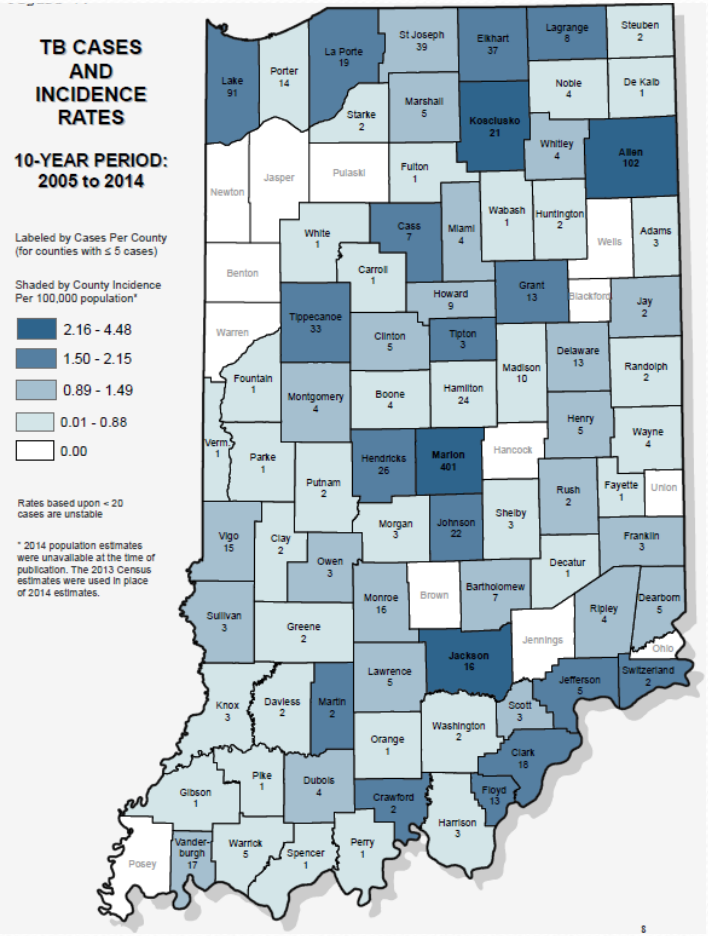
Background

- Approx. 100 cases of TB annually (2014=108)
  - Largest Burmese population outside of Burma
- State PHL tests approximately 2000 specimens/year
  - Majority of samples submitted by county health departments



2014 ISDH TB Annual Report

Background, (con't.)

- TB Control regulated by the Indiana Communicable Disease Rule (CDR)
  - Investigation must be performed immediately by local health officer
  - Infectiousness determined by set of 3 sputa
    - 3 smear negative sputa=release from isolation

- **As a direct result of the CDR, timeliness of smear results is critical**

AFB Testing at ISDH

- AFB and culture performed for every specimen
  - Heat fixed on slide warmer for 2 hours
  - Stained with Auramine o-phenol
  - AFB rated with CAP scale
    - >50/field, >10/field, 1-10/field, <1/field, <1/smear (very few)
- PCR performed for smear positives and high risk smear negatives

April 2012—Diagnostic Mycobacteriology Course

- ***False-Positive and false-negative smear and culture results***--Ken Jost
  - Acceptable methods
    - 2 hours at 65-75 °C
    - 20 minutes at 80 °C
    - 5% phenol in 70% ethanol for 5 minutes

80 °C Validation

- Attempted in August 2012
- Panel of 20 smear positive sputa specimens
- Tested in duplicate
  - 14/20 specimens exhibited lower AFB counts at 80 °C
    - AFB not adhering sufficiently to slide?
- Additionally, problems observed with hot and cold spots on slide warmer

Chedore *et. al.*

Journal of Clinical Microbiology

Method for Inactivating and Fixing Unstained Smear Preparations of *Mycobacterium tuberculosis* for Improved Laboratory Safety

Pamela Chedore, Cecelia Th'ng, Dennis H. Nolan, George M. Churchwell, David E. Sieffert, Yvonne M. Hale and Frances Jamieson

J. Clin. Microbiol. 2002, 40(11):4077. DOI: 10.1128/JCM.40.11.4077-4080.2002.

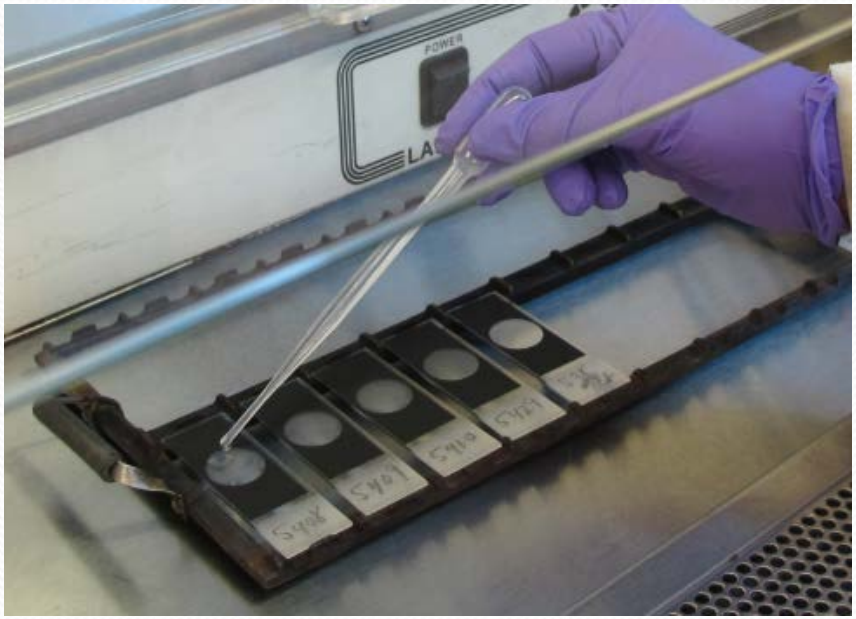
Phenol/Alcohol Validation

- August 2012
- Panel of 40 samples tested in duplicate
  - 30 previously tested sputa
    - 20 smear positives with a range of AFB counts
    - 10 smear negatives
  - 10 AFB cultures
    - 5 MGITs
    - 5 7H11 plates

Number of Samples	Previous AFB Smear Results
7	>10/field
1	8-9/field
2	5-6/field
1	4-5/field
2	3-4/field
3	1-2/field
4	<1/field
10	AFB negative
3	MGIT pos
2	MGIT pos w/cording
5	7H11 solid medium

Smear Fixing Procedure

- Step 1: Pipette a few drops of processed sputa onto the slide
- Step 2: Place the slide on the slide warmer for 10 minutes to dry
- Step 3: Place the slides in a staining rack over absorbent paper
- Step 4: Flood the slide with 5% phenol in 70% ethanol
- Step 5: Let the slide soak for 5 minutes
- Step 6: Immediately stain the slide



Step 4: Flooding the slide with phenol/alcohol

Validation Results

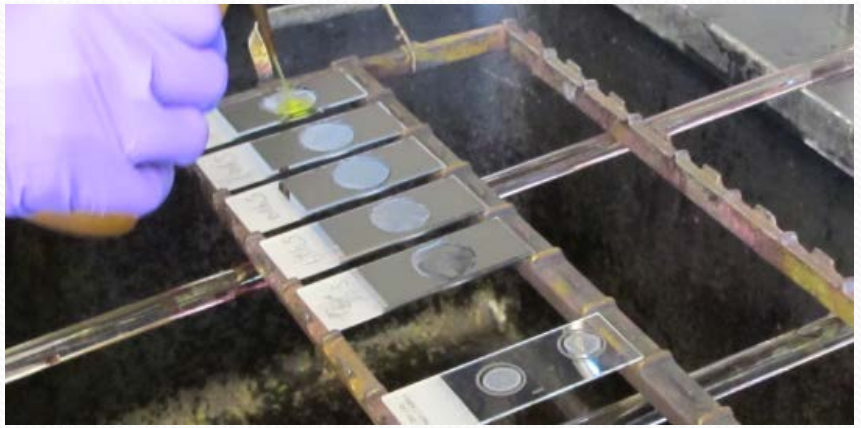
- 100% correlation
- Slightly improved smear counts for 13% of smear positive samples

Sample	Sample Type	65-75 °C	Phenol/alcohol
7	Sputa	<1/field	1-2/field
14	Sputa	<1/field	1-2/field
18	Sputa	8-9/field	>10/field
26	7H11 plate	Pos (very few)	Positive

- Could potentially result in the detection of AFB in samples with very low bacillary loads—the difference between a positive and a negative result

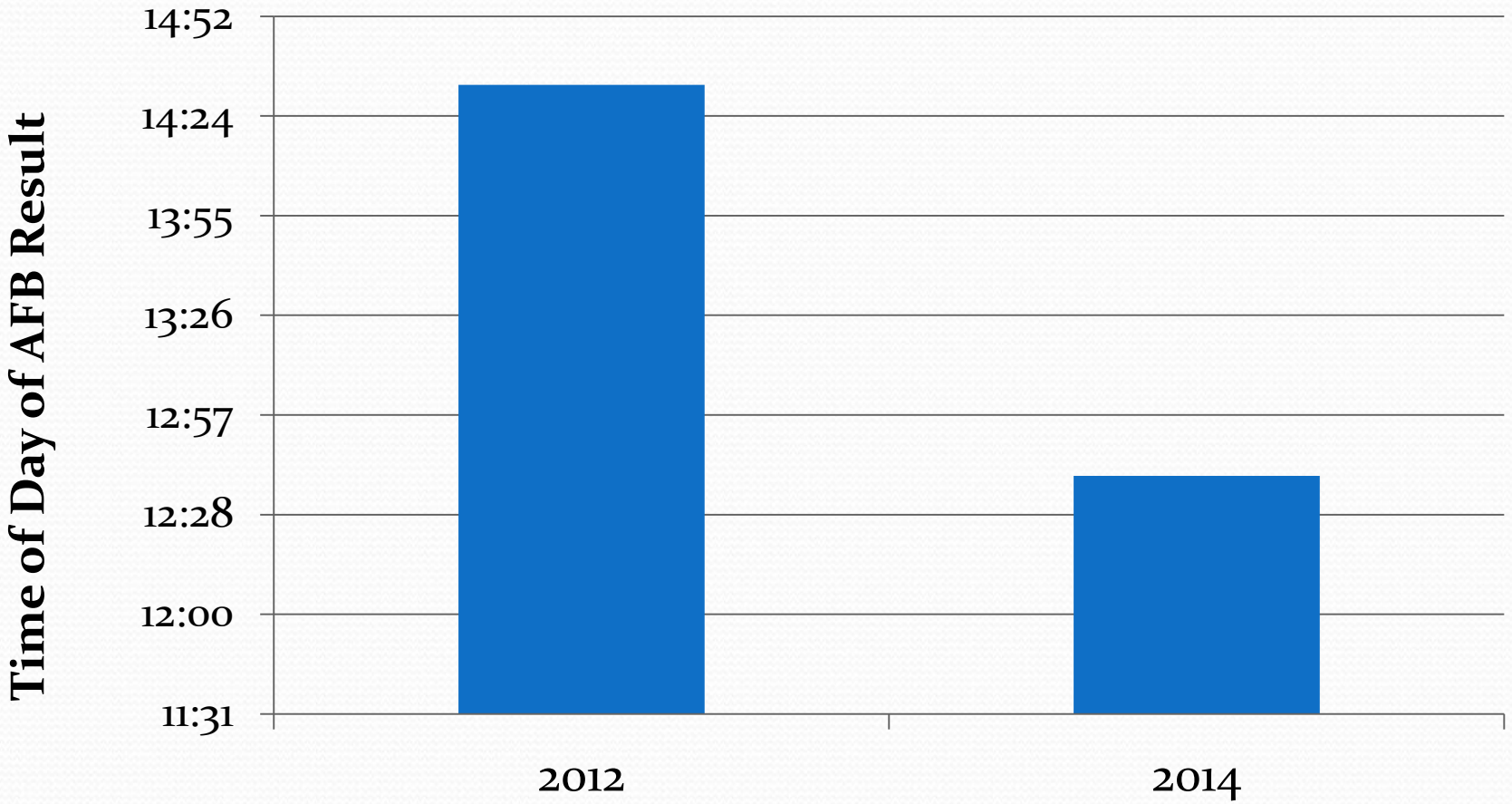
Improvement in Safety

- TB infection is one of the most common forms of lab acquired infection (Harding *et. al.*, 2000)
- Reducing the risk of infection is very desirable
- All steps of procedure are performed in the BSC until stain is applied
- Chedore *et. al.* paper demonstrated that flaming slides was insufficient to kill MTBC (10/10 were viable)
  - After 5 minutes in 5% phenol in 70% ethanol, all samples were nonviable

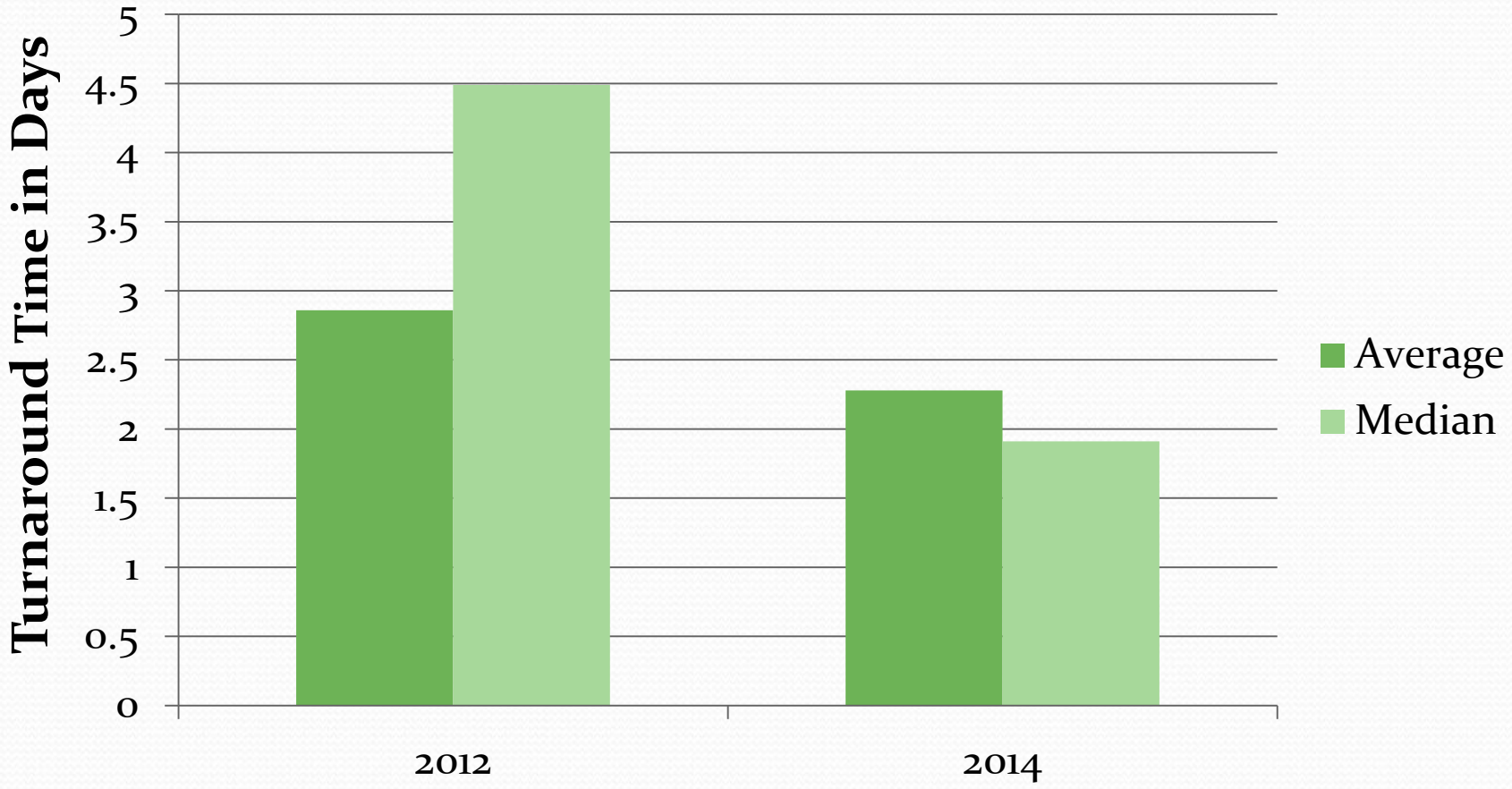


Applying Auramine o-phenol stain

AFB Smear Turnaround Time



PCR Turnaround Time



Acknowledgements

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Questions?

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