

# Validation of a Two Week Agar Proportion Drug Susceptibility Test for INH & Rifampin

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# Background (1)

- CDC recommends rifampin DST results be reported within 17 days after MTBC culture positive ID
  - Most labs use a rapid commercial broth-based system (MGIT or VersaTREK)
  - Agar Proportion (AP) is the “gold standard” method for phenotypic DST
  - AP is not a rapid method; conventional AP method takes 21 days (3 weeks)
- CDC MPEP surveys & literature reports show commercial broth DST methods miss some clinically significant rifampin and ethambutol resistance detected by AP

## Background (2)

- A rapid report of INH & rifampin susceptible is the single most common & important DST report issued by the TB lab
  - ~90% of U.S. cases are RIPE-S
  - INH & RMP susceptible predicts RIPE Rx efficacy (Rx often already started empirically)
  - INH & RMP susceptible allows discontinuation of relatively toxic EMB & PZA in treatment continuation phase

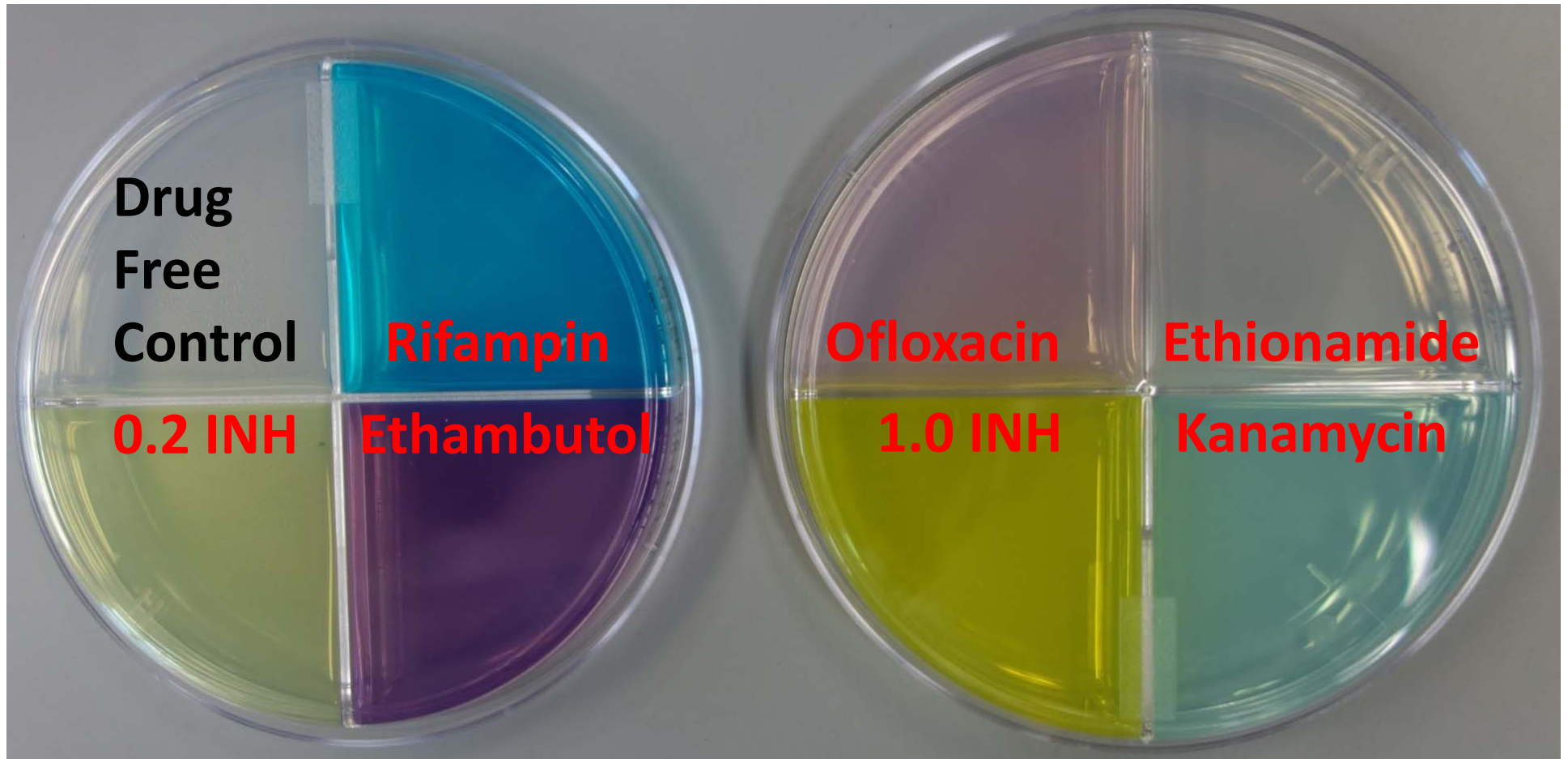
# Hypothesis

MTBC drug susceptibility to highly tuberculocidal drugs like isoniazid & rifampin can be determined at 2 weeks by microscopic observation of growth on drug-free control & drug-containing Middlebrook 7H10 agar.

# Agar Proportion

- Pro
  - Multiple dilutions plated
    - Reduces under- or over-inoculated tests
  - Colonial growth visualized
    - Confirm resistance
    - Confirm purity
  - Detects RMP and EMB resistance missed by broth methods
  - Concurrent 2<sup>nd</sup> line DST easily added (FQNs & injectables)
- Con
  - Some drugs like EMB & FQNs require 3 weeks to declare susceptibility
  - Some MTBC strains do not grow on 7H10 agar
  - LDT Validation required
  - Labor intensive/Subjective
    - Requires expert personnel

# Texas DSHS 7H10 Agar Proportion Plate Panel



# Two & Three Week 0.2 mcg/ml INH

All Tests: Oct 26, 2015 – Mar 30, 2016

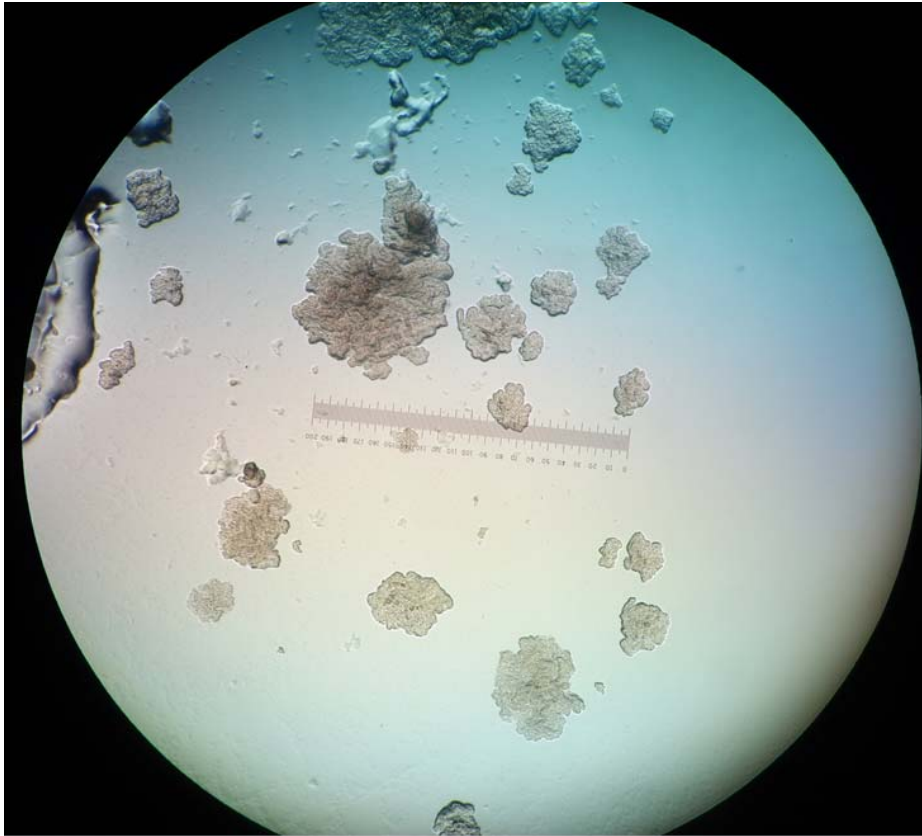
Two Week 0.2 mcg/ml INH Result	Three Week 0.2 mcg/ml INH Result			Total
	Inconclusive	Resistant	Susceptible	
Inconclusive	20*			20
Resistant		52		52
Susceptible	1**		468	469
Wait for 3 week result	3	7	2	12
<b>Total</b>	<b>24</b>	<b>59</b>	<b>470</b>	<b>553</b>

\* Tests were inconclusive due to insufficient control growth or contamination

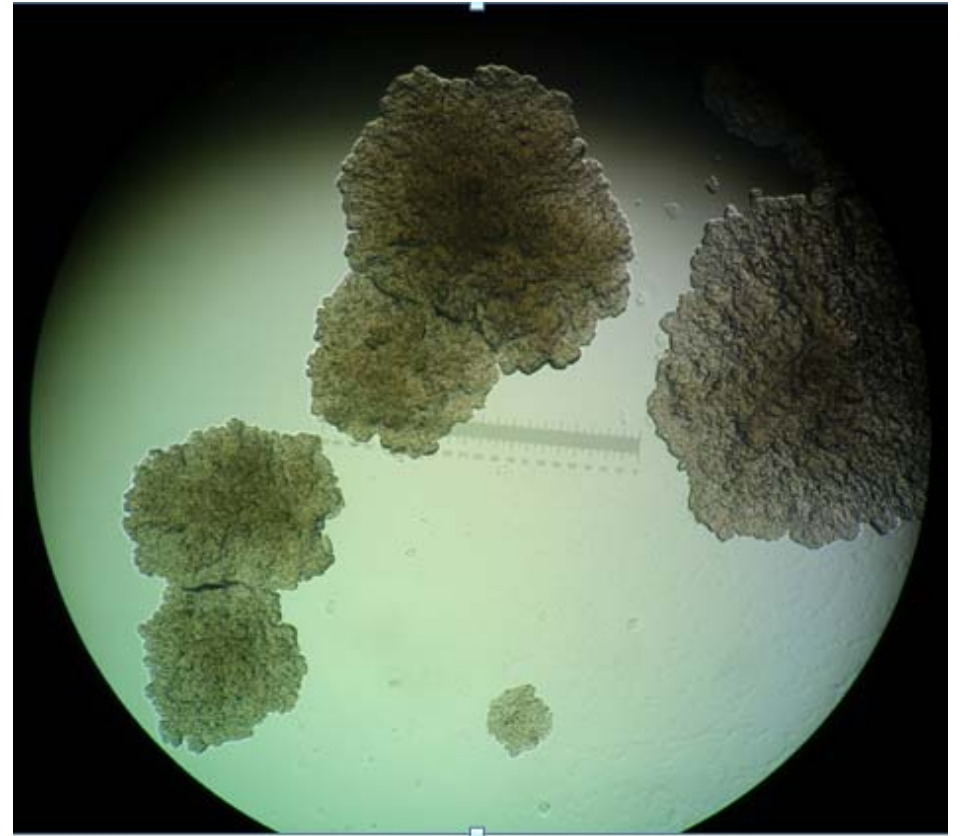
\*\*Repeat test was susceptible at two and three weeks

Incubation	No. of Conclusive Results	% of Total Results
Two Week	52(R) + 469(S) = <b>521</b>	94.2%
Three Week	59(R) + 470(S) = <b>529</b>	95.7%

# Typical MTBC 7H10 Agar Proportion Growth at 50X



**Two Weeks Incubation**



**Three Weeks Incubation**



# Summary

2 week AP was an accurate, practical, & rapid method for INH and RMP DST

- $\geq 98.5\%$  of INH & RMP tests could be declared S or R at 2 weeks with 100% accuracy
- Only a small proportion of tests required a third week of incubation
- 2 Week AP allowed CDC DST TAT guidelines to be met

Thank You!

