FDA Workshop on Development of New Tuberculosis Treatment Regimens
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TB Biomarkers and Clinical Utility

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advancing health worldwide

Relevant Disclosures

- Funding from Centers for Disease Control and Prevention, TB Trials Consortium contract
- Funding from NIH/NIAID TB biomarker discovery and qualification awards

Overview

1. Current methods and endpoints for TB drugs/regimen testing
2. Challenges of culture-based systems
   - Uncertainties around prediction and surrogacy
   - Technical and specimen-related issues
3. Mycobacteriology in TBTC Study 31/A5349
4. Alternative biomarkers on the horizon and opportunities
   - Sputum LAM
   - GeneXpert Cycle Thresholds
   - Time to positivity on MGIT 960 and EBA
   - Sputum Mtb transcriptomic profiling
   - Consortium for TB Biomarkers (CTB2) Repository

Current methods and endpoints: All phases rely on culture

Phase of Drug/Regimen Development	**Critical Test**
Phase 3 Endpoints	
TB disease-free survival at 12 months after study treatment assignment. **Seeking high sensitivity and specificity**	
Phase 2 Endpoints	
Proportion culture negative at completion of 8 weeks of treatment (solid and liquid media considered separately)	
Time to stable sputum culture conversion (solid and liquid media considered separately)	
Speed of decline of sputum viable bacilli by automated liquid MGIT culture days to detection	
EBA Endpoints	
Logarithms of daily CFU counts per ml sputum over 14 days of treatment, as compared to baseline counts	

Challenges of culture-based systems

a. Uncertainties around prediction and surrogacy in early and middle drug development
b. Technical and specimen-related issues

EBA not predictive of sterilizing activity or long term outcomes

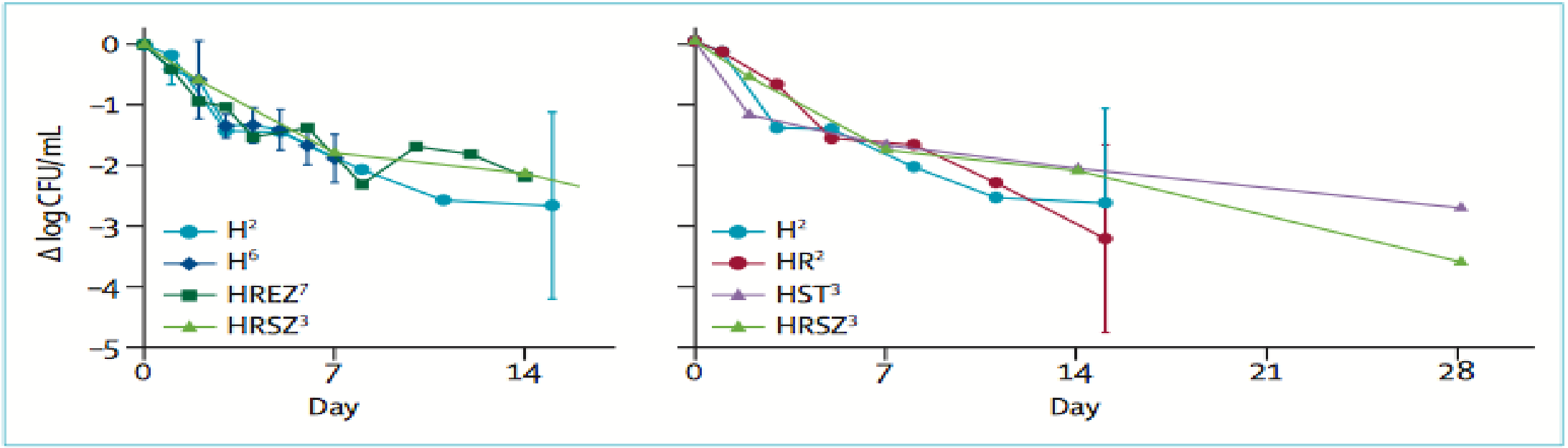


***Figure:* Published trials of quantitative sputum microbiology in patients with pulmonary tuberculosis of 7–28 days' duration**

Left: Across-trial comparisons of similar treatments, showing similar results. Right: Within-trial comparisons of distinct treatments, showing similar results during first 14 days. H=isoniazid; R=rifampicin, E=ethambutol; S=streptomycin, Z=pyrazinamide; T=thiacetazone. CFU=colony-forming units.

Wallis letter, Lancet, 2012

Drugs with modest to no EBA

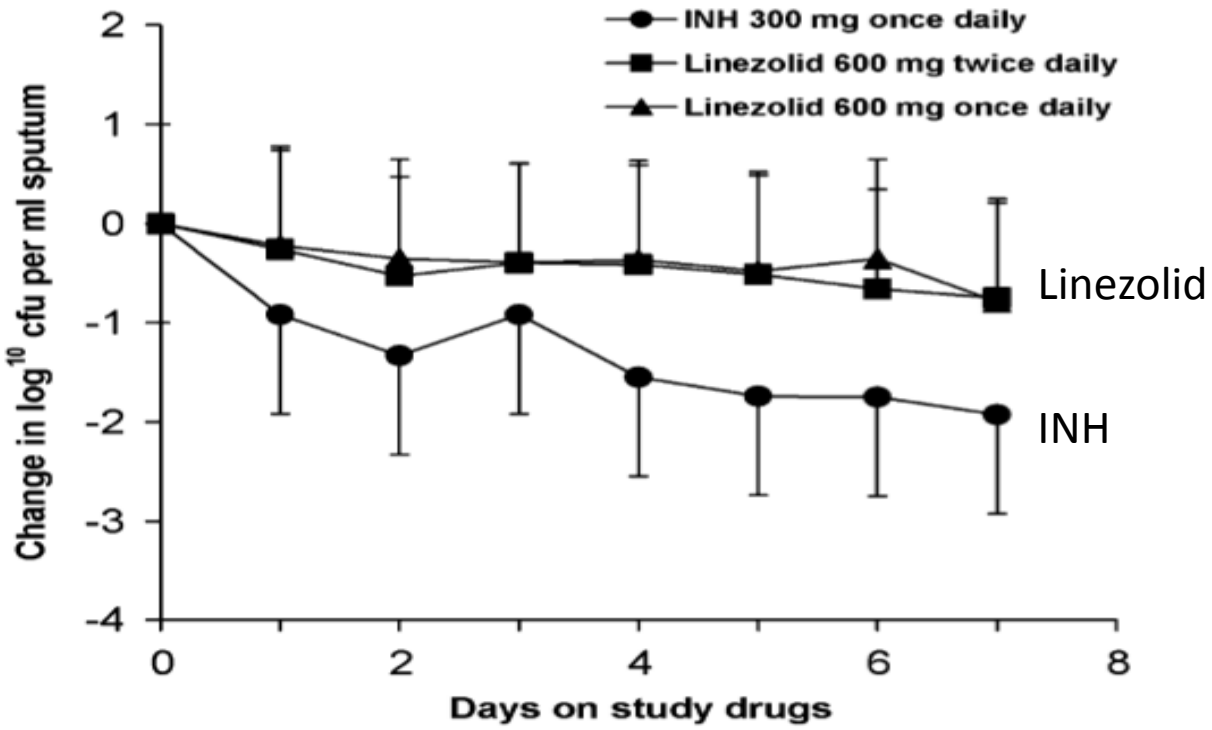
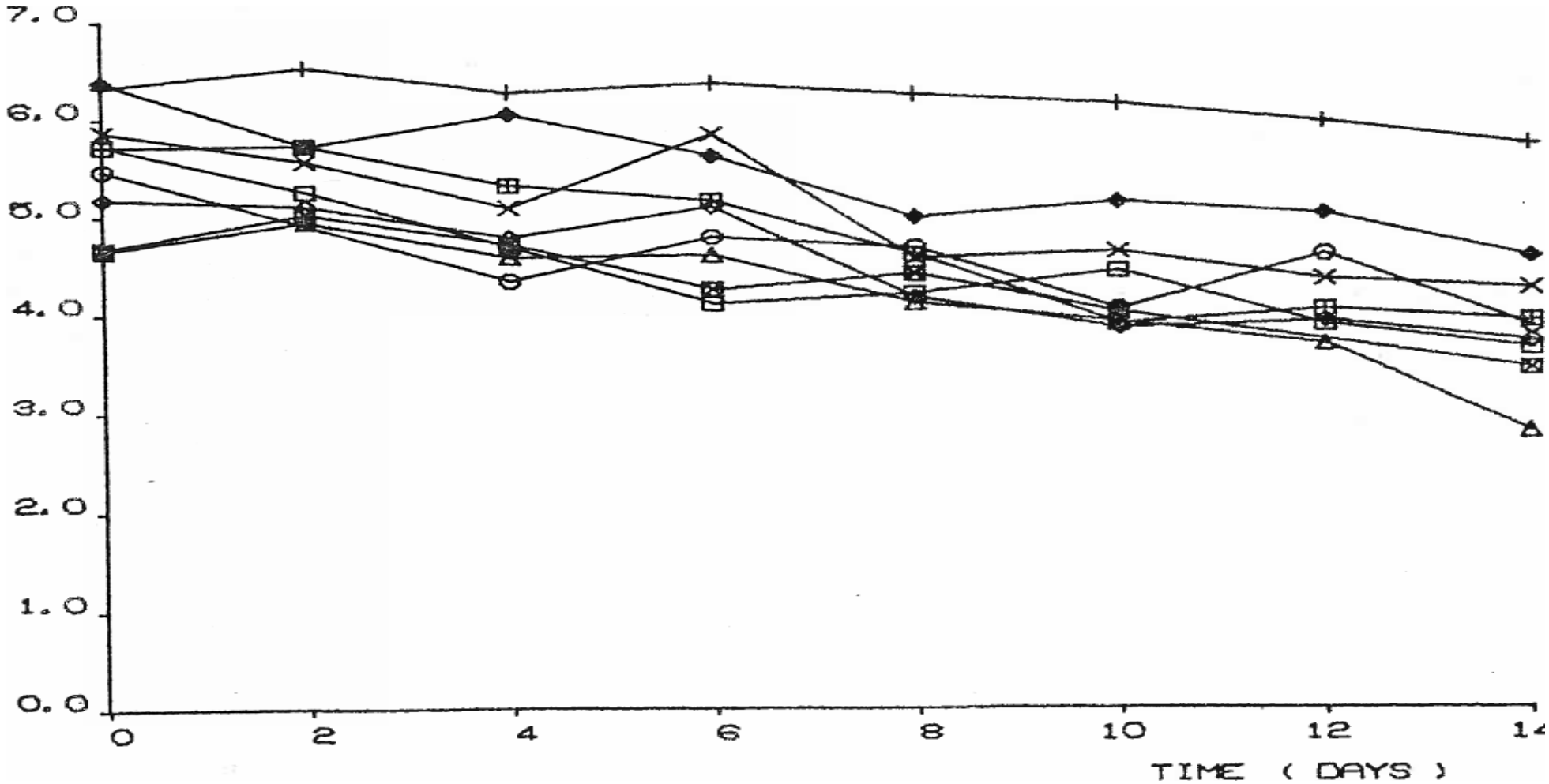


Figure 1. Change in colony-forming units (cfu) in sputum before and during 7 days of study drug administration with isoniazid (INH, 300 mg once daily) and linezolid (600 mg, once or twice daily). Sputum was collected for 12 hours for 2 days before and daily during 7 days of drug administration. Data represent the mean change in $\log_{10}$ cfu/ml of sputum ± SD for each of the 7 days of study drug administration. Mean baseline colony-forming unit counts for each treatment group are listed in the text.

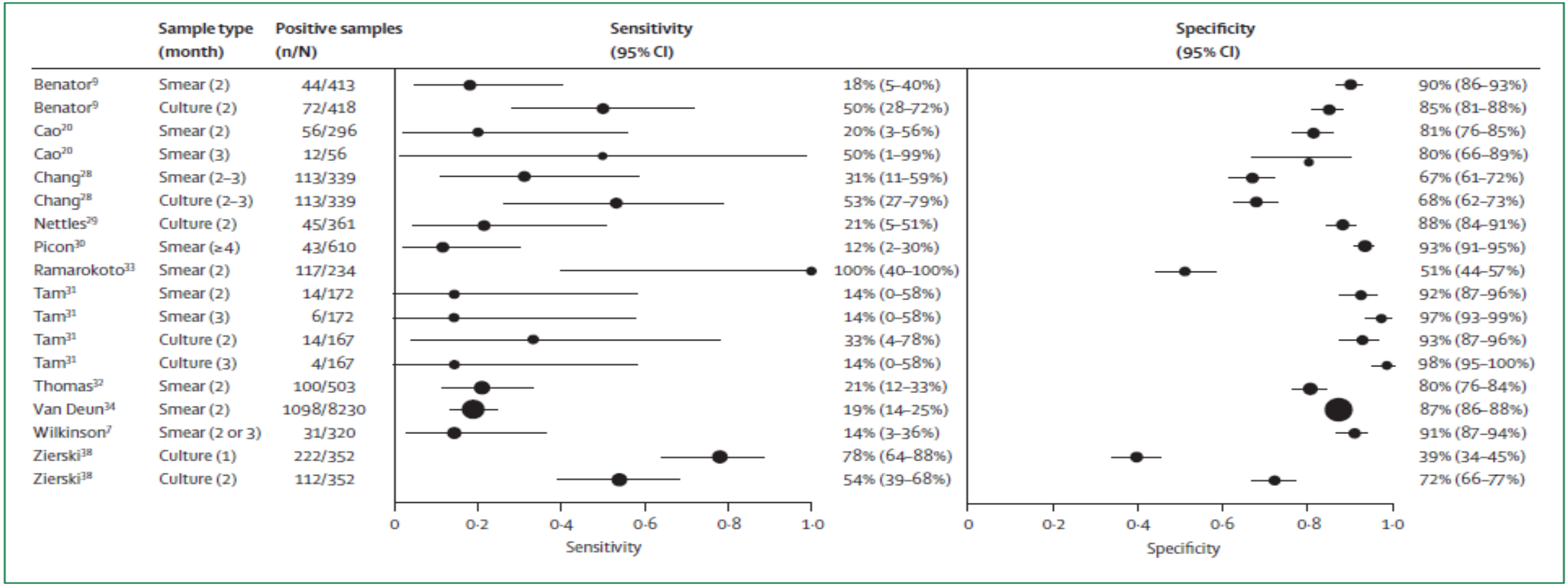
Dietze, AJRCCM 2008

Mean decrease in $\log_{10}$ CFU in PZA Group



Jindani, Am Rev Respir Dis. 1980

2-month culture and outcomes (failure/relapse)

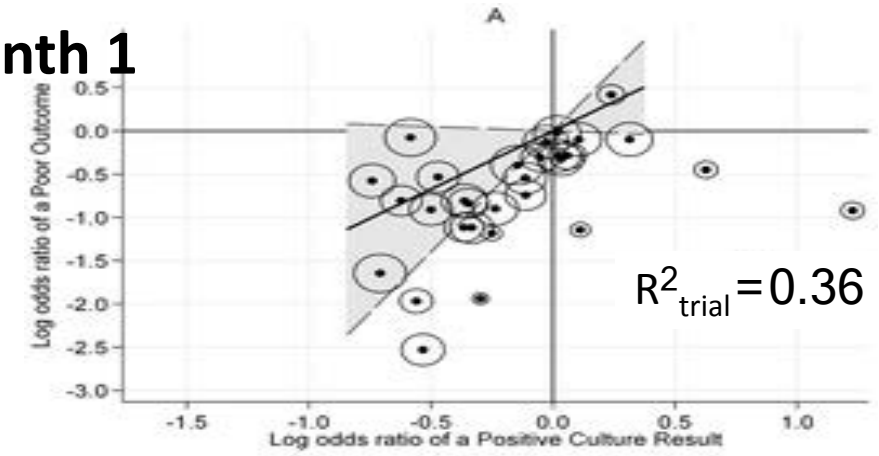


Low positive predictive value for individual level prediction

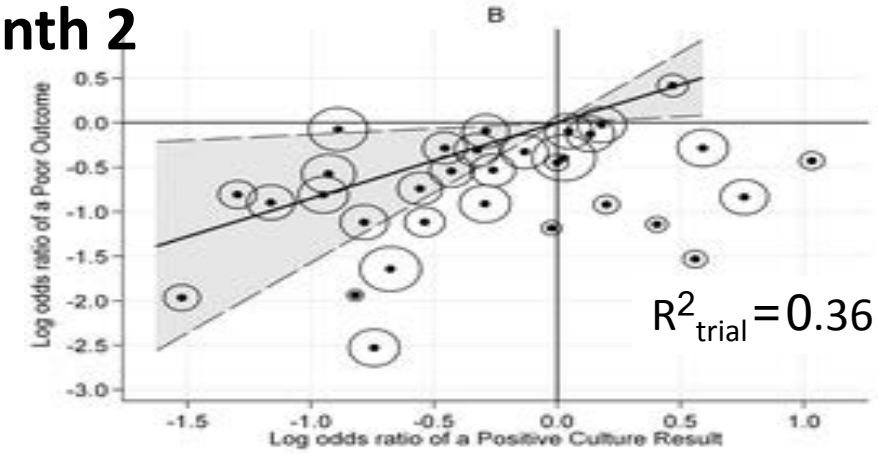
Horne DJ *et al.* Lancet Infect Dis 2010

Evaluation of Culture Results during Treatment for Tuberculosis as Surrogate Endpoints for Treatment Failure and Relapse

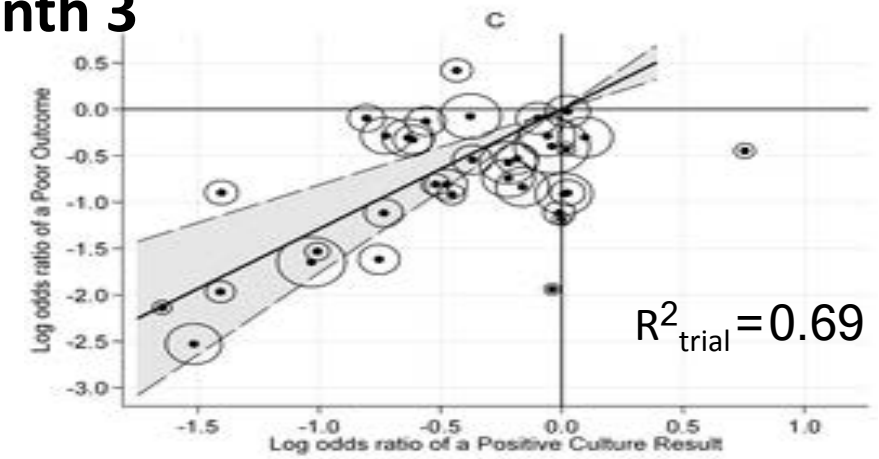
Month 1



Month 2



Month 3



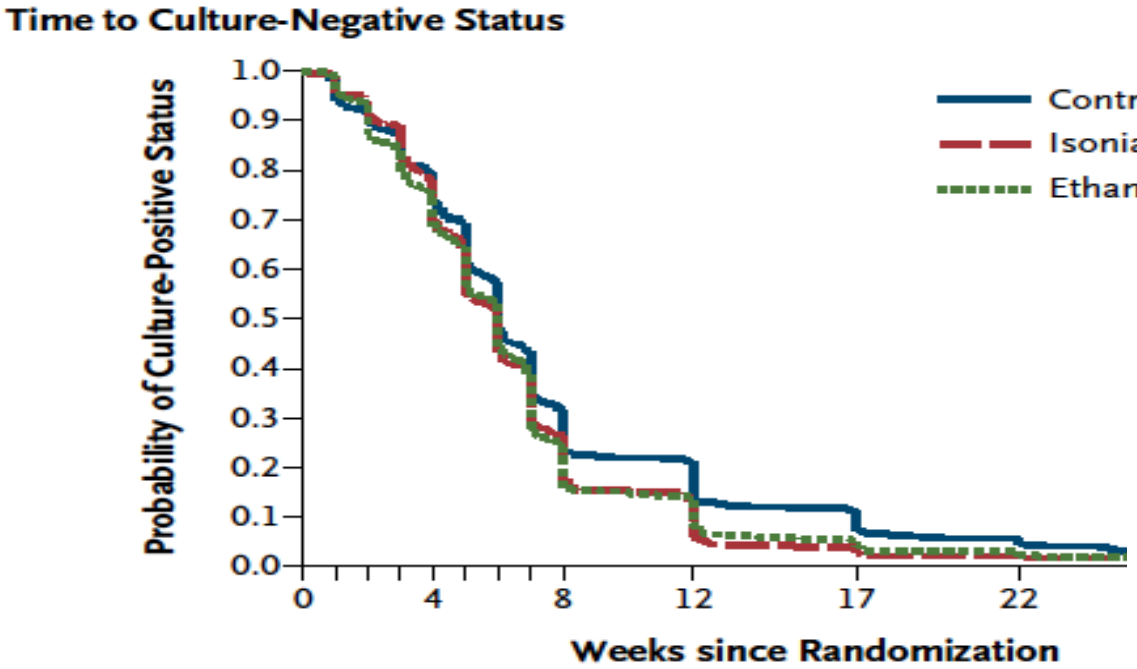
Using 37 total possible treatment comparisons from 49 trial arms

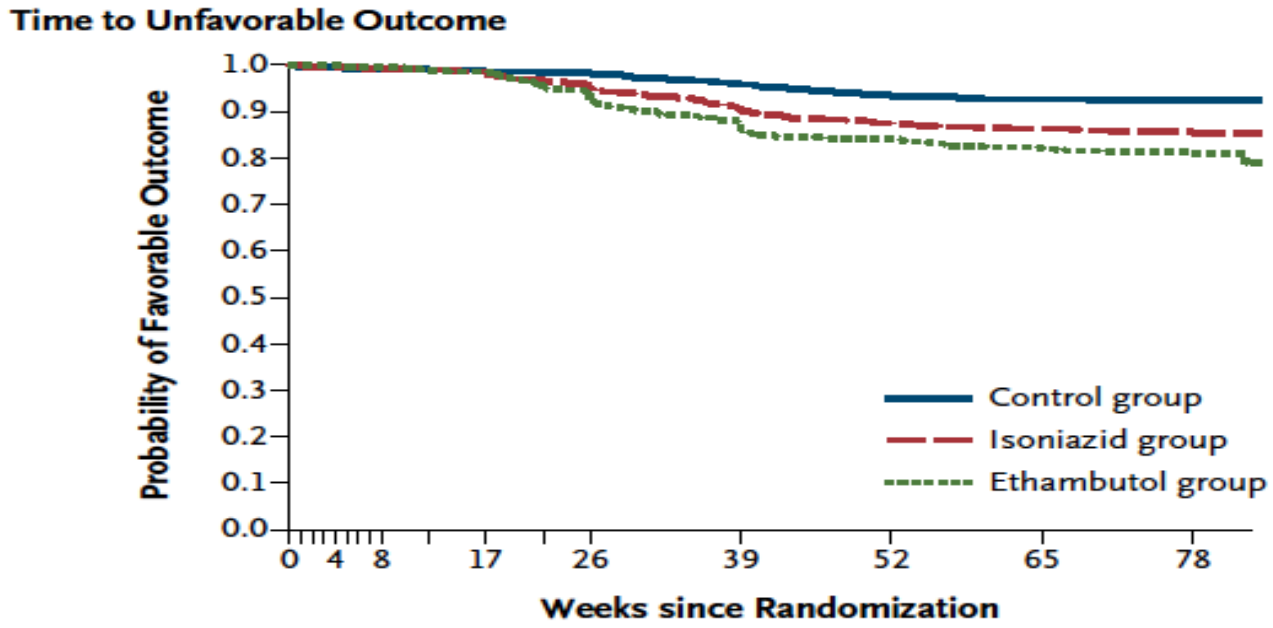
Logs odds ratio of a poor outcome plotted against log odds ratio of a positive culture.

The dotted line represents the 95% confidence interval on the slope.

Phillips PPJ, PLoS ONE, 2013

Time to stable culture negative status within REMox TB also did not predict outcome of the trial

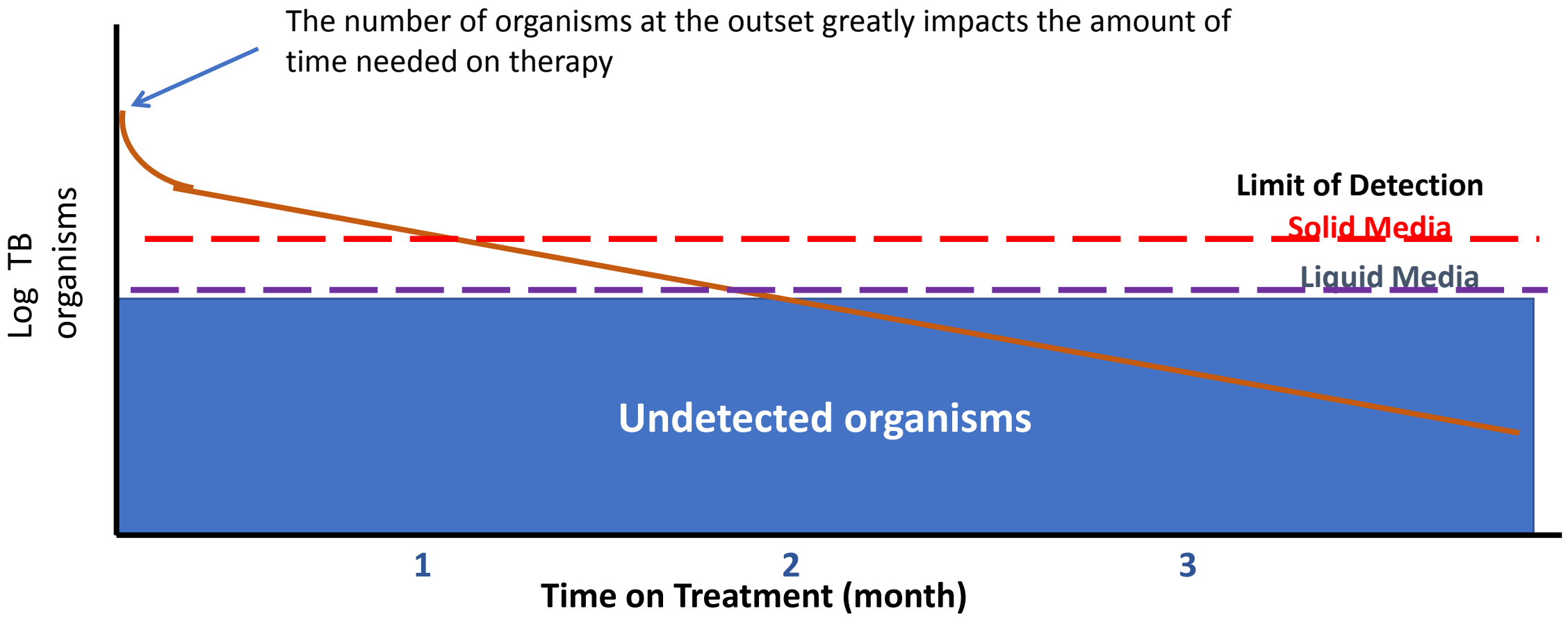




Gillespie et al NEJM 2014

Challenges in the level of detection

A negative culture on solid or liquid media during treatment does NOT equate with absence of viable MTB in the host (LOD issue).



Month 2 Culture Status and Treatment Duration as Predictors of Recurrence in Pulmonary Tuberculosis: Model Validation and Update

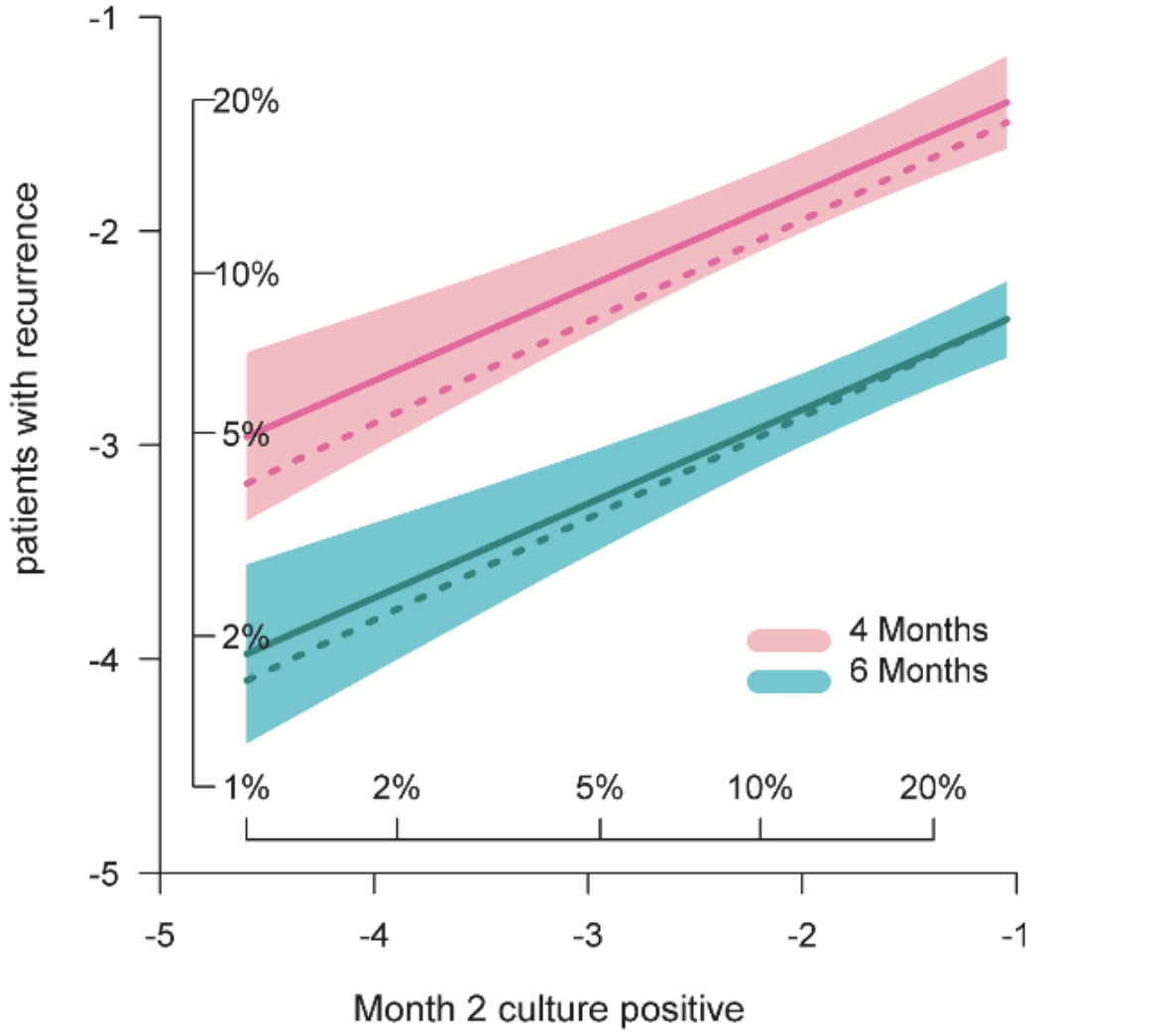
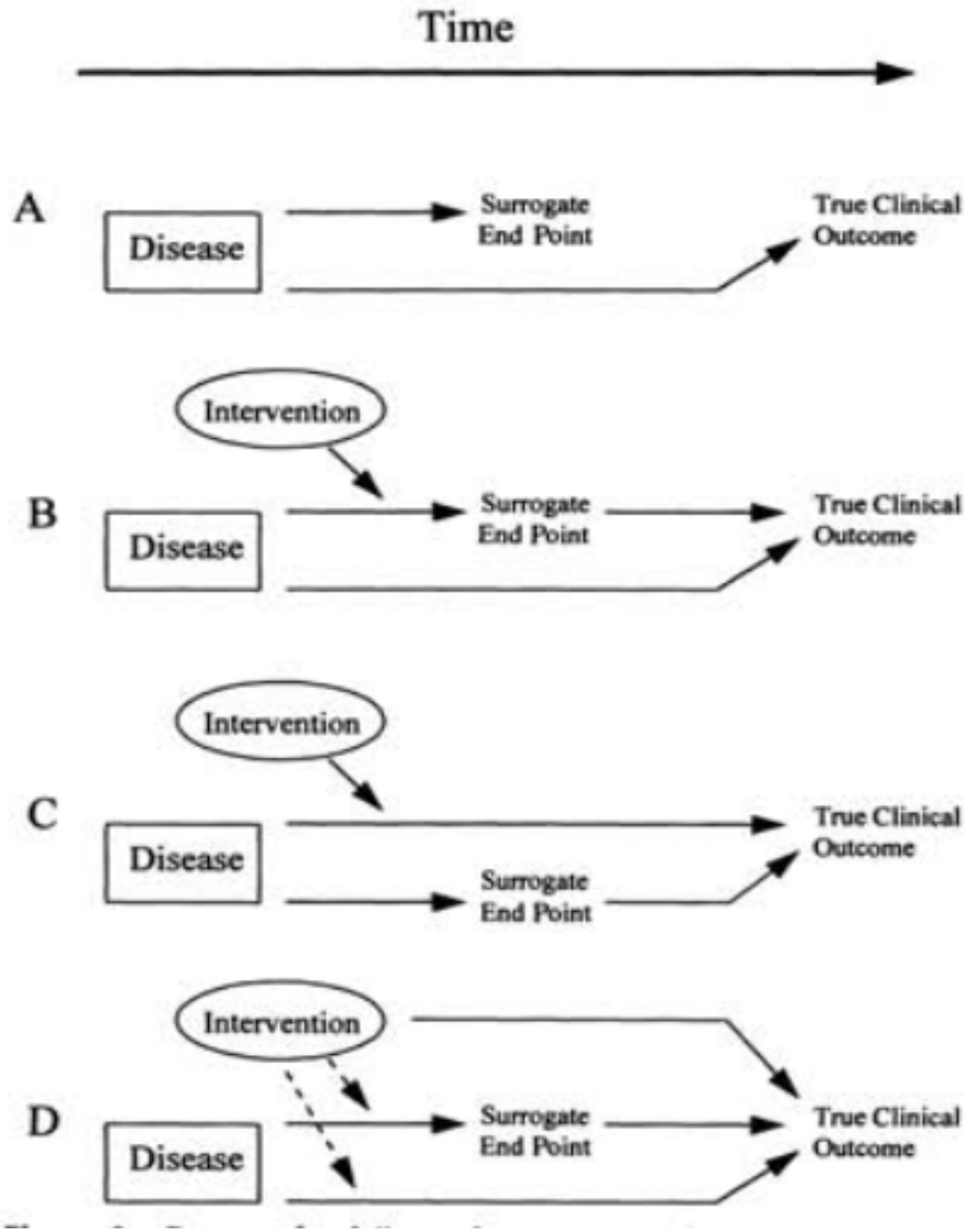


Fig 2. Predicted proportion of patients with recurrence based on the proportion positive after 2 months of treatment, for regimens of 4 and 6 months duration. Axes indicate logit-transformed proportions; inset scales indicate corresponding percentages. Solid and dotted lines indicate updated and original model predictions, respectively. Shading indicates 80% confidence intervals for the updated estimates.

Wallis et al.,
PLOS One, 2016

Surrogate endpoint is 'used as a substitute for a clinically meaningful endpoint. . . changes induced by a therapy on a surrogate endpoint are expected to reflect changes in a clinically meaningful endpoint'

Reasons for failures of putative surrogate endpoints:



Fleming TR, DeMets DL, Annals Internal Medicine, 1996

Fleming TR, J.H. Powers Stat Med. 2012

Technical and specimen-related issues

We may already be working with the most informative surrogate marker, but are our technical methods imperfect?

- Technical challenges with sputum as a sample type and with culture, requiring training laboratory staff, and maintaining proficiency
- Difficulty in specimen collection, transport, processing
- Lack of standardization in mycobacteriologic methods
- TB trials occur where TB is… in resource-limited countries
- TB trials sponsored by not-for-profit networks with limited resources
- Limited number of laboratories with expertise for culture (one laboratory in country of Kenya)

A comparison of identical specimens at baseline

	Lab A
Transport Time	1 hour
Transport Temp	4°C
Decontamination	1.5% NaOH
Centrifuge	3000 x g at 4°C for 20 min.
Resuspension vol.	1.5 mL
MGIT inoculum	0.5 mL
Baseline TTP	**7 days**

A tale of identical specimens after 8 weeks of study treatment

	Lab A	Lab B
Transport Time	1 hour	3 days
Transport Temp	4°C	21°C
Decontamination	1.5% NaOH	2% NaOH
Centrifuge	3000 x g at 4°C for 20 min.	3000 x g, ambient temp. for 15 min. with cold PBS
Resuspension vol.	1.5 mL	2.5 mL
MGIT inoculum	0.5 mL	0.5 mL
Baseline TTP	**7 days**	**12 days**
8 week TTP	**21 days**	**Negative Culture**

Courtesy Dr. Anne Purfield, PhD

Is mycobacteriology challenging to standardize? Yes, however…

- Specimen is not sterile at collection
  - Opportunity for contaminants to affect culture results
- Specimen must be manipulated, processed and decontaminated before culture
  - Tedious methods require technical expertise, many steps and time (1.5 hours to decontaminate a sputum specimen)
  - Critical steps include centrifuging specimen and resuspension of pellet; lack of precision affects Mtb recovery and affects intra- and inter-lab variability
  - Harsh chemicals used to reduce likelihood of contaminants, but also destroy Mtb and reduce culture yield!

Is mycobacteriology challenging to standardize? Yes, however...

- An expected rate of contamination of cultures and resultant specimen loss is 2-5% for solid media and 5-10% for liquid media
  - Two culture media used to prevent specimen (time point) loss due to contamination
  - Types of solid media (e.g., LJ, 7H11S, 7H10) used by labs varies
    - Which solid media is best for clinical trials remains uncertain
  - Contamination rates may be higher for specimens after weeks of treatment, possibly due to reduced sputum quality

Mycobacteriology activities in TBTC Study 31/A5349

- Harmonization across networks and across sites
- Adoption of laboratory minimum standards

TBTC Study 31 / ACTG A5349

- Randomized clinical trial to assess safety and efficacy of two 4-month daily, high-dose, rifapentine-based treatment regimens compared with standard 6-month regimen
- 2500 participants
- Duration: 18 months

Study 31 approach to limitations of culture-based endpoints

- TB laboratory methods have been harmonized across both trial networks using “Key Elements”
  - Standardized 20 key steps in TB lab methods, focusing on those likely to impact endpoint measures
  - This required within-lab validation at some sites prior to adoption of Key Elements
- Real time monitoring for deviations from standard methodology and reporting to assure quality data are collected
- Data collected using CDISC for transferability and pooled analyses

Study 31 approach to limitations of culture-based endpoints

- Both liquid and solid media used
  - Automated MGIT 960 used by all, except one site (using manual MGIT)
    - Reduces variability, uses standard commercial media, and automates TTD
  - Solid media type is not prescribed; sensitivity and specificity of various solid media types will be assessed
    - Thus far: 75% of specimens cultured on LJ, 24% on 7H11S, 1% on 7H10
- TBTC and ACTG leadership have strongly supported pursuit of technical training for laboratorians as well as lab-focused site visits

Study 31/ACTG 5349 Key Elements of Mycobacteriology Laboratory Procedures

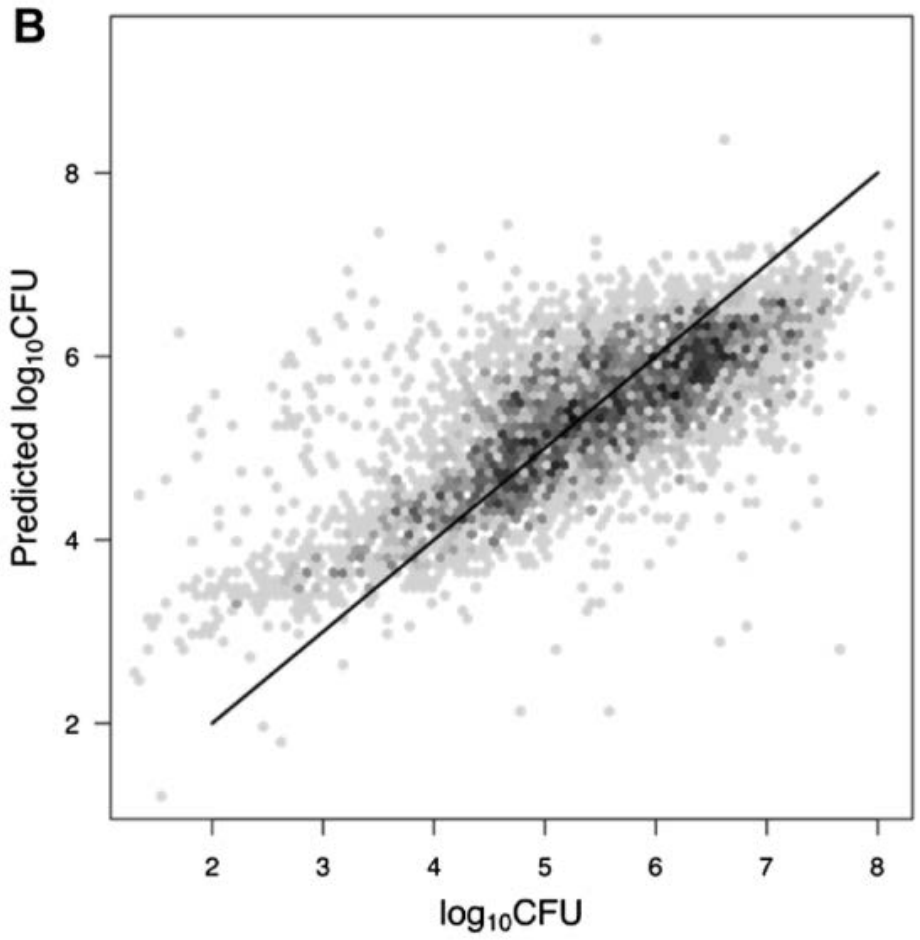
Table 1: Key Elements of Mycobacteriology Laboratory Procedures

	Laboratory Procedure	Key Element in Procedure	Potential Affect/Impact
1	Sputum Collection & Transport	Participant is to rinse mouth with boiled/sterile/bottled or distilled water prior to sputum collection	Quality of specimen
2	Sputum Collection & Transport	Collect at least 3 to 5 mL of sputum. If larger volumes cannot be obtained, a minimum of 1 mL is acceptable[a]	Quality of specimen
3	Sputum Collection & Transport	Transport sputum specimen to the laboratory in a cool box as soon as possible after collection. Store sputum in a refrigerator or cool box (2-8°C) if not received by to the laboratory within 1 hour of collection[b]	Integrity of specimen
4	Sputum Receipt & Storage	Store sputum specimen in a refrigerator or cool box (2-8 °C) if not processed within 1 hour of receipt at the laboratory	Integrity of specimen
5	Sputum Processing	Decontaminate sputum specimen with a final sodium hydroxide (NaOH) concentration of 1.0 to 1.5% for 15 to 20 minutes prior to adding phosphate buffered saline (PBS) (pH 6.8)	Isolation of MTB
6	Sputum Processing	Centrifuge specimen with a relative centrifugal force (RCF) of 3000x*g*, for at least 15 minutes[c]	Isolation of MTB
7	Sputum Processing	Resuspend the digested decontaminated specimen to final volume of 1.5 to 2.0 mL with PBS (pH 6.8)[d]	Comparability of results
8	Sputum Processing	Include positive controls at least once per week or with each participant batch, and negative controls daily or with each participant batch	Isolation of MTB and Detect Cross-Contamination
9	Smear Microscopy	Positive and negative control slides must be included with every batch of participant slides	Quality of smear results
10	Smear Microscopy	Report results according to WHO/IUATLD grading scale as per the Global Laboratory Initiative (StopTB Partnership) Sputum Microscopy Handbook[e]	Comparability of results
11	Rapid Molecular Testing	Perform rapid molecular test (e.g., GeneXpert) according to the manufacturer's product insert	Comparability of results
12	Rapid Molecular Testing and Smear Microscopy	Report results of screening tests used for subject eligibility to clinic staff within 48 to 72 h of sputum specimen receipt	Turnaround time
13	Solid Media Culture	Inoculate solid media (slant or plate) with 0.2 mL of resuspended sputum sediment[f]	Comparability of results
14	Solid Media Culture	Incubate solid media for at least 6 weeks before reporting a negative result; or at least 8 weeks for drug resistant TB trials	Isolation of MTB
15	Solid Media Culture	Test appropriate controls before media is used, regardless if purchased commercially or prepared in-house[g]	Isolation of MTB
16	MGIT Culture	Inoculate each MGIT tube with 0.5 mL of the resuspended sputum sediment	Comparability of results
17	MGIT Culture	Work up all MGIT cultures (positive and negative) according to the FIND MGIT Manual and MGIT culture algorithms/flow charts included in the study-specific laboratory reference manual[h]	Isolation/Detection of MTB
18	Identification of MTB	Confirm the presence of *M. tuberculosis* complex (MTBC) vs. non-MTBC at each trial time point when culture is positive[i]	Isolation of MTB
19	Identification of MTB	Include positive and negative controls at least once per week or with each batch of participant specimens and with each new lot or shipment of testing kits/reagents	Accuracy of MTB ID
20	Drug Susceptibility Testing (DST)	Include a drug susceptible quality control (QC) strain at least once per week or with each batch of participant specimens	Quality of DST results

Courtesy Dr. Anne Purfield, PhD

Alternative biomarkers and opportunities

Ability of TTP to predict CFU counts in EBA studies up to the first 14 days of treatment.



CFU=colony forming units, TTP=time to positivity

Using 5754 sputum samples from 487 patients

The observed number of CFU (log10CFU) of M. tuberculosis plotted against the predicted number of log10CFU using TTP with the formula:

Log10CFU = 16.41 – 5.17 * log10(TTP).

Diacon et al., Tuberculosis (2013)
Diacon et al., Clin Microbiol Infect, 18 (2012), pp. 711-717

Express 31: Sputum Transcriptomic Expression Profiling in Study 31

“Study 31A” of Study 31/ A5349

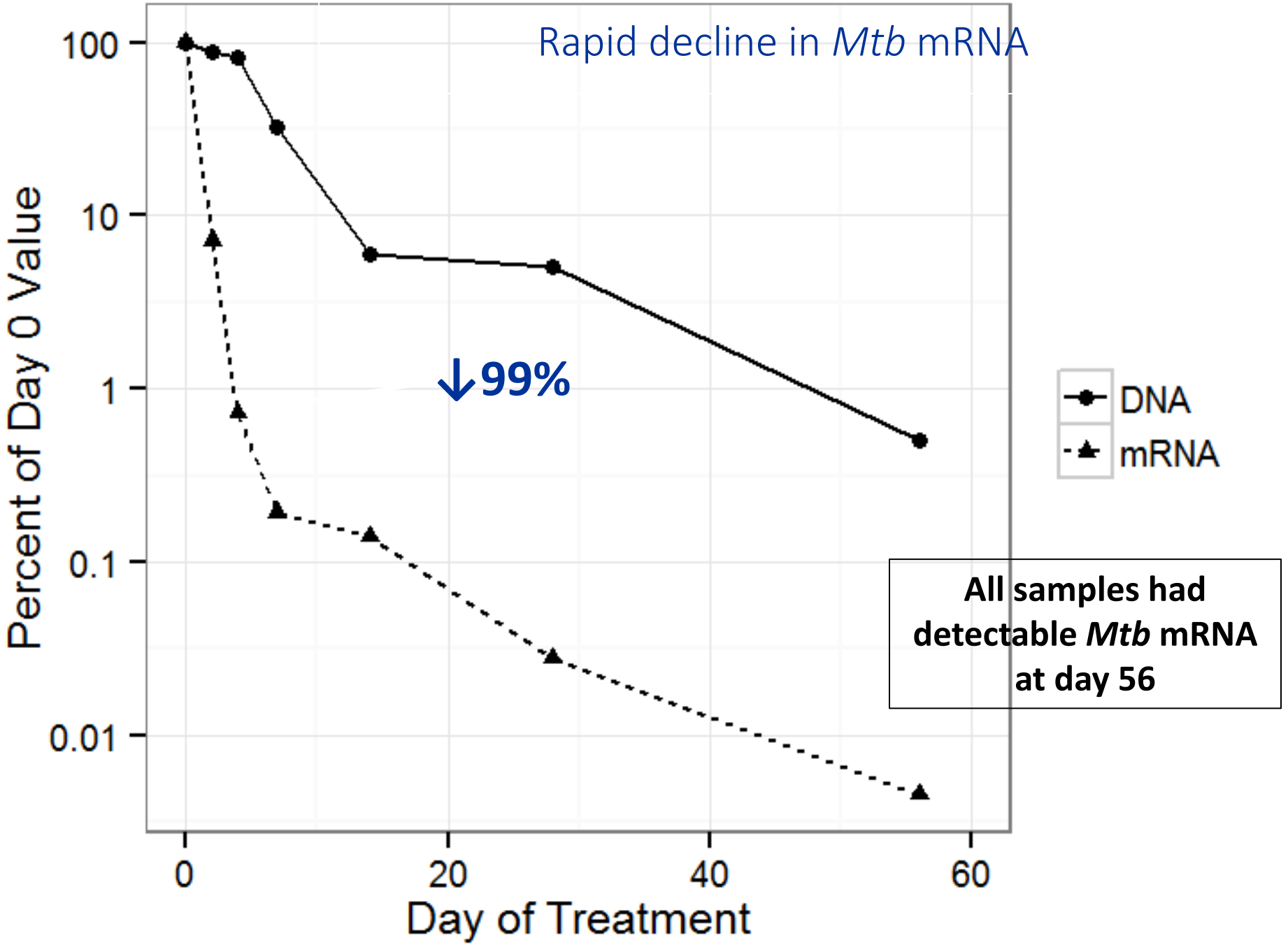
Alternative to enumeration… Focusing on the *Mtb* physiologic state

- Dynamic
- Adapted to immunity & tissue micro-environments
- Affects drug effectiveness
- Differs *in vitro* and in humans

Mtb-transcriptional profiling in sputum

- Nested qRT-PCR for 2,400 *Mtb* mRNA transcripts
  - 60% of genome
- Transcriptome: pattern of mRNA present in a sample
- mRNA half-life is minutes-long
- “Biological snapshot” of *Mtb* physiologic state

Rapid decline in *Mtb* mRNA



All samples had detectable *Mtb* mRNA at day 56

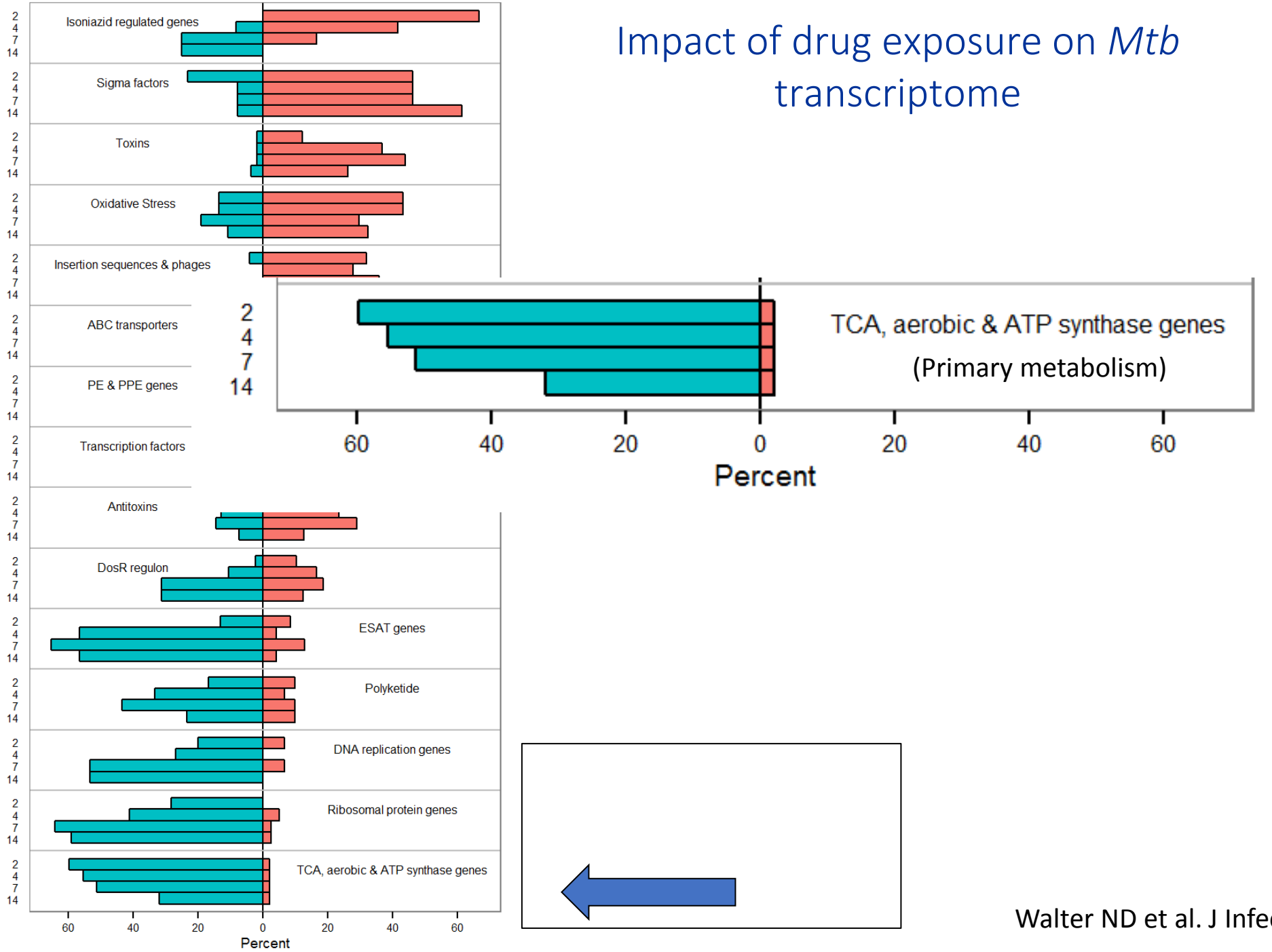
Impact of drug exposure on *Mtb* transcriptome

Massive alteration of MTB transcriptome

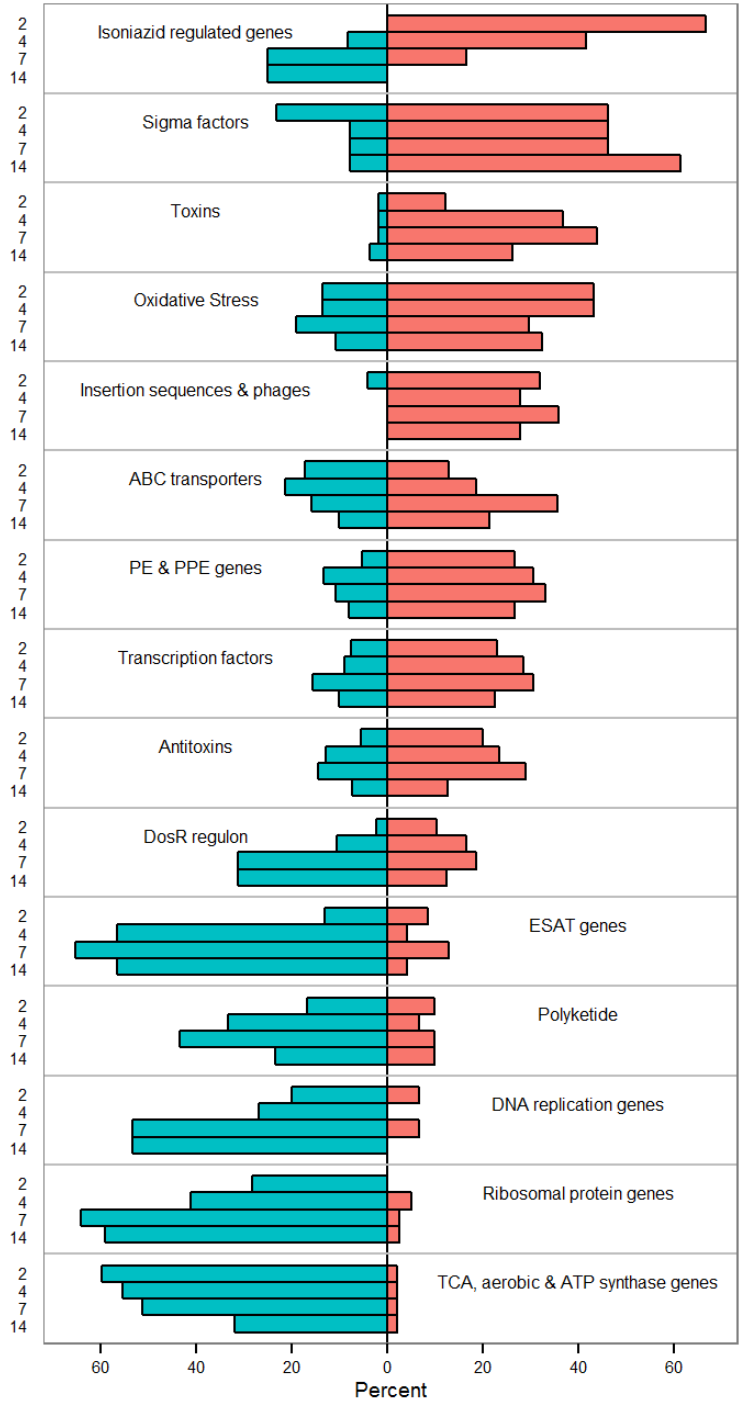
At least 20% of genes differentially expressed at each day

Walter ND et al. J Infect Dis 2015.

Impact of drug exposure on *Mtb* transcriptome



Walter ND et al. J Infect Dis 2015.



Increased oxidative stress response

Transcriptional regulation

Reduced expression of ESAT genes

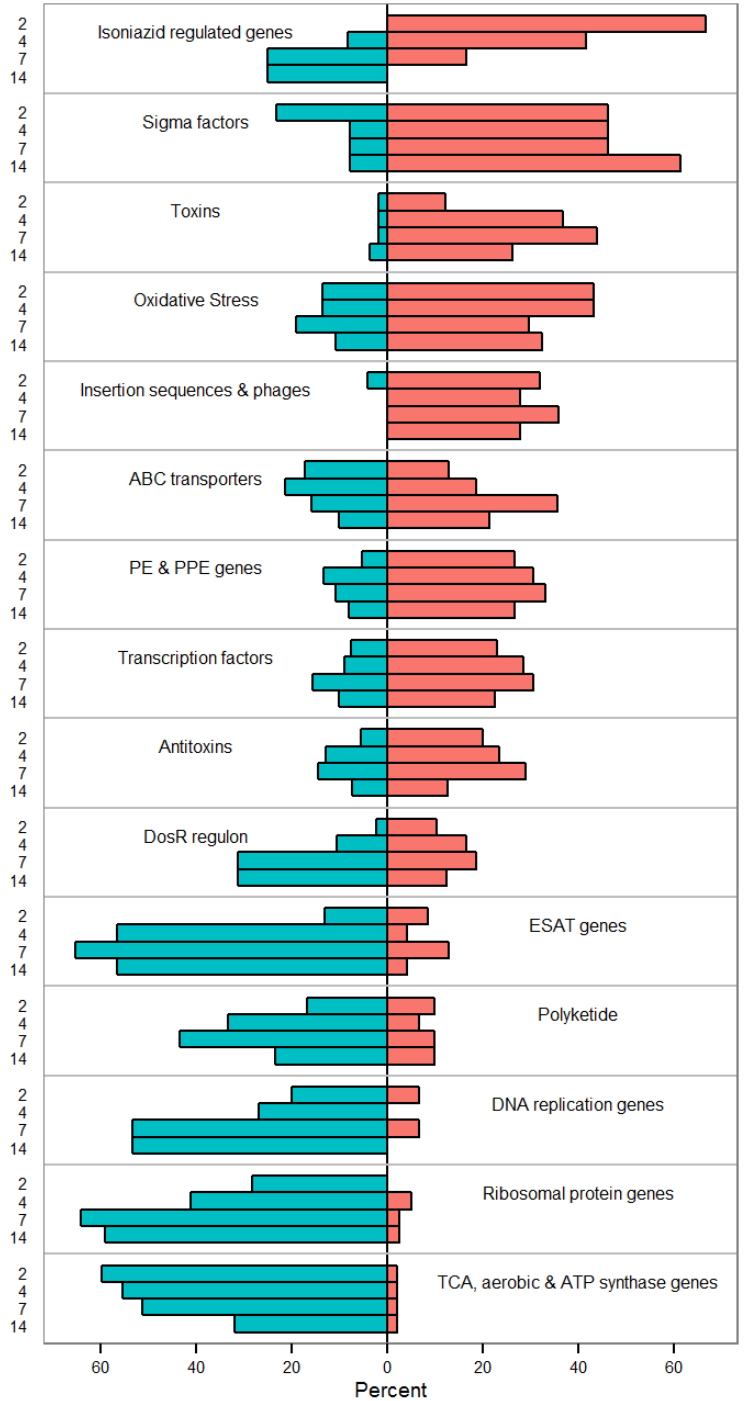
Reduced lipid synthesis

Reduced DNA synthesis

Reduced protein translation

Reduced energy production

Walter ND et al. J Infect Dis 2015.



Isoniazid stress signature

Increased transcriptional initiation factors

Increased translational regulators

Increased oxidative stress response

Transcriptional regulation

Reduced expression of ESAT genes

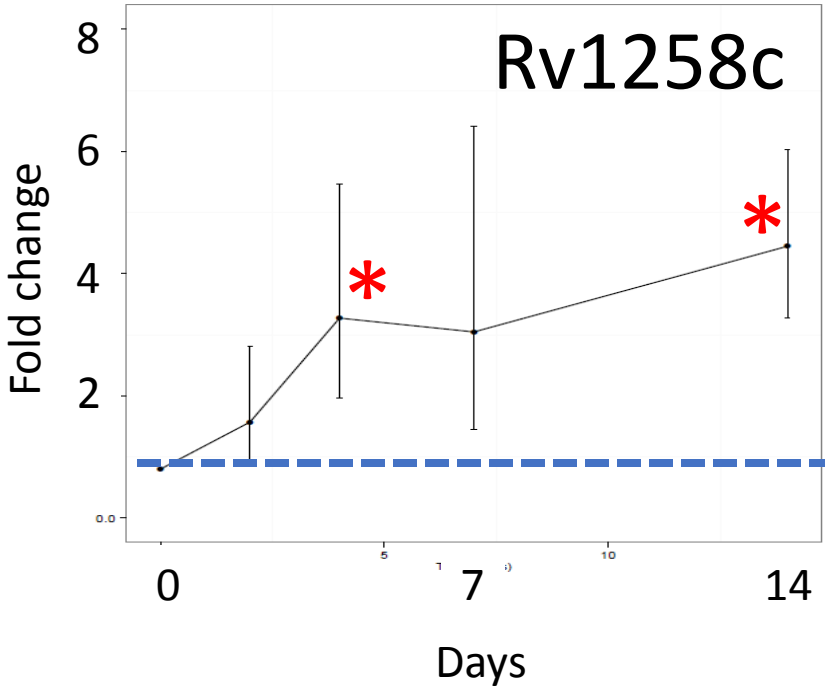
Reduced DNA synthesis

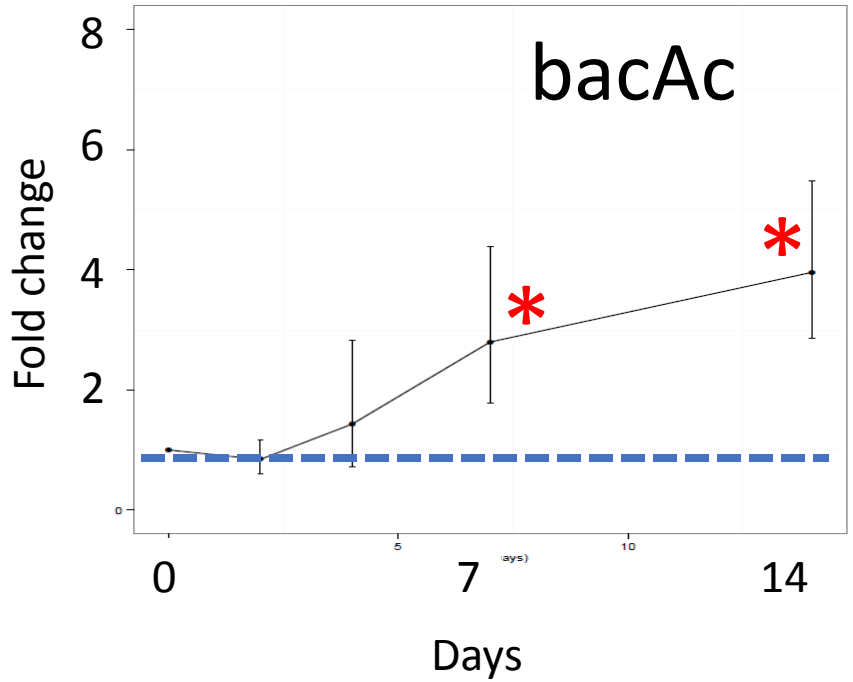
Reduced protein translation

Reduced energy production

Walter ND et al. J Infect Dis 2015.

Efflux Pumps





Walter ND et al. J Infect Dis 2015.

Consortium for TB Biomarkers Biorepository (CTB2)

IDENTIFYING TB BIOMARKERS, ACCELERATING TREATMENTS

CTB2 is a collaborative biobank accelerating development of new TB cures by validating biomarkers of response to TB drug treatments.

- **Goal: 1,000 culture-confirmed TB patients contributing 300,000 longitudinally collected specimens for research on TB biomarkers of treatment effect**
- Samples & clinical data provided at 7 scheduled times; baseline through 12 months post-treatment, plus at recurrence/withdrawal
- Samples provided:
  - RNA from whole blood (PAXgene)
  - QuantiFERON from whole blood (QFT nil, mitogen, TB antigen)
  - Sputum
  - Urine
  - Plasma

www.tbbiorepository.org

Email: tbbiorepository@tballiance.org

Deadline for next submission: November 10, 2017

CTB2 is supported by:

CTB2 is comprised of:

Summary

- All phases of TB drug and regimen development rely on culture
  - Sensitivity is a priority in phase 3. Accuracy and precision in enumeration paramount for EBA and phase 2
- Uncertainties persist regarding prediction and surrogacy
  - Mechanisms of relapse exist that are not fully captured by culture-based intermediate markers (as non-culturable bacilli persist).
- Standardization of methods is feasible and essential
  - Standards will reduce noise, increase precision, accuracy and sensitivity
- Harmonization across networks and sites is also essential
  - Will enhance ability to conduct multi-site and multi-trial pooled analyses
- Biomarkers that move the field beyond simple enumeration hold some promise,
  - Provide insights into the physiologic adaptations of Mtb in response to treatment
  - Potentially identify mechanisms of persistence that are in causal pathway to relapse

Study 31 / A5349 Protocol Team (v. 2.0, May 2015)

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