Update on the Mycobacteriology Laboratory Sourcebook for Harmonization and Support of TB Clinical Trials

IMPAACT Annual Meeting 2018

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Plan

1) Overview of Sourcebook
2) Version 1
3) How it will be used for Phoenix
4) Updates

Variations in TB lab procedures

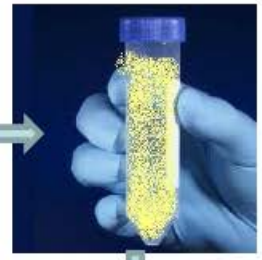
- TB drug trials are performed at multiple sites in different geographical settings
- TB laboratories worldwide use a variety of methods for diagnosing TB
- Many procedures are not automated and can be affected by the individuals performing them (e.g., smear reading, sputum processing)
- These variations can affect microbiology endpoints, comparability of results and quality of results for participant safety

Sputum specimen

NALC/NaOH

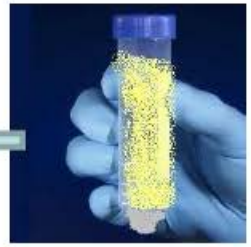
15 minutes

Add buffer to 50 ml



Centrifuge rotor

Centrifuge 15 minutes

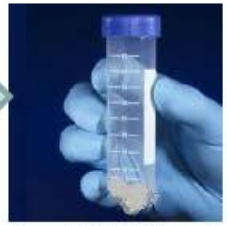


Supernate and sediment

Pour off

Pellet

Reconstitute with saline or buffer



Ready to inoculate

Broth tube

Slide for smear

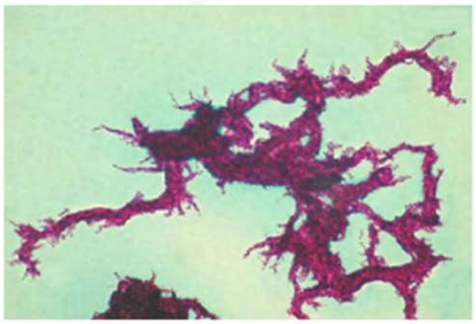
NAAT

Solid media

- Mycobacterial Growth Indicator Tube
- Automated liquid culture system but manual steps for identification once growth detected by instrument: these steps may vary from one lab to another

Identification test

Ziehl-Neelsen



ZN: serpentine cords of varying length or district linear clumping

Blood agar plate

Harmonization of TB testing

- To minimize variations and achieve high quality, comparable testing results across TB laboratories participating in the clinical trial
- Since TB labs already use validated standard operating procedures (SOPs) and participate in trials sponsored by different networks, efforts were directed towards harmonizing Key Elements
- Lab procedures should be reviewed for the presence or absence of these necessary Key Elements, and incorporated as necessary

Harmonization Based on Key Elements

- Key Elements in TB lab procedures are those that
  - Have the greatest impact on microbiology endpoints of clinical trials
  - Allow for comparison of results among all trial sites (or from one study to another) and
  - Provide accurate test results to ensure safety of trial participants
- Dr Kathy Eisenach
- First version of the Key Elements was implemented in the DMID 13-0057/TBTC Study 32 and second version in TBTC Study 31/ACTG 5349
- ACTG TB Core Lab Team

Checklists and Guides

- Checklists developed for each test procedure
  - Key Elements
  - Important technical points
    - Do not directly affect the microbiology endpoints, comparability of results among labs or participant safety, but are important in overall performance of the test and strongly recommended
- Background information added
- Combined into guides that were piloted in a small group of network laboratories
- All guides, accompanying checklists and Key Elements have been revised and combined in the Sourcebook

Sourcebook Sections

- Introduction
- Biosafety
- Quality Assurance with examples of Quality Indicators
- Specimen Collection, Transport and Laboratory Receipt
- Main Procedures
  - Specimen Processing
  - Smear Microscopy
  - MGIT Culture
  - Solid Media Culture
  - MPT64 Antigen Identification
  - MGIT Drug Susceptibility Testing (DST)
  - Solid Media DST
  - Hain Line Probe Assays
  - Cepheid Xpert MTB/RIF
  - Storage
- Checklists and Appendix (Study Protocol Review Form)

Section content

- Background
  - Historical use of the method/assay, how test results are used, why certain test methods are required, preferred or optional, all in the context of TB clinical trials
- List of related Key Elements
  - How they impact the quality, reproducibility, and comparability of results, and participant safety
- Reporting terminology for study data
- Quality controls (QC)
- Checklists: Key Elements and critical technical points
  - All grouped at the end of the Sourcebook

Example

7 Specimen Processing

7.1 Background Information

7.1.1 Introduction

This section focuses on processing respiratory specimens for Acid Fast Bacilli (AFB) smear, MTB culture, and drug susceptibility tests (DST, phenotypic or genotypic). Respiratory specimens included here are the most commonly used in TB drug trials, i.e. expectorated sputum, induced sputum and more specifically in children, gastric aspirates. The NALC (N-acetyl L-cysteine)-NaOH (sodium hydroxide) method is widely used and validated with the BACTEC™ Mycobacteria Growth Indicator Tube (MGIT) TB System. Since MGIT culture is used in all TB drug trials the NALC-NaOH method is the standard.

Table 7-1.Key Elements of Respiratory Specimens Processing Procedure

Key Element	Affect	Impact
Use all respiratory specimen up to 10 mL in processing	Isolation of MTB	Microbiology endpoints
Decontaminate respiratory specimen with a final sodium hydroxide (NaOH) concentration of 1.0 – 1.5%	Isolation of MTB	Microbiology endpoints
Decontaminate respiratory specimen for 15 to 20 minutes prior to adding phosphate buffered saline (PBS) (pH 6.8)	Isolation of MTB	Microbiology endpoints
Centrifuge specimen with a relative centrifugal force (RCF) of 3000x*g*, for at least 15 minutes. A refrigerated centrifuge is preferred	Isolation of MTB	Microbiology endpoints
Re-suspend the digested, decontaminated specimen to final volume of 1.5 – 2.0 mL with PBS (pH 6.8)	Standardization of suspension used for inoculating culture	Comparability of results
Set up cultures immediately following the suspension of decontaminated, concentrated specimen	Isolation of MTB Speed of confirming TB diagnosis and DST results	Microbiology endpoints Participant enrollment and safety
Include positive control and negative controls at least once each day that specimen processing is performed	Isolation of MTB Detect cross-contamination and contaminated reagents	Microbiology endpoints

Table 7-2. Specimen Processing Internal Quality Controls

Element	Description
Positive control	The positive processing control measures the extent NaOH killing of MTB. When subjected to smear and culture, these results are monitored to ensure the extent of killing does not deviate from the norm. An ideal positive control yields a MGIT TTD of 6-10 days, which is equivalent to a 3+ AFB smear, 10^6 CFU/mL, or 1:500 dilution of 0.5 McFarland standard. Positive control options: • Suspension of MTB H37Rv or MTB H37Ra • AFB positive sputum specimen from a known TB case, homogenized or digested • Sputum specimen to which MTB H37Rv or MTB H37Ra has been added • Artificial sputum sample to which MTB H37Rv or MTB H37Ra has been added
Negative control	The two main purposes of the negative processing control are to: 1) detect cross-contamination events and 2) check the sterility of the processing reagents.

17.3 Specimen Processing Checklist

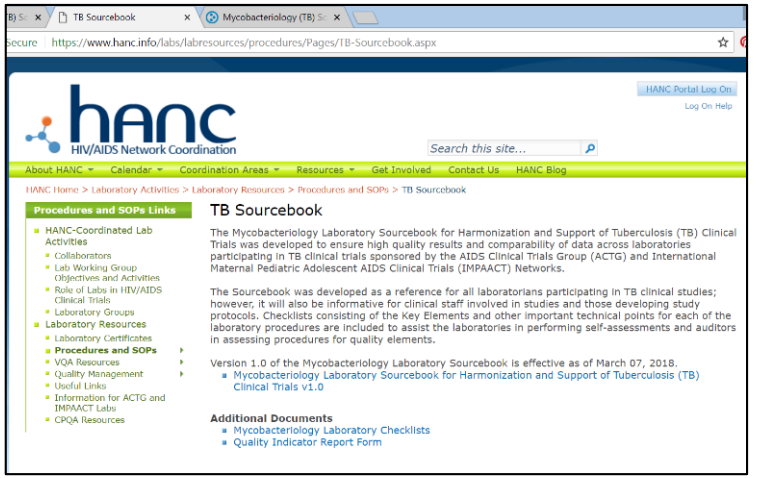
A. Laboratory Information	
Laboratory Name:	
Laboratory Address:	
Completed By:	
Date Completed:	

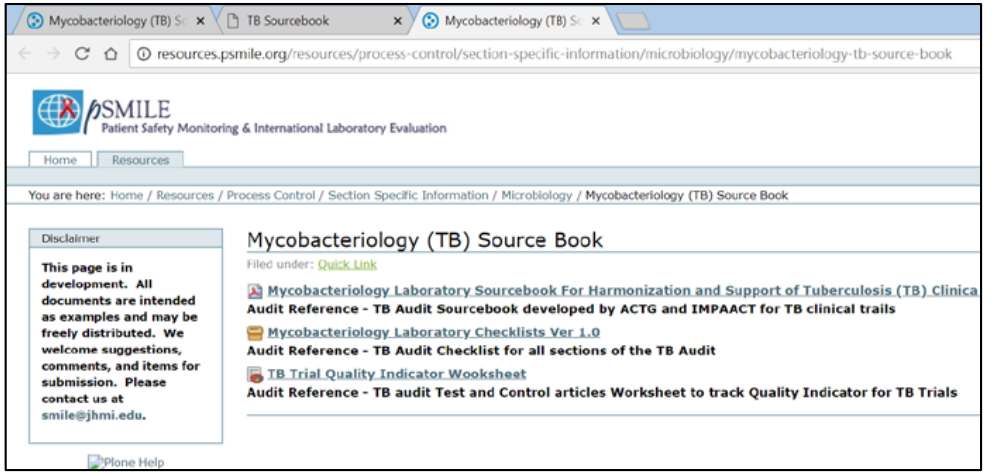
B. Relevant Standard Operating Procedures (SOPs)		
SOP No.	**SOP Title**	**Version No.**

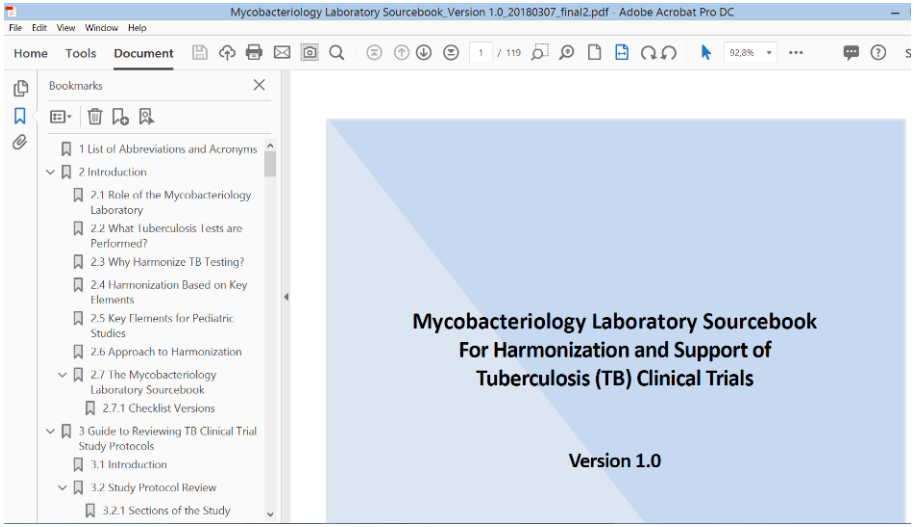
C. Do your laboratory SOP(s) include instructions for the following? *Indicate, "Yes (Y)", "No (N)" or "Not Applicable (NA)" for each response below. Justify or explain all "N" or "NA" responses in the "Comments" column.*				
Key Elements	**Y**	**N**	**NA**	**Comments**
1. Use the entire respiratory specimen in processing. If more than 10 mL, a procedure is used to reduce the starting volume.				
2. Decontaminate specimens with a final sodium hydroxide (NaOH) concentration of 1.0 – 1.5%.				
3. Decontaminate specimens in NaOH-NALC for 15-20 minutes at room temperature prior to adding phosphate buffered saline (PBS, pH 6. 8).				
4. Centrifuge the decontaminated specimen at				

2) Finally, version 1!

- Version 1.0 March 2018
- Available on Hanc and pSMILE websites
- Sourcebook document
- Word version of check-lists
- Examples of Quality Indicators Report Form







Letter to network

Date: April 24, 2018

TO: ACTG and IMPAACT Laboratories

FROM: Dr. Grace Aldrovandi, ACTG & IMPAACT Network Laboratory Principal Investigator
ACTG Laboratory Science Group
IMPAACT Laboratory Center

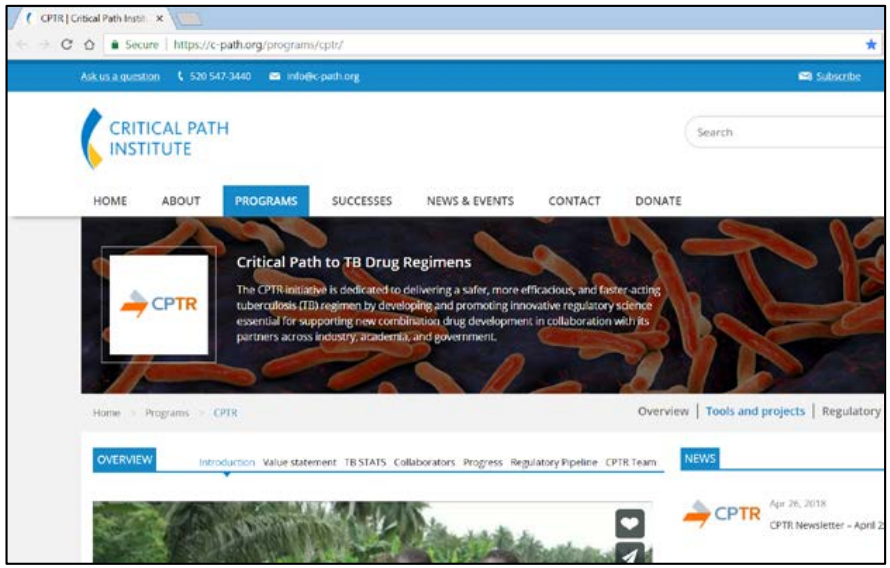
Cc: ACTG Laboratory Science Group
IMPAACT Laboratory Center
Dr. Kathleen Eisenach
Dr. Anne-Marie Demers
Fatima Jones

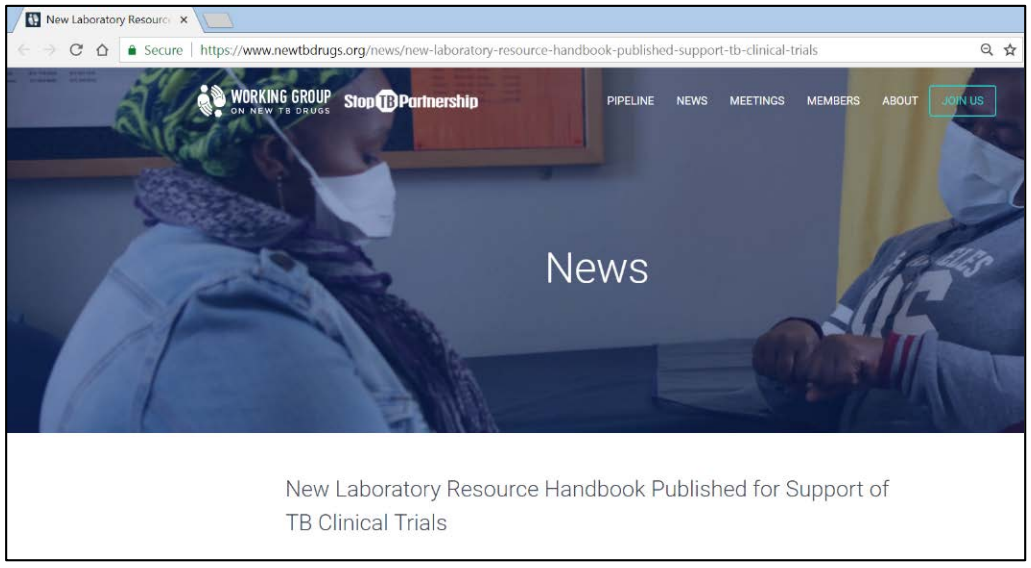
Re: IMPAACT/ACTG Memo: Mycobacteriology Laboratory Sourcebook for Harmonization and Support of Tuberculosis (TB) Clinical Trials

****Please forward this information to the relevant clinic and laboratory personnel at your site**

Also:

- NIAID Funding News
- WHO Workshop on TB clinical trials design
- Working Group on New TB Drugs News
- CPTR website





3) Using the Sourcebook for A5300B/I2003B/PHOENIx

- TB laboratory SOE table
- Protocol specific TB testing to address study objectives and endpoints: details of TB tests and procedures to be performed at time points throughout the study

NC-005-(J-M-Pa-Z)			Screening			Treatment										Relapse or culture+ D56
Day Number (when specimen collection started) – micro testing designation	Sample Type		-9 to -3	-2	-1	1	3	7	14	21	28	35	42	49	56	
Visit Number (day/week subject attended study visit)			-9 to -3	-2	-1	1	4	8	15	22	29	36	43	50	57	
Laboratory Assessment/Week Number	Coached Spot	Pooled Overnight	-1			1			2	3	4	5	6	7	8	
Sputum Collection	X		X	X	X	X	X	X	X	X	X	X	X	X	X	
Sputum AFB Smear[A]	X		X													
MTBDR*plus*[B]	X		X													
MTBDR*sl* [B, C]	X		X													
MGIT Culture and TTP[D]	X			X	X	X	X	X	X	X	X	X	X	X	X	
Quantitative Culture	X			X	X	X	X	X	X	X	X	X	X	X	X	
Sputum Collection		X		X	X	X	X	X	X	X	X	X	X	X	X	
Sputum AFB Smear[A]		X		X	X											
MGIT Culture with TTP[D]		X		X	X	X	X	X	X	X	X	X	X	X	X	
Culture Id with MPT64 Ag test[E]		X		X	X	X	X	X	X	X	X	X	X	X	X	
Quantitative Culture		X		X	X	X	X	X	X	X	X	X	X	X	X	
MGIT DST for SIREZ[F]		X		X												X[G]

3) Using the Sourcebook for A5300B/I2003B/PHOENIx (2)

- TB laboratory SOE table in appendix of LPC
- References are made to the Sourcebook for information however all study specific testing is detailed in the Table
- Checklists of relevant technical procedures have been combined into one study specific document
- Participating TB laboratories are expected to have all elements of the checklists in place for the start of the study. Lab procedures should be reviewed for the presence or absence of these necessary Key Elements, and incorporated as necessary.

3) Using the Sourcebook for A5300B/I2003B/PHOENIx (2)

- If some items cannot be addressed in time for the start of the study, “N” will be indicated for the item with comments in the corresponding comment box.
- The completed checklists will be uploaded in the MiLAB system (along with completion of the MiPAL).
- The ACTG and IMPAACT Lab Centers, and the PHOENIx protocol team will follow-up if necessary based on responses received.

4) Updates

- WHO critical concentrations for TB drug susceptibility testing recently updated
- Addressed in the TB laboratory SOE table for Phoenix
- Useful information will be added to the Sourcebook in upcoming update

Critical concentrations (CCs)

- World Health Organization. Technical report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis. WHO/CDS/TB/2018.5 2018.

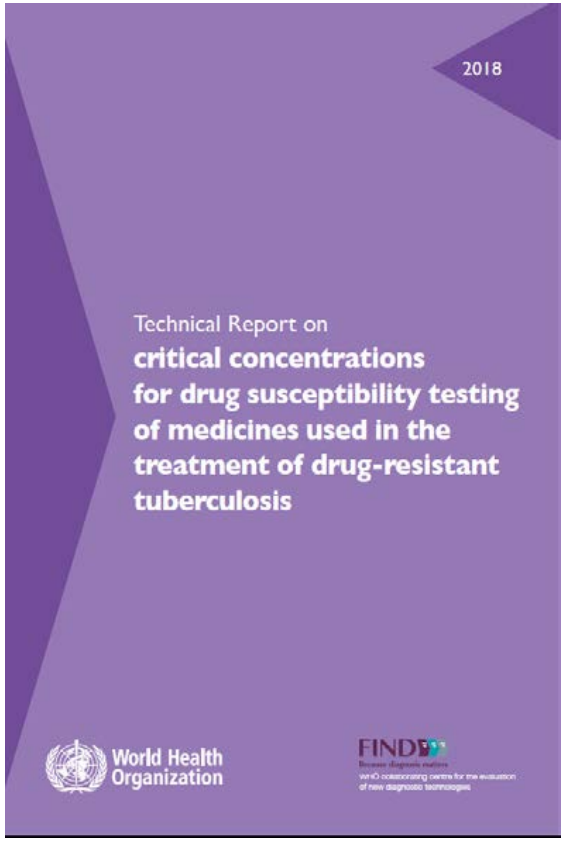


Table 1. Critical concentrations and clinical breakpoints for medicines recommended for the treatment of rifampicin-resistant and multidrug-resistant TB.

Drug groups	Drug	LJ	7H10	7H11	MGIT [1]
A. Fluoroquinolones [2]	Levofloxacin (CC) [3]	2.0	1.0	–	1.0
	Moxifloxacin (CC) [3]	1.0	0.5	0.5	0.25
	Moxifloxacin (CB) [4]	–	2.0	–	1.0
	Gatifloxacin (CC) [3, 5]	0.5	–	–	0.25
B. Second-line injectable agents	Amikacin	30.0	2.0	–	1.0
	Capreomycin	40.0	4.0	–	2.5
	Kanamycin [6]	30.0	4.0	–	2.5
	(Streptomycin) [7]	4.0	2.0	2.0	1.0

- No changes in CCs for second-line injectable agents (kanamycin, amikacin, capreomycin, streptomycin) for MGIT
- For fluoroquinolones, CCs have been changed for levofloxacin, moxifloxacin and gatifloxacin. A clinical breakpoint for the higher dose of moxifloxacin (800 mg/day) was established for the 1st time.
- Testing of ofloxacin is not recommended as it is no longer used to treat drug resistant-TB and laboratories should transition to testing the specific fluoroquinolones used in treatment regimens.
- During this transition, testing of ofloxacin at the CCs of 2.0 mg/L in MGIT may be performed instead of testing at the CCs for levofloxacin or moxifloxacin, but not for the clinical breakpoint for moxifloxacin.

Antimicrobial specs of first-wave NLAs (New Lyophilized Antimicrobials)

NLA	Catalog No.	Quantity per vial µg	Vial Reconstitution Volume ml*	Final reconstituted concentration µg / ml	WHO critical concentration µg / ml	Usage volume ml
Amikacin	215350	332	4	83	1.0	0.1
Kanamycin	215348	830	4	207.5	2.5	0.1
Capreomycin	215351	830	4	207.5	2.5	0.1
Ethionamide	215355	1660	4	415	5.0	0.1
Ofloxacin	215352	664	4	166	2.0	0.1
Moxifloxacin	215349	498	6	166	1.0	0.1
			24	41.5	0.25	0.1
Growth supplement	245116	Same as OADC Enrichment or SIRE Supplement				

* Reconstitute with sterile, distilled water

The current BD moxifloxacin-0.5/2.0 laboratory use reagent vial will be discontinued.

Previously:

Moxifloxacin	215349	498	3	166	2.0
			12	41.5	0.5

Conclusion

- Mycobacteriology Laboratory Sourcebook for Harmonization and Support of TB Clinical Trials version 1
- Developed to ensure high quality results and comparability of data across laboratories participating in TB clinical trials
- Laboratories involved in Study 31/A5349 are already familiar with the concept of Key Elements
- Key Elements and Checklists will be used for A5300B/I2003B/PHOENIx
- Checklists can also be used during lab audits or visits
- Eventually also used by other networks and groups

Acknowledgements

- TB Lab Core Team: Kathy Eisenach, Fatima Jones and Frances Whalen
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