Study 31/ACTG 5349 Key Elements of Mycobacteriology Laboratory Procedures:

Towards Harmonization of Mycobacteriology in TB Trials

Title	**Study 31/ACTG 5349 Key Elements of Mycobacteriology Laboratory Procedures**
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Background

The proposed approach towards harmonization of Standard Operating Procedures (SOPs) across the Tuberculosis Trials Consortium (TBTC) and AIDS Clinical Trials Group (ACTG) Network Tuberculosis (TB) laboratories included, (1) identifying Key Elements in Mycobacteriology laboratory procedures that have the greatest impact on data quality and, (2) harmonizing those elements across all Mycobacteriology Laboratories in both networks. Rather than create new SOPS, Mycobacteriology Laboratory procedures should be reviewed for the presence or absence of the necessary Key Elements. Most clinical Mycobacteriology Laboratories already have SOPS and have either been accredited or are in the process of accreditation. Creating new SOPs would be burdensome in terms of time and resources, and would duplicate the efforts of other universal lab manuals that are currently available (e.g., GLI Mycobacteriology Laboratory Manual)

Cross-network harmonization and implementation of the Key Elements of Mycobacteriology Laboratory Procedures begins with TBTC Study 31, ACTG #5349, a multi-network phase three trial to evaluate safety and efficacy of shortened TB treatment regimens for acute pulmonary TB.

The Key Elements for TBTC Study 31, ACTG #5349 are detailed in Table 1 below. These are in agreement with the Key Elements developed by the ACTG Tuberculosis Transformative Science Group (TB TSG) Lab Core Team. Good Clinical Laboratory Practices (GCLP) and quality assessment (QA) activities are not considered as key elements but **must** be a part of the Mycobacteriology Laboratory procedures, and laboratory-specific Quality Management manuals. These Key Elements are directed towards Mycobacteriology results used as endpoints in TB drug trials; however, Key Elements of rapid drug susceptibility tests are included as the quality of these results and turnaround times are important in screening for subject eligibility. Likewise, quality indirect Drug Susceptibility Testing (DST) results are needed to ensure safety for subjects enrolled in the study.

How to Use Key Elements of Mycobacteriology Laboratory Procedures

1. Review the Key Elements for Mycobacteriology Laboratory Procedures
2. Ensure your laboratory SOPs for Mycobacteriology include each Key Element
3. Sign and return signature page to TBTCStudy31@cdc.gov, with subject line “Laboratory Key Elements”

Study 31/ACTG 5349 CLINICAL STUDY TEAM AND LABORATORY AGREEMENT SIGNATURE PAGE

TBTC/ACTG Site _____ agrees that the Key Elements of Mycobacteriology Laboratory Procedures are included in SOPs that are performed for Study 31

Site Name: _____

Laboratory Name: _____

This agreement must be signed and dated prior to participant enrollment and reviewed annually thereafter until study is completed.

Principal Investigator Printed Name Signature Date

Annual review initial and date (month/year): _____/_____; _____/_____; _____/_____; _____/_____

Study Coordinator Printed Name Signature Date

Annual review initial and date (month/year): _____/_____; _____/_____; _____/_____; _____/_____

Coordinator(s) Collecting Sputum Printed Name Signature Date

Annual review initial and date (month/year): _____/_____; _____/_____; _____/_____; _____/_____

Laboratory Director Printed Name Signature Date

Annual review initial and date (month/year): _____/_____; _____/_____; _____/_____; _____/_____

Mycobacteriologist* Printed Name Signature Date

Annual review initial and date: _____/_____; _____/_____; _____/_____; _____/_____

***The individual(s) completing TBTC Mycobacteriology (MB) Form**

Table 1: Key Elements of Mycobacteriology Laboratory Procedures

	Laboratory Procedure	Key Element in Procedure	Potential Affect/ Impact	What laboratory SOP document(s) has this key element been incorporated into? (name and SOP number)?	How will you address this element if it is not already in your current SOPs? Please provide a short description of how this Key Element is implemented in your laboratory.	What part/section of the laboratory SOP reflects this key element?
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[i]	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^{o}C) if not received by to the laboratory within 1 hour of collection[ii]	Integrity of specimen			

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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[iii]	Isolation of MTB			
7	Sputum Processing	Re-suspend the digested decontaminated specimen to final volume of 1.5 to 2.0 mL with PBS (pH 6.8)[iv]	Comparability of results			

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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[v]	Comparability of results			
11	Rapid Molecular Testing	Perform rapid molecular test (e.g., GeneXpert) according to the manufacturer's product insert	Comparability of results			

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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 re-suspended sputum sediment[vi]	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[vii]	Isolation of MTB			
16	MGIT Culture	Inoculate each MGIT tube with 0.5 mL of the re-suspended sputum sediment	Comparability of results			

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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[viii]	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[ix]	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			

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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			

[i] If not possible to collect at least 1 mL expectorate sputum, use local procedures for sputum induction, when necessary

[ii] When the distance between the clinic and laboratory is great (i.e., the clinic ships the specimen to a regional laboratory), the specimen should be maintained on cold chain and received at the laboratory no more than three to five days after collection.

[iii] Use of a refrigerated centrifuge is preferred.

[iv] For guidance on how to achieve accurate and precise resuspension volumes, please see Study 31/ACTG 5349 Mycobacteriology Laboratory Reference Manual.

[v] See Section 9, "Acid-fast Bacilli Microscopy (AFB) Examination", from Global Laboratory Initiative Stop TB Partnership. Laboratory Diagnosis of Tuberculosis by Sputum Microscopy – The Handbook 2013. Available from: http://www.stoptb.org/wg/gli/assets/documents/TBLabDiagnosisSputum%20Microscopy_Handbook.pdf.

[vi] If using slants or plates where 0.2 mL of inoculum would overwhelm the surface area of the media, inoculate additional slants or plates so that the total volume of resuspended sputum sediment cultured on solid media is 0.2 mL. See Study 31/ACTG 5349 Mycobacteriology Laboratory Reference Manual.

[vii] See Section 16, "Quality Assurance", from Global Laboratory Initiative Stop TB Partnership: Mycobacteriology Laboratory Manual. First edition, April 2014. Available from: http://www.stoptb.org/wg/gli/assets/documents/gli_mycobacteriology_lab_manual_web.pdf

[viii] See Study 31/ACTG 5349 Mycobacteriology Laboratory Reference Manual.

[ix] At least one positive culture (e.g., AFB-positive MGIT) at each time point for each participant should be identified as *M. tuberculosis* or otherwise, depending on the laboratory resources. See Study 31/ACTG 5349 Mycobacteriology Laboratory Reference Manual.