Title:	Collection, Clinic Storage and Transport of Sputum (Expectorated) Specimens SOP		
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1 Purpose

This procedure describes how to collect expectorated sputum in the context of ACTG/IMPAACT clinical trials. It is a Standard Operating Procedure (SOP) that outlines standardized methods for the collection and transport of sputum to ensure a quality specimen that will yield quality data. The protocol document will include study-specific requirements, but these requirements should not differ from the procedures outlined in this SOP. A Manual of Procedures (MOP) may be used to provide the study-specific details for sputum collection; if a MOP is not used, the study-specific sputum collection procedure should be made available to the sites.

The study-specific collection requirements will also be included in the Laboratory Processing Chart (LPC). The LPC will reference the appropriate SOPs relevant to the collection, transport, processing, testing and shipping of sputum specimens and the derivatives. If an SOP is not available, the LPC will provide instructions in the appendices. All instructions will be pre-approved by MyLab Working Group of the TB Transformative Science Group (TBTSG) and the protocol team before inclusion in a LPC.

2 Scope

Users of the ACTG/IMPAACT Laboratory Manual.

3 Background

Tuberculosis (TB) is a disease caused by the organism *Mycobacterium tuberculosis*. Proper sputum collection is not only critical for optimizing detection/recovery of MTB, but it is also extremely important for infection control, since this specimen is highly infectious (11.1, 11.2, 11.3). Accurate detection of MTB through screening tests is important for determining eligibility of patients for clinical trials. Once enrolled and receiving anti-tuberculosis treatment in a clinical trial, the time when the patient converts his/her sputum from culture positive to culture negative is the basis for determining efficacy of the treatment regimen. This SOP describes how to collect expectorated sputum specimens in a safe and reliable manner to achieve consistent and quality data among all the laboratories in the trials networks and allow data comparisons from various clinical trials.

4 Authority and Responsibility

4.1 The Network Laboratory Directors (or his/her designee) have the authority to establish, review and update this procedure.

4.1.1 Include, without procedural modification, the portions of the current version of the LTC SOP that are used within the network site-affiliated laboratory

4.1.2 Reference the current version of the LTC SOP

4.2 The MyLab Working Group of the TB Transformative Science Group (TBTSG) is responsible for maintenance and control of the scientific content of this SOP. The ACTG/IMPAACT Laboratory Technologist Committee (LTC) is responsible for the maintenance and control of SOP format including CLIA requirements.

4.3 The Laboratory Director is responsible for the implementation of this LTC SOP or laboratory-specific SOP and for ensuring that all appropriate personnel are trained. A laboratory SOP must:

4.3.1 Include, without procedural modification, the portions of the current version of the LTC SOP that are used within the network site-affiliated laboratory

4.3.2 Reference the current version of the LTC SOP

4.4 All laboratory technicians are responsible for reading and understanding this SOP prior to performing the procedures described.

4.5 The site PI and designees are responsible for understanding and adhering to the patient preparation and specimen collection components.

5 Budgetary Considerations

5.1 Room/facility fees (if applicable)

5.2 Specimen collection kit costs (if applicable)

6 Schedule of Specimen Collection (Study Specific)

The protocol document or protocol specific Manual of Operations (MOP) should include the following study-specific aspects of specimen collection:

6.1 Which patients will have sputum collected

6.2 Number of specimens required per patient

6.3 Type of sputum specimens: spot vs. overnight

6.4 Time of collection: early morning, during day, overnight

6.5 Schedule of specimen collection: baseline, week 2, week 4, etc.

6.6 Site of collection: home, clinic/hospital

6.7 Target volume of sputum: 5mL, 10 mL, etc.

6.8 If specimen collected at home is unsatisfactory in quality (saliva, spit), insufficient in quantity (< 1 mL), or transported from home to clinic after delay (> 3 h), specify that sputum should be collected at the clinic

6.9 If the patient does not produce expectorated sputum, specify if sputum induction is required or if collection can be obtained at another time

7 Equipment, Consumables, and PPE

Refer to Appendix A for full list of reagents and consumables.

NOTE: Sputum collection systems such as those listed in Appendix A are available commercially. Limited data are available supporting the use of such devices. Advantages would include a possible decrease of specimen contamination as it prevents patients from touching the sample or the tube. The container tube is graduated and can help assess sputum volume. The tube is also the same used by the TB lab during processing and prevents having to transfer into another container once in the laboratory

7.1 Specimen labels or pre-printed labels

7.2 Indelible marker

7.3 Bottled drinking water (commercial source or container with boiled or sterile or distilled water)(11.6, 11.7).

NOTE: needs to be in a container when taken home by the patient

7.4 Clean disposable cups

7.5 Sterile, wide-mouth, universal specimen containers or 50 mL-conical shaped tubes

7.6 Container/tube with 5 mL, 3 mL, and 1 mL markings

7.7 Disposable gloves

7.8 Respirators (N-95 or equivalent)

7.9 Refrigerator - maintained at 2-8°C

7.10 Insulated cooler and ice packs

7.11 Leak proof biohazard specimen bags

7.12 Absorbent Material

8 Sputum Collection Procedures

The ideal sputum specimen is produced by repeated deep inhalation and exhalation of breath followed by a cough as deep within the chest cavity as is possible for the patient. Sputum should consist of thick, mucoid, white-yellow, sometimes blood-tinged, material from the lower airways and lungs (not saliva or oral/nasal secretion). Collection of early morning specimens is preferred due to overnight accumulation of secretions. However, specimens may be collected at any time from patients who have a deep cough that is readily productive.

8.1 Supervised collection by Clinic or Study Staff

8.1.1 When collected in the clinic, collection staff should remain within viewing distance of the patient during the procedure to provide assistance as needed; and to ensure that he/she is isolated from others until sputum collection is complete. Specimens should be collected in a well-ventilated area. Clinic staff collecting the sputum, regardless of the setting, must observe the appropriate infection control precautions, i.e. wear a N95 mask and wear gloves when hand-contact with blood or other potentially infectious materials is anticipated (11.8)

8.1.2 Collect sputum specimen in a sterile disposable, wide-mouth container or in a 50mL conical tube.

8.1.3 Prior to collection, label the specimen container with the appropriate identifying label; including study/screening number, patient ID, visit, protocol, and date and time of collection. Mark the container/tube ‘CLINIC’ to differentiate it from the sputum collected at home.

8.1.4 Positively identify the patient by his/her initials and patient ID number.

8.1.5 Inform the patient that saliva and upper respiratory/nasal secretions are not sputum and are not acceptable specimens.

8.1.6 Demonstrate to the patient how to properly rinse his or her mouth and how to collect a sputum specimen using a demonstrator bottle/cup of water (from commercial source, boiled, sterile or distilled) and container/tube.

8.1.7 Instruct the patient to:

8.1.7.1 Thoroughly clean his/her hands with soap and water. Provide the patient clean disposable paper towels to dry his/her hands.

8.1.7.2 Rinse his/her mouth with water (from commercial source, boiled, sterile or distilled) prior to collection of sputum(11.6, 11.7). Provide a new, clean, disposable cup for each patient.

8.1.7.3 Breathe deeply a number of times and then cough from deep down within the lungs.

8.1.7.4 Lean forward, breathe in and out slowly twice, hold breath for 2-3 seconds each time, and on third time forcefully cough to bring up the sputum.

8.1.7.5 Collect the sputum in the sterile container provided and avoid touching the inside or edge of the specimen container or lid with their fingers.

8.1.7.6 Once collection has been completed, thoroughly clean his/her hands with soap and water. Provide the patient clean disposable paper towels to dry his/her hands.

8.1.8 Repeat the above sequence until an adequate amount of sputum is collected. This may take up to 1 hour. If the patient is unable to produce enough sputum within 1 hour, decide if the patient is “unable to expectorate”, requires rescheduling for another attempt at collection, or needs to undergo sputum induction. If collection is attempted and successful at another time, do not pool this sputum (unless instructed by the protocol) with the sputum collected at a different time.

8.1.9 Tighten the lid/cap on the container/tube and to avoid leakage.

8.1.10 Estimate the volume of sputum collected by comparison with container/tube with markings. The minimum adequate specimen volume must be annotated in the protocol-specific LPC.

NOTE: As a rule, a minimum volume of 1 mL of sputum must be collected; however, the target volumes for time intervals (e.g. 5 mL sputum at screening, baseline, and at all other visits through week 4; 3 mL at all subsequent visits thereafter) should be collected if possible.

8.1.11 After the specimen is collected, place specimen container in refrigerator or a cooler with pre-chilled ice packs unless it is being transported to the laboratory within 1 hour.

NOTE: Specimens must be packaged in sealed zip lock bags with sufficient absorbent material and marked with appropriate biohazard labeling before transporting the specimen.

8.1.12 Complete all the relevant fields on the Specimen Request Form/CRF.

8.2 Collection by patient at home

8.2.1 Refer to Appendix B for visual reference.

8.2.2 Provide the patient with a labeled specimen container with the appropriate identifying information; including study/screening number, patient ID number, visit number, and date of collection. Mark container/tube 'HOME' to indicate the specimen was collected at home.

8.2.3 Provide the patient a bottle of water (commercial source) or container with boiled, sterile or distilled water.

8.2.4 Provide the patient with a zip lock storage bag and absorbent material.

8.2.5 Inform the patient that saliva and upper respiratory/nasal secretions are not sputum and are not acceptable specimens.

8.2.6 Instruct the patient to:

8.2.6.1 Collect the sputum after getting out of bed, before the morning meal, and prior to taking any medications.

8.2.6.2 Collect the sputum in a well-ventilated area such as by an opened window or outside.

8.2.6.3 Thoroughly clean his/her hands with soap and water.

8.2.6.4 Rinse his/her mouth with bottled or boiled water prior to collection of sputum.

8.2.6.5 Breathe deeply a number of times and then cough from deep down within the lungs.

8.2.6.6 Lean forward, breathe in and out slowly twice, hold breath for 2-3 seconds each time, and on third time forcefully cough to bring up the sputum.

8.2.6.7 Collect the sputum in the sterile container provided and avoid touching the inside or edge of the specimen container or lid with their fingers.

8.2.6.8 Replace the lid/cap after collection and close tightly to avoid leakage.

8.2.6.9 Once collection has been completed, thoroughly clean his/her hands with soap and water.

8.2.6.10 Store the container in a zip lock bag with absorbent material in the refrigerator or cooler with chilled ice packs, if provided.

8.2.6.11 Bring the specimen container to the clinic as soon as possible, preferably in insulated cooler.

8.2.7 Suggest placing the water bottle/container and specimen container in a place that will remind the patient to collect the specimen first thing in the morning upon rising.

8.2.8 Inform the patient that they must write down the time of collection on the specimen container or bring this information when he/she brings back the specimen.

8.3 Receipt of Specimen Container

8.3.1 Ask the patient at what time he/she collected the specimen and record their response on the container (if not already present).

8.3.2 Estimate the volume of sputum collected by comparison with container/tube with markings. If specimen volume is inadequate (< 1 mL), try to collect another specimen from the patient while in the clinic.

8.3.3 Refrigerate the specimen until transported to the laboratory.

8.3.4 Complete all the relevant fields on the Specimen Request Form/CRF.

9 Transport of Sputum

A testing or processing laboratory will be assigned to each study site and all study related sputum specimens must be forwarded to the designated laboratory for testing or processing. Consistent attention to patient identification on the specimen collection container, Specimen Request Form, and Specimen Transport Form significantly reduces error.

9.1 Guidelines

9.1.1 Sputum specimens should be placed in a leak proof biohazard bag with sealed lids and absorbent material and transported to the laboratory in a cooler with chilled ice packs as soon as possible after collection. If delay is unavoidable (> 1 h), the specimens should be refrigerated at 2-8°C to inhibit growth of undesired microorganisms (11.6, 11.9). Processing requirements for sputum testing will be outlined in the protocol. Sputum must be handled per the requirements in the protocol to ensure quality specimens are obtained for testing.

9.1.2 Sputum specimens must be delivered to the laboratory as soon as possible and within 24 h of collection; however, delays up to 3 days (11.9, 11.10) in transport from clinic to laboratory may be allowable if the transport distance is long, the sample is chilled and the extended transit time is agreed upon by the study/protocol team.

9.1.3 Notify the testing laboratory of all shipments in advance of transport. Provide the date and time that specimens are expected to be delivered. This ensures that laboratory personnel are prepared to receive and process the specimens.

9.1.4 All specimens should be transported in compliance with local and national regulations governing the transport of potentially infectious materials. These rules must be followed, no matter how short the transport distance is.

9.2 Transport Procedures

For each transport box the delegated clinic or study staff must verify that:

9.2.1 The total number of sputum containers in the box corresponds to the accompanying Specimen Request Forms/CRF (one for each sputum specimen).

9.2.2 The study or screening number and other details on each sputum container corresponds to that on the Specimen Request Form and Specimen Transport Form (if applicable).

9.2.3 There are an appropriate number of chilled ice packs in the transport box to maintain the temperature between 2°- 8°C. This may be monitored using a maximum/minimum thermometer or a temperature recording device, but is not required.

9.2.4 The Specimen Transport Form is included in an appropriate envelope (if applicable).

9.2.5 The name and signature of the driver or courier transporting the samples are recorded in the 'Transport details' section of the Specimen Transport Form (if applicable).

10 Forms

These forms should be provided to the sites; either as part of the protocol document, the MOP or as additional information posted on the Protocol-Specific Web Page (PSWP).

10.1 Sample Informed Consent Form

10.2 Sample Patient Instructions (Appendix B)

10.3 Sample Laboratory Requisition and Transport Chain of Custody Document

11 Literature and References

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11.10 Ken Jost, personal communication, March 2014

12 Acknowledgments/ Collaborators

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12.5 Sam Ogwang, Joseph Akol, Moses Joloba, Joint Clinical Research Center-Uganda (JCRC)

12.6 Christopher Lane, Vandana Kulkarni, Cheryl Jennings, ACTG Laboratory Technologists Committee

13 Appendices

13.1 Appendix A: Example Supplies

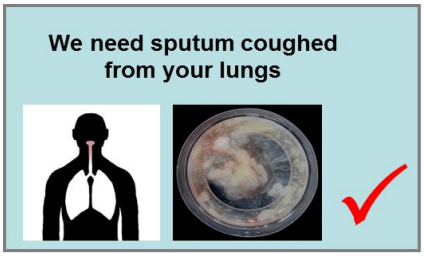
13.2 Appendix B: Patient at Home Collection Visual Guide

Appendix A: Example Supplies

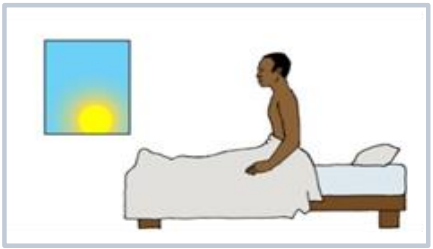
Reagent/Supply	Example(s)
Marking pens	Fisher Scientific Fisherbrand Marking Pens cat#13-379, or Nalgene® Lab Pen/Lab Marker #6310/#6311, or equivalent
Absorbent Material	Saf-T-Pak STP-151, or equivalent
Sputum collection containers	Thermo Scientific 50 ml conical centrifuge tube Cat # 12-565-804 Medi Pak Sterile Specimen Container Cat # 16-9542 Allegiance Brand Clik Seal Cat # BXTC13900
Sputum collection systems	BD Sputum Collection System Cat # 290020 Evergreen Scientific Sputum Collection Cat # 221-3996-G80 Covidien Sputum Collector – Fisher Cat # 14-375-182, Covidien Cat # 26505A

Appendix B: Patient at Home Collection Visual Guide

Instructions for Sputum Sample Collection at Home

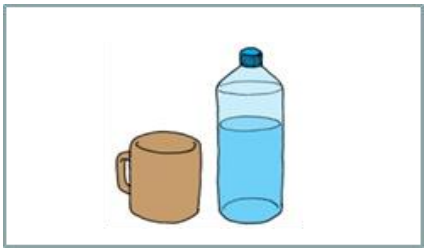


- Saliva and upper respiratory/ nasal secretions are not sputum and are not acceptable specimens.



- Collect sputum after getting out of bed, before morning meal, and prior to taking any medicine.
- Collect the sputum in well-ventilated area such as by an open window or outside.

- Thoroughly clean you hands with soap and water.



- Rinse your mouth with bottled water (or water that has been boiled) prior to collection of sputum.

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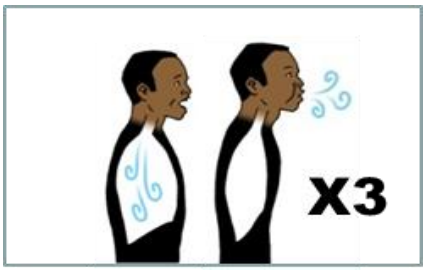
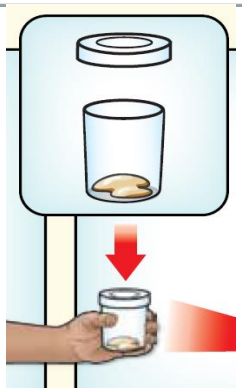
Instructions for Sputum Sample Collection at Home



- Lean forward, breathe in and out slowly twice, hold breath for 2-3 seconds each time, and on third time forcefully cough to bring up the sputum.

- Collect sputum in container provided.
- Avoid touching inside or edge of specimen container or lid with fingers.



- Replace lid after collection and close tightly to avoid leakage.
- Place container in a cooling box

- After collecting sputum thoroughly clean your hands with soap and water.

- Bring container in the cooling box to the clinic as soon as possible.

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