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UCL

STAND
SHORTENING TREATMENTS BY ADVANCING NOVEL DRUGS

LABORATORY MANUAL

MYCOBACTERIOLOGY – CLINICAL SITE

Protocol Title: A Phase 3, Open-Label Partially Randomized Trial to Evaluate the Efficacy, Safety and Tolerability of the Combination of Moxifloxacin plus PA-824 plus Pyrazinamide after 4 and 6 months in Adult Subjects with Drug-Sensitive Smear-Positive Pulmonary Tuberculosis and after 6 months of Treatment in Adult Subjects with Multi-Drug Resistant, Smear Positive Pulmonary Tuberculosis.

Protocol Number: NC-006-(M-Pa-Z)
Protocol Name: STAND (Shortening **T**reatments by **A**dvancing **N**ovel **D**rugs**)**
Version: 1.0; 16January2015

I hereby approve the above document and release it for appropriate amendment to make it Clinical Site specific and thereafter use at the Clinical Sites:

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Date:	16 Jan 2015
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Approval position:	Senior Medical Officer
Approval signature:	
Date:	16 Jan 2015

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Version History:

Master Version Number/Date	**Change**
1.0/16January2015	Initial Master Version

This Version Number/Date	**Change**
<<X.X; DDMonthYYYY>>	*<<>>*

1. ABBREVIATIONS

AFB	Acid Fast Bacilli
(e)CRF	(electronic) Case Report Form
DR	Drug Resistant
DS	Drug Sensitive
DST	Drug Sensitivity Testing
E	Ethambutol
EWD	Early Withdrawal
FQ	Fluoroquinolones
H	Isoniazid
MDR	Multi-Drug Resistant
MIC	minimum inhibitory concentration
MGIT	mycobacterial growth indicator tube
MTB/M.Tb	*Mycobacterium tuberculosis*
PZA	Pyrazinamide
R	Rifampicin
TB	Tuberculosis
TTP	Time to Positivity
UCL	University College London
Z	Pyrazinamide
Z-N	Ziehl-Neelsen

2. CONTACT DETAILS

Name	Contact Person	Contact Details		Physical and Postal Address/es
Screening: Z-N smear (AFP +/- grading), Hain MTBDRplus/GeneXpert (R resistance), Hain MTBDRsl (FQ resistance, *MTB* confirmation). All visits: MGIT (*MTB* confirmation and TTP).				
<<Local/Regional Laboratory>>	<<>>	Tel:	<<>>	<<>>
		Fax:	<<>>	<<>>
		Cell:	<<>>	<<>>
		E-mail	<<>>	<<>>
<<Local/Regional Courier>>	<<>>	Tel:	<<>>	<<>>
		Fax:	<<>>	<<>>
		Cell:	<<>>	<<>>
		E-mail	<<>>	<<>>
<<Other>>				
<<Other Laboratory>>	<<>>	Tel:	<<>>	<<>>
		Fax:	<<>>	<<>>
		Cell:	<<>>	<<>>
		E-mail	<<>>	<<>>
<<Other courier>>	<<>>	Tel:	<<>>	<<>>
		Fax:	<<>>	<<>>
		Cell:	<<>>	<<>>
		E-mail	<<>>	<<>>
Screening: pncA molecular test (Z resistance). Note: Sample prepared and couriered by <<>> laboratory.				
TASK Laboratory	Dr. M. Barnard	Tel:	+27 21 938 9556	Room F519, 5th Floor, Fisan Building Dept. of Biomedical Sciences Faculty of Health Sciences Stellenbosch University Francie van Zijl Drive Tygerberg 7505 South Africa
		Fax:	N/A	
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Post screening: MICs, resistance, speciation and strain testing				
University College London	Dr Julio Canseco	Tel:	+44 207 794 0500	University College London, Centre for Clinical Microbiology (2nd Floor), Royal Free Hospital Campus, Rowland Hill Street, London, NW3 2PF United Kingdom
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		E-mail	julio.canseco@ucl.ac.uk	
<<UCL courier>>	<<>>	Tel:	<<>>	<<>>
		Fax:	<<>>	<<>>
		Cell:	<<>>	<<>>
		E-mail	<<>>	<<>>

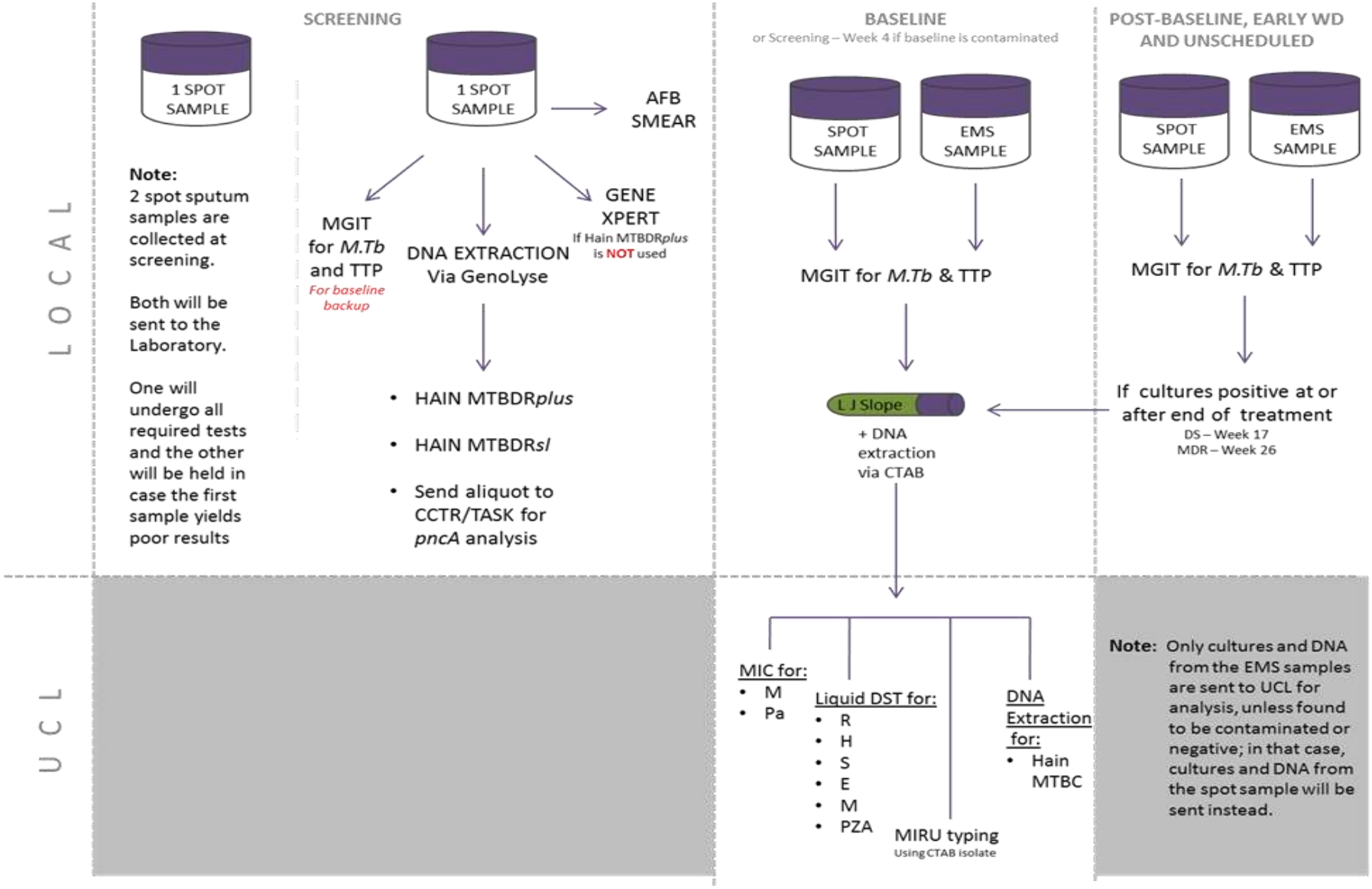
3. INTRODUCTION

The STAND/NC-006-(M-Pa-Z) clinical trial is a Phase 3 trial of the PaMZ regimen used to treat pulmonary tuberculosis. The trial is being conducted globally in approximately 15 countries, and at approximately 50 clinical sites. Microbiological assays for the trial will be conducted at a number of local or regional laboratories (labs), and some assays will be conducted at only one of two central laboratories. The diagrams and table that follow, before the start of the details of the procedures, give an overall orientation to the individual assays and their location of conduct. The local and regional labs will work with sputum samples from the sites and will conduct the MGIT cultures for *MTB* that will be the basis of the primary endpoint of the trial. These laboratories will also do the screening evaluation of sputum smears for AFB and will do rapid molecular tests to determine whether the *MTB* is susceptible to rifampicin and/or fluoroquinolones (moxifloxacin). The local and regional labs will extract DNA from the screening sample to send to the *pncA* lab at Stellenbosch University, South Africa, where that central laboratory will do a rapid molecular test to determine whether the *MTB* is susceptible to pyrazinamide. The local and regional labs will also subculture isolates on LJ slopes. An LJ slope and DNA extracted from an LJ slope will be sent to the central laboratory at University College London. At this central laboratory isolates will be evaluated for susceptibility to a standard panel of antibiotics in liquid culture and Minimum Inhibitory Concentrations will be determined to pretomanid (PA-824) and to moxifloxacin. This laboratory will also conduct a genetic analysis of DNA from the isolates of subjects with positive cultures at or after the end of treatment to evaluate if these isolates are identical or not to the baseline isolate.

4. MYCOBACTERIOLOGY LABORATORY TESTING

The Mycobacteriology Laboratory Testing that occurs at each laboratory (local plus central (UCL and CCTR/TASK (pyrazinamide resistance testing) is summarized in Figure 2.

Figure 1: Microbiology Testing for STAND Trial per laboratories



The Mycobacteriology Laboratory Testing Schedule is summarized in Figure 2.

Figure 2: Site Visit Flow Chart

Period	**Screening**	**Treatment**													**Follow-Up**						
Visit	Day (-14 (MDR)/9 (DS) to -1)	1 Day (Baseline)	Day 7 (Week 1)	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8 (Month 2)	12 Week (Month 3)	Week 17 (Month 4) End of treatment: 4 month treatment	Week 22 (Month 5)	Week 26 (Month 6) End of treatment: 6 month treatment	Month 9	Month 12	Month 15	Month 18	Month 24	Early Withdrawal [c]	Unscheduled
Early Morning Sputum		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Spot Sputum	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

The Mycobacteriology Laboratory Testing details are summarized in

Table 1: Mycobacteriology Testing Details.

Table 1: Mycobacteriology Testing Details

TIMING	SAMPLES COLLECTED	ANALYSES PERFORMED
Days (-14(MDR) /-9 (DS) to -1)(Screening):	Two spot sputum: ○ Both collected at the research site under the coaching and observation of the trial staff. ○ The second sample is collected as a back-up sample to the first sample in case it is not possible to obtain a result/s on the first sample. ○ If spot sputum smear shows an indeterminate result or is AFB negative, the test may be repeated on a freshly collected spot sputum/s and that result used.	Performed at <<Local/Regional Laboratory>> Screening Analyses: ○ Direct sputum smear microscopy using Ziehl-Neelsen stain for Acid Fast Bacteria (AFB); ○ *<<Hain* MTB*DRplus or GeneXpert>>* Rapid test for rifampicin resistance; ○ Hain Assay MTBDRsl Rapid test for fluoroquinolones resistance and confirmation *MTB;* ○ GenoLysed DNA extracted and sent to central *pncA* laboratory for *pncA* molecular test for pyrazinamide resistance. Baseline back up – not for screening purposes: ○ Liquid Culture (MGIT) for presence or absence of *MTB*; ○ TTP in liquid medium (MGIT).
• All visits from Day 1 (baseline) up to and including Month 24. • If both sputum samples at Month 2 or later are contaminated → Unscheduled visit • Positive culture at or after the end of treatment (Week 17 (4 month treatment arms)/Week 26 (6 month treatment arms)) → Unscheduled visit ≥ 7days from previous sample collection • Unscheduled visits • Early withdrawal visit	Two sputum samples: ○ One early morning collected and brought by subject from home. ○ One spot collected at the site under the coaching and observation of the trial staff.	Performed at <<Local/Regional Laboratory>> Efficacy Analyses: ○ Liquid Culture (MGIT) for presence or absence of *MTB*; ○ TTP in liquid medium (MGIT).
• Day 1 (baseline) sputum sample (or screening or out to Week 4 if the baseline is contaminated or negative); • Positive Cultures at or after Week 17 (4 month treatment arms)/Week 26 (6 month treatment arms)	N/A. Local laboratory will send LJ slopes and extracted DNA to the central UCL laboratory for above samples already collected.	Performed at University College of London Department of Clinical Microbiology The *MTB* isolates will be processed for: ○ Speciation of the infecting organisms and positive cultures after completion of treatment by HAIN MTBC to confirm *MTB;* ○ MIC against moxifloxacin and PA-824 (method to be confirmed); ○ Drug Susceptibility Testing for streptomycin, rifampicin, isoniazid, ethambutol, moxifloxacin, and pyrazinamide (MGIT); ○ Molecular strain typing (MIRU)

The Mycobacteriology Laboratory Testing flows are summarized in Figure 3, Figure 4, Figure 5 and Figure 6.

Figure 3: Screening/Enrolment Flow Chart

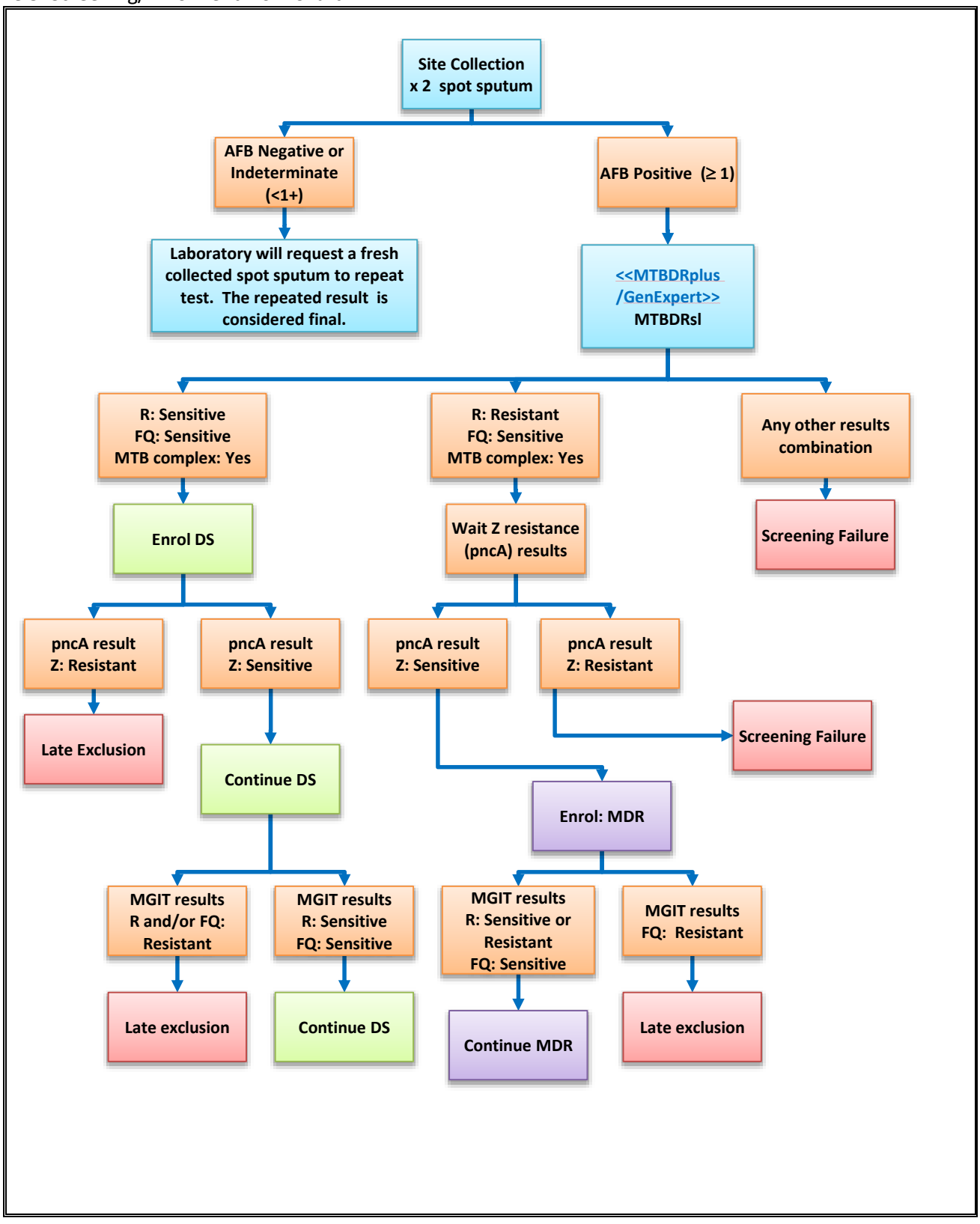


Figure 4: Conduct Flow Chart: All Scheduled Visits

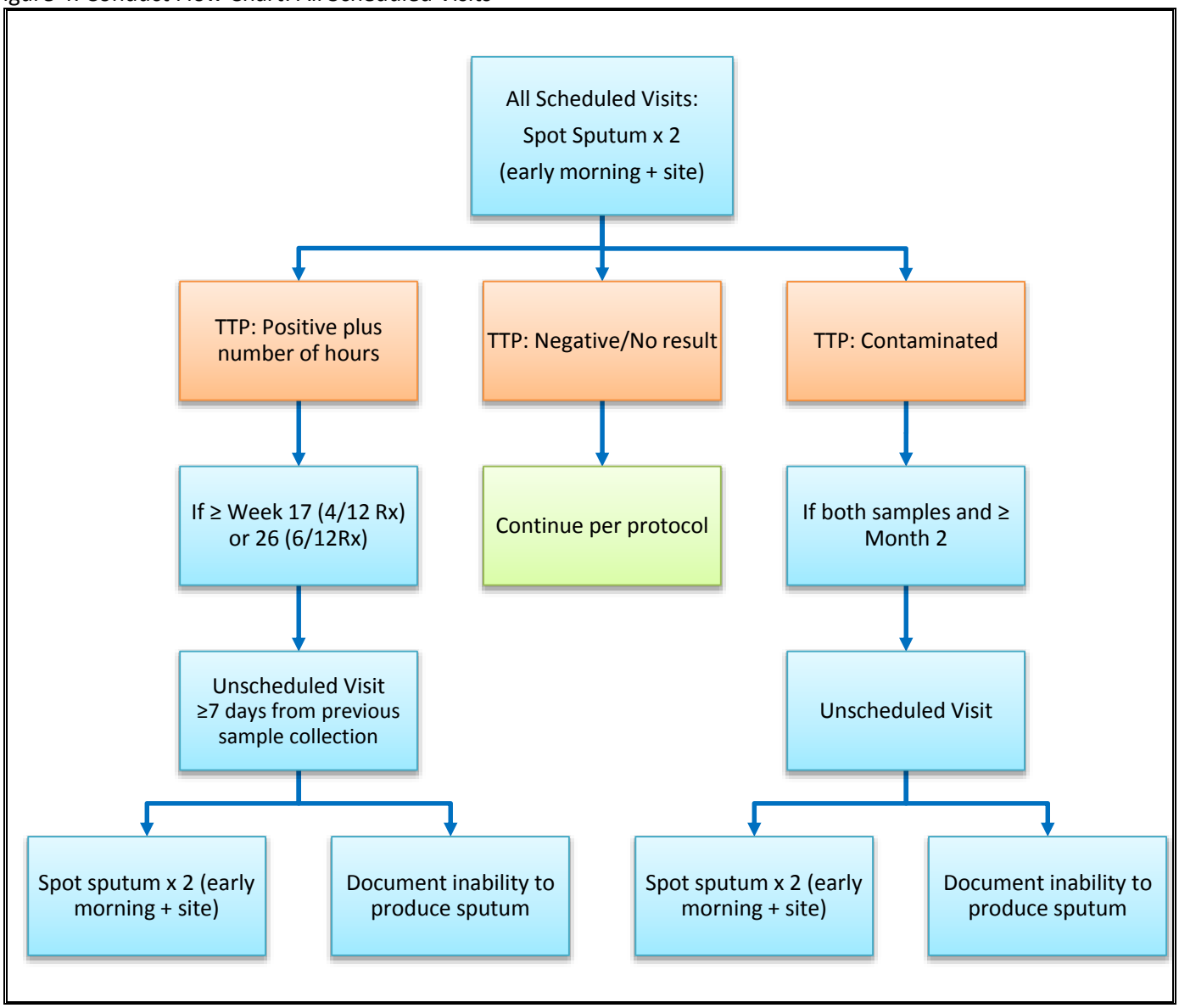


Figure 5: Conduct Flow Chart: End of Treatment/End of Follow-Up/Early Withdrawal

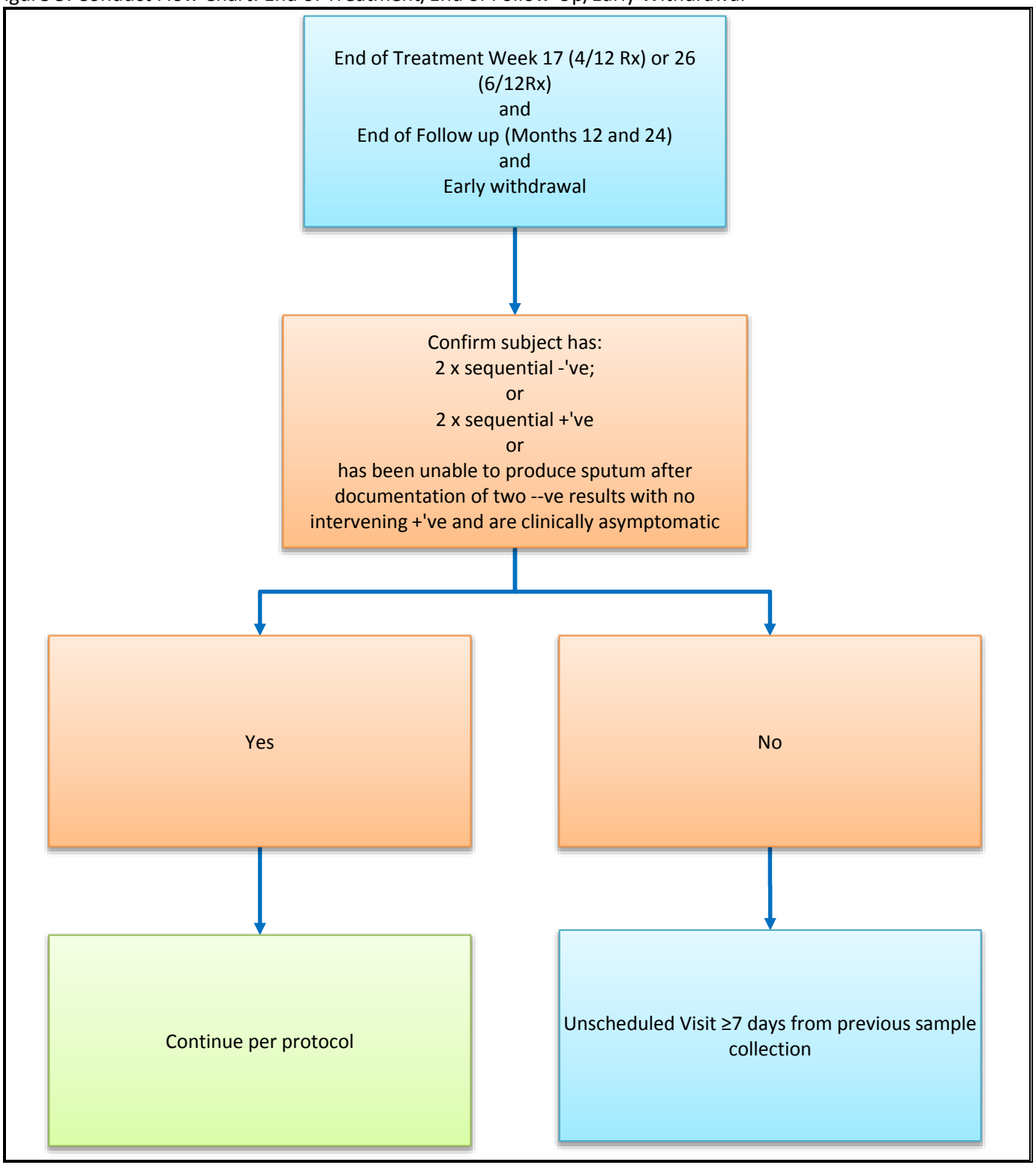
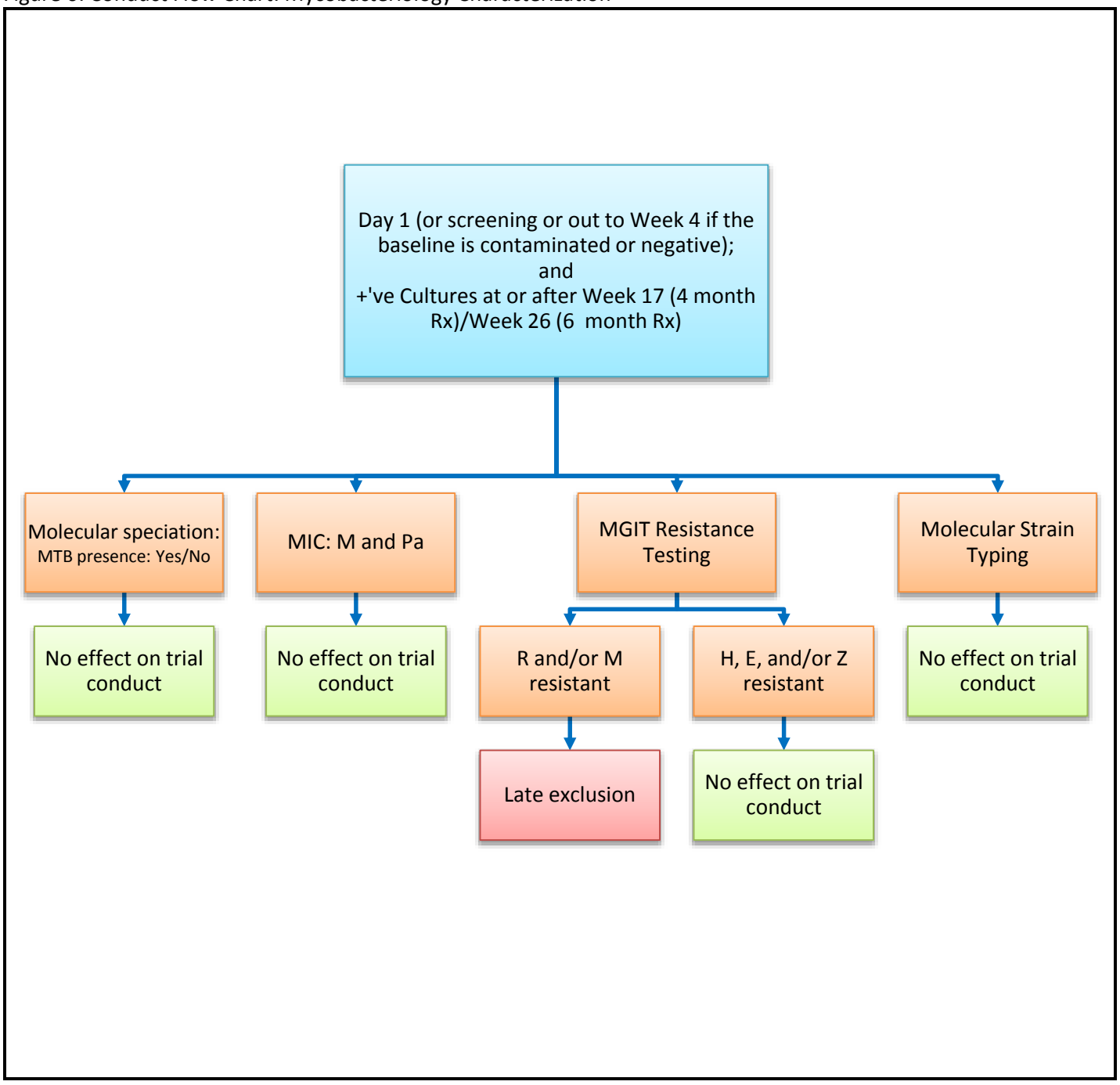


Figure 6: Conduct Flow Chart: Mycobacteriology Characterization



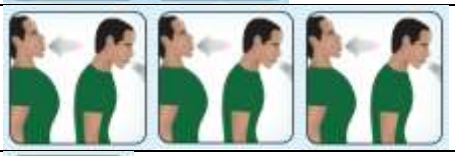
5. SPUTUM SAMPLE COLLECTION

Spot sputum samples (collected at the research site) and Early Morning sputum samples (collected in the early morning at home) are collected in this study.

5.1. Spot Sputum Sampling

The spot samples are preferably, if possible, to be collected in the early morning at the clinic.

Procedure:

1. Check the Expiry date on the Specimen container	
2. Complete the Specimen label container with: - Subject number, - Subject initials, - Sample Collection date, Sample Collection time (prior to start of collection).	
3. Inform the subject that: - nasal secretion and saliva are not sputum; - the desired specimen is produced by a deep cough and is thick, mucoid, white-yellow, and sometimes blood-tinged; - is from the lower airways and lungs.	
4. Instruct the subject on how to collect the sputum (per the below instructions). Instruct patients not to touch the inside of the collection container or inside of the lid with their fingers or other objects.	
5. Talk the participant through the collection process:	
- Rinse and clean their mouth with water:	
- Stand (if possible) and breathe in and out deeply three times:	
- Cough as hard as possible:	
- Place sputum container under their lower lip and collect the sputum:	
- Screw the lid on tightly so that it does not leak.	
6. If there is a delay in transport, specimens must be kept in a refrigerator at 2-8°C and the temperature of this refrigerator must be monitored.	

Note: Specimens that comprise of saliva or less than 2 mL (unless deemed to be a good quality sample) **must not be processed.** The attending physician/study nurse should inspect the sample for volume and quality and, before the patient leaves the clinic, another sample should be requested that does not contain mostly saliva and/or is greater than 2mL in volume.

5.2. Early Morning Sputum Sampling

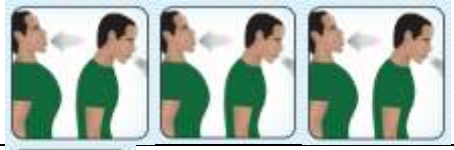
Procedure:

1. Provide the Subject with a pre-labelled Specimen container which has been checked for expiry date.

2. Complete the Specimen label container with:
- Subject number,
- Subject initials,
- Date of their next visit.

3. Inform the subject that:
- nasal secretion and saliva are not sputum;
- the desired specimen is produced by a deep cough and is thick, mucoid, white-yellow, and sometimes blood-tinged;
- is from the lower airways and lungs;
- not to touch the inside of the collection container or the inside of the lid with their fingers or other objects.

4. Instruct the subject on how to collect the sputum (per the below instructions and the Participant Booklet provided to them which describes this methodology for their reference).
- Take the clean sputum collection bottle that they received from the study site and keep it close to them, for example at their bedside.
- Start collecting sputum as soon as they feel the need to cough occurs on waking on the day they are going to visit the study clinic. **This is the first sputum of the day after they have woken up.** It is to be collected as follows:

- Rinse and clean their mouth with water:	
- Stand (if possible) and breathe in and out deeply three times:	
- Cough as hard as possible:	
- Place sputum container under their lower lip and collect the sputum:	

- Screw the lid on tightly so that it does not leak. Put it inside the plastic bag, closing the plastic bag properly.
- Take this with you to the study site when you visit them. Keep it in a cool place, away from direct sunlight and heat.

5. On receipt of the sputum samples at the site, ensure they are correctly labelled. If there is a delay in transport, specimens must be kept in a refrigerator at 2-8°C and the temperature of this refrigerator must be monitored..

6. SPUTUM SAMPLE PACKING AND TRANSPORT

Local health and safety risk assessment recommendations on the transport of dangerous goods must be followed when packing specimens for transport. <<For air transport of samples, the packing of the specimens must be performed in collaboration with the <<>> courier company responsible for the transport according to the International Air Transportation Association (I.A.T.A.) regulation.>>

The laboratory specimen receipt times are as follows:

<<Laboratory Hours, including any weekend, public holidays and other closures>>

6.1. Procedure

1. Pre-arrange transport with the <<driver/<<>> courier company>> and notify the <<>> laboratory that samples are going to be delivered.
2. Complete the Specimen Transfer Form (one for each sputum specimen)(Quality Manual attachment B and Appendix 2).
3. Prepare a cool box for transporting the sputum containers to the laboratory. Ensure that there are an appropriate number of frozen cool packs in the container in order to maintain the temperature between 2°-8°C. The temperature should be 2 – 8°C before sputum samples are added to the cool box. The Sputum containers should not come into direct contact with the frozen ice packs to prevent freezing of the sputum
4. Before dispatch, the attending clinical staff member must cross check the details on all sputum containers and the corresponding specimen transfer forms – samples must NOT be dispatched if they are not correctly competed and any discrepancies will be resolved.
5. Confirm the total number of sputum containers in the box corresponds to the number of accompanying Sputum Transfer Forms.
6. The maximum/minimum thermometer must be allowed to reach equilibrium in the container so that an accurate departure measurement can be taken. Enter the temperature plus the name and signature of the driver or courier transporting the samples in the 'Transport details' section onto the Specimen Transport Form and reset the maximum/minimum thermometer at time of departure, just prior to transport.
7. Make a copy of the Specimen Transfer Form to keep filed at the clinic for future reference.
8. Put the Specimen Transfer Forms in an appropriate envelope to be transported with the container.

Appendix 1: Laboratory Reference Ranges

Acid Fast Microscopy (Z-N)
Normal value: No AFBs seen
Pathological value: Scanty Positive, 1+, 2+, 3+ (IUALTLD/WHO grading)
Also possible value: Missing

Hain MTBDR*plus* Test (if GeneXpert is not performed)
Normal value: Sensitive to isoniazid and rifampicin; *MTB* confirmed Yes
Pathological value: Resistant to isoniazid and/or resistant to rifampicin; *MTB* confirmed Yes. MTB confirmed No
Also possible value: Missing

GeneXpert Test (if Hain MTBDR*plus* Test is not performed)
Normal value: Sensitive to rifampicin; MTB confirmed Yes
Pathological value: Resistant to rifampicin; MTB confirmed Yes. M.TB confirmed No
Also possible value: Missing

Hain MTBDR*sl* Test
Normal value: Sensitive to fluoroquinolone; MTB confirmed Yes
Pathological value: Resistant to fluoroquinolone, MTB confirmed Yes. MTB confirmed No
Also possible value: Missing

pncA Tests
Normal value: Sensitive to pyrazinamide
Pathological value: Resistant to pyrazinamide
Also possible value: Missing

MGIT Liquid Culture
Normal value: Negative (no growth after 42 days)
Pathological values: - Positive (growth after x days x hours) plus ZN AFB positive and Blood Agar negative (MGIT result valid) /positive (contaminated)
Positive (growth after x days x hours) plus ZN AFB negative and Blood Agar positive (contaminated).
Also possible value: Blood Agar: Other. ZN smear: Missing

Minimum Inhibitory Concentration for PA-824 and Moxifloxacin
No reference range
Concentration of antibiotic that caused near-complete inhibition is given
Also possible value: Missing

MGIT Liquid Culture: Drug Sensitivity Tests SIRE, Moxifloxacin and Pyrazinamide
Normal value: Sensitive to streptomycin and, isoniazid, and rifampicin, and ethambutol, and moxifloxacin, and pyrazinamide
Pathological value: Resistant to streptomycin, and/or isoniazid, and/or rifampicin, and/or ethambutol, and/or moxifloxacin, and/or pyrazinamide
Also possible value: Contaminated, Missing

Hain MTBC
No reference range
MTB confirmed yes/no is given

Molecular Strain Typing: MIRU
No reference range
Two strains indistinguishable yes/no is given

Appendix 2: Specimen Transport Form Quality Manual Attachment B

Attachment B: Specimen Transfer Form- **SPUTUM**

This form should accompany each sputum specimen generated from a STAND patient at the clinical site to the laboratory.

Clinical Details

This section should be completed in the clinic

Patient number __ __ __ __ **-** __ __ __	
Initials	
Date of birth (dd/mmm/yyyy)	
Type of Sputum Sample	☐ Early Morning ☐ Spot
Visit Date (dd/mmm/yyyy)	
Visit in STAND schedule * Week __ __ **OR Month** __ __ Unscheduled treatment phase ☐ Unscheduled post-treatment phase ☐	
Date specimen produced (dd/mmm/yyyy)	
Time specimen produced (hh:mm)	
Physician/nurse attending (print name)	
Physician/nurse attending (signature)	

*screening = SC, baseline = 00

Transport Details

This section should be completed by the driver, courier or person accompanying specimen

Date specimen dispatched from clinic (dd/mmm/yyyy)	
Time specimen dispatched from clinic (hh:mm)	
Temperature of transport container (°C)	
Driver/courier (print name)	
Driver/courier (signature)	

Laboratory Receipt

This section should be completed by the laboratory technician receiving the specimens.

Laboratory Name	
Date sample received (dd/mmm/yyyy)	
Time sample received (hh:mm)	
Temperature of transport container on receipt (°C)	
Sample in good condition (y/n)	
If no *please give details (detail problems, is this sample going to be processed? has another sample been requested?)*	
Sample processed within 30 minutes (yes/no)	
If no, time sample transferred to fridge (hh:mm, and give fridge ID)	
Laboratory technician (print name)	
Laboratory technician (signature)	
Laboratory Accession number	ATTACH LABEL