Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Marks GB, Nguyen NV, Nguyen PTB, et al. Community-wide screening for tuberculosis in a high-prevalence setting. N Engl J Med 2019;381:1347-57. DOI: 10.1056/NEJMoa1902129

(PDF updated October 8, 2019)

A Randomized controlled trial of community-based screening for tuberculosis (the ACT 3 studies)

Cover page

This supplement contains the following standard operating procedures and the data analysis plan

1. Protocol section of IRB submission
2. Community screening
3. Clinical follow up of people with Xpert positive result
4. Lab procedure
5. Latent TB infection survey among children born 2012
6. Latent TB infection survey among children born 2004-2011
7. Data analysis plan

Extract from Protocol Section of IRB Submission

Protocol Number 2013 / 073

Submitted: 16th January 2013

255 - Outline the theoretical, empirical and/or conceptual basis, background evidence for the research proposal with reference to the relevant literature (include at least four research citations).

Progress in controlling TB in high burden countries requires enhanced case detection (15). However, in the absence of compelling evidence, there has been little enthusiasm for actively screening for disease in at-risk populations in these countries because of concerns about low cost effectiveness relative to other strategies. On the other hand, active case finding has played an important role in higher income settings since before the chemotherapy era. There was a dramatic decline in mortality in North America and in Britain when community-wide mass radiography was implemented, along with chemotherapy, during the 1950s (16-18). Mass radiography screening was also widely implemented in Australia around this time (19) and this coincided with a period of rapid decline in TB incidence and mortality. A similar program was in place in Fiji until the mid-1980s (CI-Linh, personal communication) and may explain the lower incidence of TB in that country compared with its Pacific Island neighbours. The effectiveness of these community-wide active case finding regimens has never been formally evaluated.

There have been some attempts at active case finding in high-burden countries. These have mainly focused on screening high risk subsets of the population, in particular, known contacts of people with active TB. This is a strategy that continues to be a routine component of TB control activities in high income, low-burden settings. In our recent meta-analysis we identified 71 observational studies of contact investigations in low and middle income countries. Among 878,724 people screened the prevalence of active TB was 3.1% (14). These findings imply that contact tracing has a high yield. However, there have been no reported randomised controlled trials of contact tracing as a strategy for case finding (20). In Vietnam, we are currently conducting the first such trial (NHMRC #632781, 2010-2014) (21).

Despite the high yield of contact tracing, most newly identified cases of TB in highly endemic settings are not known contacts of patients with TB (22), implying that most transmission occurs as a result of casual contact. Hence, the impact of contact tracing alone on the pool of prevalent cases of TB, and consequently on the risk of ongoing transmission, will be relatively small. In high burden settings, it will be necessary to find and treat all, or nearly all, prevalent cases to reduce transmission. That is the rationale for community-wide active case finding. Any intervention which successfully detects and treats most prevalent cases in the population will dramatically reduce disease transmission. Since at least half of the incident cases in high prevalence settings are attributable to recent transmission (23), an intervention to detect and treat prevalent cases that is sustained over a sufficient period to substantially reduce disease prevalence will markedly reduce the incidence of disease so that only cases resulting from reactivation of long-standing latent TB infection occur. These are the conditions that exist in low burden countries, where maintenance of TB control can be sustained by contact tracing, screening of immigrants, and (limited) treatment of

latent TB infection as well as good access to care and standardised case management for newly diagnosed cases. Hence, the long-term goal of the intervention we propose to test, over a period of four years, is to transform the epidemiology of the disease from that of a high-burden, high transmission setting to that of a low-middle burden setting where the resources required to maintain TB control are much less than those required at present.

Recent reports of community-wide active case finding in high-burden settings highlight both the potential value of the intervention and its limitations using â€œoldâ€ technology. In 2005, investigators in Uganda conducted a house-to-house survey among 930 slum dwellers in Kampala (24). They collected three sputum specimens for AFB smear among the 189 (20%) who reported chronic cough

and found 33 (18% of those with chronic cough and 3.5% of the target population) with smear positive pulmonary TB. A more ambitious study was undertaken in Harare, Zimbabwe between 2006 and 2008 (25). Approximately 110,000 people were screened for chronic cough on six occasions at six monthly intervals. Those with cough for more than two weeks were asked to provide sputum for AFB smear examination. Overall, 10,177 participants submitted sputum during the study period and 392 (3.9%) of these were AFB smear positive. This intervention was associated with a 44% decrease in the prevalence of culture-proven pulmonary TB in the study population, including a 60% reduction among HIV-ve participants. Hence, large scale active case finding is feasible and can achieve impressive results in high burden settings. However, sputum AFB smear may not be the best available tool.

Tools for active case finding (screening) for TB

A range of tools are available for screening for TB but newer tools are the most promising. Sputum smear microscopy for AFBs is a cheap and widely available diagnostic tool for TB and it is for this reason that it has been used in the recent surveys in Africa referred to above (24, 25). The main limitation of sputum smear examination is lack of sensitivity: ranging from 20% to 80% in various studies (26, 27). A further limitation is the number that a single operator can perform in a single day: usually 20 to 25 slides. If the study in Zimbabwe had used a more sensitive diagnostic tool, the yield, and hence the reduction in prevalence, would have been greater. This observation was highlighted in the accompanying editorial by the head of the WHO STOP TB Department (15).

Sputum culture is not a suitable screening tool for TB in high burden settings because of the long turn-around-time (at least three weeks), the relatively complex procedure and the need for specialised (reference) laboratory staff and facilities, with high-level biosafety precautions.

Historically, mass radiography was used as a screening tool in high-income settings (16). It has also been used in low-middle income settings for prevalence studies (28). However, problems with infrastructure and logistics, human resources, cost, and radiation exposure all limit the utility of radiography as a tool for repeated community-wide screening in low income and remote settings The development of a fully automated rapid nucleic acid amplification test (XpertÂ® MTB/RIF, Cepheid, Sunnyvale, CA, USA) for TB diagnostics represents a quantum leap in the technology

available for TB diagnostics. Its importance is reflected in the speed with which it was adopted and recommended by the WHO Technical Advisory Group (TAG) (29) and the prominence given to publications reporting the initial findings (30-34). It incorporates nucleic acid amplification testing (NAAT) with

advanced fluid dynamic technology to allow automated processing of sputum specimens. It has good sensitivity (77%) and excellent specificity (99%) for the diagnosis of culture-proven M. tuberculosis and detects rifampicin resistance (and hence multi-drug resistant (MDR-)TB status) with sensitivity 94% and specificity 98% compared with drug susceptibility testing (31). The test has a turn-around time of 2 hours. It is suitable for operation at District level facilities with a minimal level of training and technician handling of specimens. No special biosafety precautions are required. It has been developed in collaboration with the Foundation for Innovative New Diagnostics (FIND, funded initially by the Gates Foundation) and, as a consequence of this, the manufacturers have agreed to make the product available at very substantial discounts for low and middle income countries. The projected discount for 2014, compared with North American prices, is 86%. The device is being rapidly adopted as a diagnostic tool in District-level facilities in developing countries (35).

There has been considerable debate about the proper place of this test in the diagnostic pathway for TB (36). However, in our view its real potential, as a tool for community-wide active screening, has been overlooked. In this context the Xpert MTB/RIF platform has the capacity to perform first stage screening, diagnostic confirmation and detection of MDR-TB in one test.

Human genetic susceptibility to TB:

Genetic risk factors are thought to contribute to 30% of the risk of tuberculosis in humans A number of promising candidate genes have been identified in the international literature. Recent studies performed by Professor Britton (an AI on this project) have examined the association of TB with two molecular targets: the puringenic P2X7 receptor2 (37) and SP110, a nuclear body protein, variants of which have been associated with human TB and progressive TB in mice. Other associations have also been identified. Unpublished data from a recent study we have performed in Vietnam demonstrates an association between TB and polymorphisms for one of these genes, SP110 (G Fox, unpublished data).

This proposal seeks to extend this study to include known variants in other components of host immunity, and new candidate genes. By identifying genetic linkages, it will be possible to increase an understanding of the genetic susceptibility of patients to tuberculosis and influence developments of future treatment. We propose to collect blood from subjects, for DNA extraction and storage. This will be done in a deidentified manner. We propose that we will apply for further ethical approval in the future, specifying the nature of the genetic tests to be undertaken.

Biomarker studies:

Biomarkers have been an increasingly important focus for research of TB in humans. These may provide prognostic information, either for individuals or cohorts, and provide new opportunities to enhance case detection of TB or latent tuberculosis infection. Translation of existing biomarkers into

clinical practice, and investigation of possible new biomarkers, promises to make an important contribution to TB diagnosis and management (38).

We propose to collect blood from patients and control subjects, nested in the larger screening cohort, which will be subequently tested. The specific plan for biomarker analysis will be presented again to an ethics committee in the future, before proceeding with the testing.

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256 - Outline the methodology for the research proposal.

Summary of methodology

We will conduct a cluster randomised controlled trial of active case finding in Ca Mau province of Vietnam. This is the southern-most of Vietnamâ€™s 58 provinces and has a population of 1,212,000. Eighty percent of the population live in rural areas and 74.2% are aged 15 and over. There were 1,459 cases of TB notified in 2011, including 993 infectious (smear positive, pulmonary) TB cases. Patients with TB are managed according to the standard NTP regimen. Currently, all patients diagnosed with MDR-TB in Ca Mau are referred to nearby Can Tho Province for initial treatment and then referred back to their local facility for ongoing management. There are plans to scale up PMDT into Ca Mau and other provinces during 2012.

Randomisation

Sub-communes will be the unit of randomisation for this study. These are hamlets, small villages or neighbourhoods, known as â€œApâ€, comprising approximately 1000 adults (aged 15 years and over). We will obtain (from the local Peopleâ€™s Committee) a list of sub-communes together with their population size for persons aged 15 years and over. Sub-communes will be randomly selected as intervention or control clusters with a probability proportional to their population size.

All clusters (intervention and control)

Patients diagnosed with TB through the routine health care system will be managed according to standard procedures, as described above, in both active intervention and control clusters. The only change to usual management is that we will arrange for patients diagnosed with TB through the DOTS centres, hospitals and (where possible) private clinics to have two additional e

arly morning sputum specimens collected. These specimens will be transferred to our provincial laboratory in Ca Mau where they will be stored in a refrigerator until once weekly batch transfer to the NLH reference laboratory in Hanoi using established transport procedures. At this laboratory direct smear examination, culture (BACTEC MGITÂ® 960, BD Diagnostic Systems, Sparks MD USA), identification and drug susceptibility testing (DST) will be performed. Susceptibilities to INH and rifampicin (RIF), pyrazinamide and ethambutol will be tested at â€œbreakpointâ€ concentrations using the MGITÂ® method. For each participant with a positive TB culture, a sub-culture will be sent to the research laboratory at the National Institute of Hygiene and Epidemiology in Hanoi for molecular analysis.

Intervention clusters

In the active intervention clusters we will screen all persons aged â‰¥ 15 years once each year for four years.

Residents of the sub- commune will be informed about the screening by public information including media, public announcements, and leaflets. Both a mobile van and door-to-door screening will be used to recruit participants for screening. We will make the facility available from early morning to late evening, seven days per week, to ensure accessibility for all residents. Initial screening will comprise a brief questionnaire about symptoms of cough and sputum. All persons who say that they can or do expectorate sputum will be asked to produce a single sputum specimen either at the time of the visit or within the next 24 hours.

Each day all sputum specimens collected within the preceding 24 hours will be transferred to our provincial laboratory in Ca Mau where the Xpert MTB/RIF assay will be performed on that day. Positive results for MTB will be transferred electronically and immediately to the field team who will visit the participant, explain the result, arrange for the collection of two more (early morning) sputum specimens, which will be processed as described above (â€œAll clustersâ€), and refer the patient for treatment to the District DOTS (TB control) centre. All treatment for TB (including treatment for MDR-TB) will be provided free-of-charge.

Outcome measures

We will compare the prevalence of

ï‚· culture-confirmed pulmonary TB disease among persons aged â‰¥ 15 years and

ï‚· TB infection in children entering school (at age 6 years)

between the active and control clusters in the final (4th) year of the intervention.

We will also compare the transmission index between active and control clusters.

We will estimate the prevalence of culture-confirmed pulmonary TB in persons aged â‰¥ 15 years in the active intervention and control clusters. One sputum specimen will be collected from all persons who indicate that they can expectorate sputum and these will be referred for TB smear and culture at the NLH reference laboratory.

The prevalence of TB infection in children is an early epidemiological marker of the extent of TB transmission within a community. We will use an interferon-gamma release assay (an IGRA, QuantiFERONÂ®-TB Gold In-Tube, Cellestis, Carnegie, Vic) to estimate the prevalence of TB nfection in a sample of six year old children at school entry. The advantage of using an IGRA, as opposed to a Mantoux test, is that the former uses TB-specific antigens and reduces the confounding effect of BCG vaccination and exposure to other non-tuberculous mycobacteria. A sample of eligible children will be randomly selected in all active and control clusters in the final (4th) year of the intervention.

All children who are found to have latent TB infection will be offered treatment for this free-of-charge through the DOTS centres.

We will compare the rate of transmission of TB within active and control clusters using the transmission index . All isolates from cases diagnosed over the study period will subjected to mycobacterial interspersed repetitive unit (MIRU) typing according to internationally standardized methods with 15 loci. We will identify groups of related cases within the study population as those within an identical MIRU-15 type or having one repeat difference in alleles. Within each group of related cases, we will identify the first diagnosed pulmonary case as the index case and all other cases as secondary cases. We will estimate the transmission index for active and control sub-communes as the average number of secondary cases per group of related cases for groups with an index case in an active or a control sub-commune, respectively.

X-ray screening

Mass radiography has been the traditional method used for population screening for TB. We have argued that, for several reasons, it is unlikely to play a major role in future screening programs. However, it is important to be able to anchor the findings of this study against previous research in the field. For this reason, we will conduct x-ray surveillance using mobile digital x-ray equipment in the active intervention clusters to estimate the proportion of people with chest x-ray findings suspicious for pulmonary TB (a) who are able to provide a sputum specimen and (b) who have a positive result of Xpert MTB/RIF. Each year, one quarter of the active intervention clusters will have x-ray screening performed at the same time as the brief questionnaire screening survey. All active clusters will have x-ray screening once over the four years of the study.

Data management

Our existing customised, web-enabled database developed for implementation of the current trial in Vietnam will form the basis of the database for the new study. However, we will add capacity for mobile data entry and scanning of bar code labels by our field workers. This will be facilitated by the excellent 3G mobile telephone network that exists in Vietnam (including in Ca Mau). Our existing database has built-in quality assurance checks that will be expanded for the new study.

Sample size

We have made the following assumptions for the sample size calculations:

ï,· the average population (aged 15 years and over) per sub-commune cluster will be 1000 people;

ï,· the upper limit of the intra-class correlation coefficient is 0.001 (2006-07 prevalence survey).

ï,· the prevalence of culture-proven TB in the control clusters will be 350 per 100,000;

We will need 55 clusters (55,000 adults) in each group to have 90% power to detect a prevalence ratio for culture-proven pulmonary TB equal to 0.6 or lower (i.e., a 40% or greater reduction in prevalence). We plan to recruit 60 clusters for each study arm, to allow for drop-outs.

We estimate that for each 1000 adults aged 15 years and over, there will be 22 children aged 6 years and expect to test 15 children in each cluster, which give 90% power to detect a prevalence ratio of 0.70 or lower as different ($p < 0.05$).

We will x-ray all available subjects on one occasion during the four year study period and expect 800 subjects per cluster to be available. We expect 3.4% of the population will have an chest x-ray that is suspicious for TB and that 80% of these will be able to produce sputum for examination. Taking account of the design effect, we will be able to estimate the proportion of x-ray suspects who able to produce sputum with a precision of Â± 2.6%. We expect 3% of x-ray suspects will have a positive Xpert MTB/RIF test and will be able to estimate this with a precision of Â± 1.1%.

Human genetic susceptibility study:

We will collect 15mL of whole blood from:

(a) All patients with tuberculosis, diagnosed by routine NTP procedure as well as diagnosed through our screening study

(b) A random sample of healthy controls from participating sub-Communes, selected according to a randomly generated sequence of numbers electronically, will also be asked to give 15mL of blood

All subjects will provide written informed consent for the collection and storage of blood samples. All subjects will be asked about their TB history, risk factors relating to susceptibility to TB and other demographic information. Patients will be matched according to age and gender where possible. Subjects may choose to participate in other aspects of the study but decline the DNA testing.

Blood will be stored in a -70 degree freezer located in Vietnam, and DNA extracted at a later date. The DNA of patients and controls will be tested for susceptibility related to TB. Any DNA testing will be approved subsequently by submission of a modification to the Ethics Committees in Australia (HREC) and Vietnam (Scientific Committee at National Lung Hospital). Control subjects may also be used as control subjects for other case-control studies, if these are approved by the Ethics Committee in the future.

The blood samples will transported for tested in Australia, or another country which is approved to test the samples by a future modification to the Ethics committees described in the previous paragraph.

Biomarker testing

A sample of serum will be stored for each patient in whom blood is collected for DNA extraction. This serum will be used for subsequent screening for selected biomarkers for TB. These biomarkers have not yet been chosen, but there will be an application before the two ethics committees mentioned in the above section before testing these samples. These samples will be transported to Australia, or another location approved by a future ethics committee modification, for testing and analysis.

Subjects' blood will be stored in a deidentified manner, and remain securely stored in a locked -70 degree freezer, prior to testing.

Standard infection precautions will be in place for staff engaging with patients with suspected active TB, according to the usual practice within the NTP. Mtb is an infection which is treatable by available medical therapies. Laboratory handling of sputum samples will be carried out at authorized laboratories of the NTP.

A Randomized controlled trial of community-based screening for tuberculosis (the ACT 3 studies)

MANUAL OF PROCEDURES
Community Screening
Version 1.0
Last updated: 18th March 2014

Supported by:
Australian National Health and Medical Research Council (APP1045236)
Vietnam National Tuberculosis Program

Table of contents

1. Introduction

1.1. Overview

This is the Manual of Procedures for the study of the Community Screening for the ACT3 study. This document is complemented by the following separate protocols:

- Advocacy, Communication and Social Mobilisation protocol
- Community screening protocol
- Patient management protocol (for patients with positive Xpert results)
- Laboratory protocols (including sputum handling and testing)
- Data management protocol

(a) Title of study: A Randomized controlled trial of community-based screening for tuberculosis (the ACT 3 study)

(b) Duration of main study: 5 years (2013 – 2018)

(c) Program administrators: National Lung Hospital / National Tuberculosis Program

(d) Research director: Associate Professor Nguyễn Viết Nhung
Title: Vice Director of the National of TB and Respiratory Diseases
Address: National Lung Hospital,
463 Hoàng Hoa Thám Street, Ba Đình,
Hà Nội, Vietnam
Telephone: +84 4 7614673
Mobile phone: + 84 912507993
Fax: +84 4 8326162

Research Director (Woolcock Institute of Medical Research):
Clinical Professor Guy Marks
Title: Head of Respiratory and Environmental Epidemiology, Woolcock
Address:
Woolcock Institute of Medical Research,
431 Glebe Point Road, Glebe, NSW 2037 Australia.

(e) Lead Investigators

Vietnam:
Nguyen Viet Nhung
Dinh Ngoc Sy
Tran Ngoc Buu
Nguyen Binh Hoa
Nguyen Van Anh
Nguyen Nhat Linh (WHO)

Australia:
Guy Marks
Vitali Sintchenko
James Wood
Greg Fox
Warwick Britton

(f) Cooperating institutions in Vietnam:

a. National Lung Hospital, Hanoi
b. Ca Mau Social Medicine and Disease Prevention Department
c. National Institute of Hygiene and Epidemiology
d. Department of Education, Ca Mau Province
e. Pham Ngoc Thac Hospital, Ho Chi Minh City

f. Can Tho Lung Disease Hospital, Can Tho Province

(g) Cooperating Institutions in Australia:

a. Woolcock Institute of Medical Research
b. Institute of Clinical Pathology and Medical Research, Westmead Hospital, Sydney
c. Centenary Institute of Cancer Medicine and Cell Biology
d. Centre for Research Excellence in Tuberculosis (TB CRE)

(h) **Funding support**: Australian National Health and Medical Research Council Grant APP1045236

(i) **Human research ethics approval**:

a. Human Research Ethics Committee, University of Sydney. Approvals: 2013 / 073.
b. Institutional Review Board, National Lung Hospital, approval number 407/QD-BVPTW (29th August, 2013)
c. Ministry of Health, Department of Science and Training, approval number pending
d. Ministry of Health, Department of Finance and Training, approval pending.

1.2. Introduction

The Active Case finding for Tuberculosis study (ACT3) is a five-year tuberculosis (TB) community screening project supported by the Australian National Health and Medical Research Council. The project primarily involves a cluster randomized controlled trial of community based screening for TB among people living in Ca Mau Province, in southern Vietnam. The project's primary goal is to **test the effectiveness of community-wide screening for tuberculosis using sputum examination for MTB by nucleic acid amplification (using Xpert MTB/RIF) in reducing the prevalence of TB and rate of new infections with TB** in Vietnam.

In addition, there are a number of additional projects nested within this larger study. The study will provide the opportunity to enhance tuberculosis control within Vietnam, and build capacity for research within the Vietnamese health care system.

1.3. Study population

Population in Ca Mau

The main study is a cluster randomised controlled trial of active case finding in Ca Mau province of Vietnam. This is the southern-most of Vietnam's 58 provinces and has a population of 1,212,000 (38). Eighty percent of the population live in rural areas and 74.2% are aged 15 and over. There were 1,459 cases of TB notified in 2011, including 993 infectious (smear positive, pulmonary) TB cases. Patients with TB are managed according to the standard NTP regimen. Currently, all patients diagnosed with MDR-TB in Ca

Mau are referred to nearby Can Tho Province for initial treatment and then referred back to their local facility for ongoing management.

2. Integrated pilot screening study

2.1. Roles and responsibilities

2.1.1. Field work supervisor

The field work supervisor is responsible for overseeing the implementation of the field research in Ca Mau and provides management to all team members based in Ca Mau.

Qualifications:

- University degree in public health or related field, preferable having a master degree
- Preferably at least 5 years of experience in field work for research projects
- Managerial skills
- Highly motivated, willing to learn, service-oriented and able to work under high pressure and within a limited time frame
- Professional use of personal computer utilizing word processing and spreadsheet software programs
- Excellent communication skills

Job description:

- Develop and strengthen key partnerships and relationships with relevant organisations within Ca Mau Province. Liaise the local, district and provincial authorities on issues regarding fieldwork.
- Field work
  - Arrange pilot-testing and its evaluation
  - Planning field work
  - Train and supervise field team to make sure the team follows the SOP
  - Coordinate the day-to-day fieldwork
  - Monitoring of the implementation of the trial including subject recruitment and implementation of all study procedures, and dealing with queries
  - Monitor data quality
  - Supervise data management
  - Registration and management of adverse events
  - Report to the Trial Coordinator and the Country Director without delay any major problems in preparation, execution or data management of the survey
- Financial and administration
  - Establish effective procurement control and asset management

    - Oversight of budget, audit and financial accounting
    - Ensure efficient expenditure of resources
    - Ensuring appropriate financial control processes are followed
- HR:
    - Recruitment of local staff to conduct screening
    - Supervise performance of all staff.
    - Foster a community of learning and teamwork, continuous quality improvement and continuous professional development for staff
- Reporting:
    - Maintain daily communication with Hanoi office via daily email and weekly skype meeting
    - Preparing monthly reports for the Trial Coordinator and the Country Director on progress of trial implementation
    - Assisting in preparation of the final study report
    - Oversee completion of financial reporting

Report to:
Trial Coordinator

2.1.2. Field team leader

The Field team leader is responsible for overseeing the team of research staff participating in the survey. They will liaise directly with the community leaders, including the local health worker, commune leaders and other local authorities, to arrange the study and implement this protocol.

Qualifications:

- Preferably at least 2 years of experience in field work for research projects
- Managerial skills
- Survey organization and training
- Expertise in field work
- Team player and motivator
- Attention to detail and accuracy when conducting administrative procedures

Job description:

- Oversee preparatory visits to selected villages before fieldwork
- Develop the plan for screening
- Lead the field team during the implementation process
- Be responsible for logistics and organization during fieldwork
- Coordinate the day-to-day fieldwork
- Liaise with local, district and provincial authorities on issues regarding fieldwork
- Prepare the final field report to the study coordinator at the end of fieldwork in each village
- Liaise with the Trial Coordinator (and other visiting field supervisors) on a regular basis
- Report, without delay, any problems in implementing the research protocol in the field
- Conduct health promotion activities in research clusters

Reports to:
Field work supervisor

2.1.3. Field worker (WIMR)

The Field worker will work under the supervision of the Field Team leaders to implement all technical field activities.

Qualifications:

- Preferably experience in field work in a research setting
- Experience in the assigned task
- Good administration and organizational skills
- Adequate social skills to interact with the survey population

Job description:

- Participating in required training, including Good Clinical Practice training.
- Logistical support for activities
- Participate in community meetings and local engagement activities
- Assist the Field Leader with any tasks related to screening activities
- Interviewing participants, recruiting them to the study
- Collecting sputum
- Data collection and data validation

Reports to:
Field Team Leader

2.1.4. Field team staff (AP leader, village health worker)

The field team staff will accompany field worker to each household to conduct screening.

Qualifications:

- Preferably experience in field work in a research setting
- Knowledge of local language(s) spoken in the cluster
- Knowledge of the area where the activities are carried out
- Adequate social skills to interact with the survey population

Job description:

- Site preparation and health promotion
- Organization of flow of subjects in the field site
- Assistance with census taking
- Tracing of survey subjects
- Assistance with sputum collection
- Transport of sputum samples
- Feedback of positive laboratory results

Reports to:
Representative of Ca Mau Center for Social Medicine and Disease Prevention

2.1.5. Medical advisor

The medical advisor is a clinical doctor who is responsible for providing appropriate medical advice for subjects identified to have any medical problem, including TB and other issues, during the survey. This is a part-time position.

Qualifications:

- Medical degree, preferably with post-graduate training in lung disease
- At least 5 years experience working as a doctor in tuberculosis and lung disease
- Ability to communicate regarding complex medical problems with other individuals within the health care system.
- Experience of the local health care environment in Ca Mau
- Experience conducting research or field surveys

Job description:

- Advise Trial Coordinator and Team Leader about management of medical conditions identified among study participants
- Assist arranging follow-up of individuals with likely TB or other respiratory diseases either in the household or at District TB Units
- Follow up subjects who are commenced on treatment, to ensure they take appropriate therapy for the appropriate duration
- Report to the Trial Manager regarding diagnosis and follow-up of individuals
- Discuss complex medical issues with a specialist physician, according to clinical need.

Reports to:

Field work supervisor and Trial Manager; Principal Investigators

2.2. Inclusion and exclusion criteria for community screening

The inclusion criteria for community members included in screening will comprise the following, for focus group discussions:

Participant	Inclusion	Exclusion
Community member in Aps	○ Age 15 or above ○ Residence at the screened AP time of screening ○ Able and capable of providing informed consent	○ Aged 14 or younger ○ Not residence at the screened AP time of screening ○ Unwilling to answer questions or give verbal informed consent

2.3. Community screening procedure

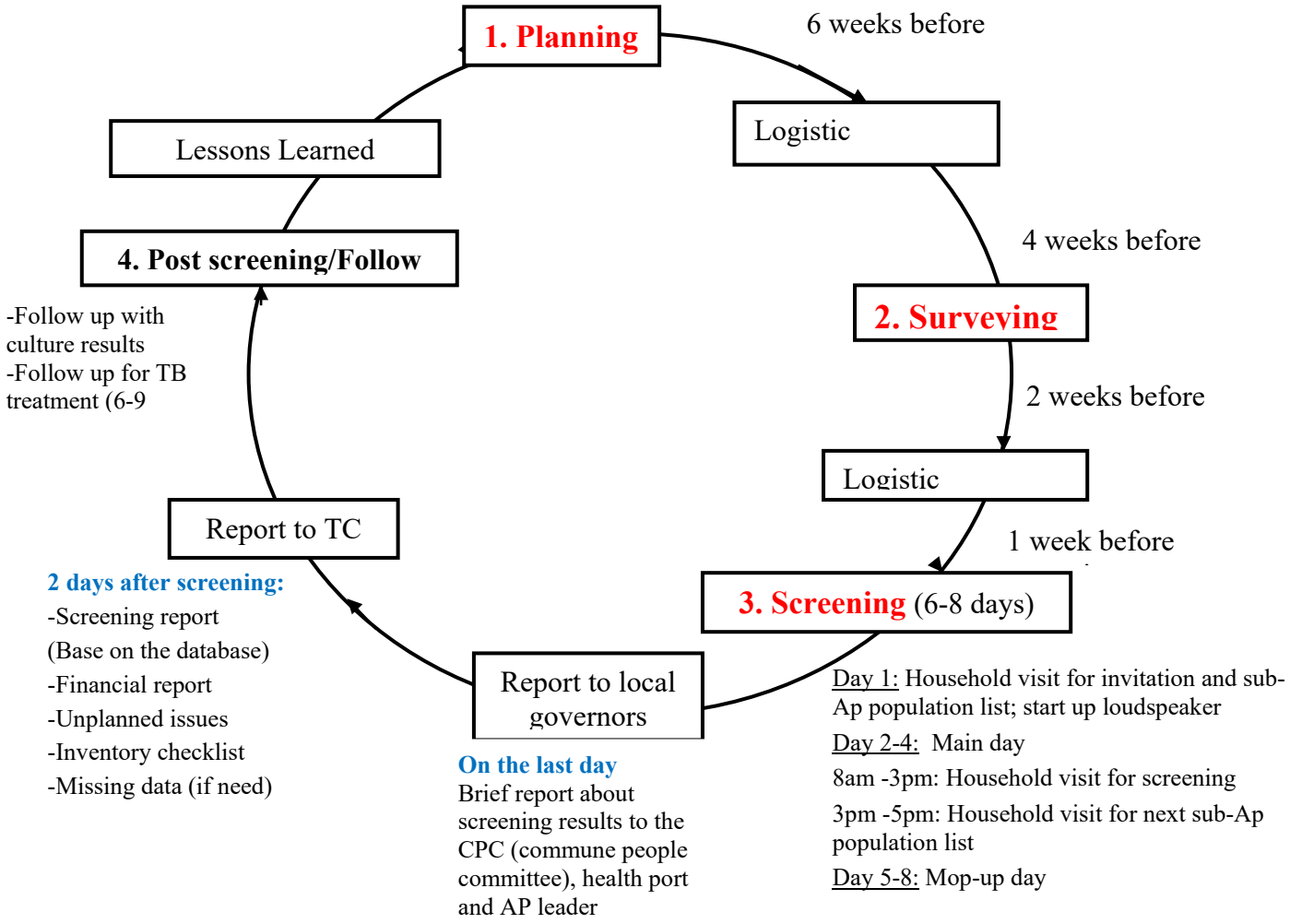


Figure 2.5.1: Overview of stages of screening in each village

IMPLEMENTATION PROCEDURE

Responsible for all activities: Field team Leaders (LBK, NTN)
Total for 1 AP: 15 – 17 days

#	Activities	Timeline		Note
		Duration	***Before (3.2)***	
1	**Plan building** – Implementation plan in designed Ap – Estimate budget for all activities in 1 Ap and approval (in line with approved budget) – Send correspondences/documents from CSDP to District Health Center and Commune People's Committee (CPC)	1 day	6 weeks	Only fieldwork supervisor (TNP) responds directly to CSDP.
2	**Surveying in AP**		4 weeks	
2.1	***Logistics preparation for the pre-screening visit*** – Confirm the meeting time with contact person in commune – Preparation list for surveying: MoH approval, PPC agreement, List of 120 clusters, ACSM material (Flyer #1 & #2, Loud speaker, flipchart), CSDP's official document to CPC & district health center, Checklist for observation, appointment letter and all of needed financial document.	1 day		
2.2	***Surveying in AP/commune*** Meeting with CPC, Commune Health Post (content attached): – ACT3 introduction – Determine precise date and time of the screening – Send template for appointment letter to the CPC for their official approval (sign & stamp) – Build up local collaborators and describing their responsibilities; collecting contact information; Guiding from house to house (100,000 VND/person/day) – Identify sputum transportation method – Determine to use mobile loudspeaker or current loudspeaker Understanding about the local community through some household visits: – Map AP's: road, number household per sub-AP – 3G infrastructure	1 day		
2.3	***Report for surveying*** – Prepare and submit meeting minute – Make surveying report, fill in observation checklist – Prepare plan for next stage	1 day		
3	**The main screening in AP**			
3.1	***Logistic preparation for the screening***			

#	Activities	Timeline		Note
	– Advance for the trip – Prepare communication material: banner, poster, flyer, instruction panel – Prepare sputum collection material: sputum cup, dry-ice, elastic band, tissues, Ziplock bag, disinfected hand washing, gloves, discard yellow/black bag… – Prepare forms: sputum transportation forms, screening form (applicable only to pilot), Ap's population list, QRcode stickers, printer, paper for barcode printer, chairs… – Personal stuffs: life jacket, blouse, drinking water, tablet… – Prepare financial form (for expenses access 2 million VND): inventory form, expenses form, report….	1 day	1 week	
3.2	***Screening implementation at Ap:*** – Day 1: Household visit for invitation and sub-Ap population list; start up loud speaker – Day 2-4: Main day 8am -3pm: Household visit for screening 3pm -5pm: Household visit for next sub-Ap population list – Day 5-8: Mop-up day Looking for the subjects have been not screening, yet – Quick report to CPC on the last day **Note:** Database checking for no missing; transporting sputum samples to CSDP and flowing up samples with GeneXpert (+), come to get two more sputum specimens.	6-8 days	Ngày 0	Daily access to database.
3.3	***Screening report*** – Report based on index (automatically updated from database) – Report Adverse event/unplanned issues/problems (attached) – Clear Advance for all activities in 1 AP. – Check for missing data (if any) – Inventory screening material (ASAP) – Lesson learn & next year recommendation	2 days		
4	**Post screening** (via email, phone or visits (if needed)) – Tracking on culture results for all case in AP has Xpert (+) – Following up cases has TB treatment. – Reporting treatment status (form)	1 day	2-8 weeks after screening	After 8 weeks, all of culture results will be available.

2.4. Screening in Households

Screening in the households will be done by teams of 1-2 field workers and 1 local person.

When the team approaches a household, they will identify number of people who are eligible to participate into the screening. Each person will be registered and interviewed according to the following diagram.

Participant will be given the flyer #1 (ACT3 introduction) and flyer #2 (information sheet about TB)hich provide basic information about the study. The study is introduced using the following words:

- *We would like to invite you to participate in the Ca Mau Lung Health study.*
- *We would like to speak to all adults (age 15 and over) in this Ap.*
- *We are looking to identify people with lung or breathing troubles and help to connect them to the appropriate treatment services.*
- *Some people have lung problems that they do not yet know about but early detection of these problems can prevent serious illness later. Therefore it important that you allow us to speak with you whether or not you think you have lung or breathing problems.*
- *The screening will include collection and testing of a sputum specimen, if you are able to produce one.*
- *Both the interview and the sputum test are provided free-of-charge. If you are found to have TB, treatment for this disease will also be free-of-charge.*

People who agree to participate will provide answers according to the questionnaire in the tablet. Instructions for what to do with sputum samples are included below.

2.4.1. Requirements for space for participant to produce sputum

The minimum requirement for the sputum production space is a well ventilated space. The target for review of all individuals will be a wait of less than 15 minutes per person.

2.4.2. Sputum collection

The sputum collection tool is the project flip chart. The field worker will explain key steps for collecting sputum

1. Introduction:
   - Explain purpose of the test:
   - this is a test to screen for a lung infection called tuberculosis
   - Timing of sputum sample – spot sample (or early morning, before eating or smoking) – for staff video only

2. Method of collection:
   - Label the sputum cup with 2 QRCODE stickers and date of collection
   - Open sputum container – without touching inside
   - 3 deep breaths –
     - Breathe in, then out
     - Breathe in then out hard
     - Breathe in then out a third time and have a deep cough from your lungs
     - Expectorate into cup
     - Explain difference between sputum and saliva
     - Sputum comes from the lungs, where lung infections can occur
     - We don't want samples from the nose or the mouth
     - Close lid of the cup tightly without touching sides
   - Wash your hands (TB worker must sterilize hands with antiseptic)

3. What to do with the sample after collection
   - Fill in the screening form
   - Stick QRCODE in the sputum transportation list
   - Triple packaging sputum cupKeep in a cool box with ice until the sample arrives at the laboratory
   - Transport out of the light, and deliver to the Ca Mau CSDP as soon as possible
   - The Medical Officer will inform the subject if the test is positive and will tell them to go to a clinic

2.4.3. 24 hour follow up visit

When a participant's Xpert result for their screening sputum sample is TB positive (MTB detected), they will receive a follow up visit at which the fieldworker/ team leader will return GeneXpert result; give PIS 01 form; request signature on consent 01; and capture the information in the 24 Hour Follow Up Form

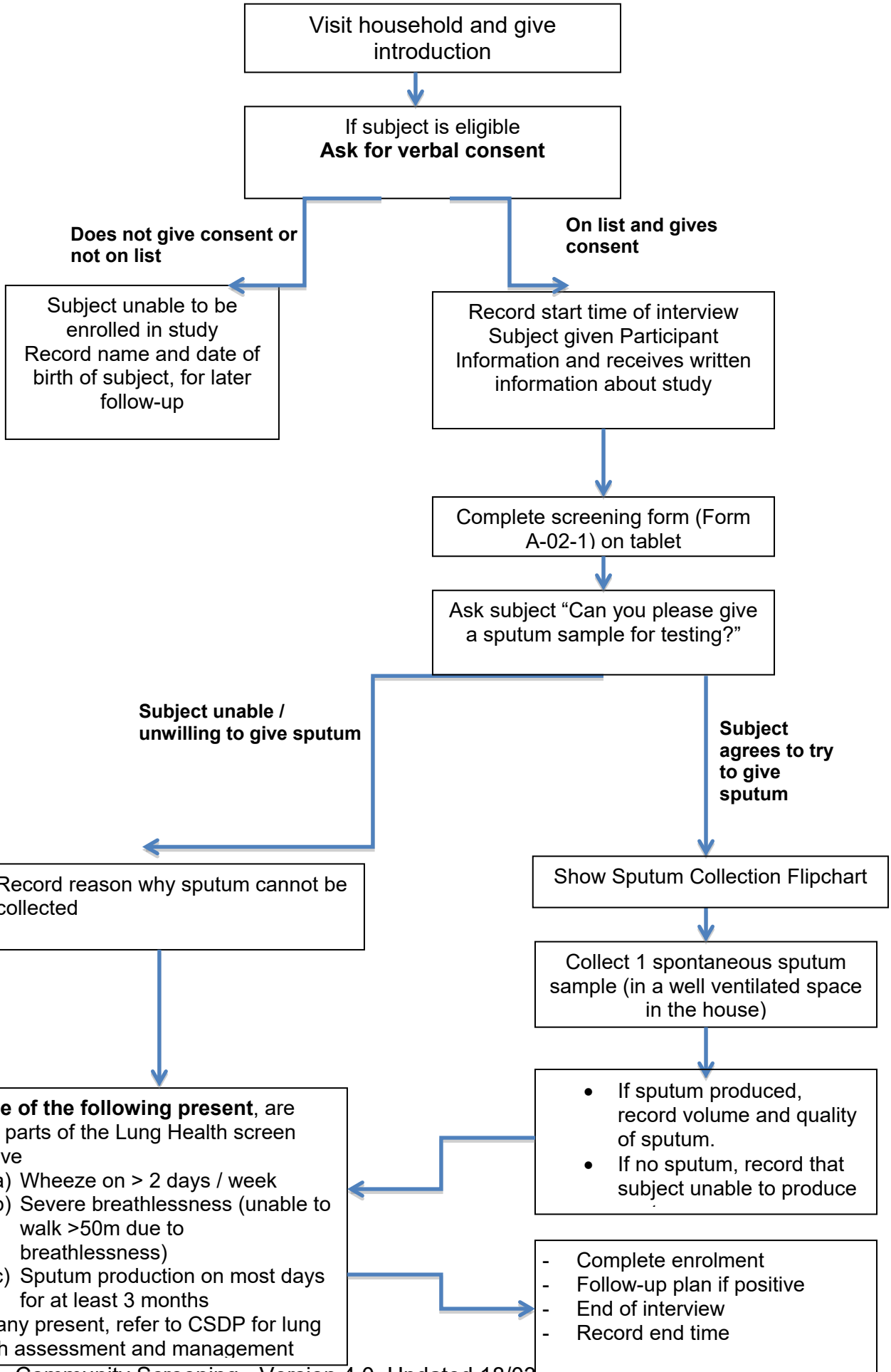
Before leaving the participant, the field worker will ask the participant to:
- Try and produce the first sputum sample later on if they were unable to produce it at the visit.
- Produce a second sputum sample the following morning which will be collected the following day.

The next day, the fieldworker will return to collect and capture the details of sputum 1 (only if outstanding), sputum 2, and the blood sample (only if outstanding):

1. Sputum specimen IDs as well as sputum type (morning/spot) will be captured using the FU Sputum 1 and FU Sputum 2 forms respectively.
2. Blood specimen IDs will be captured using the FU Blood Form.
3. If any of the specimens cannot be collected after numerous attempts, this should be captured into the applicable form:
    a. Refused.
    b. Unable.

The follow up specimens (blood and sputum) and X-ray IDs will be added to the Lab Samples in Transit report as was done for screening sputum samples.

Flowchart A-02-1: Flowchart for Screening at household



2.5. Handling of sputum samples

2.5.1. Receipt and logging of samples

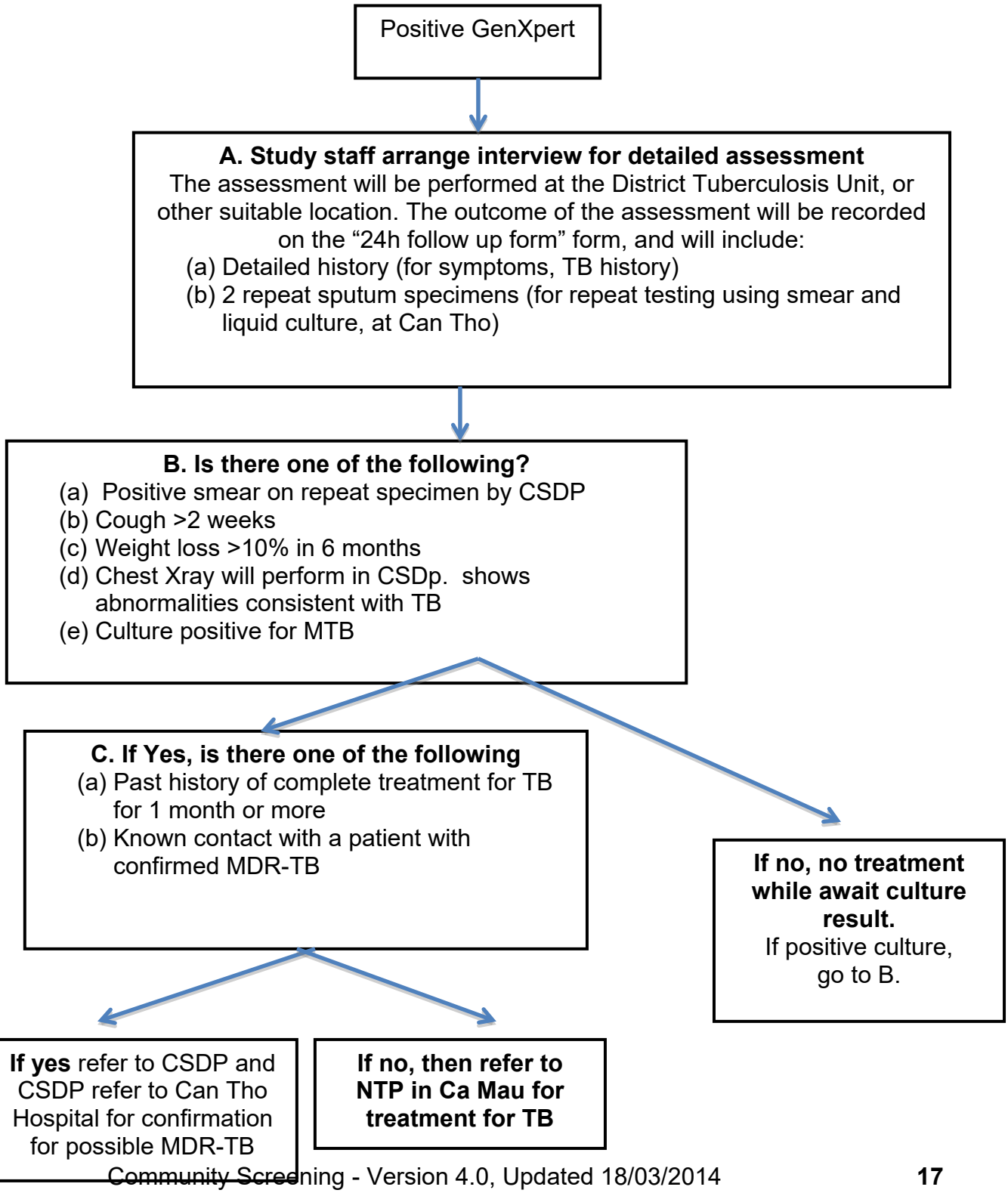
- If a sputum sample is collected, there will be (a) a list of samples' with QRCODE (b) the QRCODE will be attached to the sputum tube; (c) the sputum tube will be placed in a sealed plastic bag (single wrapped); and one spare QRCODE for lab usage.
- Samples will be stored in a closed box, in a cool location.
- Each day, the samples will be transported to Ca Mau City (except for a remote location, when these will be transported every 2 days).

2.5.2. Receipt of sputum samples at CSDP and testing

- At CSDP, the Manual for handling of sputum will be followed (see separate protocol).

3. Management of subjects with positive GeneXpert results

Protocol for subjects with a positive GeneXpert result



4. Reporting of adverse outcomes

The Field site Leader will record any adverse outcomes in an Adverse Outcomes form. This will be submitted to the Trial Coordinator each week. For urgent clinical outcomes, the Trial Coordinator will be contacted on the same day.

These will be used to develop protocols for managing adverse outcomes, and so are very important to record.

Adverse events would include:

- Abnormal symptoms during sputum collection: e.g. chest pain, difficulty breathing, any other adverse outcome of screening
- Accidents that happen to staff or research participants during the activity (e.g. falls, motorbike accidents etc)
- Any complaint made by study participants or local leaders

For each adverse event, an OUTCOME must be recorded. A staff member must take responsibility for following up the adverse event within a reasonable period of time (less than 1 week, or less if appropriate).

NATIONAL TUBERCOLOSIS PROGRAM

WOOLCOCK INSTITUTE OF MEDICAL RESEARCH

MANUAL OF PROCEDURE

ACT3 PROJECT

CLINICAL FOLLOW UP FOR RESEARCH PARTICIPANTS WITH GENEXPERT TB POSITIVE

VERSION 1.5

Index

Definitions

TB treatment outcomes	The following definitions will be used for either index patients or contacts that are treated for TB. A treatment outcome will be recorded for each index patient for the baseline episode, and for up to one episode of incident TB (defined below) for index patients and contacts. (a) **Cured**: A pulmonary TB patient with bacteriologically confirmed TB [smear and/or culture confirmed TB] at the beginning of treatment who was smear- or culture-negative in the last month of treatment and on at least one previous occasion (b) **Treatment completed**: A TB patient who completed treatment without evidence of failure BUT with no record to show that sputum smear or culture results in the last month of treatment and on at least one previous occasion were negative, either because tests were not done or because results are unavailable (c) **Treatment failed**: A TB patient whose sputum smear or culture is positive at month 5 or later during treatment. (d) **Died**: A TB patient who dies for any reason before starting or during the course of treatment. (e) **Lost to (treatment) follow-up**: A TB patient who did not start treatment or whose treatment was interrupted for 2 consecutive months or more. (f) **Not evaluated**: A TB patient for whom no treatment outcome is assigned. (This includes cases "transferred out" to another treatment unit as well as cases for whom the treatment outcome is unknown) to the reporting unit. (g) **Treatment success**: The sum of cured and treatment completed.

Definitions of TB diagnoses (WHO, 2013)

New TB	New TB is TB in patients that have never been treated for TB or have taken anti-TB drugs for less than 1 month.
Previously treated TB	Previously treated patients have <u>received 1 month or more</u> of anti-TB drugs in the past. They are further classified by the outcome of their most recent course of treatment as: (a) Relapse patients (see definition below) (b) Treatment after failure: patients are those who have previously been treated for TB and whose treatment failed at the end of their most recent course of treatment. (c) Treatment after loss to follow-up: patients have previously been treated for TB and were declared lost to follow-up at the end of their most recent course of treatment. (These were previously known as treatment after default patients.) (d) Other previously treated patients: those who have previously been treated for TB but whose outcome after their most recent course of treatment is unknown or undocumented.

Relapse TB	Relapse patients have previously been treated for TB, were declared cured or treatment completed at the end of their most recent course of treatment, and are now diagnosed with a recurrent episode of TB (either a true relapse or a new episode of TB caused by reinfection) [4].
Incident TB	New and relapse cases of TB.
Smear positive TB	Smear positive pulmonary TB was defined by the presence of at least one positive smear in combination with an abnormal chest radiograph, or one positive smear plus a positive culture.
Smear negative TB	Smear negative TB was diagnosed if contacts had radiographic changes consistent with TB, no response to broad-spectrum antibiotics and a response to anti-tuberculous drug treatment.
HIV positive patient	HIV-positive TB patient refers to any bacteriologically confirmed or clinically diagnosed case of TB who has a positive result from HIV testing conducted at the time of TB diagnosis or other documented evidence of enrolment in HIV care, such as enrolment in the pre-ART register or in the ART register once ART has been started.
HIV negative patient	HIV-negative TB patient refers to any bacteriologically confirmed or clinically diagnosed case of TB who has a negative result from HIV testing conducted at the time of TB diagnosis. Any HIV-negative TB patient subsequently found to be HIV-positive should be reclassified accordingly.
HIV status unknown	**HIV status unknown TB patient** refers to any bacteriologically confirmed or clinically diagnosed case of TB who has no result of HIV testing and no other documented evidence of enrolment in HIV care.
Bacteriologically confirmed TB	A **bacteriologically confirmed TB case** is one from whom a biological specimen is positive by at least **ONE** smear microscopy, culture or Xpert MTB/RIF. All such cases should be defined as such, regardless of whether TB treatment has started.
Clinically diagnosed TB	A **clinically diagnosed TB case** is one who does not fulfil the criteria for bacteriological confirmation but has been diagnosed with active TB by a clinician or other medical practitioner who has decided to give the patient a full course of TB treatment. This definition includes cases diagnosed on the basis of X-ray abnormalities or suggestive histology and extrapulmonary cases without laboratory confirmation. Clinically diagnosed cases subsequently found to be bacteriologically positive (before or after starting treatment) should be reclassified as bacteriologically confirmed.
Presumptive TB	Presumptive TB (also referred to as “a TB suspect”) refers to a patient who presents with symptoms or signs suggestive of TB.
Multidrug resistant TB	Multidrug resistance: resistance to at least both isoniazid and rifampicin.

(confirmed)	
Rifampin resistant TB	Either meeting the definition of multi-drug resistant TB (confirmed), or with a positive result for RIF resistance on Xpert MTB/RIF (i.e. rifampicin resistant).

Roles of research staff and institutions for the ACT3 Clinical follow-up protocol

Position: Chief Investigators

Overview

The Chief Investigators are responsible for overseeing study design and supporting the Trial Coordinator and other staff as they implement the project. The CIs will oversee the development of Manuals of Procedures. The lead Chief Investigator (CIA) will authorize expenditures in accordance with to the delegations policies of the Woolcock Institute in Vietnam.

Position description for Clinical follow-up protocol.

- Oversee development of SOP in consultation with WIMR staff, NTP staff and other staff.
- Review summary of clinical follow-up for all Xpert positive subjects once each month.
- Provide formal report for chest Xrays (selected investigators).

Position: Country Director

Overview

The Country Director is responsible for oversight of the project in Vietnam. This person will manage the Trial Coordinator, who will in turn be responsible for the day-to-day running of the project.

Position description for Clinical follow-up protocol.

- Support Trial Coordinator to train staff and implement protocol.
- Approve budget and expenditure, according to Woolcock Institute financial delegations policy.
- Assist with staff recruitment
- Perform periodic monitoring visits to evaluate performance against SOP.
- Monitor and support other staff in Vietnam to meet routine deadlines for this project.
- Reports for this research to.

The Country Director will report to the Principal Investigator in relation to this project.

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Position: Trial Coordinator (Woolcock Institute)

Overview:

The Trial Coordinator is a full-time member of the Woolcock Institute who will oversee the design and implementation of the ACT3 study. This person will be responsible for ensuring staff working on this project comply with a high quality of research standards, and will be responsible for the integrity of research data. This person will manage all staff employed under the ACT3 project. The Trial Coordinator will report directly to the Country Director, and through them to the Chief Investigators of the Woolcock Institute.

Position description

The Project Coordinator will fulfill the following key roles in the Clinical Follow-up project:

- Develop and implement this procedure, in consultation with Chief Investigators
- Recruit and train staff to perform this part of the study
- Support Clinical Follow-up officer to prepare the clinical follow-up report each month, and develop an action plan to address any issues arising from the report.
- Perform periodic monitoring visits to compare reported data with original medical records, laboratory records etc

Position: Project Officer (Clinical Follow-up)

Overview

This person will assist the Trial Coordinator in the conduct of the follow up of all subjects with Xpert positive sputa. In particular, this person will manage the implementation of this Manual of Procedures at the local sites.

Position description

- Train and support staff from the WIMR, NTP and other partners to comply with the procedures in this manual
- Coordinate and oversight Research Assistant to refer WIMR research participants who have been referred to attend CSDP to the appropriate rooms.
- Follow-up all test results of subjects with Xpert MTB positive sputum
- Coordinate and conduct post-diagnosis interviews
- Coordinate and oversight interviews with project's patients who completed TB treatment course.
- Contact Can Tho laboratory/ACT2 staff in Can Tho to receive test results and upload results to Mobenzi system
- Update the Clinical Follow-up report weekly (using data from Mobenzi and other sources)
- Store chest Xrays (original copies and also digital copies) in a systematic way, which are available for clinical review by Investigators or Expert Clinical Review Panel members
- Compile patients profile and share to the Treatment Committee, follow up to make sure treatment advice by the Committee is implemented
- Liaise with staff at the CSDP to ensure subjects with Xpert positive sputum are managed according to study protocols, and that all necessary data is being collected
- Have weekly meetings with the Medical and Laboratory consultant to ensure clinical and laboratory procedures are implemented according to appropriate standards.
- Prepare a monthly report for the Chief Investigators about the clinical management and results of all subjects with Xpert MTB positive sputum and coordinate monthly and non-periodical meetings with CIs/PIs in regards to treatment decisions and follow up.

Position: Medical and Microbiology Consultant (Clinical Follow-up)

Overview

This person will provide support to the Project Officer in implementing the Clinical Follow-up protocol. They will provide advice about the laboratory procedures relating to testing samples from subjects with Xpert positive sputum. They will review all clinical data from each subject and make recommendations (either according to protocols, or referring subjects to the Expert Clinical Review Panel). This person will review and develop this Manual of Procedures, in consultation with the Trial Coordinator and Investigators.

Position description

- Develop this Manual of Procedures, in consultation with the Chief Investigators and Trial Coordinator

- Support the Project Officer to train staff from the WIMR, NTP and other partners to comply with the procedures in this manual
- Conduct regular monitoring visits of the laboratory where culture is performed, to ensure compliance with the study protocol and reporting
- Support the Project Officer to prepare the monthly Clinical report for all Xpert positive patients
- Advice regarding technical aspects of bacteriological testing and radiographic testing of subjects.
- Collaborate with partners at the CSDP to develop appropriate clinical algorithms for assessing subjects, and completing clinical assessment forms.

Position: CSDP Medical Officers

Overview

The CSDP medical officers will assess subjects with positive Xpert, and evaluate all relevant data to make a decision about the diagnosis and treatment of subjects. They will provide documentation about their decisions, and work with the WIMR staff to follow-up subjects.

Position description

- Clinically evaluate all subjects with Xpert positive sputum in the CSDP
- Arrange for chest Xray and additional testing, as required, to make a clinical diagnosis
- Refer subjects according to agreed protocols, consistent with the NTP policy
- Provide clinical advice to the Project Officer and Trial Coordinator regarding management of patients.
- Participate in training regarding the study methods and reporting processes.
- Support monitoring and evaluation visits by the Medical and Laboratory Consultant.

Position: Culture laboratory Coordinator for ACT3 (Can Tho lab)

Overview

This person is working for the NTP within the culture laboratory. This person will act as the focal point for the ACT3 team members, when obtaining results and conducting monitoring visits. This person will be responsible for the timely documentation of results and conducting tests according to the Manual of Procedures for this research.

Position description

- Participate in training by the ACT3 staff relating to laboratory procedures
- Receive sputum specimens from WIMR lab in Ca Mau
- Conduct direct smear (Ziehl Neelsen) and liquid culture (MGIT) of sputum in accordance with the Manual of Procedures
- Conduct TB identification by BD MGIT TBc ID kit.
- Send all of the liquid positive MGIT to NIHE monthly
- Record test results in a timely fashion
- Complete financial payment forms, according to the Manual of Procedures and appropriate financial regulations of the NTP and WIMR.
- Inform the Project Officer of any problems with samples in a timely fashion
- Support monitoring and evaluation visits by other members of the ACT3 team.

Position: DST laboratory Coordinator for ACT3 (NIHE)

Overview

This person is working for NIHE. This person will act as the focal point for the ACT3 team members, when obtaining results and conducting monitoring visits. This person will be responsible for the timely documentation of results.

Position description

- Receive samples sent from the culture laboratory
- Perform drug susceptibility testing for first line tuberculosis drugs
- Perform additional molecular (genetic) testing as agreed
- Store cultured isolates in a -80 freezer, according to study protocols
- Extract and store mycobacterial DNA for transportation from Vietnam to Australia, for subsequent genetic analysis
- Participate in external quality assurance activities to evaluate compliance with relevant laboratory standard

Overview of follow-up procedures

Chart 1 ***Clinical follow-up of subjects with MTB+ on GeneXpert in ACT3 study***

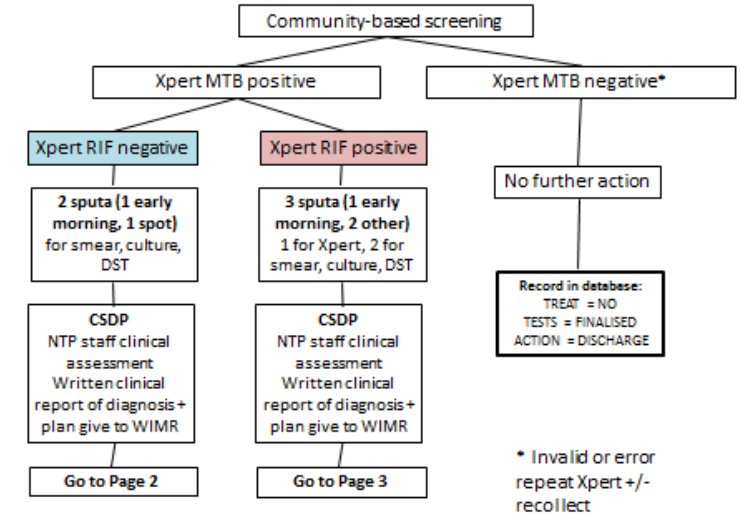


Chart 2

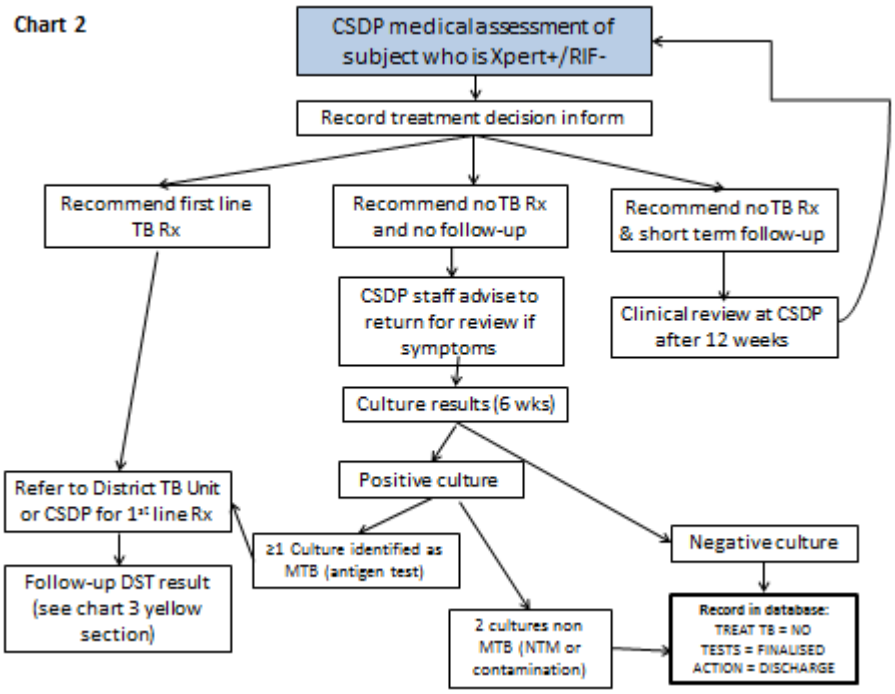
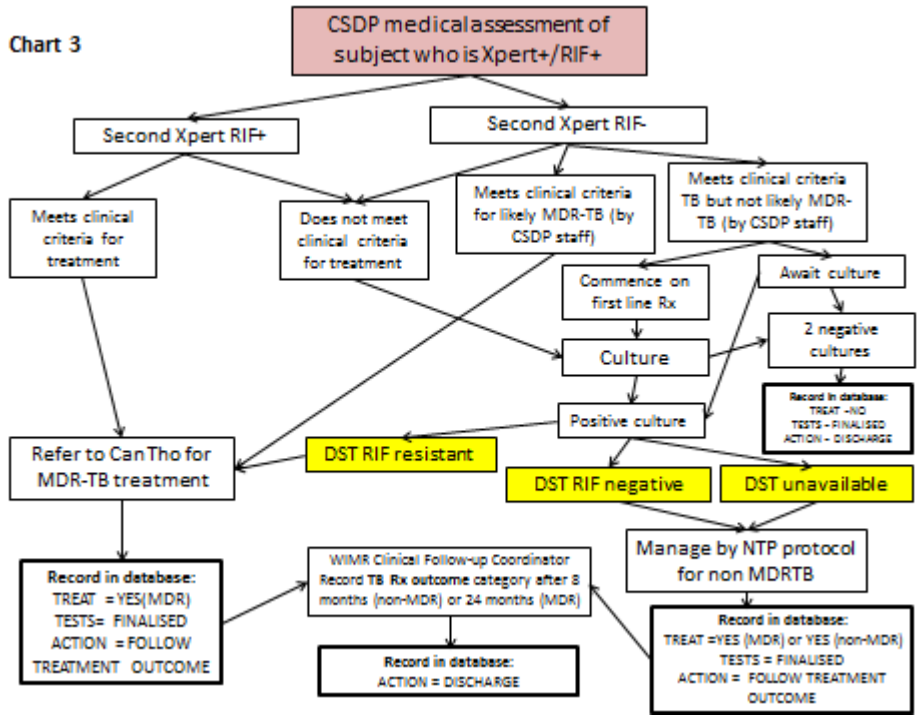


Chart 3



Instructions for Woolcock staff after a positive Xpert MTB result

Step 1: Notification of subjects and collection of sputum

- If a subject screened during the ACT3 study has a sputum Xpert that is positive for MTB, the following steps will be performed:
  - Laboratory staff will inform: sample ID, PID, Ap where patient lives via e-mail to: (i) Vice-team leader/Team Leader; (ii) Project Co-ordinator; (iii) Project Officer (clinical follow-up)
  - The Team leader or vice-team leader will:
    - Visit the household of the subject, and inform them of the Xpert MTB result (no RIF results will be notified)
    - Collect 2 follow-up sputum samples: spot and early morning sputum sample the next day
    - Ask them to attend the CSDP for further evaluation
    - Provide a map and written instructions, and discuss financial support available for travel and/or accommodation.
  - If the team has already finished screening in the Ap, the team leader or vice-team leader will:
    - Call subject and notify of them of the Xpert MTB result
    - Ask them to attend CSDP for further evaluation
    - Inform that 2 follow-up sputum samples will be collected at CSDP: early morning and spot

Step 2: Further investigations and interview of subjects at CSDP

- At the CSDP, the subject will go to the Interview room (room #7) to meet Project Assistant who will explain the plan for further investigations and interviews. The subject will complete the following at CSDP:

Step 2A. Interview room #7: The Project Assistant or Lab Coordinator will

- Return Xpert MTB paper-based test result to patients who have not received it
- Collect the first sputum sample in the sputum collection area(if not already done) and

- Label with subject name and QR Code
  Scan the QR code into the Mobenzi database
- Transport this specimen to the laboratory

- Collect bloods
  - Obtain consent for biomarker study
  - Collect routine bloods
    - **FBC (1 EDTA tube)**
    - **LFTs (1 clotted serum tube)/ Chức năng gan**
    - Biomarker bloods (1 Paxgene and 1 EDTA tube)
  - Apply QR codes to each collected tube
  - Transport routine FBC, LFT to CSDP lab
  - Transport biomarker bloods to WIMR lab
- Complete post-diagnosis interview (if not already done)
  - Explain the questionnaire and obtain written consent to complete the questionnaires
  - Administer post-diagnosis questionnaire
- Instruct patients to go
    - To Consultation room #9, followed by
    - CSDP reception (to pay and to keep the receipts), followed by
    - radiology (to have chest x-ray) and then
    - back to room #7 for reimbursement and further instructions
      .

Step 2B. Consultation room #9: CSDP doctors will conduct

- a health exam
- write medical test requests including: Posterior-anterior (PA) chest x-ray and blood tests (FBC, LFTs, HIV).
- send patient to CSDP reception with these forms to pay for the tests

Step 2C: CSDP-Reception: Patient will pay for the chest x-ray and blood tests and receive red receipts.

Step 2D: CSDP Radiology staff willdo the following

- perform aPA chest x-ray
- make a copy of the chest x-ray
- give both the original and the copy to the patient.
- patient then goes to back to CSDP lab

Step 2E. Patient returns to laboratory to collect written copy of blood test (FBC, LFT, HIV) results and takes these to Room #7,

Step 2F. Interview Room 7 (2^{nd} visit): The patient will return to room 7 with the two copies of the chest x-ray and the blood test results. Project Assistant will:

- Keep one copy of the chest x-ray and
  - apply QR code sticker to the x-ray
  - scan this into the relevant Mobenzi field.
  - label the chest x-ray with name, date and PID
  - take a photograph of the chest x-ray
  - upload the photograph of the chest x-ray to online repository (Dropbox or Amazon Cloud)
- Enter blood results into Mobenzi lab results form
  - Apply QR code to the written results form
  - Photograph the written results form
  - Upload the photograph of the written results to online repository (Dropbox or Amazon Cloud)
  - return written results to patient

  Reimburse the subject for
  - their travel costs and overnight accommodation costs (if required) according to the cost norms

- cost of chest x-ray and blood tests (in return for receipts).
- Collect the second sputum of subject in the sputum collection area(if not already done) and
  - labeled with subject name and QR Code
  - scan the QR code into the Mobenzi database
  - transport this specimen to the laboratory
- Administer the self-efficacy questionnaire

- Inform subject of next steps: Project Assistant will inform the subject of the follow-up plan. This will include advice about:
  - Any further follow-up appointments that are required
  - Inform subject of contact details of the Team Leader in case of any questions
  - Other information as required

Step 2G. Consultation Room #9 (2):

- CSDP doctor will conduct clinical assessment: The Project Assistant will take the subject to the room#9 for clinical assessment by CSDP staff. Project Assistant will provide the Clinical staff with:
  - Chest x-ray film
  - Written report of blood test results
  - Clinical Assessment form A1 (Visit 1), with the subject's PID and name recorded on the form and results of Xpert MTB and RIF tests
  - The CSDP doctors will complete this form and return it to ACT3 staff and place them in CSDP WIMR office.

  CSDP doctor will make treatment decision and refer ACT3 subject to appropriate clinic for treatment

Finally, at WIMR office, the Project Officer (Clinical Follow-up) will review all study documents, including the Clinical Assessment Form, and enter the following Clinical Assessment Form(A1) into the Mobenzi database:

Step 3: Follow-up of subjects with a positive culture

- If a subject has a positive Mtb culture result, this will appear on the Mobenzi Clinical summary. The Project Officer (Clinical follow-up) will review the Clinical Summary weekly and identify participants who have a positive Mtb culture who are not yet on treatment for TB.
- The Project Officer (Clinical Follow-up) or team leader
  - will invite these participants for a further clinical assessment at the CSDP
  - complete the first part of Form A2 (with the microbiology results)
  - give this form to the CSDP doctor to complete
- The CSDP doctor will be asked to complete Form A2 (culture positive clinical follow-up form) and the Project Officer will enter this form into the Mobenzi database.
- Project Office will reimburse costs of transport and accommodation as required.

Step 4: Follow-up of outcomes at end of treatment

- Nine months after the commencement of treatment (for non-MDR patients) and 24 months after the commencement of treatment (for MDR patients) the Project Officer will call to the place where TB treatment is being administered. This will be identified from the Clinical Summary (with an alert generated automatically at 9 months). The Project Officer will ask
  - For the final treatment routcome (according to NTP / ACT3 definitions above). This outcome will be recorded on the Mobenzi database.
  - A photograph of the treatment dispensing record. This photograph will be uploaded to the Amazon Cloud/Dropbox (with the PID).
- The Project Officer will use the information in the photograph to complete the Mobenzi 9month or 24 month follow-up form.

Instructions for CSDP clinical staff

Clinical assessment of subjects with positive sputum Xpert MTB results

- Subjects will attend the clinic with the following information:
  - Chest X-ray
  - Routine blood test results
  - Xpert (MTB and RIF) results (this will be written on form A1)
- CSDP staff will make a clinical assessment, and record the findings of this assessment, and a clinical decision, on the Clinical Assessment Form A1 (which will be pre-completed with patient's name and PID.
- The subject should be referred to the appropriate treatment provider, according to routine clinical practice of the National TB Program.
- If the CSDP staff have any questions about the test results, they can discuss this will the Project Officer (clinical follow-up) who will seek advice from the Medical and Laboratory Consultant
- The CSDP staff will then inform the subject about any follow-up plans.
- At the completion of the assessment, the CSDP staff will submit the Clinical Assessment form to an ACT3 staff member

Instructions for staff at culture laboratory (Can Tho)

Step 1: Receipt and testing of specimens

- Open sample receipt page on Mobenzi database and scan the QR code to record receipt of the specimen at the Can Tho lab. This step will be performed by ACT2 staff in Can Tho on Tuesday and Friday each week.
- Place the forms with QR codes in the ACT3 study folder
- Record the date and time of receipt of the samples on the form with QR codes.
- Record all samples in the Department of Microbiology register books, according to usual laboratory practice
- Perform MGIT testing and sputum smear on each sample according to standard procedures. Apply QR code to MGIT tubes.
- All positive cultures will be tested with antigen kit, according to procedures described in a separate flowchart

Step 2: Record results of culture and informing Project Officer of positive results

- Record in paper record: Record the results of the culture in the ACT3 Laboratory Book. Negative results should also be recorded in the book.
- Enter MGIT culture results into Mobenzi database. This step will be performed by ACT2 staff in Can Tho on Tuesday and Friday each week.

Step 3: Test positive cultures for MTB using antigen kit

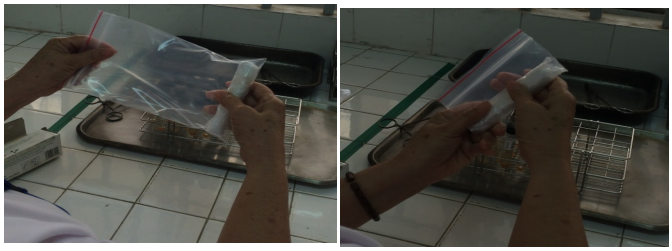
- All positive cultures should be tested using an antigen detection kit for M. tuberculosis.
- The Ag test result should be recorded in the ACT3 Laboratory Book
- Enter TB Ag test results into Mobenzi database. This step will be performed by ACT2 staff in Can Tho on Tuesday and Friday each week.
- All samples (including each sample for subjects where 2 cultures are positive) should be tested BEFORE being sent to NIHE..
- Write the antigen kit test result on the Form with the samples to be sent to the DST Laboratory (either positive, negative or not done).

Step 3: Send samples to NIHE

- Until cultures are sent to NIHE, they should be stored at room temperature in their culture bottles (they should NOT be frozen or refrigerated).
- Positive cultures shoulý be batched and sent by post to NIHE, monthly. The tubes should be triple wrapped according to the following instructions:

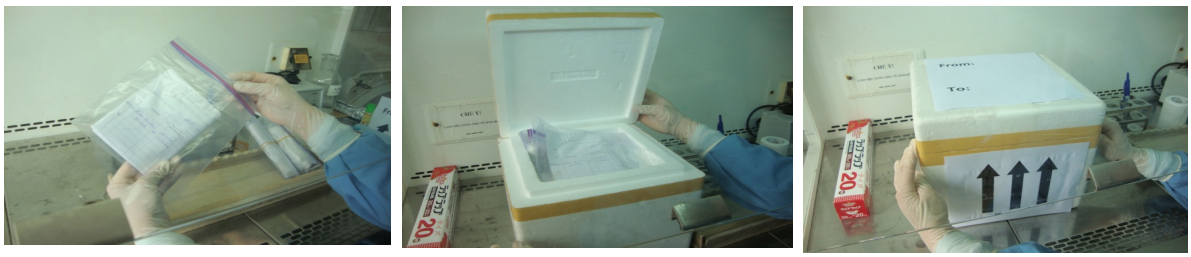
1. Wrapping the ***primary container*** with toilet paper, wrapping film/

2. Place into plastic ziplock bag, ***secondary packaging***



3. Place vertically into the ***tertiary /container***

4. Place the request-form, transportation-list into the tertiary container and close tightly, label on the box



Samples should be addressed to:

Dr: Nguyễn Thị Vân Anh (098 858 2678)

Phòng vi khuẩn lao (phòng 309),

Khoa vi khuẩn, Viện Vệ Sinh Dịch Tễ Trung Ương

Số 1-Yecxanh, Hai Bà Trưng, Hà Nội,

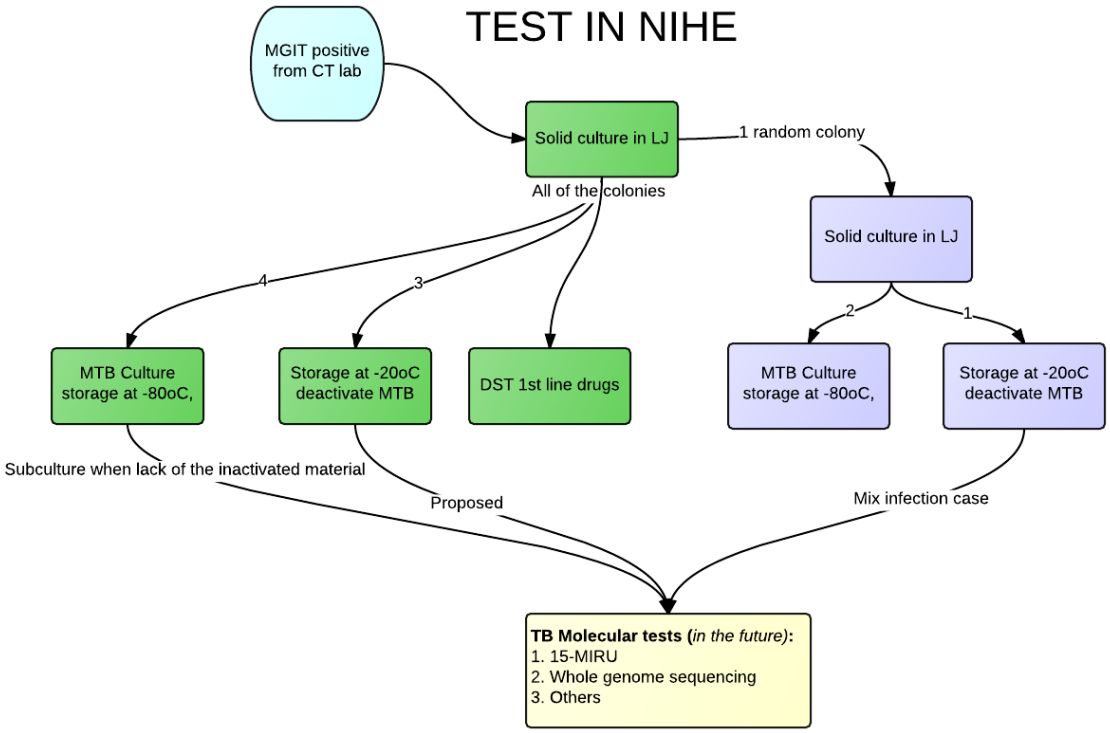
- After posting a sample, send an email (or SMS) to: (a) the DST Laboratory Coordinator (Van Anh Nguyen - vananhtdt@gmail.com) and (b) The Project Officer. This email should state the time and date of shipping and the number of samples sent.

Instructions for staff in DST and molecular testing laboratory

Step 1: Receipt and testing of specimens

- Open sample receipt page on Mobenzi database and scan the QR code to record receipt of the specimen at the NIHE lab
- Place the forms with QR codes in the ACT3 study folder.
- Record the date and time of receipt of the samples on the form with QR codes.

- Record all samples in the Department of Mycobacterial Disease register books, according to usual laboratory practice
- For each sample, perform the following steps:
    - Solid culture according to laboratory protocols
    - Phenotypic Drug susceptibility testing (DST) on a randomly selected colony from the solid culture medium
    - Store cultured isolates in a -80 degree freezer



Step 2: Record results of DST and inform Project Co-ordinator of MDR-TB positive results

- Enter DST results into the relevant form on the Mobenzi database when completed. This will trigger email notification of relevant WIMR and CSDP staff.
- Notify project coordinator by phone about MDR-TB results as soon as they are available.
- Any questions about the results can be discussed with the Medical and Laboratory Consultant.

Instructions for monitoring, evaluation and reporting (Project Officer, Clinical follow-up)

Daily tasks

Each day, the Project Officer will perform the following duties:

- Review the Mobenzi Clinical Summary website to find any new culture or DST results.
    - If new positive culture, where patient is not yet on treatment, then arrange an appointment for the patient for clinical review at CSDP.
    - If a new DST result that shows resistance to RIF, where the patient is not currently on MDR-TB treatment, contact the patient for clinical review at CSDP.
    - For each subject being followed up, review the follow-up status. Amend if required, according to laboratory and clinical information.
- Provide a list of subjects due to attend the CSDP to the Project assistant/laboratory staff at CSDP. This list should include the names of all subjects, and any instructions for when they arrive.

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- Meet with subjects arriving at the CSDP and perform the interview (24 hour follow-up interview). This may be delegated to other ACT3 staff.
- Check all Clinical Assessment forms from that day have been entered into the Mobenzi website, and the original forms stored in the office (WIMR office in CSDP) in a Clinical Assessment Folder.

Weekly tasks

- Each Friday evening, update the Mobenzi Clinical Summary
- Each Monday morning, send a copy of the Mobenzi Clinical Summary to the CSDP Medical Officer responsible for overseeing the CSDP Clinical reviews. This should include all subjects who may require clinical review during that week.
- Arrange any appointments for follow-up of subjects, based upon the culture and DST results.
- Meet with the Medical and Laboratory Consultant to discuss each patient that is being followed up
- Contact the ACT3 heads at each District TB Unit, or other treatment facilities, of any patient who has completed their treatment (at 9 months for non-MDRTB, and at 9, 18 and 24 months for MDR-TB). Document the current treatment outcome on the Mobenzi website.
- Ensure adequate copies of the Clinical Assessment Form are available
- Make digital copies of each chest Xray. Store each digital image in the folder on the Dropbox/Amazon Cloud. The file should be named according to the PID of the participant (e.g. 103442 – Xray 28 02 2014.jpg).
- Take all chest x-rays films to the Medical and Laboratory Consultant for reading.

Monthly tasks

- **Prepare and submit report to Investigators:**
  - On the last day of each month, check the completeness of the Clinical Summary on Mobenzi, and export the file to Excel.
  - Create a formatted Clinical Summary Report that includes all subjects with positive Xpert, either treated or untreated.
  - **The names of each individual must be remove before sending the report** (i.e. the data must be de-identified).
  - The report will go to: the NTP Director and other Chief Investigators, Head of the CSDP.
- **Send a progress report to the Director of the Can Tho National TB Program** (including % of cultures >8 weeks after collection that have been entered in the Mobenzi website). This can be sent by email and copied to the Medical and Laboratory Consultant.
- Check there is an adequate supply of antigen kits for the next 3 months, and order additional kits if necessary.

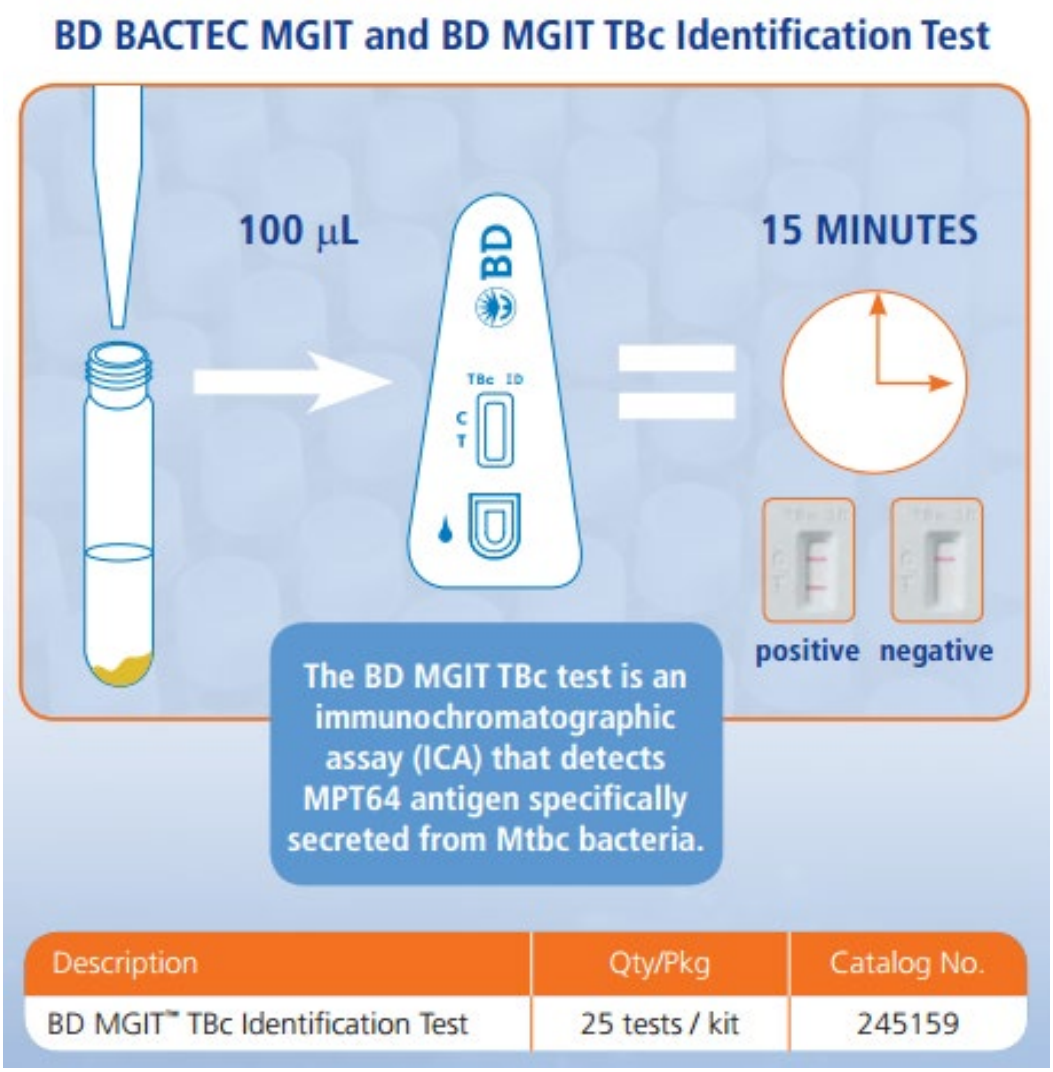
Quarterly tasks

- Check the financial request has been submitted from the Culture Laboratory to the ACT3 financial staff in Ca Mau.
- Meet with the Chief Investigators, Country Director, Trial Coordinator and Medical and Laboratory Consultant by Skype to discuss all subjects and any difficulties.

Appendix 1: Sample Clinical Summary Form (exported from Mobenzi)

DST results	FU Sputum 2
Specimen ID:	2000004
PID:	92208319
Ca Mau Received Date:	2014/04/08 14:46:32 PM
Result Lab Received Date:	2014/07/21 13:11:31 PM
DST Result:	Sensitive / Resistant H (isoniazid) ○ ○ R (rifampicin) ○ ○ Z (pyrazinamide) ○ ○ E (ethambutol) ○ ○
Comments:	
Date time resulted: ◉ Auto-record ○ Manual (back-capture)	(to be auto recorded on saving)
	Save Results »

Appendix 2: MTB antigen detection kit instructions



A1

ACT3 Clinical Assessment Form – Visit 1 Completed by ACT3 staff before interview

Key information for CSDP doctors [Completed from Mobenzi database]**:**
GeneXpert result 1: [ ] MTB detected, quantity _____ [ ] RIF resistance detected
Date of test: __/__/_____
GeneXpert result 2: [ ] Not necessary[ ] MTB detected, quantity _____ [ ] RIF resistance detected
Date of test: __/__/_____

Subject name:		
PID code		
Today's date (dd/mm/yyyy)		__ / __ / _____
Information to be completed by CSDP Doctors		
1.	Chest Xray result	☐1 = Consistent with tuberculosis ☐2 = Not tuberculosis ☐3 = Not available / not interpretable
2.	Was the patient started on TB treatment before today?	☐1 = Yes ☐2 = No ☐3 = Don't know
3.	Doctor's diagnosis (select one)	☐1 = Likely TB (low risk of MDR-TB) ☐2 = Likely TB (high risk of MDR-TB) ☐3 = Not TB ☐4 = Require further test results before diagnosis
4.	Treatment decision today (select one)	☐1 = Treat for TB (new TB regimen) ☐2 = Treat for TB (re-treatment regimen) ☐3 = Treat for TB (refer for MDR-TB treatment) ☐4 = Treat for TB (other)- specify:_____ ☐5 = No treatment required today, further review &/or test results necessary → go to Q7 ☐6 = No treatment required today, **no** further review necessary →go to Q9
5.	Subject agrees to treatment	☐1 = Yes ☐2 = No ☐3 = Not applicable
6.	Place where TB treatment will be provided during intensive phase	☐1 = CSDP ☐2 = District TB Unit → Specify:_____ ☐3 = Private facility → Specify: _____ ☐4 = Other (specify):_____
7.	Which further test results are required before a treatment decision will be made? (select one or more)	☐1 = No further test results pending ☐2 = Sputum smear ☐3 = Sputum culture ☐4= Drug susceptibility test ☐5 = GeneXpert ☐6 = Other (specify):
8.	Is another review needed at CSDP?	☐1 = Yes → if yes, in how many weeks? _____ weeks ☐2 = No ☐3 = Maybe / Don't know
9.	Full name of CSDP staff completing form	

Thank you for completing this form.
Please give this form to the patient to return to an ACT3 Staff member.

A2 ACT3 Clinical Assessment Form – Visit 2*

*** This form must be accompanied by the completed A1 ACT3 Clinical Assessment Form - Visit 1**

Completed by ACT3 staff before interview

Key information for CSDP doctors [Completed from Mobenzi database]**:**

GeneXpert result 1: [ ] MTB detected, quantity _____ [ ] RIF resistance detected
Date of test: __/__/_____
GeneXpert result 2: [ ] MTB detected, quantity _____ [ ] RIF resistance detected
Date of test: __/__/_____
Culture result 1: [ ] Positive MTB [ ] Negative MTB [ ] Contamination
Date of test: __/__/__
Culture result 2: [ ] Not done [ ] Pending [ ] Positive MTB [ ] Negative MTB [ ] Contamination
Date of test: __/__/__
DST: [ ] Not done [ ] Pending [ ] INH resistant [ ] RIF resistant [ ] EMB resistant
Date of test: __/__/___

Subject name:	
PID code	
Today's date (dd/mm/yyyy)	__ / __ / _____

Information to be completed by CSDP Doctors

1.	Has a repeat CXR been performed since visit 1	☐1 = Yes ☐2 = No 3 = Not sure
2.	If Yes Q1: 2nd Chest Xray result	☐1 = Consistent with tuberculosis ☐2 = Not tuberculosis ☐3 = Not available / not interpretable
3.	Doctor's diagnosis (select one)	☐1 = Likely TB (low risk of MDR-TB) ☐2 = Likely TB (high risk of MDR-TB) ☐3 = Not TB ☐4 = Require further test results before diagnosis
4.	Treatment decision today (select one)	☐1 = Treat for TB (new TB regimen) ☐2 = Treat for TB (re-treatment regimen) ☐3 = Treat for TB (refer for MDR-TB treatment) ☐4 = Treat for TB (other)- specify:_____ ☐5 = No treatment required today, further review &/or test results necessary → go to Q7 ☐6 = No treatment required today, **no** further review necessary →go to Q9
5.	Subject agrees to treatment	☐1 = Yes ☐2 = No ☐3 = Not applicable
6.	Place where TB treatment will be provided	☐1 = CSDP ☐2 = District TB Unit → Specify:_____ ☐3 = Private facility → Specify: _____ ☐4 = Other (specify):_____
7.	Which further test results are required before a treatment decision will be made? (select one or more)	☐1 = No further test results pending ☐2 = Sputum smear ☐3 = Sputum culture ☐4= Drug susceptibility test ☐5 = GeneXpert ☐6 = Other (specify):
8.	Is another review needed at CSDP?	☐1 = Yes → if yes, in how many weeks? _____ weeks ☐2 = No ☐3 = Maybe / Don't know
9.	Full name of CSDP staff completing form	

Thank you for completing this form. Please give this form to the patient to return to an ACT3 Staff member.

ACT3

LAB MANUAL

Contents

1. Laboratory safety

- Authorized staff only should be permitted to enter the laboratory. Study participants must not be given sputum production instructions inside the laboratory. If this is necessary, this should be performed in the CSDP's specific room (near TB examination room).
- No smoking, drinking or eating in the lab.
- No storing of food or drink in the laboratory
- No storing of personal items (bag, hat, clothes…) in the lab
- Long-sleeved laboratory coat long pants, and gloves that overlap the wrist band on the laboratory coat shall be worn when handling laboratory specimens
- Footwear should cover the toes and have a heel strap
- Watches and jewellery should be removed before entering the laboratory
- Careful decontamination of equipment and material before using (e.g. inside the sample processing cabinet, GeneXpert system etc)
- Wash hands with soap before and after working with samples and before leaving the laboratory
- Handle sputum specimens inside the cabinet: Opening/unpacking triple packaging, sputum processing etc
- Understand the Material Safety Data Sheet – MSDS
- Keep chemical containers and materials off the floor
- Discard chemicals and specimens in accordance with safety regulations
- At the end of each working session in the cabinet, wipe down cabinet with 70% alcohol
- Wipe down bench surfaces with 70% alcohol at the end of each working day
- Use mop with **0.1**% hypochlorite solution to **clean** the floor everyday; Don't leave the floor wet
- In cases of burns with acid or base, wash with cold water. In case of severe burns, apply first aid to the wound and go to the nearest health centre.
- Check electricity and water before leaving the lab. Set air conditioner at 27°C overnight.

2. Handling safety in the lab

At the start of the day

- Open the door, window (if possible), turn off AC (which is on overnight), turn on the ventilator
- Throw away garbage bags and replace with a new one (yellow bag for infected material; blue bag for normal garbage)
- Use mop with **0.1**% hypochlorite solution to **clean** the **floor**.
- After 30 minutes, close the door, window and turn on the air conditioner
- Check the electricity in the UPS and turn on GeneXpert system

When working in the Cabinet

- Turn on the light and Vortex
- Do not turn on the Laminar Flow Cabinet fan
- Unpack the screening sputum samples; Complete Form #1 "GeneXpert MTB/RIF register book"
- Process sputum following the GeneXpert MTB/RIFSOP
- Triple package the follow-up sputum (if needed)

When finishing for the day

- Pack away material in the cabinet; Disinfect the surface/inside cabinet and vortex using 70% alcohol
- Pack away material on the bench and decontaminate surface by 70% alcohol
- Wash all of the trays
- Adjust air conditioner to 27°C for overnight running
- Dispose of the garbage

Garbage

- The garbage can lined with a yellow bag should be used for infected material e.g. triple packaging material, sputum specimen containers, pipettes, paper towel and cartridges.
- The garbage can lined with a blue bag should be used for normal garbage.
- Dispose of the garbage in CSDP's designated disposal sites.

3. Handling broken/leaking sputum containers or spillage of sputum

Spill kit contents: gloves, paper towels, cotton, 70% alcohol. Spill kit should be contained in 1 bag and in an easy to reach place.

- In cases where the laboratory receives a broken or leaking sputum container
  - Sputum containers that are broken/cracked or in cases where the majority of the sputum has leaked outside the container, these specimens should not be processed. If it is still inside the plastic bag, do not remove from the bag but discard the entire sample in the biohazard waste container, after noting down study participant details.
  - The team leader should be notified for appropriate investigation and sputum recollect if possible
  - If the sputum container is leaking slightly, decontaminate the outside of the container with paper towel soaked with NaClO (Javen). If there is enough remaining sputum carefully open the container and continue sputum processing.
- In cases where sputum spillage occurs in the laboratory
  - All staff to leave the laboratory area immediately
  - Paper towel soaked with NaClO (Javen). should be placed over the spillage area
  - Add additional disinfectant starting from the outside of the paper towel and move inwards to the centre: leave for at least 15mins.
  - Place contents into a yellow plastic bag
  - Wipe the area clean with more soaked paper towel prior to wiping the area dry. Place paper towels into the yellow plastic bag and then seal the bag
  - In cases where spillage of GeneXpert cartridge solution containing sputum occurs, these spills should be treated similarly to above (although technically the reagent should deactivate most live microorganisms)
  - Dispose of the sputum container and waste in the appropriate biohazard waste container
  - Advise lab coordinator immediately of spill incident
  - The team leader should be notified for sputum recollect if possible
- Adverse report and lab-record book: Date, name and detail description Report to the responsible person (Ca Mau chief of office and trial coordinator)

4. Receiving sputum specimens in the LAB

Receiving: The lab staff must check all specimens that arrive in the lab to ensure they have arrived in safe conditions and are appropriate for testing

Rejection criteria:

1. Specimen has leaked out of container or container is broken
2. Insufficient (<0.5ml) specimen
3. Non-standard specimen container
4. Patient name on request form does not match that on the specimen container

Requirements: PPE must be worn when handling specimens .

a) Transportation forms will be kept in a separated bag to the sputum specimens. The specimens arriving in the lab must be counted and signed in, compared with the number of specimens noted on the transportation form and recorded in the "Screening sputum Receiving Book".

Email: Total number of specimens received will be sent to the Vice-team leader/Team leader and cc phong.trannhu@sydney.edu.au&phuong.nguyen@sydney.edu.au

b) Storing all of sputum specimen into refrigerator with note received date & cluster name (Ap-name) and in the order First-come first-served.

c) Unpacking: If leakage found –discard the entire specimen into the bin lined with a yellow bag; if the specimen is intact - unpacking the layers. Place the sputum cup vertically into the tray.

- Sputum specimens will be registered on Form #1"GeneXpert MTB/RIF register book".
- Check/estimate sputum volume and note in the Mobenzi database. If < 0.5 ml do not test with GeneXpert; Discard the sample; Stick QRcode on Form # 1 and write "not tested = NT"; because the quantity is not enough.
- Assess sputum quality using the colour chart - Score 1-6 (see below), and enter into Mobenzi database. Test all sputum samples regardless of colour.

Step 1: Sputum samples were collected in a cup

Step 2: Slide the **colorimetric scale** under the cup thereby allowing the approximate colour identification.

Step 3: Each colour is coded with a number, which can later be compared with other sputum samples.

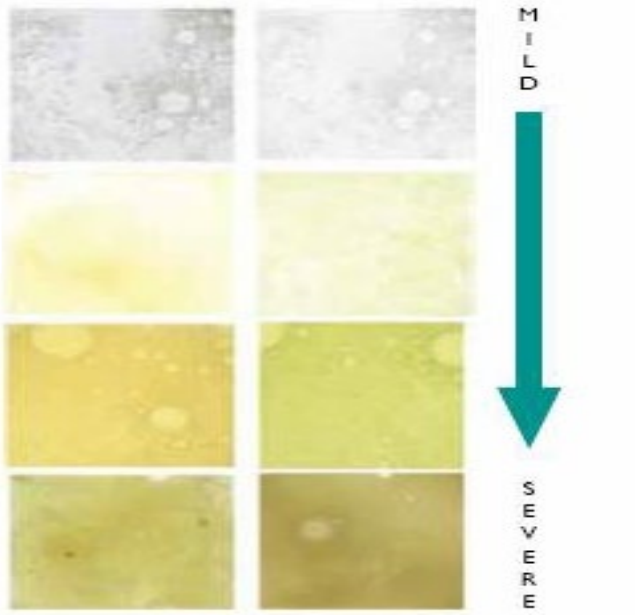
Step 4: Specimen with white or gray preponderance (number 0 and 1, respectively) were considered as mucoid, whereas shades of yellow, green, and brown were considered as purulent (#2-4). Pink or reddish colours were designated blood-stained (#5). The **colour of the sputum** when coughing up can be described.

1.Mucoid: mainly mucus (clear, white or grey; thin, frothy)

2.Muco-purulent: a combination of mucus and pus (yellow, green or brown)

3. Purulent: mainly pus (yellow, green or brown; thick, viscid, offensive odor (pus)

4. Prune-juice: dark brown, muco-purulent, offensive odor



5. Blood stained: tinged with pink or red
6. Saliva

d) Discard all of the packing material in the bin lined with a yellow bag. The transportation box (inside & outside) should be decontaminated by hypochlorite follow by70% alcohol. If a specimen has contaminated the inside of the box, wipe down with hypochlorite and leave for 15 minutes before wiping down with 70% alcohol

7. GeneXpert MTB/RIF Procedure

a) Sample preparation

Before processing the sample, CHECK:

- If leakage found on specimen container - clean the container with 1% hypochlorite and leave for 15 minutes (don't lose information on label)

- If tobacco or food particles are present - proceed but be careful not to add food or tobacco particles to the Xpert cartridge.

- If less than 0.5 ml on visual estimation - request a second specimen.

- If more than 4ml - split the specimen and select 2ml of the good part of the sample.

1. Enter the sputum specimen into the laboratory log book/computer system (Form #1)
2. Open the lid of the collection container and pour **SR buffer** (supplied in Xpert kit) to the sputum specimen in a 2:1 (SR buffer: specimen) volume ratio
3. Close the lid of the container tightly and vortex vigorously in 30" then stand for 5-7 minutes. (One back and forth movement is a single shake) After 5-7 min, vortex again once and then stand for 8-10 min.
4. Allow the mixed specimen to stand for 15 minutes.

Note:

- The sample: SR buffer mixture can be stored in the refrigerator (2-8°C) for up to 8 hours should the test need to be repeated

- Unprocessed sputum specimens (without SR buffer) can be stored at 2-8°C for 10 days and at 35°C for maximum of 3 days!

- Specimens should be maintained at 2–8°C whenever possible including during transportation to the laboratory

b) Loading the specimen into the Xpert Cartridge

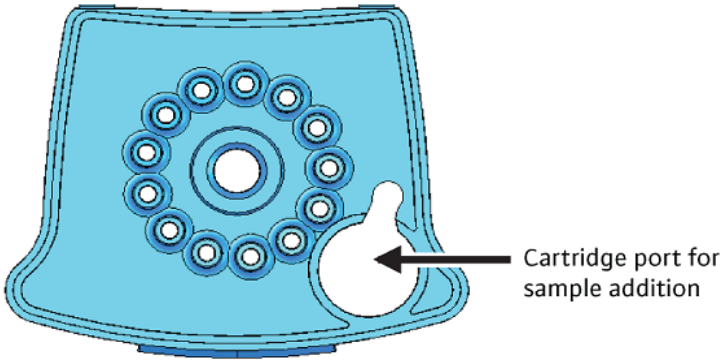
1. After 15 minutes of addition of the SR buffer, remove an MTB/RIF Xpert cartridge from its wrapper.

Note:

- Do not touch
    - centre part of the top of the cartridge
    - barcode area
    - detection probe

- Do not use a cartridge that has been opened for more than 30min
- Do not re-use a processed cartridge.
- Do not refrigerate a cartridge once sample has been loaded.

2. Stick the sample ID (QRcode) on the side of the Xpert cartridge. **Do not write on the barcode or the lid.**
3. Use the sterile pipette provided in the cartridge kit to aspirate the liquefied sample until the liquefied sample mark is above the minimum mark. Do not process further if there is insufficient volume i.e. 2ml. You can put more than 2 ml in the cartridge.
4. Ensure that the liquefied sample being transferred to the cartridge has no bubbles as this may cause an error.
5. Ensure the sample is absolutely liquefied after 15 minutes (no viscosity). If the sample is still viscous after 15 minutes, please shake and stand for 15 more minutes.
6. Open the cartridge lid being very careful only to touch the side of the lid, never the central area
7. Transfer the processed sample into the sample port of Xpert MTB/RIF cartridge. Place the tip of the pipette against the inner wall of the sample port and slowly expel the contents of the pipette
8. Close the cartridge lid tightly.
9. Dispose of the specimen collection container; pipette and leftover SR buffer into a suitable biohazard waste bin **(Do not use the left over SR buffer for another specimen).**
10. Take the Xpert cartridge to the bench with the GeneXpert instrument.



Note

- After opening the foil of the cartridge - must add the specimen to the cartridge within 30 minutes
- After addition of the specimen to the cartridge - must load the cartridge into the Xpert instrument within 30 minutes

- The sample:SR buffer mixture can be stored in the refrigerator (2-8^{0}C) for up to 8 hours should the test need to be repeated

c) Running the assay in GeneXpert

In the first running of the day: Switch on the GeneXpert at the back of the instrument; Switch on the computer.

1. Enter your windows password.
2. Double click **'GeneXpert'** icon on desktop.
3. Log onto the GeneXpert software using your username and password.
4. When asked whether or not you would like to **'Perform Database Management Tasks'**, such as backing up the database, checking the database integrity etc., choose '**Yes**' or '**No**'. A database backup should be performed once a week.

   4a) If you selected '**Yes**', see SECTION C under MAINTENANCE – Database Maintenance

   4b) If you selected '**No**', you will be prompted again when you close the GeneXpert Software
5. Click on '**Create test'** on the GeneXpert system toolbar.
6. The dialog box '**Scan Sample ID QRcode"** appears and then the dialog box & **"Scan Sample ID Barcode'** appears. Scan in the QRcode & barcode by placing the X of the barcode scanner in line with the code and hold until it beeps. Once the all the codes are entered, select '**OK**'.
7. The software automatically fills in the **Reagent lot ID, Cartridge SN**, and **expiration date**, as well as **Select Assay**, **Assay version number**, **Test Type** and **Sample type**.
8. Click on **'Start Test'**
9. A dialog box will appear, enter your password again and press **ENTER**.
10. A green light will start flashing above the empty module. Open the instrument module door with the blinking light and load the cartridge. The cartridge is seated into the machine with the detection probe facing into the machine (the barcode must be facing out of the machine).
11. Close the module door gently but firmly.

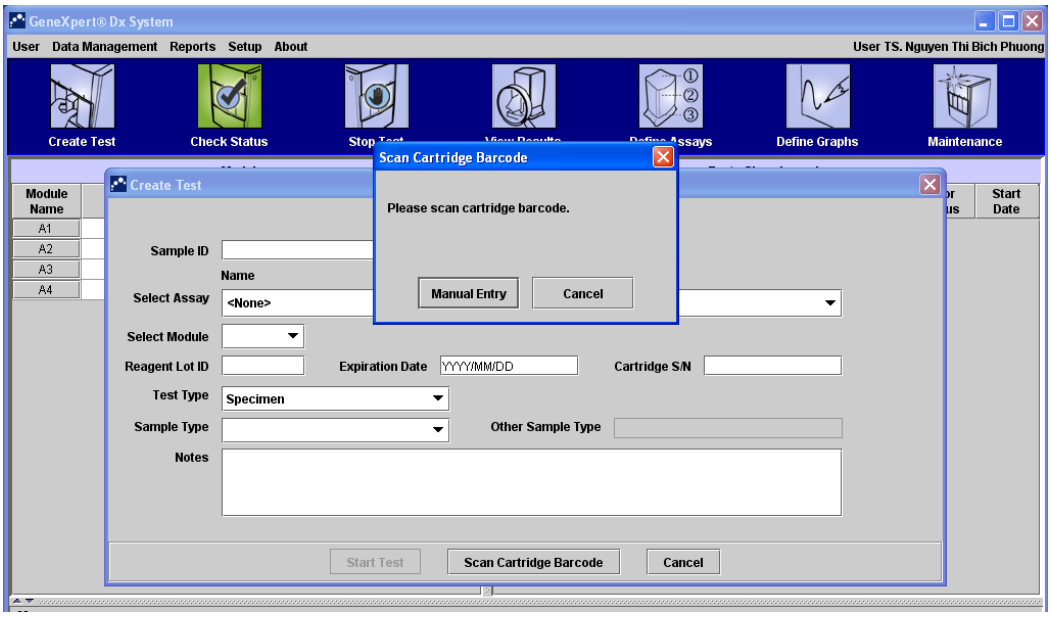


Figure 7.1
Create Test window and Scan Sample ID Barcode dialog box

12. After a few seconds, the green light will stop blinking means that the test has started.
13. Check the computer screen that the test has started and confirm the timer countdown has begun
14. After completion of the run, the green light will switch off and the module door will open automatically
15. Remove the cartridge and dispose into a suitable biohazard medical waste bin (yellow bag).

d) Interpretation of results

1. After completion of the run, click on the '**View Results'** icon on the system toolbar.

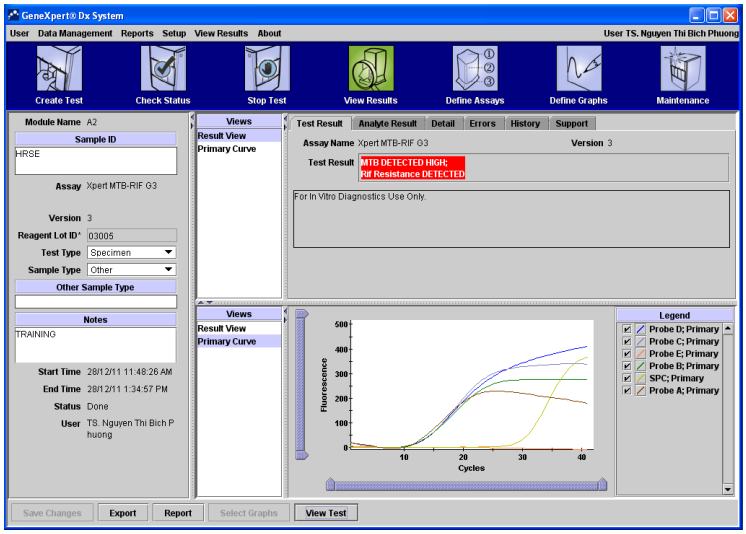


Figure 7.2
View Results window

2. Click on '**View Test'** at bottom of the result screen toolbar.
3. Select the patient test by clicking on the patient ID field. This will highlight the test.
4. Click '**OK'** and the result screen will be displayed as one of the following:

MTB Detected

MTB DETECTED: MTB target DNA is detected. The MTB result will be displayed as High, Medium, Low or Very Low

Probe Check—PASS: all probe check results pass.

MTB Detected & Rif Resistance DETECTED

A mutation in the rpoB gene has been detected that falls within the valid delta Ct setting.

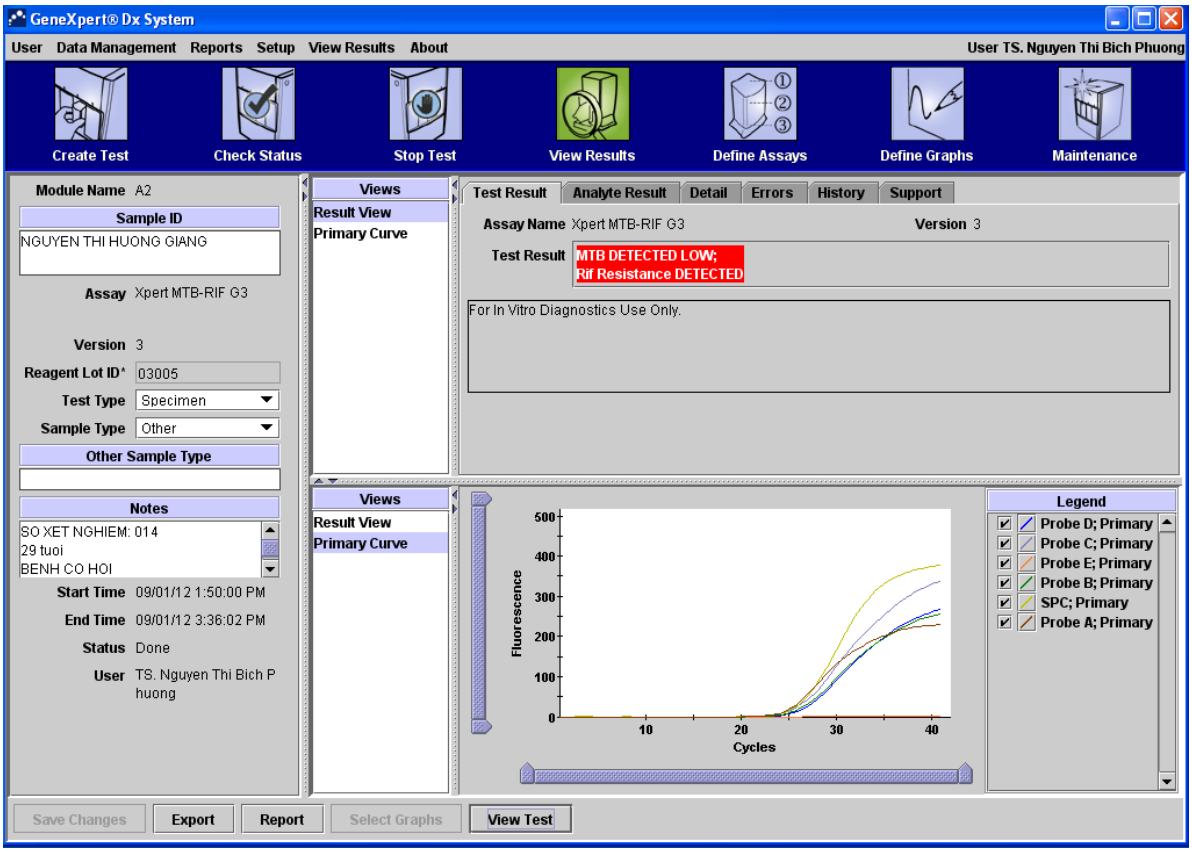


Figure 7.3MTB detected Rif resistance detected

*MTB Detected &*Rif Resistance NOT DETECTED

Rif Resistance NOT DETECTED: no mutation in the rpoB gene has been detected.

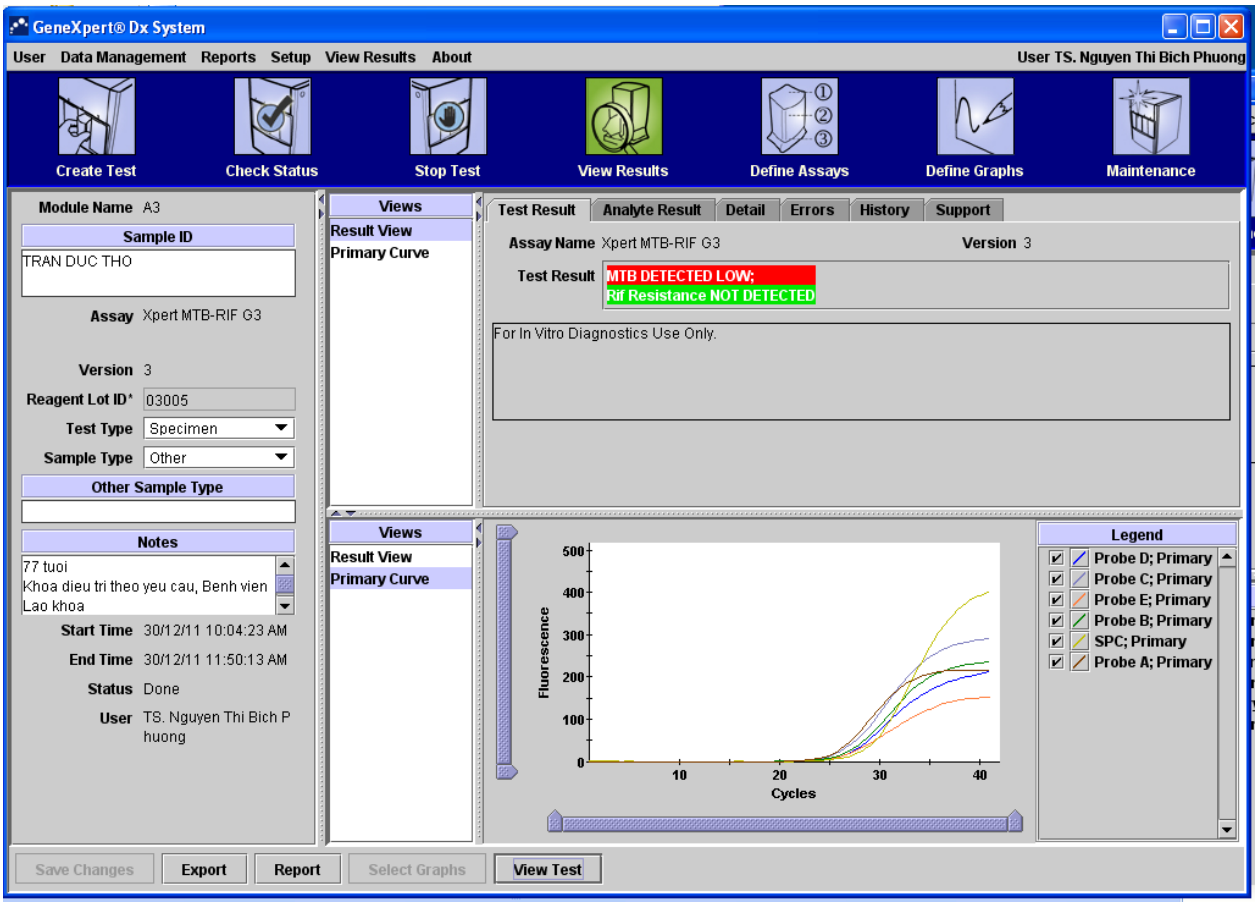


Figure7.4 MTB detected Rif resistance NOT detected

MTB Detected &Rif Resistance INDETERMINATE

Rif Resistance INDETERMINATE: the amount of MTB in the sample was very low and resistance could not be determined.

MTB Not Detected

MTB NOT DETECTED: MTB target DNA is not detected

SPC- Pass: SPC has a Ct valid range and endpoint above the endpoint minimum setting.

• **Probe Check-PASS:** all probe check results pass.

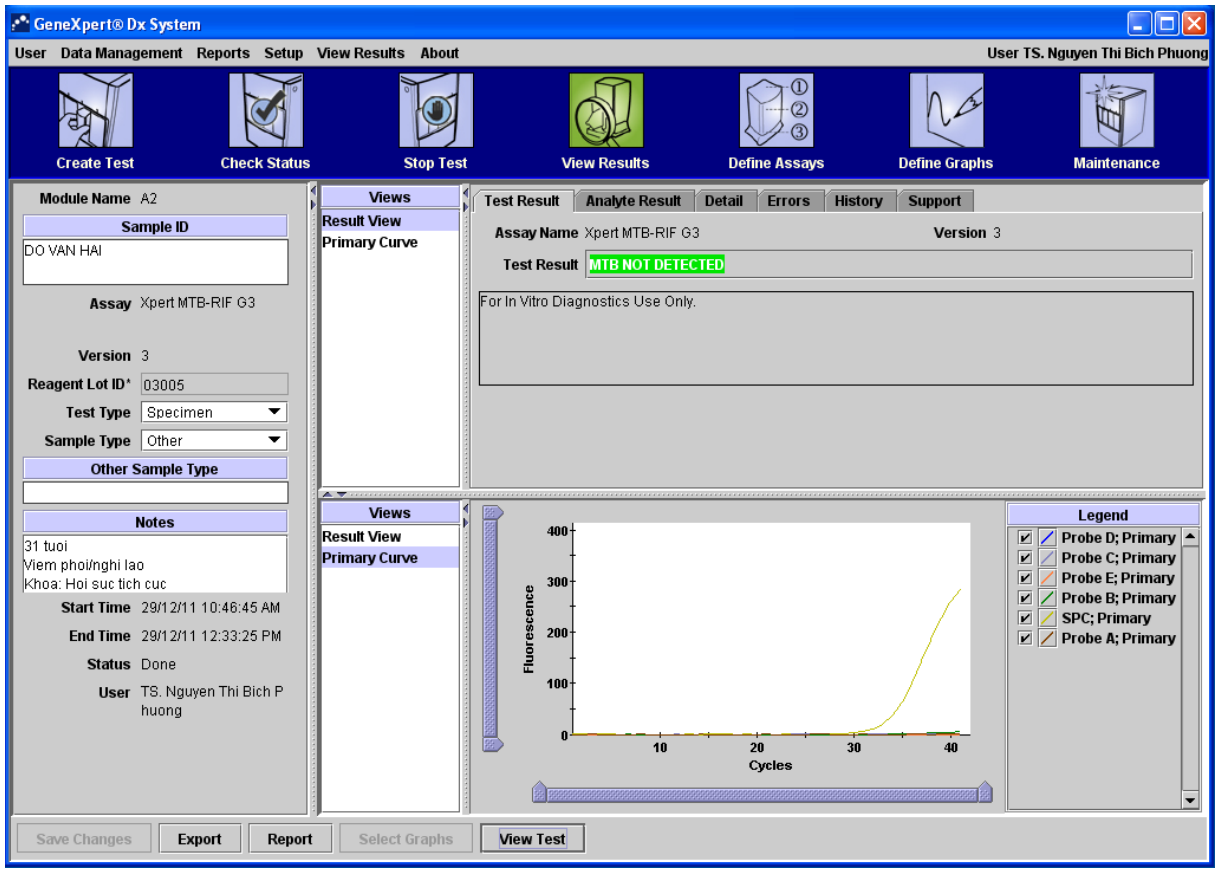


Figure 7.5 MTB NOT detected

UNINTERPRETABLE

INVALID

Presence or absence of MTB cannot be determined, repeat test with extra specimen. SPC does not meet acceptance criteria, the sample was not properly processed, or PCR is inhibited.

MTB INVALID: Presence or absence of MTB DNA cannot be determined.

SPC-FAIL: MTB target result is negative and the SPC Ct is not within valid range.

Probe Check-PASS: all probe check results pass.

ERROR

MTB-NO RESULT

SPC-NO RESULT

Probe Check-FAIL/PASS: One or more of the probe check results fail/All probe check PASS

NO RESULT

MTB-NO RESULT

SPC-NO RESULT

Probe Check-NA (not applicable)

A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress

e) Generating Test Result Reports

1. On the result screen, click on '**Report**' at the bottom of the screen.
2. On the test report window, select the result you want to view by ticking the box next to the **sample ID** and then click on '**OK**'.
3. Click on '**Preview PDF'** at the bottom of the test report screen.

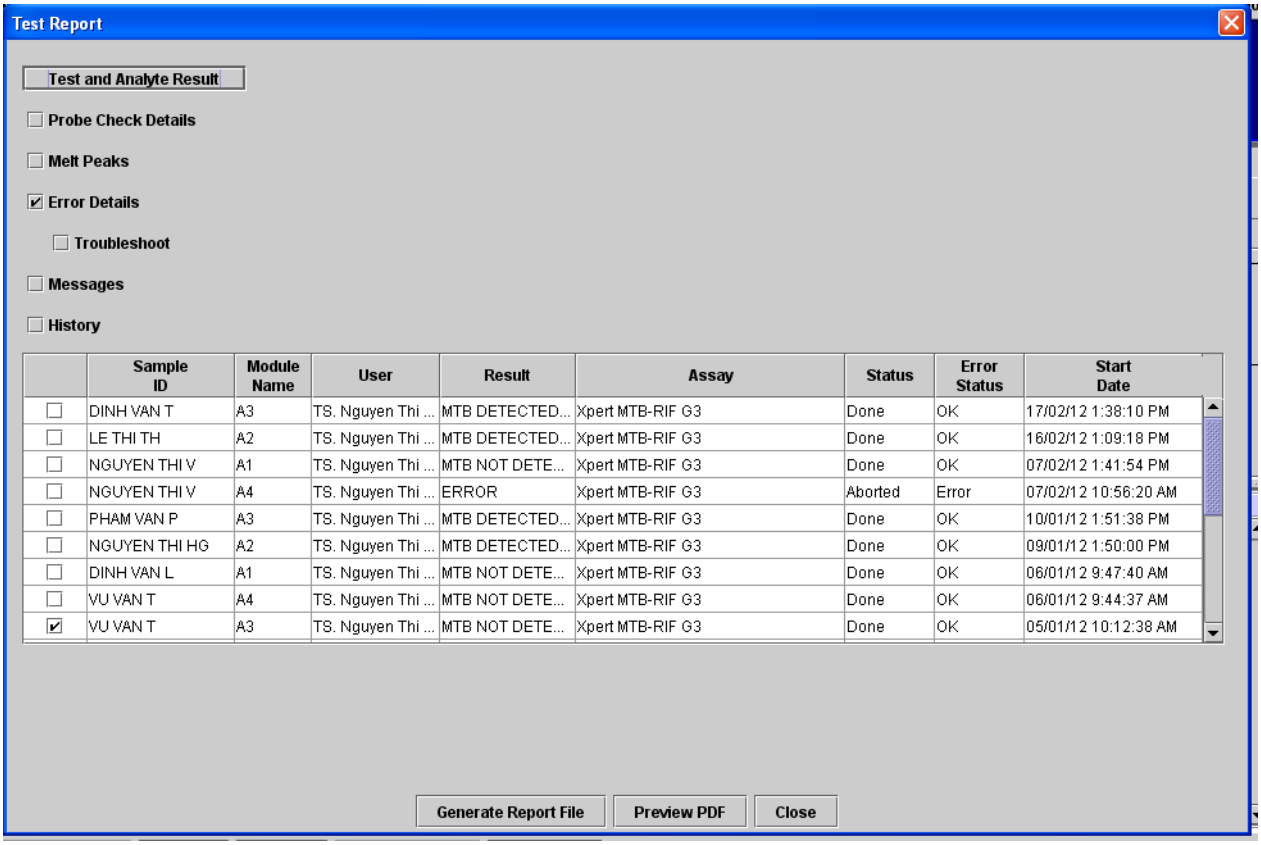


Figure 7.6 The Test Report dialog box

4. A report will be generated in PDF format. Click on '**Save a copy'.**
5. Double click on **Results** folder to open it.
6. Click '**Save'.**
7. Close the report window by clicking on the '**X'** on the top right hand corner of the toolbar and again on the next test report window.

Result maintenance

Archiving the tests (weekly)

1. Test data should be **archived** on weekly basis. This will help to keep the GeneXpert software efficient.
2. In the GeneXpert software, click on the **Database** menu.
3. On the dropdown list, click on **Archive test**.
4. An **archive test** dialog box appears. Highlight the tests you want to archive and proceed

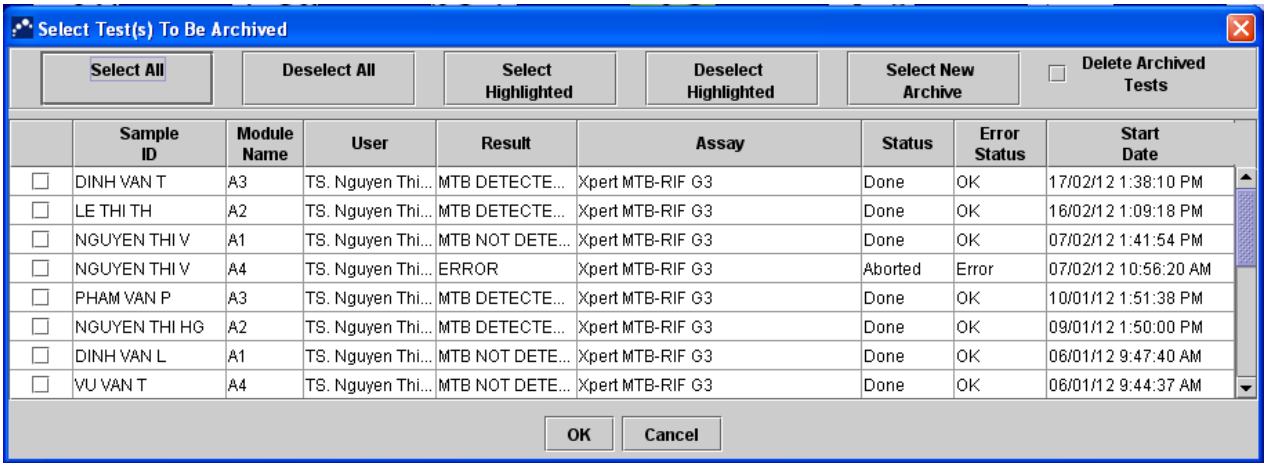


Figure 7.7 The Select test(s) to be Archived dialog box

Backup results

1. Save all PDF result reports to two USB memory stick/RW-CD weekly (monthly back-up by support laboratory).
2. Store the USB stick/CD's in two safe places: in Hoa Lu office & CSDP office.

f) Database maintenance

Click on **Database Management Tasks**, you will found these two options: **Database Backup, Database Restore, Compact Database, and Check Database Integrity**. Choose and "**Click**", you wish to perform

Database Backup

1. Select '**Database Backup'** and click '**Proceed'**. This should be performed weekly.
2. A window will appear asking you to change the backup location. The default location is C:\GeneXpert\Backup, **please do not change this default location.**
3. The file name should be automatically filled in with the backup details. **Please do not change this file name**.

4. Click '**Save'** to backup the database.
5. Again a window will display showing the status of the backup.
6. Once completed, a '**Backup Completed Dialog'** will appear showing that the database has been backed up successfully.

Database Restore

Only select this option under the direct instruction of **local service provider**. **Do not select this option, otherwise**, the current database will be **erased and all data lost** and replaced with older data! This option must **NOT** be selected unless a recent database backup has been performed

Compact Database

1. Select '**Compact Database'**
2. A dialog box will appear, select '**Cancel'** to return to the previous menu or '**Proceed'** to continue
3. A dialog box showing the status will appear
4. Once completed, a dialog box showing '**Compact Database Completed'** will appear.
5. Select '**OK'** to return to the menu.

Check Database Integrity

1. Select '**Check Database Integrity'** and click '**Proceed'.**
2. A dialog box will appear asking you to confirm this action. Select '**Proceed'** if you would like to continue, or **'Cancel'** to return to the previous screen.
3. If '**Proceed**' was selected, a new dialog box will appear stating the status of the database. The default message should appear as "**Database Integrity Check completed. No issues Found**". If the message indicates that an issue was found, please contact technical support.
4. Click '**OK'** to remove the dialog box.

g. Dealing with GeneXpert system ERROR

Any issue/error related to GeneXpert system:

- Report directly to Camau Chief of office: TNPhongphong.trannhu@sydney.edu.auand Trial coordinatorphuong.nguyen@sydney.edu.au
- Record in the Lab-Dairy

Common Errors

Common error	Explanation	Corrective action
5006/5007/ 5008/5009	Incorrect reagent volume: Too Little or too viscous	Repeat test
2008/2009	Pressure error due to sample too Viscous or blocked filter	Sample inadequately liquefied. Use stored sample:SR buffer mix if 2ml remains. Bring to room temperature and check if liquefied. Vortex and wait 15 minutes. Add more SR buffer if available Repeat test
INVALID	SPC Control did not amplify	Repeat test from different sputum
NO RESULT	Manual stop or Power interruption	Repeat test
ERROR	Reagent/cartridge error	Repeat test
Barcode Reader Failure	NO BIP sound when you connect it to the computer	Connect the barcode reader USB to another USB port of the computer?
	Barcode reader failure	Check the Assay Definition file imported - Reconfiguration the barcode reader Try cleaning the barcode window with a swab wetted with 70% alcohol [DO NOT LOOK DIRECTLY INTO BARCODE LIGHT]

6. ACT3 Mobenzi database

a) Login

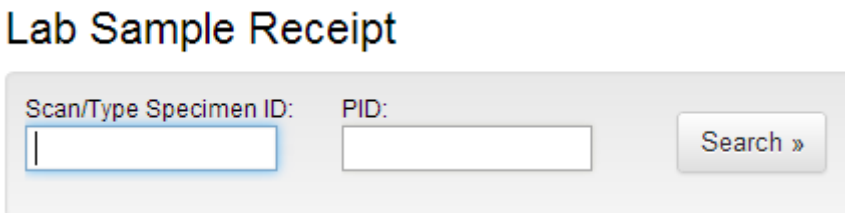
Open Mobenzi database on computer: https://outreach.mobenzi.com

Login:

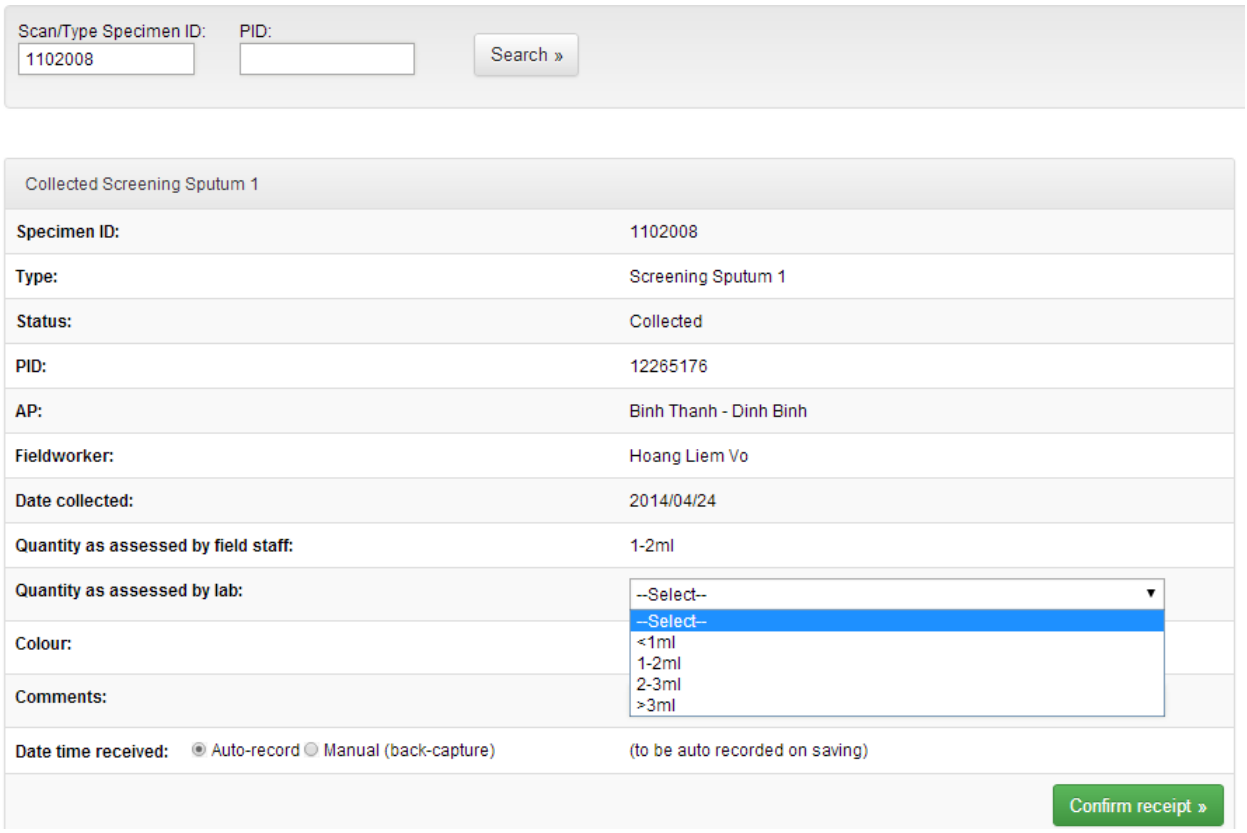
Pass:

b) Lab sample receipt

Scan/Type specimen (QRcode) on the Cartridge



In put data into the table below:



If PID & AP: N/A as table below:

- Check sample ID information when scanning
- Email notification to vice team leader/team leader cc phong.trannhu@sydney.edu.au,phuong.nguyen@sydney.edu.au

Lab Sample Receipt

Scan/Type Specimen ID: 1101271 PID: Search »

The Specimen ID entered has not yet been received by the system. Please confirm that the ID has been entered/scanned correctly before proceeding with receipt confirmation.

Not yet received Screening Sputum 1

Specimen ID:	1101271
Type:	Screening Sputum 1
Status:	Not registered
PID:	N/A
AP:	N/A
Fieldworker:	N/A
Date collected:	N/A
Quantity as assessed by field staff:	N/A
Quantity as assessed by lab:	--Select--
Colour:	--Select--
Comments:	
Date time received:	◉ Auto-record ○ Manual (back-capture) (to be auto recorded on saving)

Confirm receipt »

c) Lab samples in transit

Check “Lab Samples in Transit Report”at the completion of screening for eachAp.

- Email notification to vice team leader/team leader cc phong.trannhu@sydney.edu.au, phuong.nguyen@sydney.edu.au

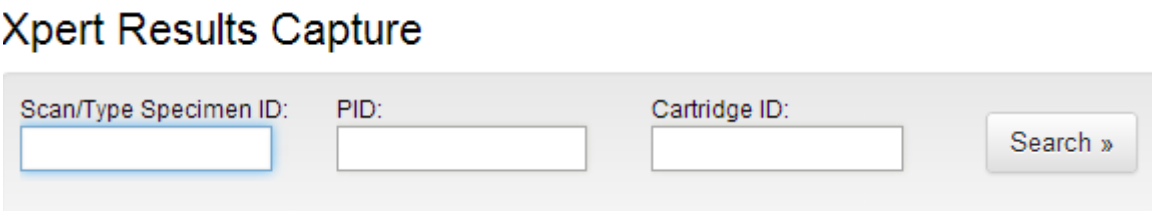
Lab Samples in Transit Report

District: All, CÀ MAU city, CÁI NƯỚC district — AP: All, Binh Thanh - Dinh Binh, Khom 3 - Phuong 4 — ☐ Include Uncollected — Search »

Participant ID	Specimen ID	Sample Type	Form Received	Days since Form Upload	Received At Lab	Days since Received At Lab
92258649	1101430Duplicate	Screening Sputum 1	Yes	9	No	-
12265237	1101918	Screening Sputum 1	Yes	4	No	-
12265298	1101919	Screening Sputum 1	Yes	4	No	-
12265730	1101920	Screening Sputum 1	Yes	4	No	-
52260996	1101642	Screening Sputum 1	Yes	4	No	-
12265028	1101921	Screening Sputum 1	Yes	3	No	-
12265005	1102053	Screening Sputum 1	Yes	3	No	-
12265113	1101922	Screening Sputum 1	Yes	3	No	-

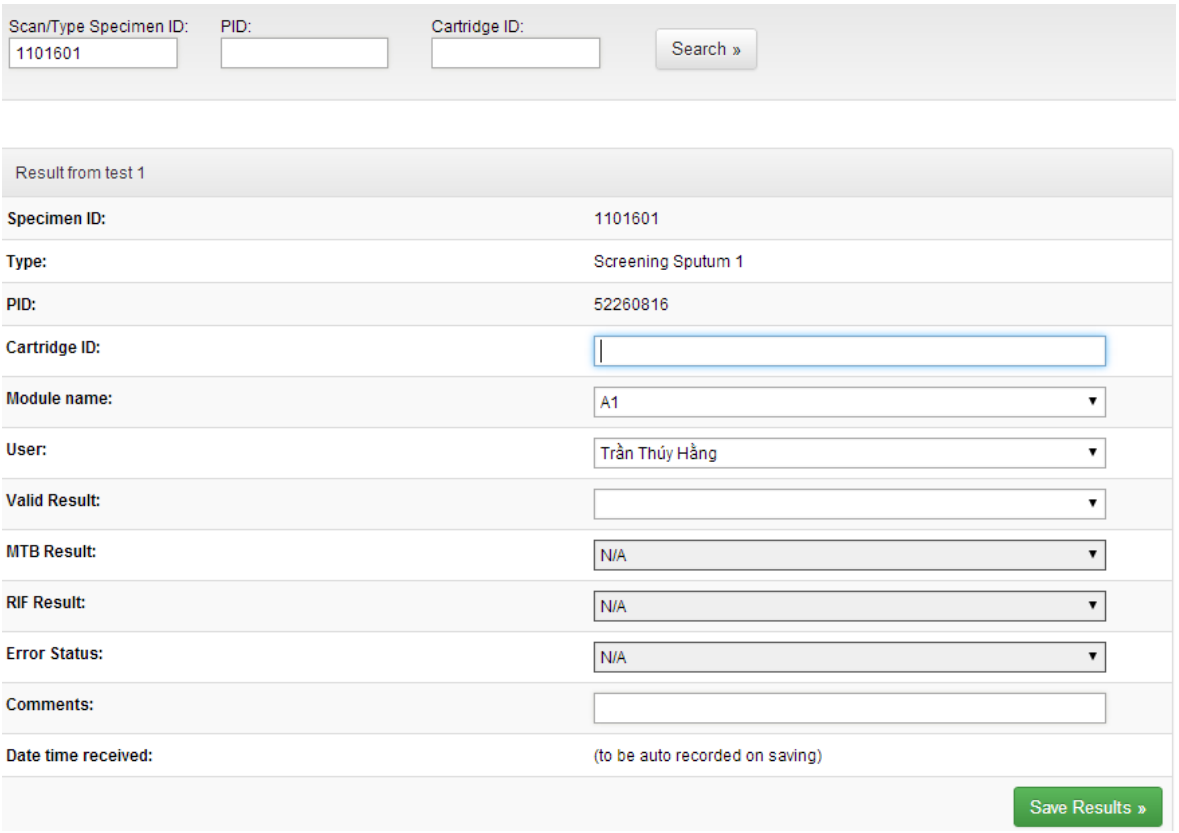
d) Xpert Results Capture

Scan/Type specimen ID (QRcode) on the Cartridge



- Scan Cartridge ID
- Select Module
- Valid Result: "Yes" → "MTB result"

  "No" → "Error status"



Note GeneXpert result in Mobenzi database:

Email notification to vice team leader/team leader cc phong.trannhu@sydney.edu.au, phuong.nguyen@sydney.edu.au for MTB detected, ERROR and INVALID results.

- MTB detected. Contact fieldworkers to collect a 2 more sputum samples (24h followup).
- Invalid result. Contact fieldworkers to collect a 1 more sputum sample in the cup with yellow lid.
- Error/No result – note the number of the error result in Mobenzi database under "comments". Use the remaining processing-sputum sample (sputum with buffer added) to repeat the test using a new cartridge.

e) CompleteForm #1in the "GeneXpert MTB/RIF register book"

Cartridge ID: 218628536

Xpert result:

f) Lab report

At the completion of testing for 1 AP, send an email notification to the vice team leader/team leader cc phong.trannhu@sydney.edu.au, phuong.nguyen@sydney.edu.au

Subject: Ap name-commnue_**LAB report**

+ # Receive:

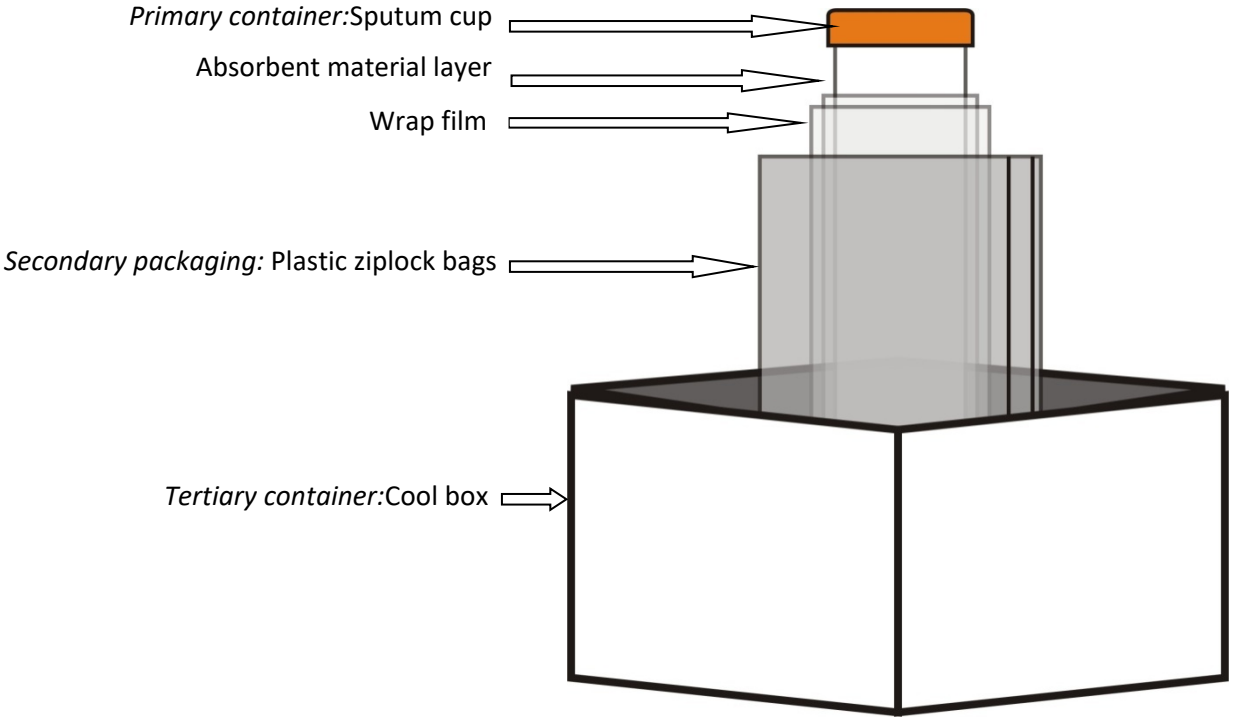
+ # Test:

+ # Not Test:

7. Transportation sputum specimens

Triple packing of specimen is an essential safety feature for the transport of infectious materials and is an international requirement. Specimens should be transported to the laboratory as soon as possible after collection. Specimens must be packaged to withstand leakage of contents, shock, pressure changes and other conditions

*Material:*Absorbent paper, Wrap film, Plastic bag with ziplock, Rubberband, QRcode sticker, Permanent Marker, Cold box with ice, Adhesive tape, Arrow Up, Biohazard sign and Transportation list.



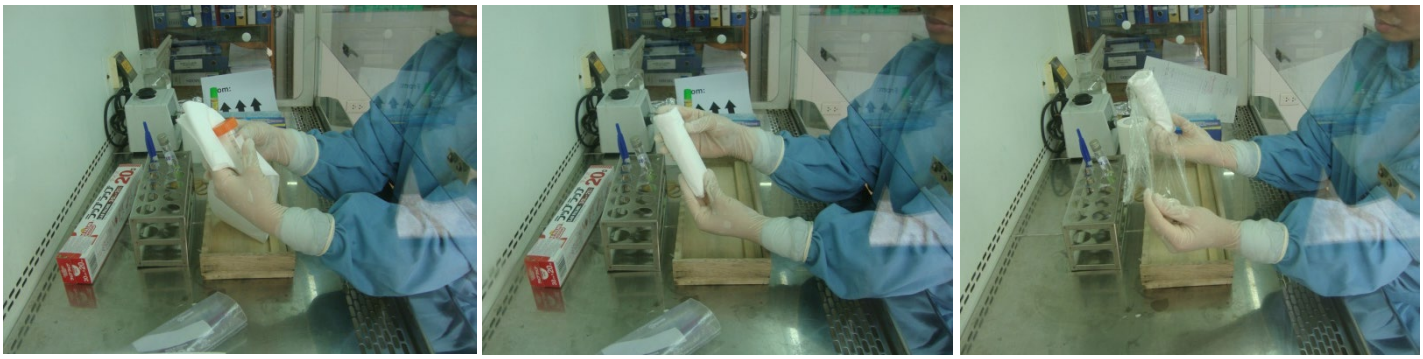
Procedure

- Wrap the specimen container in absorbent material (e.g. toilet paper) to cushion it and absorb any leakage.
- Place the primary container wrapped with the absorbent material into a plastic Ziplock bag. Then tie this secondary container with a rubber band.
- Place the secondary container into a tertiary container, e.g. an insulated cool box.
- Place the request form and list for transportation into a plastic ziplock bag and place into the tertiary container. Then close the container and seal with adhesive tape securely.

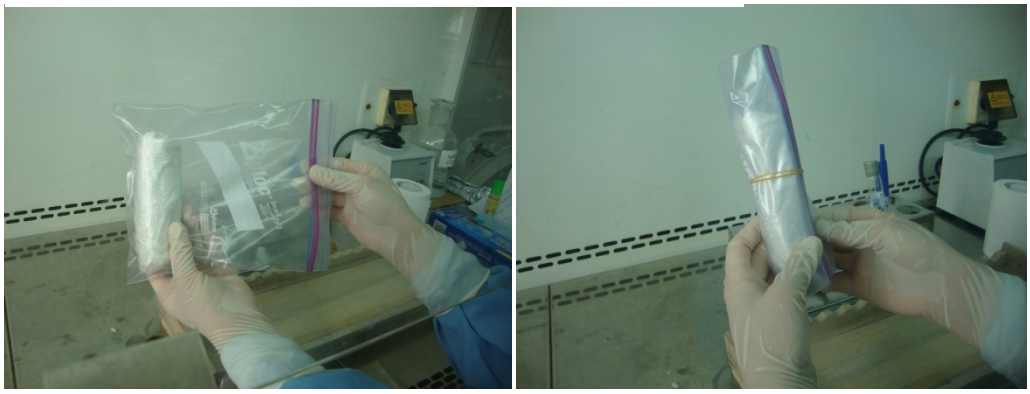
Every referral tube must be entered into the attached list specifying type of sample and from which patient the sample was collected.

- Label the tertiary container with the date, name of the screening Ap and the destination laboratory.

1. Wrapping the ***primary container*** with toilet paper, wrapping film



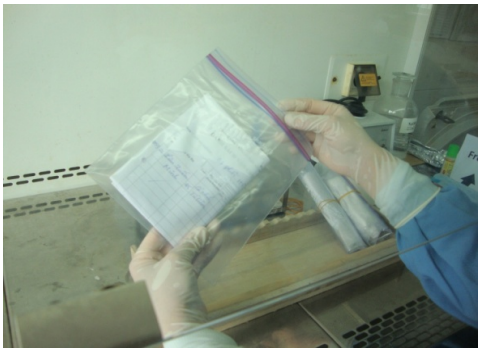
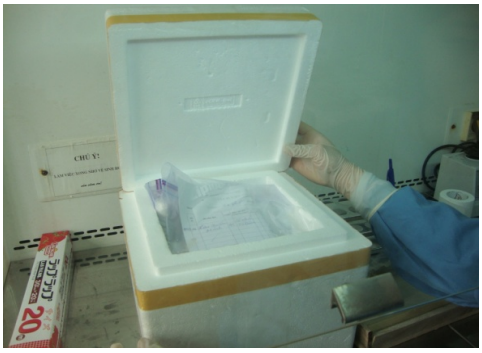
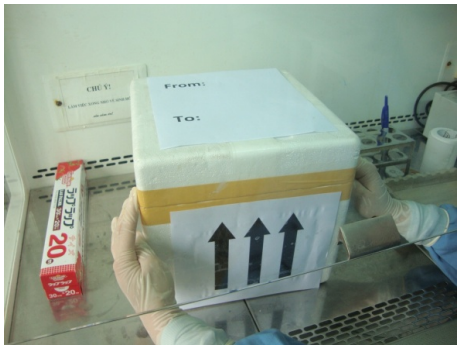
2. Place into plastic ziplock bag, ***secondary packaging***



Lock the bag securely and tight with rubber band

3. Place vertically into the ***tertiary container***

4. Place the request-form, transportation-list into the tertiary container and close tightly, label on the box

Transportation:

- The specimens will be shipped in triple packaging.

- Identification number on each specimen container corresponds to the identification number on the transportation forms.

- Make sure that the transport temperature is maintained at 2-8°C and the samples are shipped to CSDP within 48 hours (ASAP).

8. Consumable inventory management

GeneXpert MTB/RIF kit (Cartridges, RS buffer, pipette)

Daily: Lab staff will update total number of cartridges used and remaining in the "Stock log sheet"

Weekly: Admin (VTNQúi) to complete an inventory of cartridges used and send report to Trial coordinator.

Chemical & consumable material

#	Items	Unit	Using in 1 month	Note
1	70% Alcohol	litter	5	
2	Bleach	litter	1	
3	Cotton swap(50 per box)	box	1	
4	Hand washing solution	botte	1	
5	Washing solution	botte	1	
6	Yellow garbage bag	bag	140	
7	Blue garbage bag	bag	60	
8	Cellophane bags	bag	20	
9	Mask	box	6	
10	Mask M3	box	2	
11	Glove, S size	box	15	M size when need
12	Transportation box	box	8	
13	Falcon type (yellow lid)	tuyp	25	
14	Absorbent paper	piece	5	
15	Wrap film	piece	1	
16	Dry ice	bag	48	
17	White tapes	piece	2	
18	Blue tapes	piece	1	
19	Marker	piece	5	
20	Pen	piece	2	
21	Form #1	page	100	
22	Cover paper	page	20	

9. Maintenance of laboratory equipment

Equipment in CSDP's lab:

#	Equipment	Daily maintenance
1	GeneXpert systems: 4 GX-4 GeneXpert + 1 laptop + Morderm + UPS	Wipe external surface with 70% alcohol
2	Electricity stabilizer (Lioa)	Check working
3	Generator	Ensure sufficient fuel for the day's activities
4	Refrigerator	Check the temperature [2-8°C]
5	Cabinet	Decontamination with 70% alcohol
6	Air conditioner	Check working
7	-80°C Freezer	Check the temperature [78-85°C]
8	Centrifuge	Decontamination with 70% alcohol
9	37°C Incubator	Check the temperature [36-38°C]

***a. GeneXpert** Maintenance*: : 4 GX-4 GeneXpert + 1 computer + Mordem + UPS

Prepare0.5% Javen (NaOCl)**:** To be made up as needed; In a wash bottle: Add 1l Javen + 11l tap water

Daily maintenance

1. Take the cartridge out of the instrument module
2. Clean the station place
3. Record on a maintenance chart under '**Daily Maintenance'**.

Weekly maintenance

1. In the no-usage week, turn the system on. The system wills auto-check software and hardware.
2. Shut down the computer and switch off the GeneXpert at the back of the instrument.

3. Remember to tick off the procedures performed on the maintenance Logbook and sign 'Weekly **Maintenance'.**

Monthly maintenance

At the end of every month, perform the following:

1. Switch on the GeneXpert instrument.
2. Switch on the computer.
3. When prompted, log onto the computer with your **unique** password.
4. Double click 'GeneXpert' icon on the desktop.
5. Log onto the GeneXpert software using your username and password.
6. Using a small piece of paper towel, carefully wipe the outside of the GeneXpert instrument and inside of the modules with 0.5% Javen

   DO NOT WIPE the reaction chamber situated at the inside/back of the module
7. Wait 10 minutes and then repeat step 6 with 70% ethanol.
8. Disinfecting the **Cartridge Bay Module GX**: Dip a swab into the 0.5% Javen solution. Press the swab against the inside wall of the container to remove excess solution. Open the instrument module door. Wipe the surfaces inside the cartridge bay with the swab. Do not touch the slit on the I-CORE module into which the cartridge reaction tube is inserted.
9. After 10 min, Dip a new swab into the 70% alcohol solution. Press the swab against the inside wall of the container to remove excess solution. Wipe the same surfaces with the new swab. Repeat steps 5 and 6 two times. Close the instrument module door.
10. **Disinfecting the Plunger Rod**, In the GeneXpert Dx System window, clicks **Maintenance** on the toolbar. The Maintenance window appears (Figure 9.2)
11. On the Maintenance menu, click Plunger Maintenance. The **Plunger Maintenance** dialog box appears.

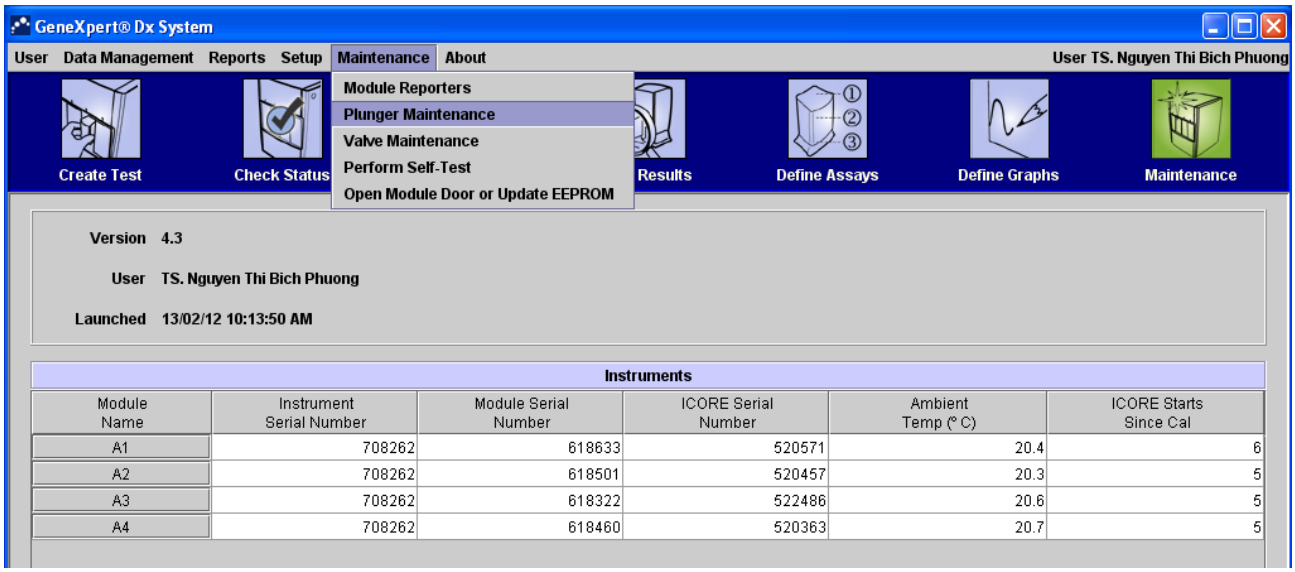


Figure 9.2 The Maintenance window

12. In the Module **table**, select the module you want to clean, and then click **Clean.** In the Plunger Maintenance dialog box, the clean button changes to Move Up. In the instrument, the plunger rod in the selected module lowers into the cartridge bay.
13. Dip a number of swabs in the 0.5% Javen solution. Press the swabs against the inside wall of the container to remove excess solution. Wipe the plunger rods with the swabs. Use a fresh swab for each plunger rod

Figure 9.3

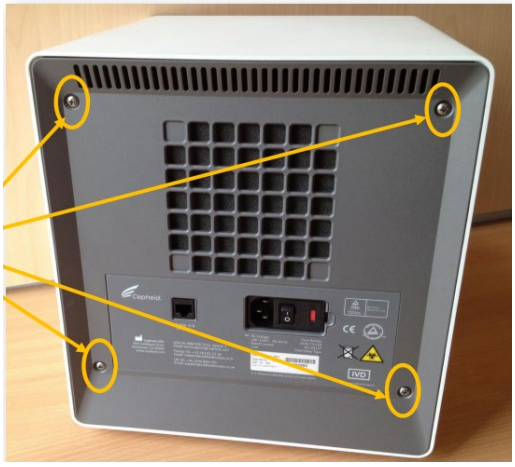
The plunger rod, lowered into the Cartridge bay

14. Wait 10 minutes.

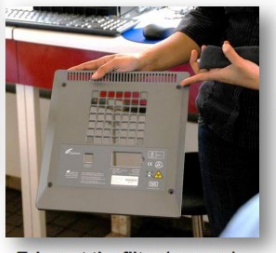
15. Dip a number of swabs into the 70% alcohol solution. Press the swabs against the inside wall of the container to remove excess solution. Wipe the plunger rods with the swabs. Use a fresh swab for each plunger rod.
16. In the Plunger Maintenance dialog box, click **Plunger Up**. The plunger rod moves back up to its resting position.

NOTE: GETTING LIQUID INSIDE THE I-CORE MODULE CAN DAMAGE THE MODULE

17. **Cleaning the fan filter:** Unscrew the 4 screws on the rear grey panel



Remove the grey cover at the back of the instrument



Take out the filter (sponge)

Wash the filter with water and soap
Let it dry and put it back

Replace the filter if necessary-
Available upon request

18.
19. **Performing a Manual Self-Test:** In the GeneXpert Dx System window, click **Maintenance** on the toolbar. The maintenance window appears.
20. On the Maintenance menu, click **Perform Self-Test**. The Module Self-Test dialog box appears. (Figure 9.4).

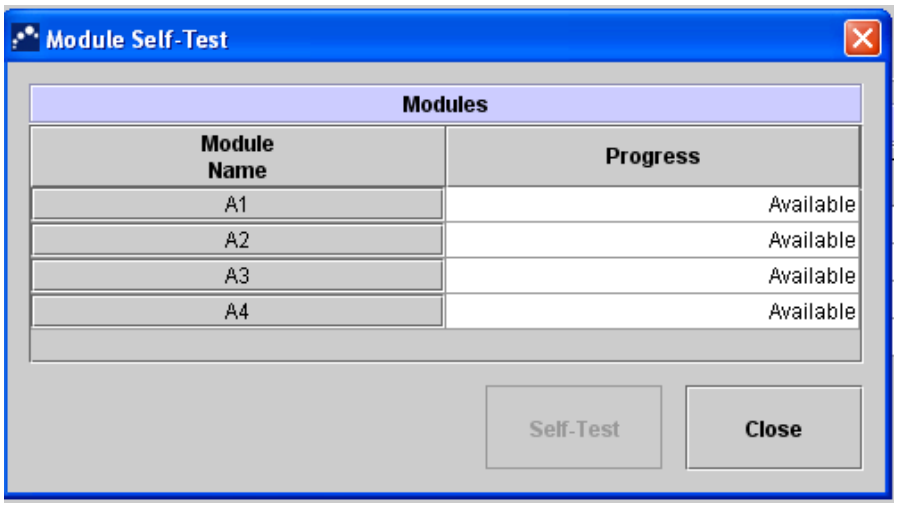


Figure 9.4. The Module Self-Test dialog box

21. Select the module you want to check.

22. Click Self-**Test**. When the self-test finishes, the software changes the progress to "Available", indicating the self-test passed. If the message indicates the self-test failed, contact Cepheid Technical Support. See the Assistance section in the preface for the contact information**.**

23. Remember to tick off the procedures performed on the maintenance Log book '**Monthly Maintenance"**

Procedure for yearly maintenance (will be performed by Trial coordinator)

1. As soon as number of runs on the GeneXpert instrument nears 2,000 a reminder appears on the GeneXpert software to make you aware.
2. Once the reminder window appears', arrange for calibration to be done.
3. If no warning window appears and machine has been running for close to 1 year, arrange for calibration to be done.
4. Contact your local service provider.

Laboratory Forms

References

1. TB lab Bio-safety Manual http://apps.who.int/iris/bitstream/10665/77949/1/9789241504638_eng.pdf
2. Triple Packaging Systemhttp://www.phls.gov.bt/downloads/Laboratory%20Guidelines%20for%20DRS.pdf
3.

NATIONAL TUBERCULOSIS PROGRAM

WOOLCOCK INSTITUTE MEDICAL RESEARCH

MANUAL OF PROCEDURES

Latent Tuberculosis Infection For Child Study (ACT3C)

Version 2.0
Last updated: 7th February 2017

Table of Contents

List of Abbreviations

Ap	Village
CSDP	Center for Social Disease Prevention and Control
ELISA	Enzyme-linked immunosorbent serologic assays
HCMC	Ho Chi Minh City
IGRA	Interferon gamma release assay
LTBI	latent tuberculosis infection
NHMRC	Australian National Health and Medical Research Council
NTP	National Tuberculosis Program
PO	Project officer
PNT	Pham Ngoc Thach
QFT-Plus	QuantiFERON®-TB Gold Plus
QRcode	Quick response code
SOP	Standard operating procedure
TB	Tuberculosis
VND	Vietnam Dong
WIMR	Woolcock Institute of Medical Research

Introduction - Overview

This is the Manual of Procedures for the ACT3C or latent tuberculosis (TB) infection (LTBI) study for children which will be conducted currently within an existing randomized controlled trial of active case finding for TB (ACT3) in Ca Mau Province, Vietnam. The ACT3C study will measure the prevalence of LTBI for children born in 2012 in Ca Mau province, using an interferon gamma release assay (IGRA) – QuantiFERON®-TB Gold Plus (QFT-Plus).

The objective of this study is to estimate the prevalence of TB infection among children born in 2012 in active and control clusters from the main ACT3 study. ACT3C will estimate required resources to treat LTBI in children in Ca Mau.

1. **Title of study**: The ACT3C study

2. **Duration of study**: 1.5 years (2017-mid 2018)

3. **Investigators**
   - Professor Guy B. Marks (Woolcock Institute of Medical Research, WIMR)
   - A/Professor Nguyen Viet Nhung (Director, National Lung Hospital of Vietnam)
   - Dr Nguyen Thu Anh (Woolcock Institute of Medical Research)
   - Dr Nguyen Thi Bich Phuong (Woolcock Institute of Medical Research)

4. **Cooperating institutions:**
   - Ca Mau Center for Social Disease Prevention and Control
   - Pham Ngoc Thach Laboratory, Ho Chi Minh City (HCMc)
   - Woolcock Institute of Medical Research
   - Centenary Institute of Cancer Medicine and Cell Biology
   - Australian National Health and Medical Research Council (NHMRC) Centre for Research Excellence in Tuberculosis Control

5. **Funding support**:
   - Australian National Health and Medical Research Council Centre for Research Excellence in Tuberculosis Control
   - Vietnam National Tuberculosis Program

6. **Human research ethics approval**:
   - Human Research Ethics Committee, University of Sydney. Approvals: 2013 / 073.
     - Including modification approval dated 12 Dec 2016.
   - Institutional Review Board, National Lung Hospital, approval number 407/QD-BVPTW (29th August, 2013)
   - Ministry of Health, Department of Science and Training, approval number 4443/QD-BYT
   - Ministry of Health, Department of Finance and Training

Roles and Responsibilities

i. Chief Investigators

The chief investigators will have the overall authority and responsibility for the research. Responsibilities include:

- Planning the implementation of the study, arranging human resources, and developing the SOPs and budget for the project
- Overseeing implementation and quality assurance

ii. Project Officer

This project officer will be responsible for oversight and supervision of all the activities related to the ACT3C from the field to lab. The project officer will work closely with the project co-ordinator and medical/scientific adviser to ensure that all procedures are carried out correctly. Any issues/problems are discussed and addressed promptly. Responsibilities include:

- Preparing training, implementation, data management and monitoring of progress and data quality
- Working with the field & laboratory staffs in training and supervision of tasks
- Day to day oversight of study preparation and implementation
- Communicating test results and further necessary investigations to study participants
- Co-ordinating and supervising communication (verbal, SMS and telephone), to the study participants about test results and necessary further investigations
- Checking all consent forms received at the Hoa Lu office after each Ap.

iii. ACT3 Team Leaders and Vice-team Leaders

The team leaders and vice team leaders will be responsible for directly supervising the field staff and ensuring their tasks (listed below) are completed satisfactorily. They will assist the field staff in completing the project tasks when necessary. They will follow the manual of procedures and receive guidance from and report to the chief investigators and project officer.

iv. ACT3 Field staff

The field staff will be responsible for recruiting participants in the ACT3C study. This will involve tasks such as: explaining the study to the participants, obtaining consent for ACT3C and referring those participants to CSDP for blood collection, taking blood samples, ensuring blood samples arrive safely in the CSDP laboratory, entering results into the Mobenzi database, preparing weekly reports, reporting adverse events, arranging appointments for patients, liaising between the field team and the laboratory staff, as well as discussing problems or other work related issues with the team leaders, project co-ordinators and chief investigators when required. They will follow the manual of procedures and receive guidance from and report to the chief investigators and project officer.

v. ACT3 Laboratory staff

The ACT3 laboratory staff in this project will be responsible for ensuring prompt and appropriate handling and processing of blood samples and correct storage of samples. They will ensure sufficient stock of consumables in the laboratory, report any problems with equipment in laboratory or aberrant results, liaise with field staff and CSDP staff, report adverse events and discuss problems or other work related

issues with the project co-ordinators and chief investigators when required. They will follow the manual of procedures and receive guidance from and report to the chief investigators and project officer.

vi. Non-WIMR staff

1. Radiographer in CSDP
   The radiographer in CSDP will be responsible for taking chest x-rays for participants in the ACT3C study with positive QFT results who gave consent. The radiographer will receive information about this study and guidance from the project officer.

2. Doctor in CSDP to clinically assess participants with abnormal chest x-ray
   Participants with potentially clinically significant abnormalities on chest x-rays will be invited to see the CSDP doctor (or their own doctor). Those who choose to see the CSDP doctor will be clinically assessed in the context of the chest x-ray findings and the Xpert test result. The doctor will be remunerated by WIMR for this service.

Study Population

Ca Mau is the southern-most province of Vietnam's 63 provinces with a population of 1,212,000. In the main ACT3 study, adults ≥15 years residing in 60 randomly selected Aps in Ca Mau Province (55,069 adult population) have been screened continuously for TB disease for 4 years; adults ≥15 years residing in the other 60 randomly selected Ap (approximate adult population 65,769) will be screened ony in the Year 4. All the children within these 120 screened Ap who were born in 2012 will be eligible for screening "latent tuberculosis infection". Commune health port will provide the list of children on the district meeting.

Methods

A. <u>Equipment and Materials</u>

i. Blood collection equipment

- QFT-plus tubes x 4 (nil control, TB1, TB2 & mitogen control) Catalogue Number: 622526. Storage conditions for QFT-plus tubes (store tubes upright): 17-25°C
- ELMA cream & tape (for covering the cream), pen
- Timer x 2
- Thermometer x 4 (for 2 portable incubators & lab incubator).
- Vacutainer Safety-lok Blood collection set: Wingset 23G, Holder, "purge" tube
- Tourniquets
- Alcohol pads
- Urgosterile
- Band-Aids or adhesive tapes
- Gloves
- Gauze or cotton balls
- Alcohol hand rubs
- Portable sharps containers
- Racks to hold blood tubes inside the portable incubator & esky
- Portable incubators (37°C) x 2
- Esky for storing QFT-plus tubes to the field (17-25°C) x 2
- Thermometer x 4 (for 2 portable incubators & lab incubator)

ii. For laboratory in CSDP
- Centrifuge
- Laboratory incubator (37°C)
- Electronic Thermometer for lab incubator x 1
- Racks to hold and process blood tubes in laboratory/field
- Gloves, lab coat and for lab staff when handling blood

iii. For laboratory in Pham Ngoc Thach Lab
- QuantiFERON®-TB Gold Plus (QFT®-Plus), Catalog no. 622120
- QuantiFERON® Control Panel, REF: 0594-080
- Consumable for ELISA: filter tips, alcohol,

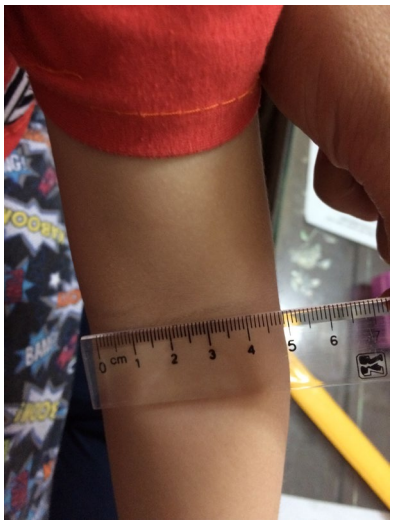
B. Procedure for recruiting ACT3C participants

- Project officer will make appointment/invitation with the parent for the date/time & location to collect the blood.
- On one day of screening in the Ap, field-staff will be assigned for QFT-plus blood collection. They will provide information about the study, both verbal and written information (child study introduction) and obtain written consent from the parent or caregiver. The written consent form will be stored at the Hoa Lu office.
- The field staff will record in the Mobenzi database whether the participant consents to study participation.

C. Procedure for collecting blood for QFT testing in the field: http://tb.gnowee.net/videos; https://www.youtube.com/watch?v=TOXF6CzPJYA

The trained field staffs will collect blood for QFT testing in the field, as follows:

1. Field staffs will identify the vein and the location on which they will put the cream.
2. Cleanse the skin with 70% alcohol. Swab concentrically, starting at the centre and progressing outwards.
3. Squeeze the ~1g ELMA cream into a mound where the needle is going to put in. Cream applied to a circular area with diameter of about 18mm (a pence coint). Put ELMA cream on both arms. This provides the option of using the second arm if blood collection in the first arm is problematic.
4. Remove the cover of the tape and then place carefully over the mound of cream. Do not spread the cream under the dressing.
5. Write the time of application on dressing.
   Note: Recommend field staff apply EMLA to their own skin and check the degree of anaesthesia after 10 mins, 20 mins. So that they have a sense of how it will feel for the child.

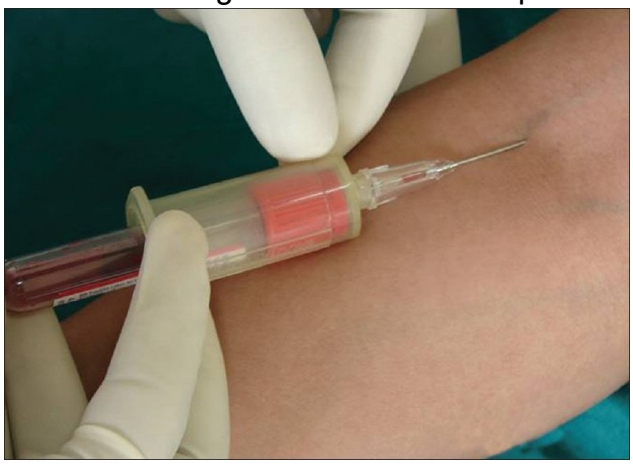


6. QFT-plus tubes should be kept in Thermocol Packaging Boxes with dry ice. The temperature should be between 17ºC and 25ºC at the time of blood filling.

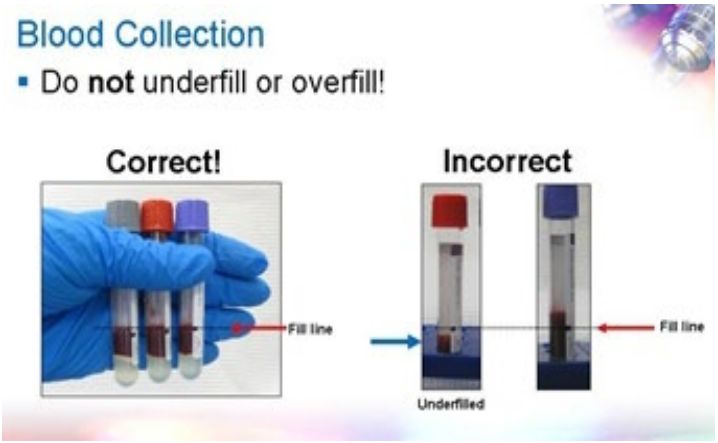
7. Label tubes with name, blood collection time, and place Qrcode stickers on each of the four tubes prior to use (use the same code for each tube).
8. Confirm the participant's name and age before drawing blood sample.
9. Ensure that the participant is sitting/holding with parental or caregiver.
10. Talk to the child to reduce his/her anxiety before a draw.

11. Using the vacutainer system, collect 1ml of blood into each QFT tube (x4)

a. A "purge" tube should be used to ensure that the tubing is filled with blood prior to the QFT-Plus tubes being used.



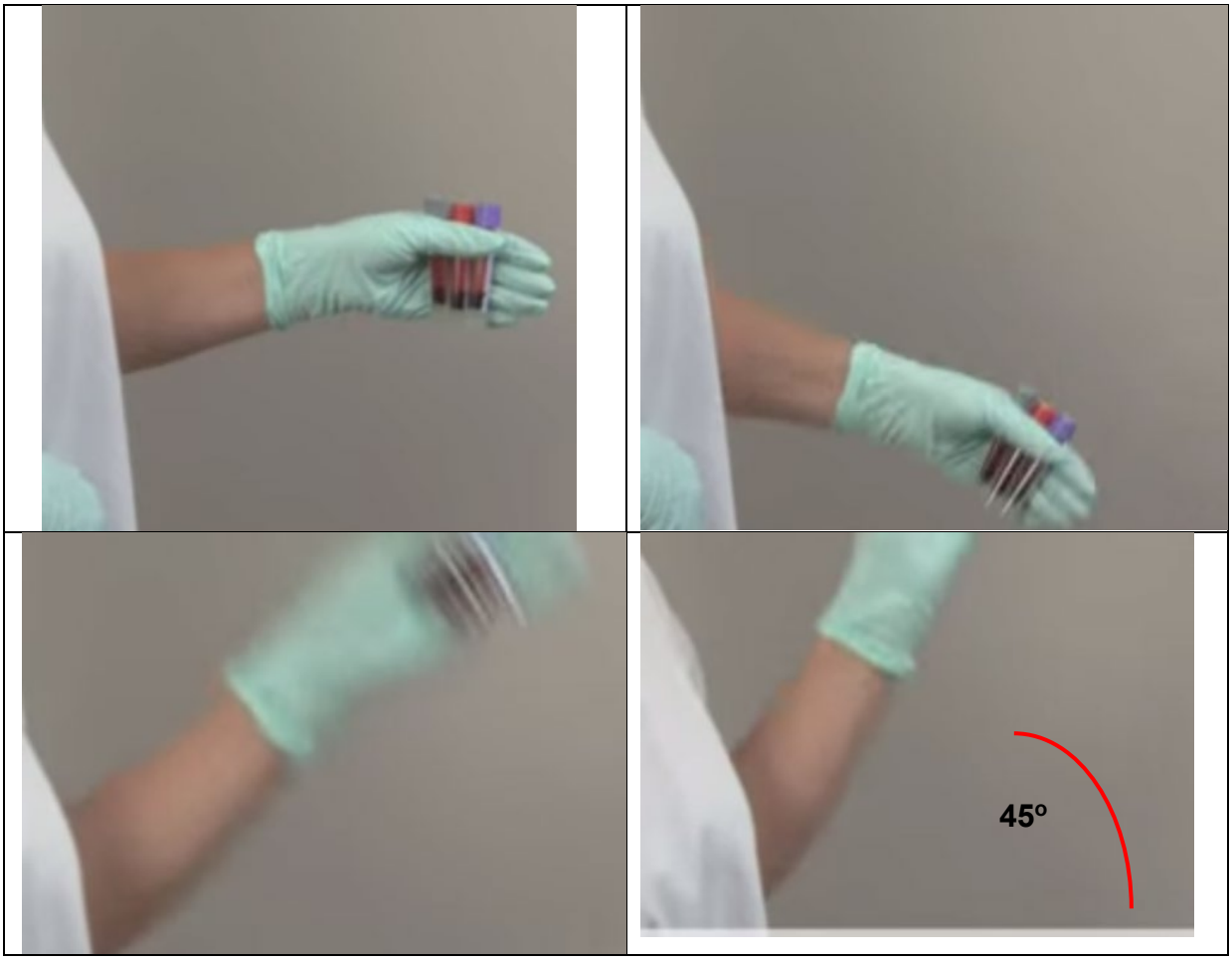
b. Tubes fill slowly, as 1ml tubes draw blood relatively slowly. Keep the tube on the needle for 2-3 seconds once the tube appears to have completed filling, to ensure that the correct volume is drawn. If blood level is not close to the **black mark** on the side of the tube label, obtain another tube (it is very important that the **correct amount of blood is drawn into these tubes**)



c. If the initial attempt is unsuccessful and the participant agrees to another attempt then re-try the other arm. Do NOT have more than two attempts. If two attempts are unsuccessful or if one attempt is unsuccessful and the participant refuses another attempt then record outcome "unsuccessful".

12. Dispose the vacutainer system into the biohazard sharps container.
13. Immediately after filling shake tubes ten (10) times just firmly enough to ensure that the inner surface of the tube is coated in blood to dissolve antigens on tube walls.

a. After gently shaking, the entire inner surface of each tube should be coated with blood.
b. Proper shaking will lead to frothing of the blood, which is required for correct performance of the test.
c. Over-energetic shaking may cause gel disruption and could lead to inaccurate results.



14. Scan Qrcode on the blood tubes into the Mobenzi database and place Qrcode on the transportation list.
15. Place tubes UPRIGHT into Thermocol Packaging Boxes with dry ice. The temperature should be between 17ºC and 25ºC within 16 hours
16. All of the tubes will be placed into kit-rack. The thin-film will be covered whole tubes to keep inline inside Thermocol Packaging Boxes for transportation.
17.
18. Clean hand with disinfection liquid.

See section 5 regarding safety when collecting blood in the field.

Transport Thermocol Packaging Boxes to the laboratory as soon as blood collection is complete, using motorbike +/- other necessary transport means.Tubes must be incubated at 37°C for 22 hours (no longer than 24h).

D. Processing and storage of blood tubes in the CSDP laboratory

Once blood samples have been received in the laboratory, cross-check these samples with the transportation list and inform the field team of the total number received.

Process the QFT blood tubes as follows: http://tb.gnowee.net/videos https://www.youtube.com/watch?v=mpj_q6PDjnk

1. Once tubes have arrived in the laboratory in the portable incubators, transfer the tubes to the laboratory 37°C incubator and continue incubation for a total of 22 hours (no longer than 24h).
2. Tubes will be divided into groups by collecting time. The time table sheet will be filled and placed on the incubator door. This will be used to guide staff about when to remove tubes from the incubator.
3. Scan blood samples (QR code) into the Mobenzi database to record laboratory receipt of sample at the time they are removed from the laboratory incubator, just prior to centrifuging. That will serve to both record receipt in the lab and date/time stamp the time of centrifuging.
4. After incubation, centrifuge the tubes for 15 minutes at 2500 RCF (g) (***in accordance with the specific centrifuge and rotor used). The **gel plug will separate the cells** from the plasma. If this does not occur, re-centrifuge the tubes at 3500 RCF. The blood tubes should be centrifuged immediately (within 30mins) after removal from the incubator.
5. After centrifugation, store the QFT tubes at 2 – 8°C (Mon/Thurs), prior to sending to Pham Ngoc Thach (PNT) laboratory for ELISA testing.

See section 5 regarding safety aspects of handling blood in the laboratory.

E. Sending QFT tubes to Pham Ngoc Thach laboratory in Ho Chi Minh City

- Packing: All of the tubes will be placed into kit-rack. The thin-film will be covered whole tubes to keep inline.
- Put in tubes fix in the rack.
- Pack the Thermocol Packaging Box.
- Label the Box with signal: upward signal, biohazard, address & telephone number of HCMC staff.
- Attach a log book with lists of samples and QR codes, a log of temperature during incubation (automatically recorded by thermometer in the incubator, need to plug into computer to get information).
- Inform PNT-HCMC staff of the time (sending & receiving), name & telephone number of the driver.
- The samples will arrive in HCMC on the Tue/Wed morning every week. PNT lab will sign to confirm they have received samples on the database.

Items	Ca Mau Post Office
Sending time: Every Mon/Thurs	At 4:00 PM
Receiving time (with warranty)	Before 9: 00 AM every Tue/ Fri

F. ELISA procedure in PNT https://www.youtube.com/watch?v=eFl2KiU6e4g

G. TEST RESULT

– Within one week, PNT lab will carry out QFT test and send the raw data and calculated QFT results to project coordinator (phuong,nguyen@sydney.edu.au).

FIGURE 2. Recommended Sample Layout for Nil, TB Antigen & Mitogen Tubes (28 tests per plate)

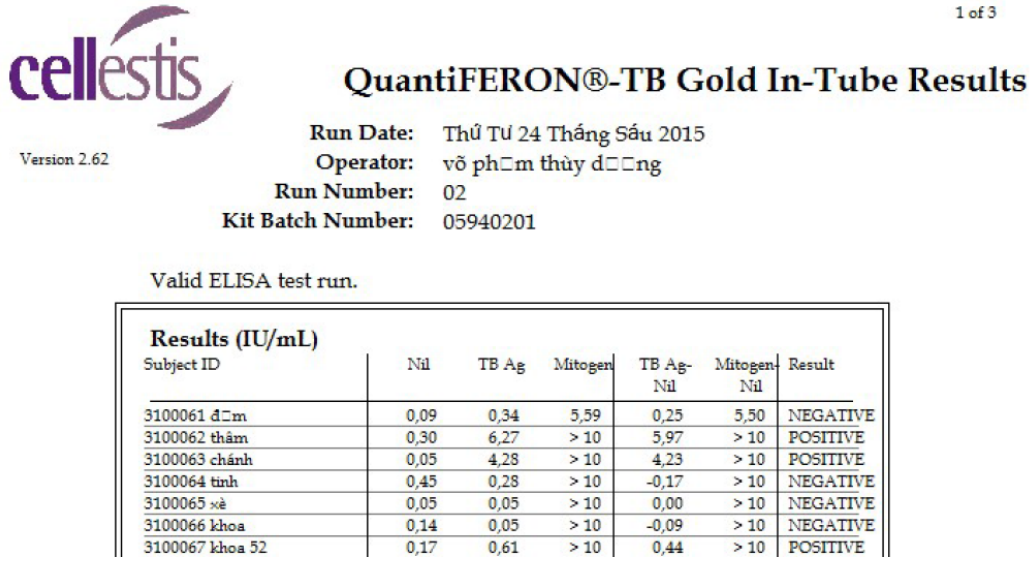
Row	1	2	3	4	5	6	7	8	9	10	11	12
A	1N	1A	1M	S1	S1	S1	13N	13A	13M	21N	21A	21M
B	2N	2A	2M	S2	S2	S2	14N	14A	14M	22N	22A	22M
C	3N	3A	3M	S3	S3	S3	15N	15A	15M	23N	23A	23M
D	4N	4A	4M	S4	S4	S4	16N	16A	16M	24N	24A	24M
E	5N	5A	5M	9N	9A	9M	17N	17A	17M	25N	25A	25M
F	6N	6A	6M	10N	10A	10M	18N	18A	18M	26N	26A	26M
G	7N	7A	7M	11N	11A	11M	19N	19A	19M	27N	27A	27M
H	8N	8A	8M	12N	12A	12M	20N	20A	20M	28N	28A	28M

- S1 (Standard 1), S2 (Standard 2), S3 (Standard 3), S4 (Standard 4).
- 1N (Sample 1. Nil Control plasma); 1A (Sample 1. TB Antigen plasma); 1M (Sample 1. Mitogen Control plasma).

Within one week, PNT lab will enter the results of the QFT assays into the Mobenzi database:

+ Precise output number

+ Calculated QFT results from TB Gold IT analysis software (version 2.62). The software will automatically analyze the raw data; performs a quality control assessment of the assay, generates a standard curve and provides a test result for each participan. The “Interpretation of Results” as below:



1 of 3

cellestis

Version 2.62

QuantiFERON®-TB Gold In-Tube Results

Run Date: Thứ Tư 24 Tháng Sáu 2015
Operator: võ ph□m thùy d□□ng
Run Number: 02
Kit Batch Number: 05940201

Valid ELISA test run.

Results (IU/mL)

Subject ID	Nil	TB Ag	Mitogen	TB Ag-Nil	Mitogen-Nil	Result
3100061 đ□m	0,09	0,34	5,59	0,25	5,50	NEGATIVE
3100062 thâm	0,30	6,27	> 10	5,97	> 10	POSITIVE
3100063 chánh	0,05	4,28	> 10	4,23	> 10	POSITIVE
3100064 tinh	0,45	0,28	> 10	-0,17	> 10	NEGATIVE
3100065 xè	0,05	0,05	> 10	0,00	> 10	NEGATIVE
3100066 khoa	0,14	0,05	> 10	-0,09	> 10	NEGATIVE
3100067 khoa 52	0,17	0,61	> 10	0,44	> 10	POSITIVE

H. Returning results of QFT test and chest x-ray to participant’s parental/caregiver

- Once per week (on Thursday) the PO will check the results of the QFT testing and the chest x-ray results in the recently screened Aps.
- For participants with a negative result, ACT3 will send out a SMS result notification as follows:

“The blood test result does not show evidence of TB infection at this time.”

- For participants with a indeterminate or positive QFT result, the PO will telephone the participant and explain the result as follows:

“The blood test we have performed suggests that you have been exposed to someone with TB in the past and may be at risk of becoming unwell with TB in the future. We would like you to come to CSDP to have a chest x-ray to look for signs of disease in your lungs that may need treatment now. We

will pay for this x-ray and also subsidise the cost of travel to CSDP according to ACT3 cost norms"

- Further investigations and interview at CSDP: The participant together with parental/caregiver will complete the following:

1. Room #8 (Interview room): Lab coordinator who will explain the plan for further investigations and interviews.
2. Room #9 (CSDP consultant room): CSDP doctors will conduct: (1) A health exam; (2) Write chest x-ray requests and (3) Send patient to CSDP reception with these forms to pay for the tests
3. CSDP Radiology staf will perform chest x-ray and give the result to participant's parental/caregiver
4. CSDP doctor will conduct clinical assessment follow NTP decision 2599/BVPTU'-DAPCL on 29 December 2016
5. Finally, the Project Officer will review all study documents, and entry Xray milestone & Clinical Assessment Form into the Mobenzi database:

Safety when collecting and handling blood

1. Collecting blood in the field
   - All blood samples should be considered and handled as potentially infectious (i.e. universal precautions).
   - All open cuts and abrasions should be covered when collecting blood.
   - Gloves should always be worn when collecting blood and discarded appropriately after blood collection for each participant.
   - Alcohol based hand wash should be used after collecting blood from each participant after removal of gloves.
   - After blood collection, the needle should be discarded immediately in the biohazard sharps container (by the person who collected the blood). The sharps container must be within an arm's length of the blood collector.
   - Any needle stick injuries must be reported immediately to the field staff leader and an adverse event report completed. Every needle stick injury event will be assessed by a medical officer (part of ACT3 team) for appropriate management in accordance with the WIMR SOP.

2. Handling blood in the laboratory
   - Lab coats, gloves and eye protection should be worn when handling blood samples.
   - All blood samples should be considered and handled as potentially infectious (i.e. universal precautions).
   - All open cuts and abrasions should be covered when handling blood.
   - Use disposable equipment wherever possible and dispose of equipment in the appropriate biohazard waste containers
   - Use sealed tubes for centrifuging blood samples. Use sealed rotors to minimise contamination in the event of tube failure
   - Laboratory benches and other areas where blood has been handled must be cleaned and decontaminated at the completion of work with disinfectant e.g. 1% sodium hypochlorite or other appropriate solution
   - Tubes arriving from the field that are broken or leaking should not be processed, but should be discarded in the appropriate biohazard container. The team leader should

be notified for appropriate investigation and a note made in the Mobenzi database that this sample could not be processed.

Cleaning blood spills in the laboratory

- Any blood spills on floors, benches or equipment must be cleaned up immediately with disinfectant e.g. 1% sodium hypochlorite or other appropriate solution.
- For spills on a flat surface, cover the spill with a paper towel soaked with disinfectant and allow to stand for 10-15 mins before discarding and wiping the area again until clean
- In the event of a failure of tubes in the centrifuge, the centrifuge rotor and bowls should be disinfected e.g. with 1% sodium hypochlorite or other appropriate solution.

Reporting of adverse events

The team leader or vice team leader will record any adverse events in an Adverse Events form. This will be submitted to a PO after each Ap's screening. For any urgent clinical adverse event, a PO will be contacted on the same day.

Adverse events would include:

- Abnormal symptoms in participant during blood collection: e.g. fainting, excessive bleeding, excessive trauma to the venipuncture site, etc.
- Needle stick injury to staff
- Accidents that happen to staff or research participants during the activity (e.g. falls, motorbike accidents, etc.)
- Any complaint made by study participants or local leaders

For each adverse event, an outcome must be recorded. The team leader or vice team leader must take responsibility for following up the adverse event within a reasonable period of time (less than 1 week, or less if appropriate).

Consent Forms and Information Sheets

Form code	Description	Details	Note
3C-AI	Child_introduction & Appointment_	Child_introduction & Appointment_give to all participants	
3C-PIS	Participant information sheet	To provide study information to potential participants of the ACT3C study	
3C-CF	Consent form	Consent form for participants in the ACT3C study if they agree to participate in the study and have blood collected	
AE	Adverse events form	Report of adverse events during study	
3C-A3	Clinical Assesment	Clinical Assesment for children (Online form)	
TP1	Incubator temperature sheet	Log sheet to keep daily record of lab-incubator temperature	
BR1	Laboratory blood sample receipt book	Forms compiled into a booklet that records date and number of blood samples received in the lab	
3C-QFT1	QFT-Plus kit	Logbook for QFTPlus kit storing in Lab	
3C-QFT2	QFT blood tubes	Logbook for QFT Blood tube receive & Transfer in Lab	
3C-QFT3	QFT transportation list	Log sheet for QFT blood collection in the field. Time of blood collection and start time of incubation	
3C-QFT4	QFT transportation list	List of participants for processed QFT tubes to be send to PNT for ELISA	
3C-Field	Plan &Checklist	Plan & Checklist for ACT3C in the field	

Appendices:

1. Key forms (as per above, particularly the Adverse Event reporting form)
2. NTP/ACT3 protocol for managing Xpert MTB positive participants
3. Consider Section on monitoring and evaluation and forms for this.

NATIONAL TUBERCULOSIS PROGRAM

WOOLCOCK INSTITUTE MEDICAL RESEARCH

MANUAL OF PROCEDURES

QFT survey in older children (ACT3C2)

Version 1.0

Last updated: 8th April 2018

Table of Contents

List of Abbreviations

Ap	Village
CSDP	Center for Social Disease Prevention and Control
ELISA	Enzyme-linked immunosorbent serologic assays
HCMC	Ho Chi Minh City
IGRA	Interferon gamma release assay
LTBI	latent tuberculosis infection
NHMRC	Australian National Health and Medical Research Council
NTP	National Tuberculosis Program
PO	Project officer
PNT	Pham Ngoc Thach
QFT-Plus	QuantiFERON®-TB Gold Plus
QRcode	Quick response code
SOP	Standard operating procedure
TB	Tuberculosis
VND	Vietnam Dong
WIMR	Woolcock Institute of Medical Research

1. Title of study

QFT survey in older children (ACT3C2)

2. Duration of study:

One year: mid-2018-mid-2019

3. Investigators

- Professor Guy B. Marks (Woolcock Institute of Medical Research, WIMR)
- A/Professor Greg Fox, University of Sydney
- A/Professor Nguyen Viet Nhung (Director, National Lung Hospital of Vietnam)
- Dr Nguyen Thu Anh (Woolcock Institute of Medical Research)
- Dr Nguyen Thi Bich Phuong (Woolcock Institute of Medical Research)

4. Cooperating institutions:

- Ca Mau Center for Social Disease Prevention and Control
- Pham Ngoc Thach Laboratory, Ho Chi Minh City (HCMc)
- Woolcock Institute of Medical Research

5. Funding support:

- Australian NHMRC
- Vietnam National Tuberculosis Program

6. Human research ethics approval:

- Human Research Ethics Committee, University of Sydney.
- Institutional Review Board, National Lung Hospital, approval number 407/QD-BVPTW (29th August, 2013)
- Ministry of Health, Department of Science and Training, approval number 4443/QD-BYT
- Ministry of Health, Department of Finance and Training

7. Background

The ACT3C study was conducted in 2017 among children born in 2012 (aged approximately 5 years) who were resident in the ACT3 study sub-communes in Ca Mau province, Vietnam. It was designed to estimate the prevalence of LTBI and also to test the hypothesis that the prevalence of LTBI was lower in those who lived in the active intervention sub-communes, where adults had been screened for active TB annually during the preceding three years, than in those who lived in the control sub-communes, where they had been no intervention. The presence of LTBI was detected using QuantiFERON®-TB Gold Plus (QFT, Qiagen). The initial sample size calculation assumed that the prevalence of LTBI in the control group would be 15%, intra-cluster correlation would be 0.001 and that, with 22 children in each cluster and 60 clusters in each group, we would have 90% power to detect a prevalence ratio of 0.70 (or less) as significant ($P < 0.05$).

In fact, the prevalence of LTBI in these children was much lower than we had anticipated. The findings were as follows. There were 1551 eligible children in 60 control clusters, of whom 843 (66.4%) had QFT performed and there were 1270 eligible children in 60 active intervention clusters of whom 821 (52.9%) had a QFT. The median (IQR) number of eligible children per cluster was 21 (14 to 29). There were three “indeterminate” QFT results. The

prevalence of positive QFT was 27 (3.3%, 95% CI 2.0% to 5.6%) in the active intervention group and 18 (2.1%, 95% CI 1.3% to 3.0%) in the control group [RR 1.54, 95% CI 0.85 to 2.77, P = 0.2]. Hence, the study was under-powered to test the hypothesis.

The new study, ACT3C2, is designed to re-test the hypothesis that the active case finding intervention resulted in a reduction in TB transmission and, hence, a lower prevalence of LTBI, by comparing the prevalence of LTBI between the two groups among older children, that is, children currently aged approximately 7 to 14 years.

8. Study population - eligibility criteria

The target population will be children who

1. were born between 2004 and 2011;
2. are resident in the active intervention or control clusters during the population enumeration in 2018-2019
3. whose parents or legal guardians are capable of giving informed consent
4. who themselves are capable of giving assent.

9. Sample size and study power

Our previous published study has shown that the prevalence of LTBI among persons aged ≥ 15 years in Ca Mau province is 37% (1) and, among persons aged 15 to 28 years, the prevalence of LTBI was 21.9% (95% confidence interval 17.6–26.3). Hence, we expect that the prevalence of LTBI among children aged approximately 7 to 14 years (that is, who were born between 2004 and 2011) will be approximately 12% in the control group. Making the same assumptions as above (60 clusters in each arm and ICC = 0.001), we would need 26 child participants in each cluster to detect a prevalence ratio of 0.70 or lower as significant (P< 0.05). That is, we will need to assess 1560 in each group (or 3,120 overall). Assuming that the response rate is similar in this age group (60%), we will need to approach 5,200 eligible participants.

10. Participant selection procedure

Assuming that the number of children born in each year from 2004 to 2011 and still alive and resident in the sub-commune in 2018 is approximately the same as it was for children born in 2012, then the number of eligible children in the study population will be 22,568 (= 2821 x 8). Hence, we will need to approach 23% of all children born in these years. The procedure will be as follows:

1. We will conduct a household enumeration (census) in each cluster (sub-commune) to identify all children born between 2004 and 2011 (inclusive).
2. At the time of enumeration, using our online database (Mobenzi), we will randomly select individuals to approach for participation in the study by applying a selection probability of 0.23 to each enumerated person in the appropriate age range.
3. The parents or guardians of the randomly selected individuals will be assessed for the ability to give informed consent and the randomly selected individuals will be assessed for their ability to give assent.

4. Capable parents or guardians will be given written and verbal information about the study (see Participant Information Statement). If they agree, they will be asked to sign the consent form.
5. When the consent form has been signed, the Project Officer will an appointment the parent for the child participant to be tested and provide him/her with an appointment card showing the date/time & location of the blood collection appointment.
6. Signed consent forms will be stored in the Hoa Lu office and scanned and uploaded to the secure Cloud storage.

11. Blood collection procedure

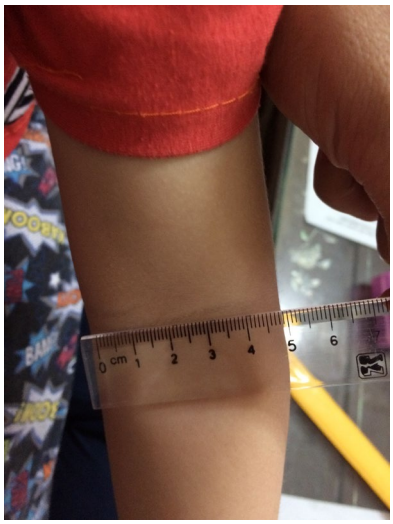
(see also: Procedure for collecting blood for QFT testing in the field: http://tb.gnowee.net/videos; https://www.youtube.com/watch?v=TOXF6CzPJYA)

i. Prior to blood collection, the field staff will explain ensure that the child is comfortable and will explain the procedure to the child. Blood will only be collected if the child verbally assents to the procedure.

ii. Field staff will identify the vein and the location on which they will put the cream.

iii. Cleanse the skin with 70% alcohol. Swab concentrically, starting at the centre and progressing outwards.

iv. Squeeze the ~1g ELMA cream into a mound where the needle is going to put in. Cream applied to a circular area with diameter of about 18mm. Put ELMA cream on both arms. This provides the option of using the second arm if blood collection in the first arm is problematic.



v. Remove the cover of the tape and then place carefully over the mound of cream. Do not spread the cream under the dressing.

vi. Write the time of application on dressing.

   a. **Note:** Recommend field staff apply EMLA to their own skin and check the degree of anaesthesia after 10 mins, 20 mins. So that they have a sense of how it will feel for the child.

vii. QFT-plus tubes should be kept in Thermocol Packaging Boxes with dry ice. The temperature should be between 17ºC and 25ºC at the time of blood filling.

viii. Label tubes with name, blood collection time, and place QR code stickers on each of the four tubes prior to use (use the same code for each tube).

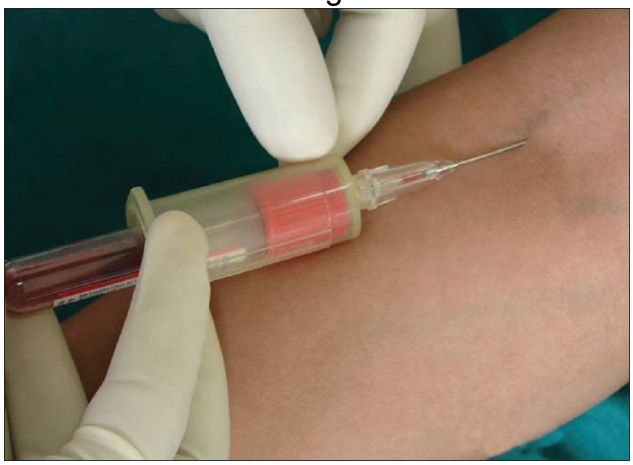
ix. Confirm the participant's name and age before drawing blood sample.

x. Ensure that the participant is sitting/holding with parent or caregiver.

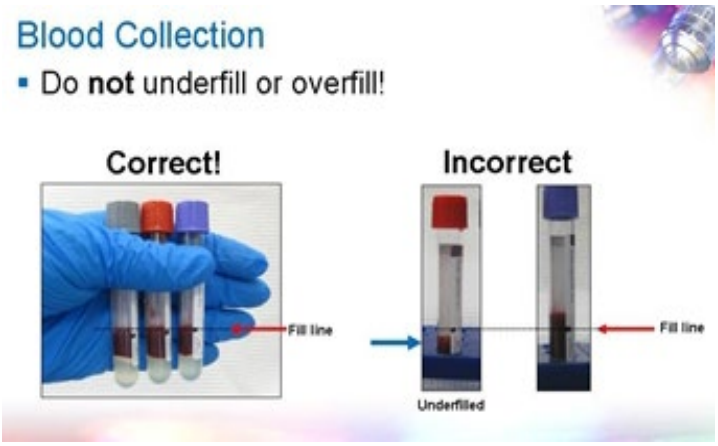
xi. Talk to the child to reduce his/her anxiety before a draw.

xii. Using the vacutainer system, collect 1ml of blood into each QFT tube (x4)

a. A “purge” tube should be used to ensure that the tubing is filled with blood prior to the QFT-Plus tubes being used.



b. Tubes fill slowly, as 1ml tubes draw blood relatively slowly. Keep the tube on the needle for 2-3 seconds once the tube appears to have completed filling, to ensure that the correct volume is drawn. If blood level is not close to the **black mark** on the side of the tube label, obtain another tube (it is very important that the **correct amount of blood is drawn into these tubes**)



c. If the initial attempt is unsuccessful and the participant agrees to another attempt then re-try the other arm. Do NOT have more than two attempts. If two attempts are unsuccessful or if one attempt is unsuccessful and the participant refuses another attempt then record outcome “unsuccessful”.

xiii. Dispose the vacutainer system into the biohazard sharps container.

xiv. Immediately after filling shake tubes ten (10) times just firmly enough to ensure that the inner surface of the tube is coated in blood to dissolve antigens on tube walls.

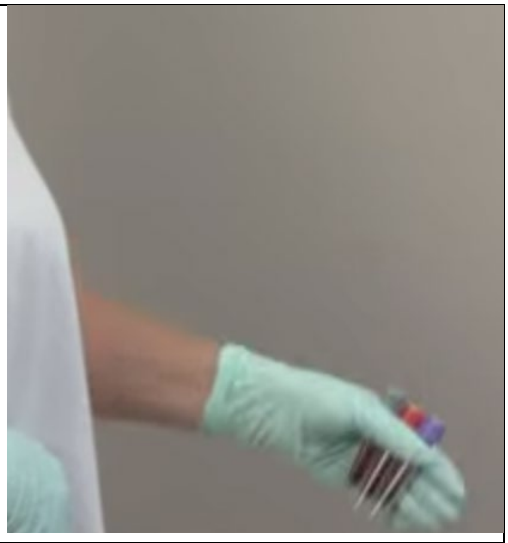
a. After gently shaking, the entire inner surface of each tube should be coated with blood.

b. Proper shaking will lead to frothing of the blood, which is required for correct performance of the test.

c. Over-energetic shaking may cause gel disruption and could lead to inaccurate results.

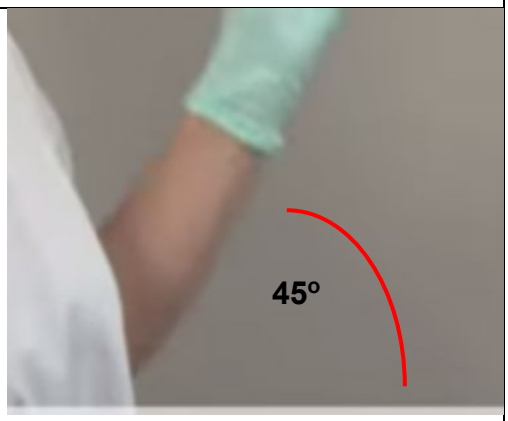
xv.

xvi.



xvii.

xviii.



xix. Scan the QR code on the blood tubes into the Mobenzi database and place QR code on the transportation list.

xx. Place tubes UPRIGHT into 37°C portable incubator as soon as possible within **2 hours after collection**. Use Mobenzi database to record time of placing tubes in the incubator (by scanning the QR code). (**do not freeze or refrigerate blood samples** prior to incubation and centrifugation). Close the incubator cover and make sure that it is tightly closed.

xxi. All of the tubes will be placed into kit-rack. The thin-film will be covered whole tubes to keep inline inside portable incubator for transportation.

xxii. Record the time in the portable incubator in the Mobenzi.

xxiii. Clean hand with disinfection liquid.

xxiv. Before transporting samples to the laboratory, one designated staff member must check list of collected specimens (in Mobenzi) and ensure that they have all be checked off as entered into the incubator. Transport portable incubator to the laboratory as soon as blood collection is complete for the day, using motorbike +/- other necessary transport means. It is very important that transportation of portable incubator should not be delayed.

12. Processing and storage of blood tubes in the CSDP laboratory

(See also Process the QFT blood tubes as follows: http://tb.gnowee.net/videos https://www.youtube.com/watch?v=mpj_q6PDjnk)

i. Once blood samples have been received in the laboratory, cross-check these samples with the transportation list and inform the field team of the total number received.

ii. Transfer the tubes from the portable incubator to the laboratory 37°C incubator and continue incubation for a total of 22 hours (no longer than 24h).

iii. Tubes will be divided into groups by collecting time. The timetable sheet will be filled and placed on the incubator door. This will be used to guide staff about when to remove tubes from the incubator.

iv. Scan blood samples (QR code) into the Mobenzi database to record laboratory receipt of sample at the time they are removed from the laboratory incubator, just prior to centrifuging. That will serve to both record receipt in the lab and date/time stamp the time of centrifuging.

v. After incubation, centrifuge the tubes for 15 minutes at 2500 RCF (g) (***in accordance with the specific centrifuge and rotor used). The **gel plug will separate the cells** from the plasma. If this does not occur, re-centrifuge the tubes at 3500 RCF. The blood tubes should be centrifuged immediately (within 30mins) after removal from the incubator.

vi. After centrifugation, store the QFT tubes at 2 – 8°C (Mon/Thurs), prior to sending to Pham Ngoc Thach (PNT) laboratory for ELISA testing.

13. Sending QFT tubes to Pham Ngoc Thach (PNT) laboratory in Ho Chi Minh City

i. Packing: All of the tubes will be placed into kit-rack. The thin-film will be covered whole tubes to keep inline.

ii. Put in tubes fix in the rack.

iii. Pack the Thermocol Packaging Box.

iv. Label the Box with signal: upward signal, biohazard, address & telephone number of HCMC staff.

v. Attach a log book with lists of samples and QR codes, a log of temperature during incubation (automatically recorded by thermometer in the incubator, need to plug into computer to get information).

vi. Inform PNT-HCMC staff of the time (sending & receiving), name & telephone number of the driver.

vii. The samples will arrive in HCMC on the Tue/Wed morning every week. PNT lab will sign to confirm they have received samples on the database.

Items	Ca Mau Post Office
Sending time: Every Mon/Thu	At 4:00 PM
Receiving time (with warranty)	Before 9: 00 AM every Tue/ Fri

14. ELISA procedure in PNT

(see also https://www.youtube.com/watch?v=eFl2KiU6e4g)

i. Within one week, PNT lab will carry out QFT test and send the raw data and calculated QFT results to project coordinator (phuong,nguyen@sydney.edu.au).

FIGURE 2. Recommended Sample Layout for Nil, TB Antigen & Mitogen Tubes (28 tests per plate)

Row	1	2	3	4	5	6	7	8	9	10	11	12
A	1N	1A	1M	S1	S1	S1	13N	13A	13M	21N	21A	21M
B	2N	2A	2M	S2	S2	S2	14N	14A	14M	22N	22A	22M
C	3N	3A	3M	S3	S3	S3	15N	15A	15M	23N	23A	23M
D	4N	4A	4M	S4	S4	S4	16N	16A	16M	24N	24A	24M
E	5N	5A	5M	9N	9A	9M	17N	17A	17M	25N	25A	25M
F	6N	6A	6M	10N	10A	10M	18N	18A	18M	26N	26A	26M
G	7N	7A	7M	11N	11A	11M	19N	19A	19M	27N	27A	27M
H	8N	8A	8M	12N	12A	12M	20N	20A	20M	28N	28A	28M

- S1 (Standard 1), S2 (Standard 2), S3 (Standard 3), S4 (Standard 4).
- 1N (Sample 1. Nil Control plasma); 1A (Sample 1. TB Antigen plasma); 1M (Sample 1. Mitogen Control plasma).

1. _____

ii. PNT lab will enter the following results of the QFT assays into the Mobenzi database:

a. ELISA assay results for each tube

b. Calculated QFT results from TB Gold IT analysis software (version 2.62). The software will automatically analyze the raw data; performs a quality control assessment of the assay, generates a standard curve and provides a test result for each participant. The “Interpretation of Results” as below:

cellestis

Version 2.62

QuantiFERON®-TB Gold In-Tube Results

Run Date: Thứ Tư 24 Tháng Sáu 2015
Operator: võ ph□m thùy d□□ng
Run Number: 02
Kit Batch Number: 05940201

Valid ELISA test run.

Results (IU/mL)

Subject ID	Nil	TB Ag	Mitogen	TB Ag-Nil	Mitogen-Nil	Result
3100061 đ□m	0,09	0,34	5,59	0,25	5,50	NEGATIVE
3100062 thâm	0,30	6,27	> 10	5,97	> 10	POSITIVE
3100063 chánh	0,05	4,28	> 10	4,23	> 10	POSITIVE
3100064 tinh	0,45	0,28	> 10	-0,17	> 10	NEGATIVE
3100065 xè	0,05	0,05	> 10	0,00	> 10	NEGATIVE
3100066 khoa	0,14	0,05	> 10	-0,09	> 10	NEGATIVE
3100067 khoa 52	0,17	0,61	> 10	0,44	> 10	POSITIVE

15. Returning results of QFT test and chest x-ray to participant’s parental/caregiver

i. Once per week (on Thursday) the Project Officer will check the results of the QFT testing.

ii. For participants with a negative QFT result, ACT3 will send out a SMS result notification as follows:
"The blood test result does not show evidence of TB infection at this time."

iii. For participants with a indeterminate or positive QFT result, the PO will telephone the participant and explain the result as follows:

"The blood test we have performed suggests that you have been exposed to someone with TB in the past and may be at risk of becoming unwell with TB in the future. We would like you to come to CSDP to have a chest x-ray to look for signs of disease in your lungs that may need treatment now. We will pay for this x-ray and also subsidise the cost of travel to CSDP according to ACT3 cost norms"

16. -Further investigations and interview at CSDP: The participant together with parental/caregiver will complete the following:

i. Room #8 (Interview room): Lab coordinator who will explain the plan for further investigations and interviews.
ii. Room #9 (CSDP consultant room): CSDP doctors will conduct: (1) A health exam; (2) Write chest x-ray requests and (3) Send patient to CSDP reception with these forms to pay for the tests
iii. CSDP Radiology staff will perform chest x-ray and give the result to participant's parental/caregiver
iv. CSDP doctor will conduct clinical assessment follow NTP decision #..... (Updated)
v. Finally, the Project Officer will review all study documents, enter chest xray milestone and the Clinical Assessment Form into the Mobenzi database:

17. Roles and Responsibilities

i. Chief Investigators

The chief investigators will have the overall authority and responsibility for the research. Responsibilities include:

- Planning the implementation of the study, arranging human resources, and developing the SOPs and budget for the project
- Overseeing implementation and quality assurance

ii. Project Officer

This project officer will be responsible for oversight and supervision of all the activities related to the ACT3C2 from the field to lab. The project officer will work closely with the project co-ordinator and medical/scientific adviser to ensure that all procedures are carried out correctly. Any issues/problems are discussed and addressed promptly. Responsibilities include:

- Preparing training, implementation, data management and monitoring of progress and data quality
- Working with the field & laboratory staffs in training and supervision of tasks
- Day to day oversight of study preparation and implementation
- Communicating test results and further necessary investigations to study participants
- Co-ordinating and supervising communication (verbal, SMS and telephone), to the study participants about test results and necessary further investigations
- Checking all consent forms received at the Hoa Lu office after each Ap.

iii. ACT3 Team Leaders and Vice-team Leaders

The team leaders and vice team leaders will be responsible for directly supervising the field staff and ensuring their tasks (listed below) are completed satisfactorily. They will assist the field staff in completing the project tasks when necessary. They will follow the manual of procedures and receive guidance from and report to the chief investigators and project officer.

iv. ACT3 Field staff

The field staff will be responsible for recruiting participants in the ACT3C2 study. This will involve tasks such as: explaining the study to the participants, obtaining consent for ACT3C and referring those participants to CSDP for blood collection, taking blood samples, ensuring blood samples arrive safely in the CSDP laboratory, entering results into the Mobenzi database, preparing weekly reports, reporting adverse events, arranging appointments for patients, liaising between the field team and the laboratory staff, as well as discussing problems or other work related issues with the team leaders, project co-ordinators and chief investigators when required. They will follow the manual of procedures and receive guidance from and report to the chief investigators and project officer.

v. ACT3 Laboratory staff

The ACT3 laboratory staff in this project will be responsible for ensuring prompt and appropriate handling and processing of blood samples and correct storage of samples. They will ensure sufficient stock of consumables in the laboratory, report any problems with equipment in laboratory or aberrant results, liaise with field staff and CSDP staff, report adverse events and discuss problems or other work related issues with the project co-ordinators and chief investigators when required. They will follow the manual of procedures and receive guidance from and report to the chief investigators and project officer.

vi. Non-WIMR staff

1. Radiographer in CSDP

   The radiographer in CSDP will be responsible for taking chest x-rays for participants in the ACT3C2 study with positive QFT results who gave consent. The radiographer will receive information about this study and guidance from the project officer.

2. Doctor in CSDP to clinically assess participants with abnormal chest x-ray

   Participants with potentially clinically significant abnormalities on chest x-rays will be invited to see the CSDP doctor (or their own doctor). Those who choose to see the CSDP doctor will be clinically assessed in the context of the chest x-ray findings and the Xpert test result. The doctor will be remunerated by WIMR for this service.

18. Equipment and Materials

i. Blood collection equipment

- QFT-plus tubes x 4 (nil control, TB1, TB2 & mitogen control) Catalogue Number: 622526. Storage conditions for QFT-plus tubes (store tubes upright): 17-25°C
- ELMA cream & tape (for covering the cream), pen
- Timer x 2
- Thermometer x 4 (for 2 portable incubators & lab incubator).
- Vacutainer Safety-lok Blood collection set: Wingset 23G, Holder, ”purge” tube
- Tourniquets
- Alcohol pads
- Urgosterile
- Band-Aids or adhesive tapes
- Gloves
- Gauze or cotton balls
- Alcohol hand rubs
- Portable sharps containers
- Racks to hold blood tubes inside the portable incubator & esky
- Portable incubators (37°C) x 2
- Esky for storing QFT-plus tubes to the field (17-25°C) x 2
- Thermometer x 4 (for 2 portable incubators & lab incubator)

ii. For laboratory in CSDP

- Centrifuge
- Laboratory incubator (37°C)
- Electronic Thermometer for lab incubator x 1
- Racks to hold and process blood tubes in laboratory/field
- Gloves, lab coat and for lab staff when handling blood

iii. For laboratory in Pham Ngoc Thach Lab

- QuantiFERON®-TB Gold Plus (QFT®-Plus), Catalog no. 622120
- QuantiFERON® Control Panel, REF: 0594-080
- Consumable for ELISA: filter tips, alcohol,

19. Safety procedure when collecting and handling blood

i. Collecting blood in the field

- All blood samples should be considered and handled as potentially infectious (i.e. universal precautions).
- All open cuts and abrasions should be covered when collecting blood.
- Gloves should always be worn when collecting blood and discarded appropriately after blood collection for each participant.
- Alcohol based hand wash should be used after collecting blood from each participant after removal of gloves.
- After blood collection, the needle should be discarded immediately in the biohazard sharps container (by the person who collected the blood). The sharps container must be within an arm’s length of the blood collector.

- Any needle stick injuries must be reported immediately to the field staff leader and an adverse event report completed. Every needle stick injury event will be assessed by a medical officer (part of ACT3 team) for appropriate management in accordance with the WIMR SOP.

ii. Handling blood in the laboratory

- Lab coats, gloves and eye protection should be worn when handling blood samples.
- All blood samples should be considered and handled as potentially infectious (i.e. universal precautions).
- All open cuts and abrasions should be covered when handling blood.
- Use disposable equipment wherever possible and dispose of equipment in the appropriate biohazard waste containers
- Use sealed tubes for centrifuging blood samples. Use sealed rotors to minimise contamination in the event of tube failure
- Laboratory benches and other areas where blood has been handled must be cleaned and decontaminated at the completion of work with disinfectant e.g. 1% sodium hypochlorite or other appropriate solution
- Tubes arriving from the field that are broken or leaking should not be processed, but should be discarded in the appropriate biohazard container. The team leader should be notified for appropriate investigation and a note made in the Mobenzi database that this sample could not be processed.

iii. Cleaning blood spills in the laboratory

- Any blood spills on floors, benches or equipment must be cleaned up immediately with disinfectant e.g. 1% sodium hypochlorite or other appropriate solution.
- For spills on a flat surface, cover the spill with a paper towel soaked with disinfectant and allow to stand for 10-15 mins before discarding and wiping the area again until clean
- In the event of a failure of tubes in the centrifuge, the centrifuge rotor and bowls should be disinfected e.g. with 1% sodium hypochlorite or other appropriate solution.

20. Reporting of adverse events

The team leader or vice team leader will record any adverse events in an Adverse Events form. This will be submitted to a Project Officer after each Ap's screening. For any urgent clinical adverse event, a PO will be contacted on the same day.

Adverse events would include:

- Abnormal symptoms in participant during blood collection: e.g. fainting, excessive bleeding, excessive trauma to the venipuncture site, etc.
- Needle stick injury to staff
- Accidents that happen to staff or research participants during the activity (e.g. falls, motorbike accidents, etc.)
- Any complaint made by study participants or local leaders

For each adverse event, an outcome must be recorded. The team leader or vice team leader must take responsibility for following up the adverse event within a reasonable period of time (less than 1 week, or less if appropriate).

21. Forms and Information Sheets

Form code	Description	Details
PIS1	Participant information sheet	To provide study information to potential participants of the ACT3C2 study
CF1	Consent form	Consent form for participants in the ACT3C2 study if they agree to participate in the study and have blood collected
QFT (+)	QFT result information sheet	Information sheet for individuals having a positive QFT test (describing their options in detail)
A3	Clinical Assessment	Clinical Assessment for children
QFT1	QFT transportation list	Log sheet for QFT blood collection in the field. Time of blood collection and start time of incubation in portable incubators.
QFT2	QFT transportation list	List of participants for processed QFT tubes to be send to PNT for ELISA
TP1	Incubator temperature sheet	Log sheet to keep daily record of lab-incubator temperature
BR1	Laboratory blood sample receipt book	Forms compiled into a booklet that records date and number of blood samples received in the lab
AE1	Adverse events form	Report of adverse events during study

22. Appendices:

i. Key forms (as per above, particularly the Adverse Event reporting form)
ii. NTP/ACT3 protocol for managing Xpert MTB positive participants
iii. Consider Section on monitoring and evaluation and forms for this.

23. Reference

1. Marks GB, Nguyen NV, Nguyen TA, Nguyen HB, Tran KH, Nguyen SV, et al. Prevalence of latent tuberculosis infection among adults in the general population of Ca Mau, Vietnam. Int J Tuberc Lung Dis. 2018; 22(3):246-51.

Community-wide screening for tuberculosis in a high prevalence setting

Data analysis plan

Defining the study population (Table S1)
The study population is derived from three datasets:

1. An initial "Household enumeration" form that included information on
    a. The household,
    b. Individuals who were present at the time of the initial enumeration
    c. Individuals who were screened at the time of the initial enumeration
2. An "Add Household member" form, which was linked to the initial household enumeration by household ID, and that included information on
    a. Individuals who were present at the time of a second or subsequent visit to the household
    b. Individuals who were screened at the time of this second or subsequent visit.
3. A "Screening data" form, which was linked to previously enumerated individuals (from the one of the two previous forms) and that included information on
    a. Individuals who were screened at a visit subsequent to their initial enumeration visit.

The first phase of defining the study population required merging the data from these three forms to create a single dataset containing all enumerated individuals and their screening data. This was done separately for each of the four years of the study.

This dataset contained the following relevant data:

- Identifying data for the household including: sub-commune, sub-sub-commune (the smallest address unit), head of household name (used to identify households)
- Identifying information for individuals within the household: a unique, anonymous PID was assigned
- Age (calculated from date of birth and date of survey) and sex
- Indicator for whether the person was age-eligible (that is, calculated age ≥ 15 years)
- Indicator for whether the person was capable of giving informed consent
- Indicator for whether the person had given verbal consent to proceed
- Responses to questions on cough, sputum and haemoptysis
- Response to question on smoking status
- Indicator variable for whether the person was randomly selected to proceed to more additional questions (on education, occupation, health insurance status and diabetes)
- Indicator variable for whether participant attempted to give a sputum specimen.
- Responses to questions on education, occupation, health insurance status and diabetes.

The following populations were defined (see Table S1):

1. Number of households enumerated
2. Number of individuals age ≥ 15 years enumerated (the eligible population)
3. Number of eligible individuals contacted to seek consent (= number of individuals in whom capacity to give consent was assessed)
4. Number of eligible individuals assessed as capable of giving consent.
5. Number eligible individuals who gave verbal consent to participate.

The last of these, the number of consenting individuals, was the denominator population for the estimation of prevalence.

Describing the study population (Table 1)
Information on the age, sex, prevalence of cough, sputum, coughing up blood and smoking status was estimated for the eligible study population in year 4, classified by randomization group. Information on education, occupation, health insurance status and diabetes was estimated for the eligible study population who were randomly selected to complete the additional questionnaire in year 4, classified by randomization group.

Defining endpoints
Xpert MTB results for each sample test were classified as positive (detected), negative (not detected), or error/invalid. As described above, where error or invalid results were obtained the specimen was re-tested or, if this was not possible, a further specimen was collected and re-test (if possible). Hence, some participants had more than one Xpert MTB result. These results were merged so that finally, only one (the last) Xpert test was used for each participant. The number of participants with valid Xpert MTB results was calculated and reported in Table S1.

The primary endpoint was the prevalence of Xpert MTB positive results. The numerator for this prevalence was the number of Xpert MTB positive results. The denominator was the number of individuals who gave verbal consent to participate.

Two sensitivity analyses were conducted using data on mycobacterial culture and chest radiographs.

Each Xpert MTB positive individual was requested to produce two additional sputum specimens, which were referred for mycobacterial culture. Culture results were classified using the following hierarchical classification:

1. If either one or both sputum cultures were reported as *M. tuberculosis*, the case was defined as “MTB”.
2. Otherwise, if either of the two sputum cultures was reported as NTM, then case was defined as “NTM”;
3. Otherwise, if either of the two sputum cultures was reported as “no growth”, then the case was reported as “no growth”;
4. Otherwise, if either of the two sputum cultures was reported as “contaminated” then the case was reported as “contaminated”;
5. Otherwise the case was reported as “inconclusive”.

Each Xpert MTB positive individual was requested to have a chest radiograph and this was reported by two readers, as described above. These were classified as follows:

1. If both readers reported the chest radiograph as “consistent with TB” the case was reported as “chest radiograph consistent with TB”;
2. Otherwise, if both readers had reported the chest radiograph, the case was reported as “not consistent with TB”;
3. Otherwise, if one or both radiographs was missing or not reported, the case was reported as missing for this endpoint.

The numerator for the prevalence of “Xpert MTB positive and *M. tuberculosis* culture positive” was the number of participants with Xpert MTB positive results who were also defined as MTB culture positive. The denominator was the number of individuals who gave verbal consent to participate. was estimated

The numerator for the prevalence of "Xpert MTB positive and either *M. tuberculosis* culture positive or chest radiograph consistent with TB" was the number of participants with Xpert MTB positive results who were either also defined as MTB culture positive or also had a chest radiograph defined as "consistent with TB". The denominator was the number of individuals who gave verbal consent to participate. was estimated

Defining covariates

Age was calculated as difference between the date of screening and the date of birth, reported at the time of household enumeration. Sex was recorded at the time of enumeration. Smoking status was classified based on self-report.

Regression modelling

In order to enable the enable the fitting of the log-binomial model using maximum likelihood with adaptive quadrature in SAS Proc Glimmix, it was necessary to fit an initial generalised linear model excluding the random effect and use the parameter values from that model as the initial values for the generalised linear mixed model. The default Newton-Raphson optimization method was used with a maximum of 10 iterations allowed. We specified the residual method for estimating the denominator degrees of freedom.

The following code was used in SAS

```
%macro glimmix(TB=, age=, gender=, cigs=, cov=);

  ods output Glimmix.ParameterEstimates=param
(where=(effect='study_group'));
proc glimmix data=Table4 startglm  inititer=10 method=quad ;
      class ap &gender &cigs;
      model &TB = study_group &age &gender &cigs / link=log dist=bin cl
ddfm=residual ;
      random  int/ subject=Ap ;
      nloptions technique=newrap;
   run;

 data param2_&TB.&cov;
 set param;
 RelRisk = exp(estimate);
 LCL = exp(Lower);
 UCL= exp(Upper);
 method = 'log-binomial with random intercept';
 TB="&TB";
 adj="&cov";
 keep RelRisk LCL UCL DF probT method TB adj;
 run;

%mend glimmix;

%glimmix (TB=Xpert);
%glimmix (TB=Xpert, age=CalculatedAgeYears, gender=gender, cov=_adj1);
%glimmix (TB=Xpert, age=CalculatedAgeYears, gender=gender, cigs=cigarettes,
cov=_adj2);
%glimmix (TB=MTB_culture);
%glimmix (TB=MTB_culture, age=CalculatedAgeYears, gender=gender,
cov=_adj1);
%glimmix (TB=MTB_culture, age=CalculatedAgeYears, gender=gender,
cigs=cigarettes, cov=_adj2);
%glimmix (TB=composite);
%glimmix (TB=composite, age=CalculatedAgeYears, gender=gender, cov=_adj1);
```

```
%glimmix (TB=composite, age=CalculatedAgeYears, gender=gender,
cigs=cigarettes, cov=_adj2);


data param2;
```