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**Incremental yield of the GeneXpert MTB/RIF Assay for the  
Diagnosis of Pulmonary Tuberculosis in congregates.  
Case of Muhanga prison/South Province/Rwanda.**

Submitted in partial fulfillment of the requirements for the award of the Degree  
of Master of Medicine in Internal Medicine.

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**March, 2014**

## DECLARATION

I declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in Internal Medicine at University of Rwanda and has not been submitted before for any degree or examination in any university.

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## ACRONYMS AND ABBREVIATIONS

‰: percentage

AFB: Acid-Fast Bacilli

ARVs: Antiretroviral drugs

BCG: Bacillus Calmette-Guérin

CHUK: Centre Hospitalier Universitaire de Kigali

CXR: Chest X-Ray

DH: District Hospital

DRC: Democratic Republic of Congo

DST: Drug Susceptibility Testing

HIV: Human Immuno-Deficiency Virus

LED: Light Emitting Diode (fluorescence microscopy)

LJ: Löwenstein-Jensen (medium)

MDG: Millennium Development Goals

MDR-TB: Multidrug-Resistant Tuberculosis

MININTER: Ministry of Internal Security

ml: milliliter

MoH: Ministry of Health

MTB: Mycobacterium Tuberculosis

NAA: Nucleic Acid Amplification

NRL: National Reference Laboratory

PCR: Polymerase Chain Reaction

PTB: Pulmonary Tuberculosis

RBC/TB & ORD: Rwanda Biomedical Center/TB and Other Respiratory Diseases

RCS: Rwanda Correctional Services

SPSS: Statistical Package for the Social Sciences

TB: Tuberculosis

UK: United Kingdom

USA: United States of America

WHO: World Health Organization

XDR-TB: Extensively Drug-Resistant TB

Xpert MTB/RIF: GeneXpert Mycobacterium Tuberculosis/Rifampicin



## **DEDICATION**

To the Only Almighty, Our God.

To my family.

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## ABSTRACT

**Background:** Tuberculosis remains a major public health problem worldwide and particularly in prison populations. Most of countries are still using conventional tests for TB diagnosis. The aim of this study was to evaluate the incremental yield of Xpert MTB/RIF assay compared to sputum microscopy for TB diagnosis in congregates case of Muhanga prison, Rwanda.

**Methods:** We conducted an analytical, prospective and longitudinal study of TB case detection among 5868 prisoners and 78 staff members of Muhanga prison from 9<sup>th</sup> December, 2013 to 16<sup>th</sup> January, 2014. Sputum samples from TB suspects (abnormal CXR, normal CXR with cough) were analysed by Xpert MTB/RIF and Auramine staining microscopy.

**Results:** Among 706 inmates and 5 staff members who were considered TB suspects, a total of 53 TB cases were detected: 20 cases were found on sputum microscopy and 50 on Xpert MTB/RIF tests. Three sputum samples positive on microscopy were not tested by Xpert MTB/RIF due to poor quality. All sputum samples positive for TB on microscopy controlled by Xpert MTB/RIF were found positive. The incremental yield of Xpert MTB/RIF compared to sputum microscopy was 66%. The prevalence rate of pulmonary TB at Muhanga Prison was 7507/100 000, approximately 65.9 times higher compared to the general population (114/100 000). 14.9% of inmates presented with more than one TB cardinal symptoms. Lung infiltrates were predominantly found on CXR (48.6%). One case (1.9%) of RIF resistance was detected by Xpert MTB/RIF. All TB cases detected were male prisoners who were incarcerated for more than 24 months. 17.7% of these prisoners were underweight. Malnutrition ( $BMI < 18.5 \text{ kg/m}^2$ ) was associated with TB disease ( $p=0.007$ ). Among TB suspects, 7.2% were HIV positive. Even if 94.1% of them have already started ARVs, 20.8% had CD4 count less than 200 cells/ $\mu\text{l}$ .

**Conclusion:** The study results showed a high prevalence of TB in Muhanga prison. Xpert/MTB/RIF clearly provided an important incremental yield.

## **CHAPTER 1 INTRODUCTION**

### **1.1 INTRODUCTION TO TUBERCULOSIS**

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB) [Koch's bacillus] in the majority of cases. The bacilli enter the organism by inhalation and reach the lungs, from which they may propagate throughout the entire organism [1,6]. Symptoms of pulmonary TB (PTB) include cough, fever, night sweats, weight loss, fatigue, anorexia, hemoptysis and chest pain. Symptoms and signs of extrapulmonary TB depend on the site of disease [2,5].

As an airborne disease, TB propagates in the crowded, poorly ventilated environments in congregates (eg prisons) in many parts of the world. In prison, the psycho-social conditions including malnutrition and stress can contribute to higher risk of developing TB disease.

In addition, poor diagnostic tools and inadequate or inaccessible medical care can lead to inaccurate treatment outcomes and acquisition of resistance [15].

Muhanga prison accommodates around 5868 (10.6 %) of all prisoners in Rwanda. Prisoners are among TB risk groups like people living with Human Immuno-Deficiency Virus (HIV) and others to whom World Health Organization (WHO) recommends the screening for active TB to improve early TB detection using tests, examinations or other procedures that can be applied rapidly [15,16].

The most common method for TB diagnosis worldwide is sputum smear microscopy which was developed more than 100 years ago. The use of rapid molecular tests for the diagnosis of TB and drug-resistant TB is increasing worldwide following recent developments in TB diagnosis [1].

WHO for TB control prioritizes earlier and improved TB case detection, including identification of smear negative disease as well as expanded capacity to diagnose multidrug-resistant tuberculosis (MDR-TB) [3,18]. For this reason, in December 2010, WHO approved and recommended the use of a new molecular test; geneXpert MTB/RIF [(Cepheid, Sunnyvale, United States of America (USA))] (hereafter referred to as Xpert MTB/RIF), that can simultaneously detect TB and rifampicin (RIF) resistance in two hours [4,5,6].

## 1.2 BACKGROUND

Tuberculosis remains a major public health problem worldwide, particularly in prison populations, especially in low and middle income countries [15,25].

According to WHO report 2011 global TB, in 2009, about 9.7 million children were orphans as a result of parental deaths caused by TB. Globally in 2010, an estimated 8.8 million incident cases of TB, corresponding to 128 cases per 100 000 population were reported; 1 to 1.2 million of them were coinfecting with HIV and the African region alone accounted for 82% of TB cases among people with HIV. In this year 35% (0.35 million) of TB patients with HIV positive and 14% (1.1 million) with HIV negative passed away. Most of the estimated number of cases in 2010 occurred in Asia (59%) and Africa (26%) whereas smaller proportions of cases occurred in the Eastern Mediterranean Region (7%), the European Region (5%) and the Region of the Americas (3%) [6].

Twelve million prevalent cases of TB were estimated worldwide in 2011, that's equivalent to 170 cases per 100 000 population. The incidence of TB in 2011 was estimated at 8.7 million among them 13% were coinfecting with HIV. In the same year, 1.4 million people died from TB, including almost one million (70%) deaths among HIV negative individuals and 430 000 (30%) among people who were HIV positive. In addition TB was one of the top killers of women, with 300 000 (30%) and 200 000 (46%) deaths among HIV negative and positive of all death respectively [8].

The five countries with the largest number of incident cases in 2011 were India (2.0–2.5 million), China (0.9–1.1 million), South Africa (0.4 –0.6 million), Indonesia (0.4 –0.5 million) and Pakistan (0.3–0.5 million). India and China alone accounted for 26% and 12% of global cases, respectively (almost 40% of the world's TB cases); 60% of cases were in the South-East Asia and Western Pacific regions whereas the African region had 24% of the world's cases [8].

The proportion of TB cases coinfecting with HIV was highest in countries of the African Region; overall, 39% of TB cases were estimated to be coinfecting with HIV in this region, which accounted for 79% of TB cases among people living with HIV worldwide in 2011 [8]. In 1995-2011, 51 million people were treated with success for TB in countries that had adopted the WHO

strategy, saving 20 million lives. In 2010, the incidence of MDR-TB was globally 290 000 cases and prevalence of 5.4% that is 650 000 cases among the world's 12 million cases of TB [8].

"High burden" countries for MDR-TB in 2011 includes China with 5.7% of estimated new TB cases with MDR-TB, Democratic Republic of Congo (DRC) (3.1%) and India (2.1 %) [10]. India, China, the Russian Federation and South Africa have almost 60% of the world's cases of MDR-TB and the highest proportions of TB patients with MDR-TB are in Eastern Europe and central Asia. Almost 80% of TB cases among people living with HIV reside in Africa [8]. The WHO reported an estimated 450 000 new MDR TB cases worldwide in 2012 and 9.6% of them had extensively drug resistant TB with 18129 (40%) notified MDR –TB cases in Africa [15].

In 2011 in the United Kingdom (UK), a total of 8,963 cases of TB were reported with a rate of 14.4 cases per 100 000 populations [7]. Whereas in 2009, the incidence of TB in Turkey was nearly 27.9 per 100 000 population and the proportion of MDR TB cases among new cases was 2.9%, that among previously treated cases was 15.5%, and that among all TB cases was 4.9% [9]. Uganda, with a population of 34 million, has a high prevalence of 7.3% and TB notification rate of 40 000 cases and thus is ranked 16<sup>th</sup> out of 22 high TB burden countries. In all Africa there are 2 500 000 TB cases over 857 382 000 population (291.6/100 000) according to WHO report 2011 on global TB control [6,10].

In Rwanda, the global prevalence survey has been conducted but the results are not yet published. However, according to 2013 WHO report, the incidence rate of TB was 290/100 000 in 1990, 513/100 000 in 1995, 106/100 000 in 2010, 94/100 000 in 2011 and 86/100 000 in 2012 where the prevalence rate of 521/100 000, 487/100 000, 128/100 000, 121/100 000 and 114/100 000 respectively [15].

The targets linked to MDGs and approved by the Stop TB partnership are to reduce the prevalence and death rates by 50% in 2015 compared with their levels in 1990 and to eliminate TB as a public health problem, defined as a global incidence of active TB of less than one case per 1 million population per year by 2050 [11,15,21].

Conventional tests for TB diagnosis include acid-fast bacilli (AFB) smear microscopy, which can produce results in 24 hours, and culture, which requires 2-6 weeks to produce results. Although rapid and inexpensive, AFB smear microscopy is limited by its poor sensitivity (45%-

80% with culture-confirmed pulmonary TB cases) and its poor positive predictive value (50%-80%) for TB in settings in which non-tuberculous mycobacteria are commonly isolated [12].

Diagnosis of TB is often delayed in resource limited settings and factors contributing to this delay include patient delays, health-system delays and delays inherent to the conventional TB diagnostic process. The consequences of delayed TB diagnosis and treatment include increased TB-related morbidity, increased mortality, and continued TB transmission. In countries with a high burden of HIV, HIV infection has reduced the sensitivity of smear microscopy contributing to delays in TB diagnosis, while concomitantly increasing the urgency in which a rapid TB diagnosis is required [13].

Currently, only 28% of expected incident cases of TB are detected and reported as smear positive. Although the culture is more sensitive than the smear microscopy, it requires biosafety measures, and needs specialized laboratory personnel. This leads to a diagnostic delay that impedes disease control, and increases healthcare costs [5].

Since the discovery of the polymerase chain reaction (PCR), a large number of molecular techniques have been developed. However, their sensitivity is greatly dependent on the efficiency of the sample preparation, deoxyribonucleic acid (DNA) extraction and the presence of PCR inhibitors. Therefore, simple, rapid and effective methods for TB diagnosis have been developed [5, 14]. One of latest assays “Xpert MTB/RIF assay” detects both MTB with sensitivity of 99% and more than 80% in patients with smear-positive and smear-negative pulmonary TB respectively and RIF-resistance by PCR amplifying five overlapping probes complementary to the rifampicin resistance-determining region (RRDR) of the MTB *rpoB* gene and subsequently probes this region for mutations that are associated with RIF-resistance. This test has sensitivity similar to culture on solid media and highly superior to that of smear microscopy. This high sensitivity of Xpert MTB/RIF makes this diagnostic tool useful in the diagnosis of TB in people with HIV co-infection, where the sensitivity of smear microscopy alone is low; 66.7% in South Africa 2011 for example [6, 19, 20] (See appendix 5).

Using this new diagnostic tool; Xpert MTB/RIF gives results in two hours, and this assay requires minimal biosafety measures allowing the provider to start antituberculous drugs earlier

whereas TB diagnosis from microscopy may take up to two days, culture and drug susceptibility testing (DST) can then take weeks for detection of TB drug resistance [14,15].

In 2012 worldwide use of Xpert MTB/RIF was associated with a 42% increase in cases eligible for treatment of MDR-TB compared with 2011 according to 2013 WHO report [15]. A recent clinical study conducted in Uganda showed a high sensitivity (79%) and specificity (96%) of Xpert MTB/RIF for culture-positive MTB while its sensitivity was low (42%) among smear-negative TB cases [17]. In Peru Xpert MTB/RIF was found to be sensitive (97.8%) and specific (97.5%) to detect MTB in HIV positive patients [17].

XpertMTB/RIF increased yield of Mycobacterium (MTB) diagnosis from 12.8% to 20% compared to smear microscopy in India [19]. In Kenya both culture and Xpert MTB/RIF showed the same detection rate of MDR-TB from 9.4% of 1171 non-contaminated specimens found TB positive on culture while Xpert MTB/RIF diagnosed 8.6% as TB patients among 824 smear negative screened [19].

Culture remains the gold standard for confirmation of TB and is necessary for isolating bacteria for drug-susceptibility testing and genotyping. In accordance with current recommendations, sufficient numbers and portions of specimens should always be reserved for culturing. Nonetheless, nucleic acid amplification (NAA) testing might become standard practice for patients suspected as having TB in order to shorten the time needed to diagnose TB from weeks to hours [12].

The Xpert MTB/RIF was introduced in Rwanda in 2012. As a new diagnostic tool its indications are limited to any TB suspected patient with HIV positive; any symptomatic household contact with a MDR-TB patient; TB suspected cases who were investigated according to the TB algorithm [chest X-ray (CXR), antibiotics, clinical evaluation] and still remain TB suspect; TB suspects from prisons and other congregates; TB suspects from diabetic patients, TB suspects from Kigali City; cases of TB re-treatment; sputum smear positive at 2 months on anti-TB drugs and TB suspect among health care providers, TB suspects from children under 15 years old, patients with severe altered general condition, suspects cases of meningitis and other extrapulmonary TB [27] (See appendix 4).



In 2011, the laboratory network in Rwanda included 192 Centers for Diagnosis and Treatment (CDT) carrying out direct smear TB microscopy. Among which 9 were using LED Fluorescence microscopy and all others were using Ziehl Neelsen method. Persons who need sputum examination are either TB suspects or patients on anti-tuberculosis drugs to monitor response to treatment. These patients are sent to the laboratories from the Out Patient Department wards (OPD), HIV or TB clinics. In 2011, 175 091 TB suspects had a smear microscopy examination and 503,029 sputum smears were done in the laboratory network, of which 97% were for diagnosis and 3% for treatment monitoring. Positivity of diagnostic smears was 2.7% while it was 13% for treatment monitoring samples. Samples from patients at higher risk for MDR-TB were sent to the NRL or CHU for culture on LJ solid medium, Genotype MTBDR Plus (Hain) and DST. By the end of 2011, solid medium primary culture had been implemented at NRL and CHUK, while liquid culture by BACTEC MGIT was implemented at NRL in 2012.

To date, Xpert MTB/RIF is used by 16 health facilities: RNL, CHUK, RMH, Biryogo Health Center, Muhima, Rwinkwavu, Kibungo, Nyagatare, Byumba, Gihundwe, Kabutare, Nyanza, Ruhengeri, Gisenyi, Kabgayi and Kibuye District Hospitals. DST is performed at RNL, CHUK and CHUB. Fluorescent microscopies are now available at all DH [34].

The prevalence of TB among prisoners in all countries is always higher than the general population, on average 100 times greater and it is the most common cause of death [16,35,36]. There are a number of factors that facilitate the spread of disease among prisoners such as a greater chance of being exposed to the TB bacilli and therefore becoming infected since the majority of prisoners come from low socioeconomic and deprived social classes, and are often homeless and/ or addicted to drugs and alcohol. Prisoners are also more likely to suffer from poor immunity due to HIV infection, diabetes, malnutrition, drug addiction, mental and physical stresses, resulting in reactivation of TB infection in this group. Other predisposing factors, such as overcrowding in prison, long-term close contact in prisons and lack of access to adequate health services lead to undiagnosed or late diagnosis of TB. Poor compliance with TB treatment or incomplete treatment when prisoners are released before end of treatment enhance the spread of MTB-TB and leads to serious concern of TB infection control in the community [15,30,31].

It is estimated that the world's prisons hold 8-10 million prisoners and the level of TB in prisons has been reported even to be up to 100 times higher than that of the civilian population. Prisons are the reservoirs of TB and MDR-TB due to poor prison living conditions, overcrowding, the concentration of people at high TB risk, limited screening procedures and access to health services inside the prison delaying in TB diagnosis. This contributes to the poor outcome and disease transmission inside the prison as well as in the community [15,26].

In Africa as well as in Western Europe, TB infection in prison was high compared to civilian population; 41% of prisoners had active TB in Tanzania (in 2000). Both studies in Turkey and France showed high rates of TB prevalence: 341/100 000 and 215/100 000, respectively [22].

In 2010, the TB prevalence rate was 2227 per 100 000 in Bangladesh prison which was over 20-fold higher than the rate in the general population whereas in Thailand the rate was 568/100 000 and in Pakistan it was 657/100 000 which are eight and 3.75 times higher than the national rate, respectively [23,24].

According to a study which was conducted as part of a comprehensive programme of TB control in the central prison in Qazvin, Islamic Republic of Iran, a programme of active case-finding was conducted from February 2004 to July 2005; from the 768 prisoners examined, 41 (5.3%) were suspected of TB and gave sputum samples. A total of 7 smear-positive TB cases were found, giving TB prevalence in the prison of 910 per 100 000, which is 113 times the total TB prevalence in Qazvin province in the same year [32].

TB incidence in Colombian prisons is 20 times higher than in the general Colombian population; 72 cases among 1305 evaluated for TB (5.5%) in a study done in four prisons between 2010 and 2012 while the incidence of TB in the general population in Colombia in 2010 was 25 cases per 100 000 inhabitants [33].

In Rwanda, between 1996-1998, 3363 prison cases of all forms of TB per 100 000 were reported compared to 79.3 of all forms civilian cases per 100 000 in 1997 [29]. Nowadays, the population of prisons in Rwanda is estimated to be 55 000 people, and during a January –December 2012 report, there was a total of 177 TB cases, all forms included, i.e, 322/100 000 [Mininter, RCS ].

Regarding this high prevalence in prisons worldwide, WHO introduced the package of measures in order to reduce the TB burden in prisons including the early diagnosis with systematic screening, use of rapid diagnostic tests, infection control system, avoid malnutrition, supervision, better management of TB cases and treatment of co morbidities such as HIV, hepatitis, diabetes and better follow up even after their release into the general population under treatment [15].

Since its introduction in Rwanda in 2012, the Xpert MTB/RIF is not yet evaluated in terms of its accuracy in diagnosis of pulmonary TB and rifampicin resistance. Therefore, we aim to evaluate the incremental yield of the Xpert MTB/RIF assay for the diagnosis of tuberculosis and rapid detection of rifampicin resistance in suspected pulmonary TB patients in congregates e.g Muhanga prison population: staff and prisoners.

### **1.3 RESEARCH QUESTION**

What is the incremental yield of Xpert MTB/RIF assay for the diagnosis of TB and rapid detection of rifampicin resistance at Muhanga prison?

### **1.4 OBJECTIVES**

#### **1.4.1 Overall objective**

To determine the incremental yield of the Xpert MTB/RIF for the diagnosis of tuberculosis and rapid detection of rifampicin resistance in suspected pulmonary tuberculosis patients in Rwandan congregates.

#### **1.4.2 Specific objectives**

1. To compare the TB prevalence among suspects of Muhanga prison with the general population.
2. To assess the yield of Xpert MTB/RIF compared to sputum microscopy to diagnose PTB
3. To evaluate the incremental yield of Xpert MTB/RIF compared to sputum microscopy in TB diagnosis.
4. To evaluate the risk factors associated with TB disease among Muhanga prison population.

## **CHAPTER 2 MATERIALS AND METHODS**

### **2.1 STUDY DESIGN**

We conducted an analytical, prospective and longitudinal study in Muhanga prison from 9<sup>th</sup> December 2013 to 16<sup>th</sup> January 2014.

### **2.2 STUDY SETTING AND POPULATION**

Muhanga prison was opened in 1973. It is located in Rwanda, Southern province, Muhanga District, with 5868 prisoners (10.6% of all Rwandan prisoners), although it has a hosting capacity for 4500 inmates. At the moment of our study, males were 5438, females were 430 with living space of 0.76 m<sup>2</sup> and 1.48 m<sup>2</sup> respectively whereas the international committee of red cross recommended minimum space per prison of no less than 3.4 m<sup>2</sup> [28, RCS].

Muhanga prison has 78 staff members.

The prison has a dispensary for the prisoners and the prison staff provides medical services including diagnosis and treatment of TB and HIV. The HIV prevalence in Muhanga prison is estimated at 1 to 1.2%.

At the time of the study, 31 inmates (528/100 000) were on antiTB drugs for either pulmonary or extrapulmonary TB. At the moment of incarceration, prisoners are screened for TB cardinal symptoms (cough, fever, weight loss, loss of appetite, asthenia and night sweats).

Every inmate admitted to the prison is screened for TB by clinical assessment and microscopy if the prisoner has sputum and records are kept in medical file.

### **2.3 STUDY POPULATION**

#### **2.3.1 Sample size**

Study included any prisoner and staff member with cough of more than 2 weeks and/or abnormal CXR.

#### **2.3.2 Inclusion criteria**

All prisoners and prison's staff member with cough and/or abnormal CXR findings who accepted to sign a written consent.

### 2.3.3 Exclusion criteria

Any prisoner or staff member on antiTB treatment or refusal to participate.

## 2.4 STUDY PROCEDURE

Before the starting of this study, from November to December 2013, all the prisoners and staff members were identified using register books available in the prison administration.

Group information sessions were conducted by nurses who already work in prison dispensary together with peer educators to assess interest and written informed consent was individually obtained thereafter.

Participants that provided written informed consent were interviewed using a questionnaire containing questions on demographic, clinical variables, history of TB and smoking habits. A stadiometer with a precision of 0.1cm was used for measuring height while the subject standing without shoes. A weighing scale with a precision of 100 g was used for measuring weight.

Body mass index (BMI), defined as the weight (in kilograms) of the individual divided by the square of the height (in meters), was used to determine the nutritional status of the patients into malnutrition ( $BMI < 18.5 \text{ kg/m}^2$ ), normal ( $BMI = 18.5-25 \text{ kg/m}^2$ ) and overweight ( $BMI > 25 \text{ kg/m}^2$ ) as recommended by WHO [39].

After completion of the interview and anthropometric measurement, all participants underwent a standard posteroanterior CXR and those with any pulmonary, mediastinal or pleural abnormality on CXR or normal CXR with sputum were selected, to give 2 sputa for Xpert MTB/RIF and microscopy. All selected participants whose HIV serology status was unknown underwent HIV screening after provider initiated counseling. Recent CD4 counts (in last 6 months) were checked for HIV positive people.

A mobile digital x-ray machine was used for taking CXR images for all selected prisoners and staff and interpretation was done by a medical doctor at the site. At the same time, the images taken were sent to the central archive at Centre Hospitalier Universitaire de Kigali (CHUK) for a second reading by the radiologist who was blinded to the results of the other investigations.

CXR images were classified as “normal”, “abnormal non suggestive of TB” and “abnormal suggestive of TB” with details (infiltrates, cavity, pleural effusion, nodules etc).

Any study participant with an abnormal CXR non suggestive of TB or an abnormal CXR suggestive of TB to whom sputum microscopy and/or Xpert MTB/RIF was negative or who did not have sputum were referred to the nearest District Hospital (DH) for further investigations.

Peer educators were helping in the orientation of participants during the all activities.

## **2.5 SPUTUM SPECIMEN COLLECTION AND PROCESSING**

Participants suspected of TB after CXR were requested to produce two early-morning sputum specimens, (> 2mL each). Sputum samples were not induced, but collection was directly observed by the TB focal person.

One direct sputum specimen from each participant was analyzed onsite using Xpert MTB/RIF (Cepheid, Sunnyvale, CA) according to manufacturer's instructions; processed by use of N-acetyl-L-cysteine and sodium hydroxide (NALC–NaOH), followed by centrifugation, and then was suspended in 1.5 ml of phosphate.

Another sputum sample was stained and tested by AFB-smear microscopy using Auramine O staining. Sputum results were given to the prisoners by the laboratory technician.

## **2.6 DATA RECORDS AND ANALYSIS**

Entry and descriptive statistical analysis of collected data were performed using SPSS version 16 and EPIDATA softwares. Microsoft Words 2007 was used for text treatment and Microsoft Excel 2007 helped for graphs. The P-value < 0.05 was considered as statistically significant.

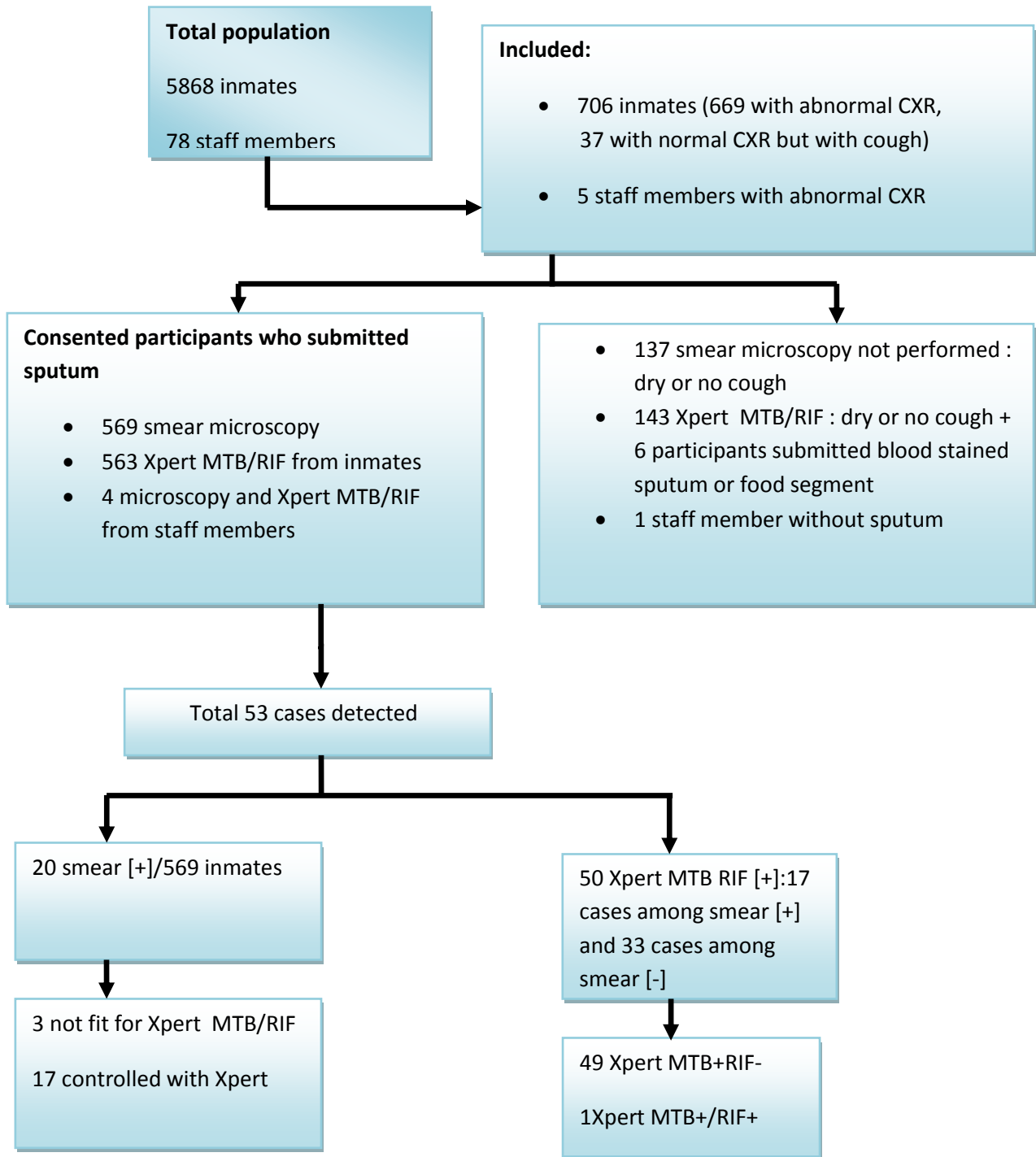
## **2.7 ETHICAL CONSIDERATION**

The study protocol was reviewed and approved by Faculty of Medicine Research and Ethics Commission. Ministry of Internal Security (MININTER), Rwanda Correctional Services (RCS), prison management staff was contacted through RBC/TB & ORD division and facilitated to carry out activities. Following the group information session, prisoners and staff expressing further interest in participation were met individually to be provided with explanation about the survey process and to seek written informed consent which contained all details about the study including her/his right of withdrawal at any time during the study. All collected information including prisoners and staff's participation status was kept strictly confidential.

## **CHAPTER 3 PRESENTATION OF THE RESULTS**

### **3.1 THE RECRUITMENT AND FINDINGS FLOWCHART OF INMATES AND STAFF MEMBERS.**

The Figure 1 provides information on recruitment of inmates and staff members who accepted to participate in our study. All participants underwent CXR. We included inmates and staff members with abnormal CXR and/or with cough and then gave sputum for Xpert MTB/RIF and for smear microscopy.



**Figure1: The recruitment and findings flowchart of inmates and staff members**



### 3.2 DESCRIPTIVE ANALYSIS

**Table 1: Demographic characteristics of inmates**

	Frequency	Percent
<b>Sex</b>		
female	18	2.5
male	688	97.5
Total	706	100
<b>Age in years</b>		
≤25	29	4.1
26-36	74	10.5
37-47	163	23.1
≥48	440	62.3
Total	706	100
<b>BMI ( kg/m<sup>2</sup>)</b>		
<18.5	125	17.7
>18.5	581	82.3
Total	706	100

97.5% of inmates were male and 2.5% were female. 62.3% of them were older 48 years and the median age was 51 years with standard deviation of 14.158. The mean BMI of the prisoners was 20.9 kg/m<sup>2</sup>. The malnutrition (BMI<18.5kg/m<sup>2</sup>) was found in 17.7% of inmates.

**Table 2: Inmates medical history and HIV status**

<b>TB in the past</b>	<b>Frequency</b>	<b>Percent</b>
no	603	85.4
yes	103	14.6
Total	706	100
<b>History of smoking</b>		
No	656	92.9
yes	50	7.1
Total	706	100
<b>HIV status</b>		
negative	655	92.8
positive	51	7.2
Total	706	100
<b>Currently on ARVs</b>		
No	3	5.9
yes	48	94.1
Total	51	100
<b>CD4 count (cells/<math>\mu</math>l)</b>		
<200	10	20.8
>200	38	79.2
Total	48	100
<b>Duration of stay in prison (months)</b>		
<24	68	9.6
>24	638	90.4
Total	706	100

14.6% of inmates reported a history of TB in the past (pulmonary or extrapulmonary TB), 7.1% were smokers before incarceration. 51 (7.1%) cases were HIV positive among them 3 were newly diagnosed so that their CD4 were unknown and had not yet started ARVs at the time of the study, 20.8% of HIV positive had CD4 less than 200 cells/ $\mu$ l. The majority of inmates (90.1%) have been in jail for more than 24 months. The median duration of stay in prison was 84 months with a minimum of 2 months and maximum of 240 months.

**Table 3: TB symptoms in inmates**

<b>Cough for more than 2 weeks</b>	<b>Frequency</b>	<b>Percent</b>
No	440	62.3
yes	266	37.7
Total	706	100
<b>Weight loss in the past 3 months</b>		
No	655	92.8
yes	51	7.2
Total	706	100
<b>Recent loss of appetite</b>		
No	648	91.8
yes	58	8.2
Total	706	100
<b>Recent chest pain</b>		
No	638	90.4
yes	68	9.6
Total	706	100
<b>Fever</b>		
No	660	93.5
yes	46	6.5
Total	706	100
<b>Night sweats</b>		
No	659	93.3
yes	47	6.7
Total	706	100

Chronic cough was found in 266 (37.7%) of prisoners. Fever, loss of appetite, weight loss and night sweats were present in 6.5%, 8.2%, 7.2% and 6.7% of examined prisoners respectively.

**Table 4: TB symptoms grouped in inmates**

<b>TB symptoms</b>	<b>Frequency</b>	<b>Percent</b>
Without symptoms	341	48.3
One symptom	260	36.8
More than one symptom	105	14.9
Total	706	100

14.9% of the prisoners were having more than one TB symptom and 48.3% reported not to have any TB symptoms.

**Table 5: CXR findings in inmates**

<b>CXR findings</b>	<b>Frequency</b>	<b>Percent</b>
Normal while coughing	36	5.1
Lung infiltrates	343	48.6
Lung nodule	53	7.5
Pleural effusion	133	18.8
Cavity	9	1.3
Hilar adenopathy	2	0.3
Sequelae	61	8.6
Infiltrates and pleural effusion	57	8.1
Infiltrates and cavity	8	1.1
Infiltrates and hilar adenopathy	2	0.3
Mediastinal adenopathy	2	0.3
Total	706	100

In our study, we found that the most common radiological abnormalities among prisoners were lung infiltrates (48.6%). 5.1% of participants had normal CXR. Pleural effusion, cavities were found in 18.8% and 1.3 % respectively and TB sequelae in 8.6%.

**Table 6: Sputum analysis results in inmates**

<b>Xpert MTB</b>	<b>Frequency</b>	<b>Percent</b>
not detected	513	91.1%
detected	50	8.9%
<b>Total</b>	<b>563</b>	<b>100</b>
<b>RIF resistance</b>		
not detected	552	98.0%
detected	1	0.2%
indeterminate	10	1.8%
<b>Total</b>	<b>563</b>	<b>100</b>
<b>Microscopy</b>		
negative	549	96.5%
positive	20	3.5%
<b>Total</b>	<b>569</b>	<b>100</b>

TB prevalence among Muhanga prisoners were 53 over 706 (7.5%) i.e 7507 per 100 000.

50 cases were positive on Xpert MTB/RIF and 20 cases on smear microscopy.

Xpert MTB/RIF was indeterminate in 10 of 563 (1.8%) tests performed. One case of RIF resistance (1.9%) was detected by Xpert MTB/RIF test.

### 3.3 EVALUATION OF RISK FACTORS ASSOCIATED WITH PTB DIAGNOSED BY XPERT MTB/RIF TEST

#### 3.3.1 Univariate analysis

##### *Gender and PTB disease*

**Table 7: Gender and PTB disease**

		Xpert MTB		Total
		not detected	detected	
<b>sex</b>	female	100		100%
	male	90.9%	9.1%	100%
<b>Total</b>		91.1%	8.9%	100%

All TB cases detected by Xpert MTB/RIF were male with a p value of 0.243.

##### *Nutritional state and PTB disease*

**Table 8: BMI and PTB**

		Xpert MTB		Total
		not detected	detected	
<b>BMI</b>	<18.5	64.3%	35.7%	100%
	>18.5	97.8%	2.2%	100%
<b>Total</b>		91.1%	8.9%	100%

There is a strong association between low BMI and PTB disease (p=0.000).

*Duration of incarceration and PTB disease***Table 9: Duration of stay in prison and PTB**

		Xpert MTB		Total
		not detected	detected	
<b>Duration of stay in months</b>	<24	91.2%	8.8%	100%
	>24	91.1%	8.9%	100%
<b>Total</b>		91.1%	8.9%	100%

There is a no statistically association between duration of stay of inmates and development of PTB disease (p=0.6).

*HIV status and PTB***Table 10: HIV status and PTB**

		Xpert MTB		Total
		not detected	detected	
<b>HIV status</b>	negative	91.3%	8.7%	100%
	positive	88.4%	11.6%	100%
<b>Total</b>		91.1%	8.9%	100%

In our study there was no statistical significance between HIV status and PTB disease diagnosed by Xpert MTB/RIF (p=0.332).

*Level of immunodepression and PTB disease***Table 11: CD4 count and PTB**

		<b>Xpert MTB</b>		<b>Total</b>
		not detected	detected	
<b>CD4 count</b>	<200	77.8%	22.2%	100%
	>200	96.8%	3.2%	100%
<b>Total</b>		92.5%	7.5%	100%

There is no association between the number of CD4 count and PTB disease (P=0.121).

*HIV management and PTB disease***Table 12: Current ARVs treatment and PTB disease**

		<b>Xpert MTB</b>		<b>Total</b>
		not detected	detected	
<b>currently on ARVs</b>	no	33.3%	66.7%	100%
	yes	92.5%	7.5%	100%
<b>Total</b>		88.4%	11.6%	100%

There was an association between TB disease and not being on ARVs treatment (P=0.03)



*Past medical history of TB and recurrence***Table 13: TB in the past and PTB disease**

		Xpert MTB		Total
		not detected	detected	
<b>Tb in the past</b>	no	89.7%	10.3%	100%
	yes	98%	2.0%	100%
<b>Total</b>		91.1%	8.9%	100%

There is a strong association between previous TB and PTB disease (P=0.003).

*Smoking and PTB***Table 14: Smoking and PTB**

		Xpert MTB		Total
		not detected	detected	
<b>history of smoking</b>	no	92.2%	7.8%	100%
	yes	76.9%	23.1%	100%
<b>Total</b>		91.1%	8.9%	100%

Smoking is strongly associated with the development of PTB with a p value of 0.004.

### 3.3.2 Multivariate analysis: logistic regression

**Table 15: Multivariate analysis of risks factors and PTB disease diagnosed by Xpert MTB/RIF**

<b>Xpert</b>	<b>Odds Ratio</b>	<b>Std. Err.</b>	<b>z</b>	<b>P&gt;z</b>	<b>[95% Confidence Interval]</b>	
<b>BMI</b>						
>18.5	1.00					
<18.5	24.84	9.824	8.12	0.000	11.45	53.93
<b>Currently on ARVs</b>						
On ARV	1.00					
Not on ARV	56.01	91.210	2.47	0.013	2.30	1362.67
<b>History of smoking</b>						
Not smoking	1.00					
Smoking	2.26	1.150	1.61	0.108	0.84	6.13

Multivariate analysis showed high risk to develop PTB disease among inmates with malnutrition, HIV positive inmates not on ARVs (OR: 24.8, p=0.00; CI: 11.5-54 and OR: 56, p=0.01; CI: 2.5-1363) respectively. Without statistical significance, history of smoking among inmates is associated with a doubled risk to develop PTB disease (OR: 2.26; p=0.1; C.I:0.8-6.13). Adjusted for age.

### 3.4 THE YIELD OF XPERT MTB/RIF COMPARED TO SPUTUM MICROSCOPY TO DIAGNOSE PTB

**Table 16: Xpert MTB/RIF versus microscopy**

		microscopy		Total
		negative	positive	
<b>Xpert MTB</b>	not detected	511	0	511
	detected	33	17	50
<b>Total</b>		544	17	561

Xpert MTB/RIF is strongly superior to microscopy in detecting PTB (P=0.00).

### 3.5 EVALUATION OF XPERT MTB/RIF INCREMENTAL YIELD COMPARED TO MICROSCOPY

50 TB cases were diagnosed with Xpert MTB/RIF test whereas microscopy alone tested 20 PTB cases among which 3 were not fit for Xpert MTB/RIF. The remaining 17 microscopy PTB positive were controlled positive by Xpert MTB/RIF. Therefore, 33 additional cases were detected by Xpert MTB/RIF compared to smear microscopy. Thus, the incremental yield of Xpert MTB/RIF compared to smear microscopy equals to 66% (33/50).

## **CHAPTER 4 DISCUSSIONS OF THE FINDINGS**

In this survey, we report for the first time, the evaluation of Xpert MTB/RIF assay in improving pulmonary TB case detection among congregates in Muhanga prison.

The performance of the Xpert MTB/RIF assay with pulmonary specimens obtained during the clinical screening was investigated.

Sample size for the staff members was 5 over 78. Abnormal CXR was found in 4 cases (3 cases with lung infiltrates and 1 case with pleural effusion) and 1 case of normal CXR with cough. Their results were not analyzed because of this small number of cases and all sputum samples examined were negative for both smear and Xpert MTB/RIF.

### **4.1 PTB PREVALENCE IN PRISON COMPARED TO GENERAL POPULATION**

Our study has identified a significant number of PTB cases in Muhanga prison. The prevalence rate was 7507 per 100 000 which is very high compared to the rate in the general population (prevalence in 2012:114/100 000); which is 65.9 times higher [15].

The TB disease rate might be higher considering the big number of inmates with extrapulmonary manifestations that were not assessed in this study. Thus, there is a need of comprehensive TB case detection in prisons.

This high prevalence is comparable to the one from Brazil in 2004 which was 8686/100 000[35]. This was explained by the poor ventilated and overcrowding of Brazil's prisons at that time, which is similar to Muhanga prison with a living space of 0.76 m<sup>2</sup> and 1.48 m<sup>2</sup> for male and female respectively; which is 4.5 and 2.3 times less than the international standards.

PTB prevalence was superior to that of Dhaka Central Jail, Bangladesh in 2010 which was 2227/100 000 compared to 111/100 000 in general population [23].

Our study found high prevalence of HIV infection among PTB inmates (11.6%) compared to countrywise HIV infection prevalence of 3%. This may be due to this overcrowding of Muhanga prison mentioned and other risk factors such as injection drugs, commercial sex worker, lower socioeconomic status.

These results are comparable to those found in Malaysia in 2012, which is among 22 countries TB burden worldwide, where the prevalence in HIV positive prisoners was 12% [37].

Our study found 1 case of Xpert MTB [+] RIF [+] over 53 PTB diseases detected (1.9%). This prevalence of MDR TB is very high in congregates compared to general population; overcrowding and some of them come from community with MDR TB and high duration of stay. In 2013 WHO report, there was an estimated 450 000 new cases of MDR-TB worldwide in 2012 among which Rwanda confirmed 58 cases, with estimated population of 11 000 000 [15,40] There was no MDR-TB case detected in a 2011 Bangladesh prison study while the TB incidence was 12% among HIV positive inmates [37].

Indeterminate results of our study of 1.8% (10/563) are almost comparable to those of Steingart KR et al's metaanalysis of 18 studies where 10 of them reported the number of indeterminate tests with a proportion of indeterminate rate of 1.1% [2].

#### **4.2 YIELD OF XPERT MTB/RIF COMPARED TO SPUTUM MICROSCOPY TO DIAGNOSE PTB**

This study confirmed a statistical significant yield of Xpert MTB/RIF in PTB detection compared to smear microscopy ( $p=0.00$ ). This might predict its high sensitivity and specificity in our setting as it was proven by several studies conducted in Turkey by Arzu N. Zeka *et al* [9].

#### **4.3 INCREMENTAL YIELD OF XPERT/MTB/RIF COMPARED TO MICROSCOPY**

Incremental yield results of Xpert MTB/RIF compared to microscopy in our study (66%), is comparable to those found in Cambodia (51%) [38] and those of Steingart KR et al's metaanalysis in 2012 (67%) [2].

#### **4.4 RISK FACTORS RELATED TO THE PTB DISEASE DEVELOPMENT IN PRISON**

As in Ethiopian prisons [36], our study showed a strong association between malnutrition and PTB development. Furthermore, HIV infected inmates not on ARVs had a high risk to develop active PTB.

## **CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS**

### **5.1 THE STUDY LIMITATIONS**

A significant number of participants with an abnormal CXR did not produce sputum for Xpert MTB/RIF analysis.

Sensitivity and specificity of Xpert MTB/RIF was not evaluated due to sputum culture not performed for all samples.

The overall active TB in our population was not assessed while there was a big number of a participant with extrapulmonary CXR findings highly suggestive for TB, e.g. pleural effusions (18.8%).

### **5.2 CONCLUSION**

In Muhanga prison, the TB prevalence is very high compared to the general population.

Low BMI and HIV infection have proven to be additional risk factors to develop active TB in this congregate. However, TB case detection might be underestimated by traditional sputum microscopy. Thus, Xpert MTB/RIF significantly improved the yield of both active PTB and RIF resistance.

### **5.3 RECOMMENDATIONS**

#### **To Ministry of Health / RBC TB & ORD division**

1. To keep up the efforts by the MoH to make available Xpert MTB/RIF diagnostic tool in other facilities for TB diagnosis especially in those serving congregates.
2. To empower TB screening in prisons by regular and systematic CXR of inmates despite the only clinical symptoms.
3. To consider a systematic provider initiated HIV testing among congregates population keeping in mind that timely ARVs start is protective against TB disease.

**To MININTER/RCS**

1. To sustain the effort made to improve nutritional condition of inmates.
2. To empower environmental TB infection control measures in prisons.
3. To keep continuous education about TB infection control measures for inmates, prison staff in regard to themselves and to the community in general.

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## **APPENDICES**

**Appendix 1 Data collection tool**

Participant names initials and code number ....

***Demographic characteristics and risk factors for active pulmonary TB.***

Age (in years)...

Gender:	0. Male	1. Female	
Body Mass Index (BMI) in kg/m <sup>2</sup> :	0. <18.5	1.18.5-25	2.>25
Duration of stay in prison (in months)....			
Recent history of smoking	0.no	1. yes	
Previous history of TB	0.no	1. yes	
Currently on ARVs (if known HIV +)	0.no	1. yes	
Recent CD4 (if HIV +) ...			

***Clinical characteristics***

Cough > 2 weeks at the time the study:	0.no	1. yes	
Weight loss in the past 3 months	0.no	1. yes	
Recent loss of appetite	0.no	1. yes	
Chest pain	0.no	1. yes	
Fever	0.no	1. yes	
Night sweats	0.no	1. yes	

***Investigations*****HIV****Chest X-Rays** findings .....

<b>Sputum smear</b> (Auramine O staining)	0. Negative	1.positive	
	0. MTB not detected	1.MTB detected	
<b>Xpert MTB/RIF</b>			
<b>RIF resistance</b>	0. not detected	1.detected	2. indeterminate

## **Appendix 2 Informed consent form in English**

### **Study on “Incremental yield of the Xpert MTB/RIF Assay for the Diagnosis of Pulmonary Tuberculosis in congregates; case of Muhanga prison”**

I, Dr Alphonse SINDAYIGAYA. Under RBC/TB& ORD division control, we are conducting a study in which we want to evaluate the incremental yield of Xpert MTB/RIF Assay for the Diagnosis of Pulmonary Tuberculosis in Muhanga prison. The purpose of this study is to evaluate the incremental yield of the Xpert MTB/RIF compared with smear microscopy for TB diagnosis.

Prison like other congregates is among the group at high risk of TB transmission (overcrowding, low immunity, etc), so that you are exposed to TB and MDR-TB, you are asked to participate in this study. Participation in this study is completely voluntary and you may refuse participate.

#### ***Background***

WHO and several studies showed that the prevalence of tuberculosis among prisoners in all countries of the world is always higher than the general population and it is the most common cause of death. In most prisons of low and middle income countries, the TB control program is limited to the diagnosis of cases among prisoners attending the prison clinic for symptoms suggestive of TB.

This study will help us to identify the active TB cases and at the same time evaluating the incremental yield of this new molecular diagnostic test (Xpert MTB/RIF).

#### ***Procedures***

We will request you to undergo a CXR and if TB suspected after interpretation, we will take venous blood sample to perform HIV test after giving 2 morning sputa for smear microscopy and Xpert MTB/RIF. Your records will be handled as confidentially as possible. All records will be coded and kept confidentially. You will have right to know your serological test result and receiving pre and post test counseling, sputum examination as well as CXR results.

***Benefits***

This study will help in diagnosis of active TB cases in a short time using a molecular test with high sensitivity and specificity and then benefit from treatment, consequently decreasing the transmission of TB, morbidity and mortality among Muhanga prisoners and staff members.

If you have understood and are willing to take part in this study, then kindly sign below, you have the right to decide to participate or to withdraw at any point in this study.

***Risks***

If you accept to participate in this study, the CXR will be done, sputum will be given and the blood sample will be taken using needles. You will be exposed to X-rays but for a short time. You will also be exposed to pain and you should have mild pain at site of puncture but this will take some minute and will be spontaneously resolved while taking blood sample with needles.

The person who conducted the informed consent discussion:

Full name:.....

Signature:..... Date:.....

***Identification of participant***

Code :.....

Names :.....

I accept to participate in this study performing a CXR, giving sputum for TB diagnosis and blood sample to test for HIV after receiving pretest counselling.

***Participant's signature***

Date .....

### **Appendix 3 Informed consent in Kinyarwanda**

**Ubushakashatsi ku “Gusuzuma indwara y’igituntu hakoreshejwe uburyo bushya bwa ‘Xpert MTB/RIF’ bugereranyijwe na ‘microscopy’ muri gereza ya Muhanga”.**

Nitwa Muganga Alphonse SINDAYIGAYA. Turimo dukora ubushakashatsi aho tugamije gupima indwara y’igituntu mu bagororwa ndetse n’abakozi ba gereza ya Muhanga hakoreshejwe uburyo bushya bwa ‘Xpert MTB/RIF’ tugereranyije n’uburyo busanzwe bwa ‘microscopy’.

#### ***Impamvu y’ubu bushakashatsi***

Ikigo mpuzamahanga gishinzwe ubuzima ndetse n’ubushakashatsi butandukanye kw’isi bwagiye bwerekana ko indwara y’igituntu ikomeze gukaza umurego cyane cyane mu magereza ugereranyije n’abandi baturage. Mu magereza menshi yo mu bihugu bikiri mu nzira y’amajyambere, basuzuma igituntu bagendeye ku bimenyetso by’igituntu gusa.

Ubu bushakashatsi buzadufasha kumenya abarwaye igituntu cyo mu bihaha kandi budufashe no kumenya akarusho k’ubu buryo bushya bwo gupima igituntu buri mu gihugu cyacu kuva 2012 cyane ko bunapima igituntu cy’igikatu, ibyo byose mu gihe kitarenze amasaha abiri.

#### ***Uko bigenda***

Dukeneye kumenya imyaka yawe, uburebure, ibiro ndetse n’igihe umaze muri gereza ndetse n’ibimenyetso by’igituntu. Nyuma tuzukunyuzwa mu cyuma cyo mu gatuza, utange ibikororwa ndetse tugufate amaraso. Amakuru yose tuzayabika mu ibanga rikomeye. Ufite uburenganzira bwo kumenya ibisubizo by’ibizamini uzakorerwa. By’umwihariko wemwerewe kuva mu bushakaantsi igihe cyose bitewe n’impamvu zawe bwite.

#### ***Umusaruro dutegereje muri ubu bushakashatsi***

Ubu bushakashatsi tuzatuma tumenya abagororwa bafite igituntu cyo mu bihaha kugira ngo bavurwe, ariko by’umwihariko tuzanamenya akarusho k’ubu buryo bushya bwo gupima igituntu bwa ‘Xpert MTB/RIF’ tubugereranyije n’uburyo busanzwe kandi bumaze imyaka myinshi bwa ‘microscopy’.



***Ingorane ushobora guhura nazo muri ubu bushakashatsi***

Uzakorerwaho ubushakashatsi azanyuzwa mu cyuma cyo mu gatuza (muri radiyo),nyuma atange ibikororwa ndetse afatirwe n'amaraso hakoreshejwe urushinge rushobora kumutera ububabare budakabije ariko bumara umwanya muto.

***Uganiriye n'ukorerwaho ubushakashatsi***

Amazina.....

Umukono.....

Itariki.....

***Uwemeye kujya mu bushakashatsi***

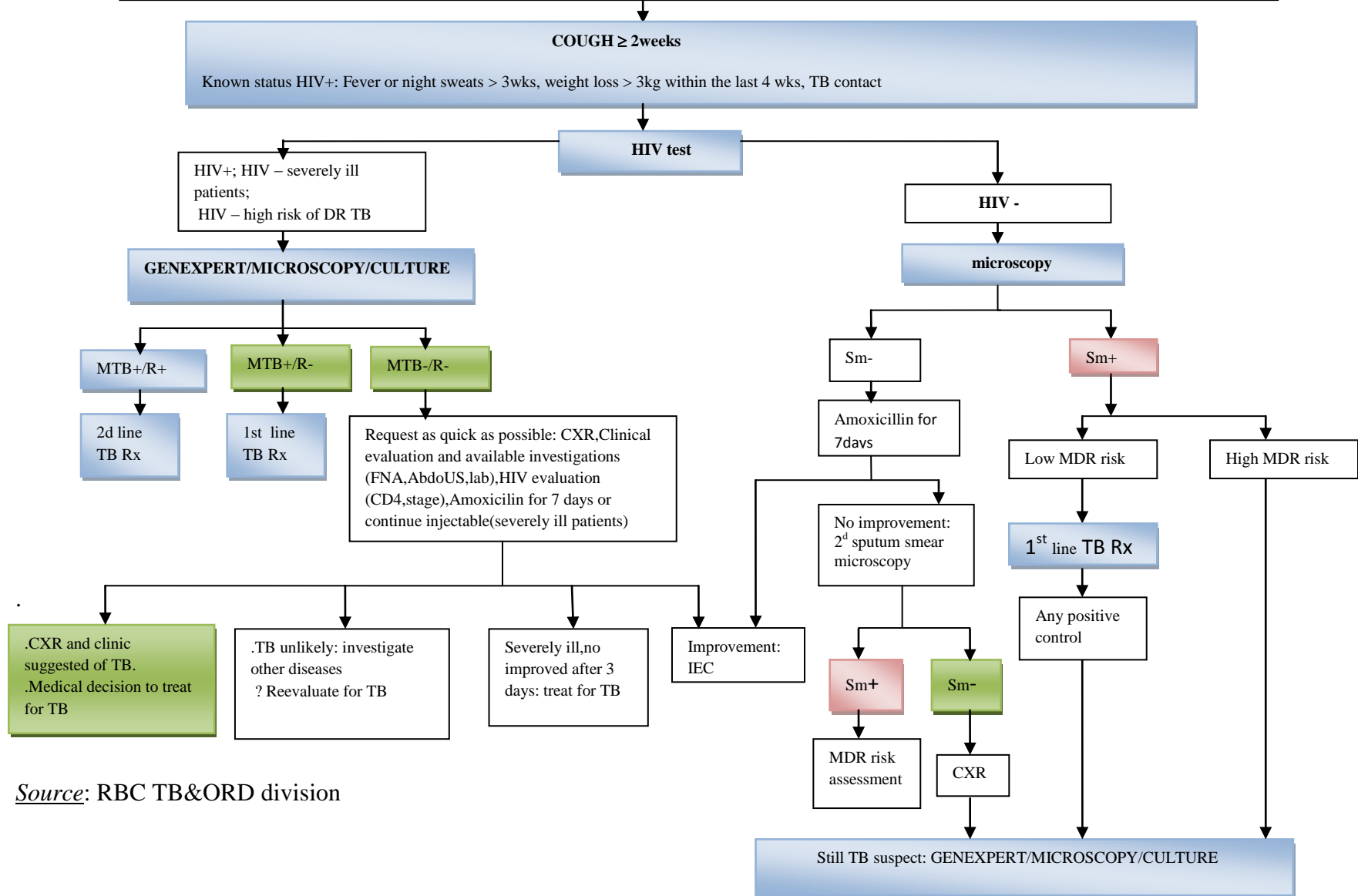
Amazina.....

Umukono cyangwa igikumwe.....

Itariki.....

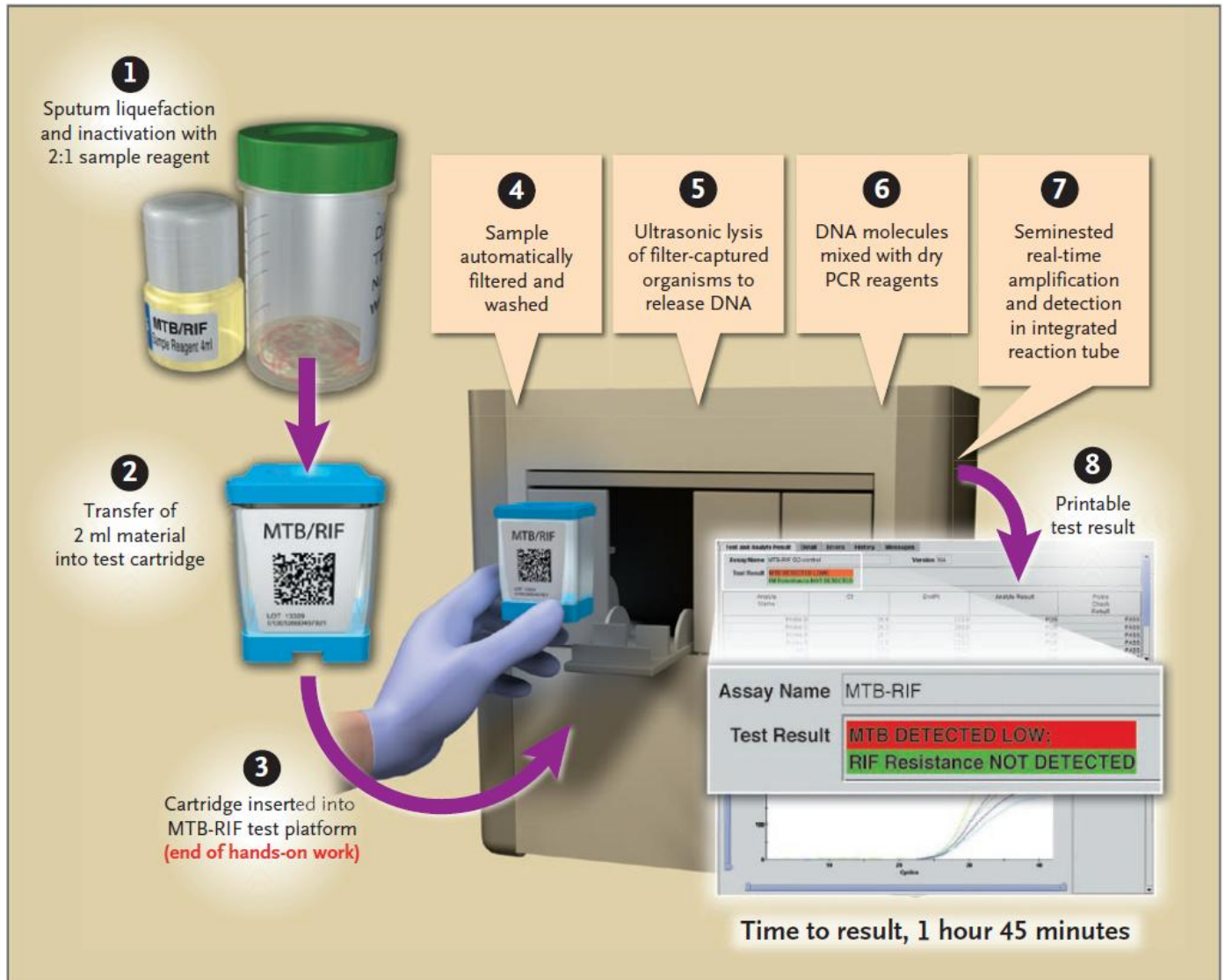
**Appendix 4 Current Rwanda national TB diagnostic algorithm**

**If danger signs (respiratory frequency >30 per minute, pulse >120/min, fever >39°C, unable to walk, confusion), give injectable ampicillin and refer to the hospital.**



Source: RBC TB&ORD division

## Appendix 5 Procedure of Xpert MTB/RIF assay



*Source:* Catharina C. Boehme et al. 2010. *Rapid Molecular Detection of Tuberculosis and Rifampin Resistance.* N Engl J Med. 10.1056/nejmoa0907847 p.6