RESEARCH AND EVALUATION REPORT

Increasing diagnosis of childhood TB in Swaziland:

Clinical utility and validity of sample collection and diagnostic methods among children

MARCH 2015

This research report on the clinical utility and validity of alternative methods for diagnosing tuberculosis in children in Swaziland was prepared by University Research Co., LLC (URC) for review by the United States Agency for International Development (USAID) and authored by Samson Haumba, Edward Broughton, and Rosanna Jeffries of URC: Themba Dlamini and Dube Gloria Sisi Nontokozo of the Swaziland National TB Control Programme; Charmaine Khudzie Mlambo of the Clinton Health Access Initiative; Marianne Calnan of URC; Eunice Ruhinda of Mbabane Government Hospital; Gugu Maphlala of Swaziland Health Laboratory Systems; and Janet Ongole of URC. The study was implemented under the USAID Applying Science to Strengthen and Improve Systems (ASSIST) Project, which is made possible by the generous support of the American people through USAID, with funding from the U.S. President's Emergency Plan for AIDS Relief (PEPFAR).

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For more information on the work of the USAID ASSIST Project, please visit www.usaidassist.org or write assist-info@urc-chs.com.

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Acronyms

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
ASSIST	USAID Applying Science to Strengthen and Improve Systems Project
AUROC	Area Under the Receiver Operating Curve
CI	Confidence Interval
ELISA	Enzyme-linked immunosorbent assay
HIV	Human Immunodeficiency Virus
LAM	Lipoarabinomannan
MDR	Multiple Drug Resistant
MGH	Mbabane Government Hospital
MOH	Ministry of Health
MTB	Mycobacterium Tuberculosis
MUAC	Mid Upper Arm Circumference
NPV	Negative Predictive Value
NTCP	National TB Control Programme
PPK	Piggs' Peak Hospital
PPV	Positive Predictive Value
QC	Quality Control
RIF	Rifampicin
RTI	Respiratory Tract Infection
SAM	Severe Acute Malnutrition
SEC	Scientific and Ethics Committee
TB	Tuberculosis
TST	Tuberculin Skin Test
URC	University Research Company, LLC
WHO	World Health Organisation
ZN	Ziehl–Neelsen

EXECUTIVE SUMMARY

The global scale of tuberculosis (TB) among children is not fully known, but in 2013 the World Health Organisation (WHO) estimated that there are approximately half a million new paediatric TB cases annually, resulting in 80,000 TB-caused childhood deaths per year [1]. In Swaziland, where annual incidence of TB is estimated to be 1,382 per 100,000 in the general population, exact childhood TB incidence is unknown [2]. However, the WHO estimates that approximately 10-20% of all TB cases are paediatric in countries with high TB burdens.

One of the biggest challenges to reducing TB deaths among children is that of diagnosing paediatric pulmonary TB, which often relies on findings from clinical examination rather than bacteriological confirmation. This is due to difficulties in obtaining a sputum sample as required for sputum-based diagnostic methods such as smear microscopy, Xpert MTB/RIF and culture, as well as the paucibacillary nature of paediatric TB, resulting in lower sensitivity/specificity of sputum-based diagnostic methods among children.

From January – August 2014, the USAID Applying Science to Strengthen and Improve Systems (ASSIST) Project collected data from 462 children (ages 0-14 years) with presumptive TB and 392 caregivers in order to improve ways to diagnose paediatric TB in Swaziland.

Specific objectives included:

1. To determine the whether the use of extracted mucus in the diagnosis of TB as a specimen collection method among non-expectoratin/'g children is more cost effective than the use of gastric lavage.
2. To determine the diagnostic accuracy of Xpert MTB/RIF from gastric aspirate and induced sputum respiratory samples compared to TB sputum culture in diagnosing TB in children.
3. To determine the diagnostic accuracy of the urine lipoarabinomannan (LAM) strip-test compared to sputum culture in diagnosing TB in children in groups disaggregated by HIV status or, if possible, CD4+ counts (or percent in children under 5 years of age).
4. To determine the diagnostic accuracy of mycobacterial blood cultures (Bactec Myco/F Lytic culture) compared to sputum TB culture in diagnosing TB in children.
5. To determine which diagnostic test is the most acceptable to the patients' caregivers.

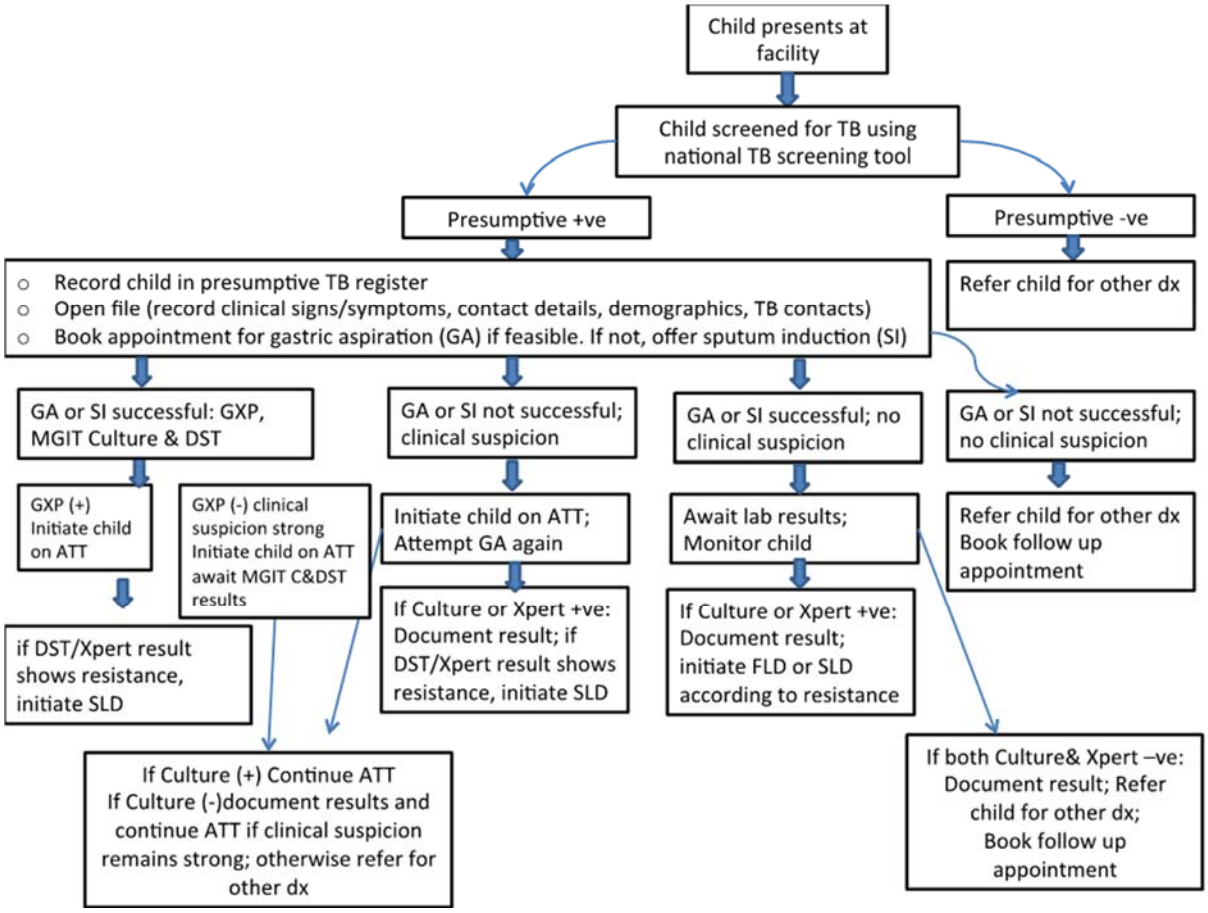
The study design used mixed-methods consisting of a prospective paediatric cohort of presumptive TB cases followed-up to confirm or exclude TB disease; quantitative questionnaires to determine acceptability of the tests to the caregivers; and a qualitative assessment of opinions of caregivers of the different diagnostic tests. All children enrolled in the study provided a urine sample (for the urine LAM test), a blood sample (for mycobacterial blood culturing) and underwent nasopharyngeal aspiration to produce two sputum samples for smear microscopy, Xpert MTB/RIF testing and culturing for MTB. In addition, those children admitted in the medical wards underwent gastric aspiration to produce gastric aspirate samples also for smear microscopy, Xpert MTB/RIF testing and culturing for MTB.

The study population was recruited from outpatients departments in two national hospitals in Swaziland: Piggs Peak Government Hospital and Mbabane Government Hospital. Of the 462 children, 4% were bacteriologically confirmed TB cases (defined as culture or smear positive). Results showed that diagnostic accuracy of the urine LAM test, the blood culture, and the Xpert MTB/RIF is very poor, emphasising the need to improve collection of samples for culturing to confirm presence of TB. This was further reinforced by the high proportion of MDR cases among the bacteriologically confirmed cases. The diagnostic accuracy of Xpert MTB/RIF from gastric aspirate and induced sputum respiratory samples compared to TB sputum culture was similar. While gastric aspiration is slightly more expensive than mucus extraction, there was no significant difference in the overall efficiency of the methods of obtaining samples in terms of confirming more cases of TB in this population.

Quantitative data from caregivers showed that the majority (73%) felt that urine collection was the easiest. A small proportion of out- and in-patient caregivers selected nasopharyngeal aspiration (9% and 19%

respectively) and 10% of the in-patients' caregivers selected gastric aspiration. This indicates that these invasive procedures may be acceptable to caregivers if administered with attention to their fears and concerns. Qualitative data showed themes of fear, pain, trauma and misconceptions being associated with gastric and nasopharyngeal aspiration balanced with the positive aspects of the speed of these procedures. There was also strong evidence of relief at having obtained a sample. Urine testing was found to be easy and painless and blood collection easy with some fears and misconceptions. Factors were highlighted that could make all procedures more pleasant for children and caregivers.

The report concluded with recommendations, including: conducting further research to investigate new diagnostic tools for TB in children; collecting samples of TB culture for all presumptive paediatric TB cases; intensifying active case finding of drug-resistant TB in children; training health care workers in conducting TB diagnostic procedures, specifically with children; amending protocols of the Swaziland Health Laboratory System; enhancing retention and care; as well as proposing a paediatric TB diagnostic algorithm.



I. INTRODUCTION

The global scale of tuberculosis (TB) among children is not fully known, but in 2013 the World Health Organization (WHO) estimated that there are approximately half a million new paediatric TB cases annually, resulting in 80,000 TB-caused childhood deaths per year [1]. This figure is likely to be an underestimate as does not include HIV-positive children nor deaths reported as HIV, pneumonia, malnutrition or meningitis where the cause of death was TB. Despite scientific and technological advances, TB remains an important cause of childhood morbidity and mortality globally.

In Swaziland, where annual incidence of TB is estimated to be 1,382 per 100,000 in the general population [2], exact incidence of childhood TB is unknown. However, according to the WHO, in countries with high prevalence of TB approximately 10–20% of all TB cases are paediatric. Although young age is a well understood risk factor for developing TB disease [3], one of the biggest challenges to achieving the WHO "STOP TB" goal of zero TB deaths among children is to accurately diagnose paediatric pulmonary TB. Accurate diagnosis is difficult because it often relies on findings from clinical and radiological examination or the tuberculin skin test (TST) [4], rather than bacteriological confirmation. This is due to difficulties in obtaining a sputum sample as required for sputum-based diagnostic methods such as smear microscopy, Xpert MTB/RIF and culture, as well as the paucibacillary nature of paediatric TB resulting in lower sensitivity/specificity of sputum-based diagnostic methods among children [5].

Non-bacteriological diagnosis is particularly problematic in this population group as clinical diagnosis has low specificity due to the non-specific nature of TB symptoms in younger children. There is subjectivity in interpretation of radiology findings and the fact that TST is a test for latent TB not active disease [4]. The WHO 2013 Roadmap for Childhood TB, acknowledging these issues, states that "there is urgent need for improved diagnostic and treatment options for children with TB. The research community needs to come forward and take action to address these challenges" [3].

This research sought to respond to this international call to action, by investigating the clinical utility of two non-sputum diagnostic tests utilising more paediatric-friendly samples: the Determine TB Urine lipoarabinomannan (LAM) test, and BD Mycolytic Blood Culture.

The Determine TB-LAM lateral flow assay, which detects the presence of the LAM antigen in unprocessed urine, has a number of advantages including lack of instrumentation, availability of results after 25 minutes and relatively low cost [6]. One South African study investigated the antigen-capture enzyme-linked immunosorbent assay (ELISA) test which is based on the detection of LAM in urine for diagnosis of pediatric TB, reporting a sensitivity of 4.8% and a specificity of 93.1% [7]. Although studies have investigated the sensitivity and specificity of the Determine lateral flow version of the assay in adults, which ranges from 13% to 93% and 87% to 99% respectively [8], no equivalent paediatric studies have been published. Given the difficulties in obtaining sputum samples from children and the need for non-sputum based TB diagnostic tools, this represents a clear research gap.

Blood culturing for TB mycobacterium provides another non-sputum based alternative sample for TB diagnosis. Although the authors found no research to investigate its use for paediatric diagnosis, there is evidence that this can be useful for non-expectorating patients, especially those who are HIV positive [9, 10]. Given that children are at greater risk for dissemination of TB, its detection in blood may be feasible and would have advantages for children without a productive cough, as with urine samples. The BD Myco/F Lytic blood culture bottles contain a nonselective culture medium suitable for the recovery of mycobacteria, yeast and fungi from blood. They are suitable for the detection of disseminated mycobacterial infections, whether M. tuberculosis or non-tuberculosis mycobacteria.

In addition to testing alternative, non-sputum based diagnostics, the research aimed to evaluate the impact of gastric aspiration and mucous extraction procedures when combined with use of the Xpert MTB/RIF automated molecular test. Several paediatric studies have investigated sensitivity and specificity

of this assay, with results varying by type of sample tested. One Zambian study compared gastric aspirate samples with sputum samples and found a sensitivity of 68.8% (95% CI 53.6-80.9) versus 90.0% (54.1-99.5) respectively, using culture positivity as the reference. The same study reports less variation in specificity at close to 99% in both sample types [11]. No similar studies have been conducted in Swaziland and, given the continued roll-out of the Xpert MTB/RIF equipment nationally, this study aims to provide evidence on the utility of this technology for paediatric TB diagnosis.

II. STUDY OBJECTIVES

The aim of this study was to identify ways of improving TB diagnosis in children, either through the use of non-sputum based samples, or through identifying the best way of obtaining a sputum samples.

Specifically, the study had the following primary objectives:

1. To determine the whether the use of extracted mucus in the diagnosis of TB as a specimen collection method among non-expectorating children is more cost-effective than using gastric lavage.
2. To determine the diagnostic accuracy of Xpert MTB/RIF from gastric aspirate and induced sputum respiratory samples compared to TB sputum Culture in diagnosing TB in children
3. To determine the diagnostic accuracy of the urine LAM strip-test compared to sputum culture in diagnosing TB in children in groups disaggregated by HIV status or, if possible, CD4+ counts (or percent in children under 5 years of age).
4. To determine the diagnostic accuracy of mycobacterial blood cultures (Bactec Myco/F Lytic culture) compared to sputum TB culture in diagnosing TB in children
5. To determine which diagnostic test is the most acceptable to the patients' caregivers

III. METHODOLOGY

A. Ethical Review

The study was approved by the University Research Co. LLC Institutional Review Board and the Scientific and Ethics Committee (SEC) of the Swaziland Ministry of Health (MOH). Written informed consent was obtained from parents or caregivers of all the participating children (see Appendix 1) and children aged over 7 years old provided additional written assent (see Appendix 2). These forms were available in Siswati and English.

All data collectors and data entry persons signed confidentiality statements.

B. Study Design

This mixed methods study had the two parts:

1. A prospective cohort of TB suspects followed-up to confirm or exclude TB disease.

2. Quantitative questionnaires to determine acceptability of the tests to caregivers and a qualitative assessment of opinions of caregivers of the different diagnostic tests.

C. Study Setting

Patient recruitment and sample collection was conducted at the outpatient and inpatient departments of two hospitals in the Hhohho Region of Swaziland, namely the Mbabane Government Hospital (MGH) and the Pigg's Peak Government Hospital (PPK). Specimen processing and analysis for all samples was conducted at the National Reference Laboratory in Mbabane.

D. Study Population

Children meeting the following inclusion criteria were considered eligible:

1. Aged between 0 years and 14 years inclusive
2. Males and females
3. Guardian informed consent provided through signing of consent form
4. The child presented at the outpatient clinics or the inpatient wards of either Mbabane Government or Piggs Peak hospitals
5. The child is a presumptive TB case

E. Sample Size

Sample size calculations were based on the following assumptions:

- An expected 95% percent of uncontaminated sputum culture gold standard results
- An expected prevalence in the sampled population of 30%
- Size of difference to detect between the gold standard and the Xpert MTB/RIF is 10% and the gold standard and Urine TB LAM is 20%
- An alpha level of 0.05 and
- A power of 0.8

To detect a difference of 0.3 (prevalence) and 0.2 (detected) with power of 0.8 requires a minimum of 376 samples. With an expected 5% contamination, the total minimum sample size was 375/0.95 = 396.

In the study, 462 patients were recruited.

F. Data Collection

From January 2014 to August 2014, 462 children aged 15 years or below and screening presumptive TB according to the National TB Screening Tool, were consecutively enrolled in this prospective, clinic-based study from the two study sites: Piggs Peak Government Hospital and MGH. Patients were recruited at these facilities' outpatient departments and paediatric medical wards and from the hospitals' HIV centres, by referral from the facilities' cough officers (staff members responsible for TB screening of all patients attending the facilities).

Upon enrolment and obtaining informed consent from the child's caregiver (and assent from children aged 7 or above), the nurse research assistants who were trained in sample collection methods collected one blood, one urine and two sputum samples from each participant. The latter were collected through sputum induction using hypertonic saline. Children were nebulized for 15 minutes with hypertonic saline and thereafter chest physiotherapy including chest percussion, vibration and deep breathing were performed to loosen the respiratory secretions. Sputum was then suctioned either from the nasopharynx or oropharynx using a sterile mucus extractor (henceforth referred to as nasopharyngeal aspiration). For young infants, urine was collected through use of a purpose-designed urine collection bag (see Figure 1).

Figure 1: Paediatric Urine Collection Bag

For in-patients, an additional two gastric aspirate samples were collected through early morning, fasted gastric aspiration. A nasogastric tube was passed before the child awoke and the gastric contents aspirated. Twenty mls of saline were then inserted down the tube

and aspirated after 5 minutes. An additional 5 – 10 ml was then inserted and aspirated until a minimum of 20 ml was obtained. All aspirates were placed in a sterile sodium carbonate containing tube and labelled.

All samples were sent daily to the National Referral Laboratory in Mbabane once per day. All sputum/gastric aspirate samples were stored on ice, whilst blood and urine samples were stored at room temperature in a cooler box, away from direct sunlight.

Upon receipt of samples by the laboratory research assistant, they were sorted and sent to the relevant departments for testing. Sputum and gastric aspirate samples were sent for Xpert MTB/RIF testing, mycobacterial culture (liquid mycobacterial growth indicator) and smear microscopy testing, in accordance with national standard operating procedures. Blood samples were incubated in the BACTEC 9050 and, those showing positive, were Ziehl–Neelsen and Gram stained to determine presence of *Mycobacteria Tuberculosis* (MTB). Due to space limitations, some blood samples were incubated for 42 days in a standard incubator (with regular manual agitation), before Ziehl–Neelsen and Gram staining. Urine samples were refrigerated and tested the morning after receipt and according to manufacturer's guidelines, with 60 microliters of urine sample pipetted onto the urine LAM test strip. Results were interpreted by the laboratory research assistant, assisted by the manufacturer's reference scale card. All test kits were stored away from direct sunlight and within the required range of 2 – 30°C (lot numbers 121211; 13110; 140722). There is no standardised quality control (QC) test provided by the manufacturer but QC was tested through weekly and batch-to-batch testing against known LAM positive and negative samples (stored in the refrigerator and replaced weekly).

Laboratory test results were stored in the Laboratory Information System, recorded (unlinked) in a spreadsheet, printed and returned to the facilities for interpretation by clinical staff. Although urine LAM results were not used for diagnostic purposes, participants who tested positive in any test were requested to see the doctor. Thus, urine LAM positive cases did undergo thorough clinical examination.

In addition to collection of samples and results data, demographic, socio-economic and basic clinical variables were collected by the nurse research assistant upon enrolment in the study. Where HIV status was unknown, participants were counselled to visit the Voluntary Counselling and Testing Centre for standard testing and counselling. All data were entered into a patient medical record document (see Appendix 3 for data collection tool), which was stored in a locked filing cabinet along with the consent and assent forms.

Table 1: List of variables collected

Child's gender
Is the child's father living?
Is the child's mother living?
Child's HIV status
Child's CD4 count or percentage (if HIV positive)
Has child had contact with someone they stay with who has confirmed TB diagnosis?
Has the child had recurrent respiratory tract infections?
Does the child have a history of asthma?
Child's region of residence
Child's height
Child's weight
Child's mid upper arm circumference (MUAC)

Child's date of birth
Date of enrolment
Date of admission (for inpatients only)
Reason for enrolment

To collect data on the caregivers' perceptions on the procedures, the nurse research assistant administered a questionnaire (see Appendix 4) to each caregiver, after the child in their care had undergone the procedures. This questionnaire collected quantitative information on which procedure they considered to be the most and least easy and which procedure they considered to be the most and least inconvenient. Qualitative data were collected on what caregivers liked least and most about each procedure, and how sample collection could be improved. Interviews were conducted in a quiet, safe location in the language of the caregivers' choice (English or Siswati). Permission was obtained to record these and they were later translated and transcribed onto a paper-based questionnaire by the nurse research assistants, and stored in a locked filing cabinet.

IV. ANALYSIS

A. Children's Data

Quantitative data collected on enrolled children were entered using Epidata version 3.1 software, then exported into excel and finally STATA Version 12.0 for cleaning and analysis.

A composite reference, 'bacteriologically confirmed' was created by combining bacteriological sputum test results from smear microscopy and culture testing for either sputum or gastric samples. These were combined to create a single dichotomous dependent variable according to the 'any positive rule' where participants are considered to have the disease if either test is indicative of disease [12]. In the absence of a true gold standard for paediatric TB diagnosis, creating this composite reference is thought to provide the closest available estimate of a true positive rate. Xpert MTB/RIF were not included as these results were concordant with culture results, but results from gastric aspirate and sputum samples were combined.

Descriptive statistics were calculated for the study population, in proportions for categorical variables and measures of central tendencies for continuous variables. A new continuous variable, Body Mass Index (BMI), was calculated for each participant using the weight and height measures provided. MUAC scores are presented for children under 60 months old, and a new variable for Severe Acute Malnutrition (SAM) was created for children in this age group, with a MUAC cut-off of 115 mm as per WHO guidelines. Data collected on whether parents were living were also combined for analysis, to create a new categorical variable of how many parents are living. Participant's age was categorised in months for analysis purposes.

Bivariate analyses were conducted using logistic regression to calculate unadjusted odds ratios for both categorical and continuous variables independent. A p-value of <0.05 was considered statistically significant.

Sensitivity and specificity, positive and negative predictive values (with 95% confidence intervals(CIs)) were then calculated for each diagnostic test and the area under the receiver operating curve (AUROC) was computed by plotting sensitivity against 1 – specificity to determine the overall performance of this tests relative to bacteriological confirmation. For TB LAM, given that it is recommended for use with TB/HIV co-infected patients, these tests were done for both the total sample as well as in HIV infected participants.

The same tests were carried out for Xpert MTB/RIF for each type of sample collected (gastric or sputum) and overall.

Only basic descriptive statistics for blood results were calculated, given that MTB was not detected in any blood sample (hence no tests of association nor sensitivity and specificity were calculated). These are presented in three ways. First, the proportion of blood samples testing positive and negative for bacterial growth (as determined by the BACTEC machine) are listed (note that not all samples went through the BACTEC machine due to limited carrying capacity).

Culture yield of the two sample collection methods were calculated by the proportion of positive cases among patients that have both a gastric and a sputum sample.

The cost efficiency of gastric aspiration versus mucus aspiration was estimated using decision-tree analysis and Monte Carlo Simulations to account for the uncertainty inherent in the model. Costs were determined from the price lists of equipment available in Swaziland and the labor costs were determined from salary information based in Ministry of Health salary bands.

B. Caregivers' Data

Data collected from caregivers were entered into a using Epidata version 3.1 software, then exported into Excel and Nvivo version 10 for analysis.

Caregiver perceptions data was both quantitative and qualitative. Basic descriptive statistics to characterise the sample were calculated for quantitative data. For qualitative data, thematic analysis was carried out with the aid of Nvivo software, divided into the four sample collection methods (blood collection, urine collection, gastric aspiration and nasopharyngeal aspiration).

V. RESULTS

A. Children's Data

1. Descriptive statistics

Sample descriptive statistics for categorical variables are shown in Table 2 and for continuous variables in Table 3.

Table 2: Categorical variable descriptive statistics

Variables	Proportions
Recruitment site, n=462	
Piggs Peak Government Hospital	51.1%
MGH	48.9%
Patient type, n=462	
In patient	16.9%
Outpatient	83.1%
Child gender, n=462	
Male	52.5%
Female	47.5%
Parents alive, n=462	
2 parents alive	83.9%
1 parent alive	13.7%
0 parents alive	2.4%

Variables	Proportions
HIV status, n=462	
Reactive	16.2%
Non-reactive	74.9%
Missing/unknown	8.9%
TB contact, n=462	
Yes	71.2%
No	22.9%
Missing/unknown	5.9%
Self-reported RTI, n=462	
Yes	90.3%
No	9.5%
Missing/unknown	0.2%
Self- reported asthma, n=462	
Yes	14.7%
No	80.1%
Missing/unknown	5.2%
Region of residence, n=462	
Hhohho	85.5%
Manzini	13.6%
Shiselweni	0.7%
Lubombo	0%
Missing/unknown	0.2%
SAM (under 5 years only), n=249	
Yes	2.8%
No	97.2%
Bacteriologically Confirmed TB cases, n=462	
Yes	4.1%
No	92.9%
Missing	3.03%
TB LAM positive, n=462	
Yes	12.6%
No	79.7%
Missing	7.8%
Xpert MTB/RIF results (sputum samples), n=462	
Detected	0.4%
Not-detected	97.6%
Missing	1.9%
Xpert MTB/RIF results (gastric aspirate samples), n=86	
Detected	1.2%
Not-detected	91.9%
Missing	7.0%

Table 3: Continuous variable descriptive statistics

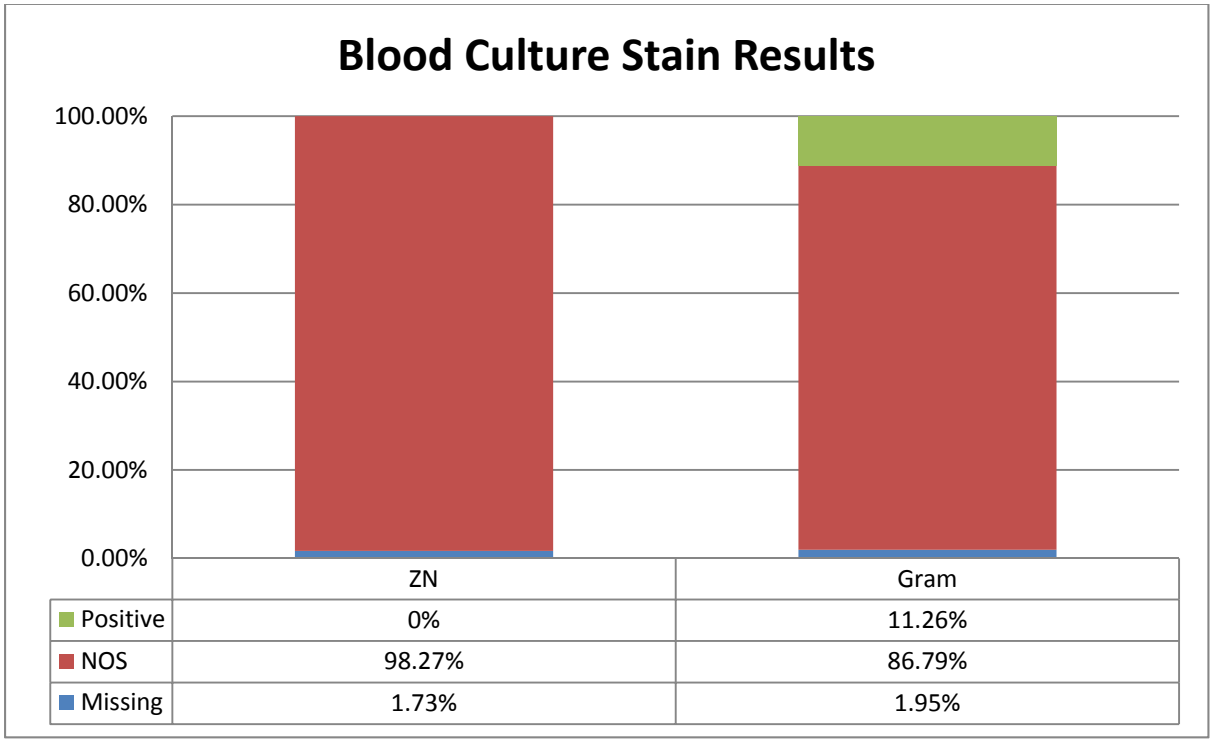
	Minimum	Median	Mean	Maximum	Standard Deviation	Number
Age in months	0	51	58.9	182	44.3	450
Under five MUAC	10	15.3	15.1	19.5	1.6	246

Approximately 16% of participants were HIV-positive. Their CD4 counts and percentages are not listed, as no bacteriologically confirmed pulmonary TB cases were HIV positive, so test of association by CD4 level was not feasible. The proportion of children with recurrent respiratory tract infection (RTI) was noticeably high, as was the proportion with TB contacts. Gender proportions are approximately equal. Of the 249 children aged under 5 years, less than 3% had a MUAC measurements indicative of SAM.

Of the total 462 participants enrolled, 448 had bacteriological TB confirmation results of which 19 were confirmed positive. Of the total enrolled, 426 had urine LAM results of which 58 tested positive. Of 453 participants with a sputum Xpert MTB/RIF result, MTB was detected in 2 samples and of the 80 that additionally had a gastric aspirate Xpert MTB/RIF, MTB was detected in one additional case.

Blood culture results are shown in Figure 2.

Figure 2: Blood culture Ziehl–Neelsen (ZN) and Gram staining results



2. Tests of association

Bivariate analysis results indicate that the only statistically significant association with bacteriological confirmed TB was the independent variable of asthma, as self-reported by the participants' caregivers (Table 4).

Table 4: Bivariate analysis results

Variable	Unadjusted Odds Ratio	p value
Sex	1.01	0.987
HIV Status	1.24	0.706
TB contact	1.75	0.379
RTI	0.54	0.351
Asthma	2.89	0.041*

Variable	Unadjusted Odds Ratio	p value
Mum alive	1.21	0.853
Dad alive	2.56	0.364
Either parent dead	1.66	0.505
Age in months ≤12 months >12 and ≤60 months >60 and ≤120 months >120 months	 5.40 1.77 6.64	 0.109 0.625 0.095
Region of residence Hhohho Manzini Shiselweni	 2.35 -	 0.114 -
Patient type	1.81	0.269
SAM	-	-
Age in months (continuous)	1.00	0.917
Under five MUAC	1.17	0.414

*denotes statistically significant associations at a 95% confidence interval

3. Diagnostic test validity

Results from the TB LAM show a sensitivity of 16.7% and a specificity of 86.6% in the whole sample and 33.3% and 88% among the HIV-infected individuals. Results from the Xpert MTB/RIF are similar, with a sensitivity of 15.8% and a specificity of 100%.

The positive predictive value (PPV) and negative predictive value (NPV) of the TB LAM are 5.4% and 95.8% in the whole sample and 11.1 % and 96.7% among the HIV-infected individuals. NPV results for the Xpert MTB/RIF are similarly high at 96.33% but PPV for Xpert MTB/RIF is 100%. CIs for all values are wide, especially for the latter results (see Table 5 and 6).

The AUROC was calculated for each of these, with values of 0.52 and 0.61 for TB LAM in the whole sample and in HIV positive cases, respectively. The value for Xpert MTB/RIF was 0.58.

Table 5: Diagnostic validity of TB LAM

TB LAM in whole sample	Value for whole sample	95% CI	Value for HIV+	95% CI
Sensitivity	17%	3.6- 41%	33 %	5.5- 88%
Specificity	86%	83- 90%	88 %	78- 95%
Positive predictive value	5.4%	1.1 - 15%	11 %	1.8- 48%
Negative predictive value	96%	93 - 98%	97%	89- 99%
AUROC	0.52	0.43 - 0.61	0.61	0.28- 0.94

Table 6: Diagnostic validity of Xpert MTB/RIF

Xpert MTB/RIF	Value	95% CI
Sensitivity	16%	3.4%-40%
Specificity	100%	99%-100%
Positive predictive value	100%	29%-100%
Negative predictive value	96%	94%-98%
AUROC	0.58	0.49-0.66

Table 7 compares the diagnostic validity of sputum and gastric aspirate samples when testing Xpert MTB/RIF against bacteriological confirmation.

Table 7: Gastric aspirate sample compared to sputum sample

Sample type	N	Sensitivity (95% CI)	Specificity (95% CI)	AUROC (95% CI)
Sputum	453	17% (0.4-64%)	100% (95.1-100%)	0.55 (0.48-0.62)
Gastric aspirate	80	11% (1.3-33%)	100% (99.1-100%)	0.58 (0.42-0.75)

4. Culture yield in gastric and sputum samples

The total number of patients with culture results for both gastric aspiration and sputum aspiration samples was just 58. Of these, one had a positive sputum culture and three had a positive gastric aspirate culture. This represents a positivity of 1.8% (95% CI: 0.0%-9.4%) and 5.5% (95% CI: 1.1%-15.1%) respectively, in the small sample of 58.

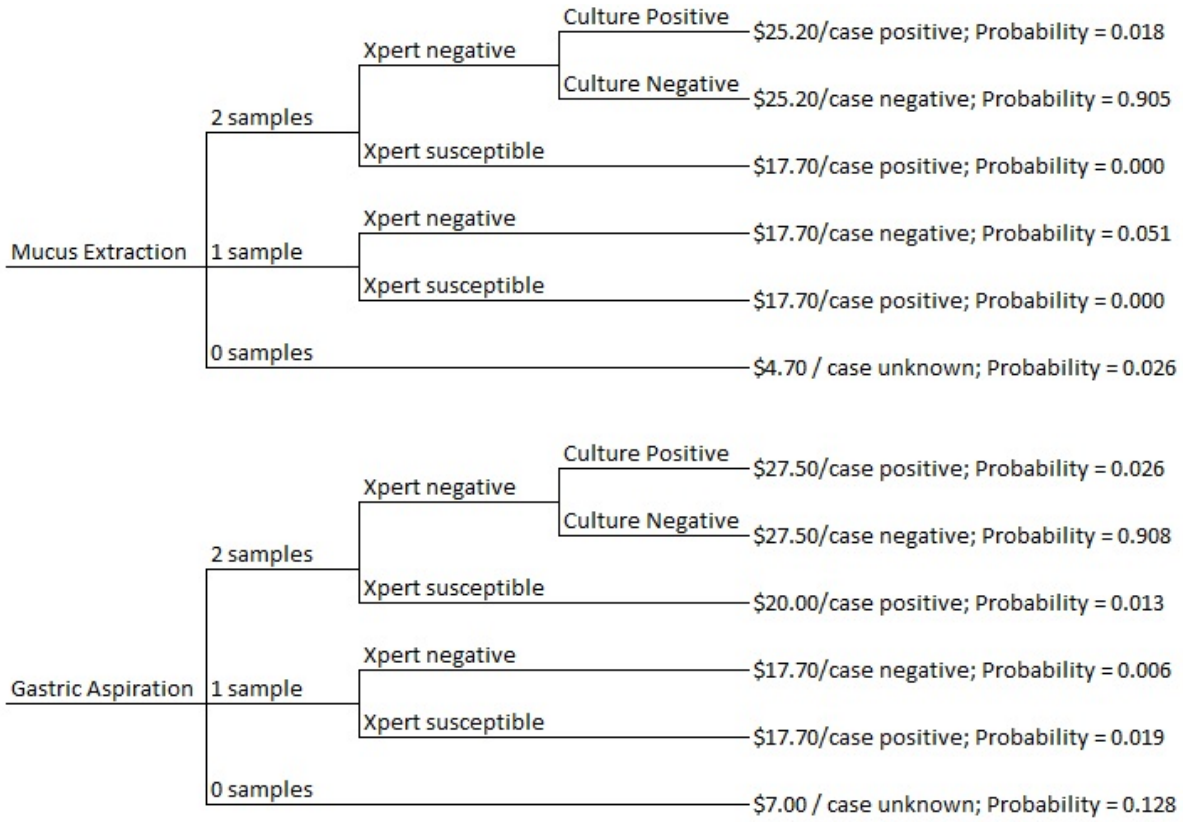
5. Cost effectiveness analysis

The decision tree compared the cohorts of participants who underwent mucus extraction and gastric aspiration to obtain samples for Xpert MTB/Rif and sputum culture. Inputs for the model are shown in Table 8. The overall result was that the cost-effectiveness for each participant undergoing mucus extraction to obtain samples (compared to the base case of gastric aspiration, was $5.09 per additional child identified as bacteriologically confirmed (95% CI: -$209 to $179) - no statistical significant difference. The costs and probabilities seen for various different scenarios are given in Figure 3. The most probable outcomes were two samples produced with a negative culture and Xpert test, whether the samples were obtained by gastric aspiration or mucus extraction. These tests pathways, which occurred in 90% of the sample, resulted in costs of approximately $25.20 when the samples were obtained by mucus extraction and $27.50 when the samples were obtained from gastric aspiration.

Table 8: Inputs for cost-effectiveness model

Variable	Point estimate	Distribution
0 samples gastric aspiration	10/78	Binomial
0 samples mucus extraction	2/78	Binomial
One sample gastric aspiration	2/78	Binomial
One sample mucus extraction	4/78	Binomial
One sample gastric aspiration, Xpert negative	1/2	Binomial
One sample mucus extraction, Xpert negative	4/4	Binomial
Two sample gastric aspiration	66/78	Binomial
Two sample mucus extraction	72/78	Binomial
Two sample gastric aspiration, Xpert negative	65/66	Binomial
Two sample gastric aspiration culture susceptible	2/65	Binomial
Two sample mucus extraction, Xpert negative, culture negative	408/416	Binomial
Two Sample Mucus extraction, Xpert negative	72/72	Binomial
Cost of sputum culture	7.50	Normal
Cost of Xpert	13.00	Normal
Cost of gastric aspiration	7.00	Normal
Cost of mucus extraction	4.70	Normal

Figure 3: Costs and probabilities of model



6. MDR case reports

Of the total number of bacteriologically confirmed cases, five MDR-TB cases were found for variables collected for these nine cases.

Table 9: MDR case reports

	Case 1	Case 2	Case 3	Case 4	Case 5
DST profile	IHN Res; RIF Susc	IHN Res; RIF Susc	IHN Res; RIF Susc	IHN Res; RIF Susc	IHN Sus; RIF Res
Facility	PPK	MGH	MGH	MGH	MGH
Patient type	In-patient	Out-patient	Out-patient	In-patient	In-patient
Sex	F	F	M	M	M
Reason for visit	TB contact; HIV positive; X-ray suggestive of TB	persistent cough; fever; rash; crepitation	non-stated	Fever and cough for 3 weeks; TB contact	Diarrhoea; appetite loss; vomiting; coughing
Age in months	71	17	22	18	15
TB history	No	No	Yes	No	No
HIV status	Positive	Negative	Negative	Negative	Negative
TB contact	Yes	No	Yes	Yes	No
Asthma	No	Unknown	No	Yes	No
RTI	Yes	Yes	Yes	Yes	Yes
MUAC	15.7	13.6	15.8	15.8	13
Sputum Xpert result	MTB not detected	MTB not detected	MTB not detected	MTB not detected	MTB not detected
Sputum culture result	Negative	Positive	Positive	Negative	Positive
Sputum smear result	Smear negative	Smear negative	Smear negative	Smear negative	Smear negative
Gastric aspirate Xpert result	MTB not detected	-	-	MTB not detected	MTB not detected
Gastric aspirate culture result	Positive	-	-	Positive	Negative
Gastric aspirate smear result	Smear negative	-	-	Smear negative	Smear negative

B. Caregivers' Data

Of the 392 caregivers who participated in this study, 92.2% were female, and 7.8% were male. The mean age of the caregivers in the study sample was 34, ranging from 18-72 years. More than half (57.3%) were unemployed and 42.7% were employed.

Quantitative results were divided into the opinions of caregivers of out-patients and in-patients. Figures 4 and 5 show selection proportions of responses to the question "which procedure did you find most easy?" and "which procedure did you find most inconvenient?" for caregivers of out-patients and in-patients, respectively.

Figure 4: Out-patient caregivers' responses

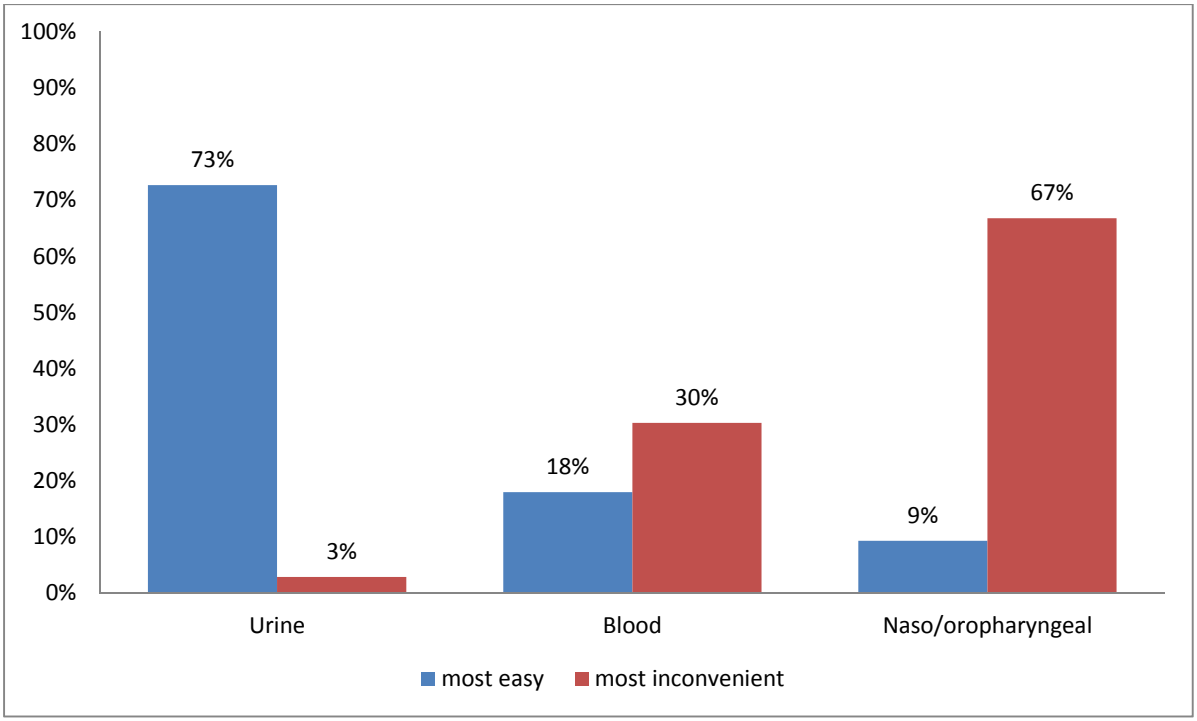
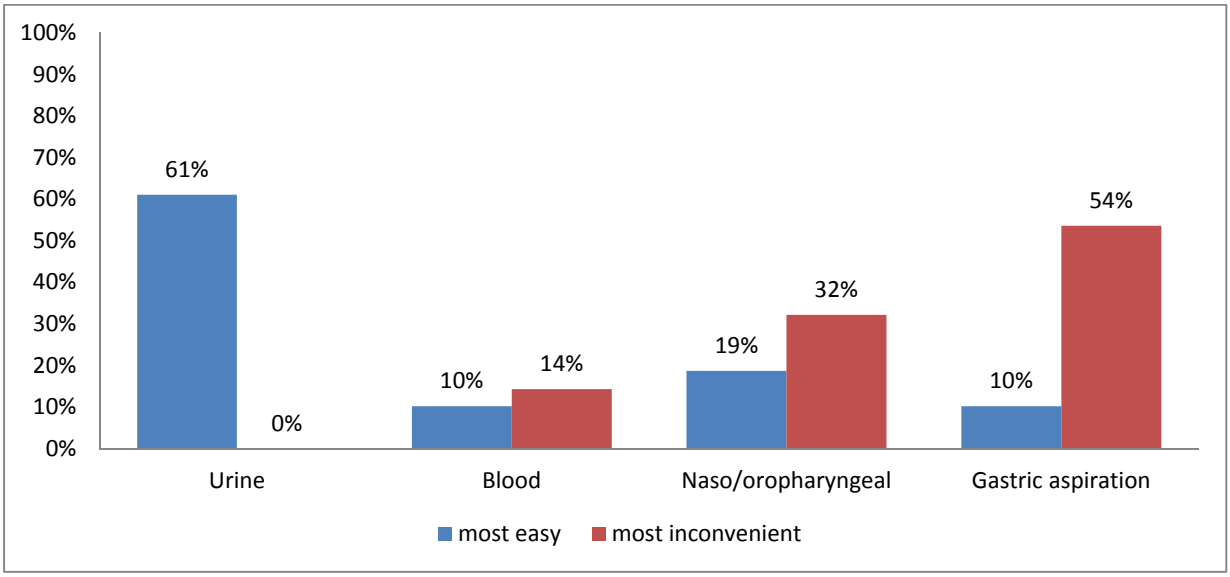


Figure 5: In-patient caregivers' responses



Qualitative findings are presented as frequencies of occurrence of themes, followed by key messages from the frequently occurring themes. Table 10 lists the five most frequently occurring themes for each sample collection procedure.

Table 10: Most frequently occurring themes for sample collection procedures

Gastric Aspiration	Nasopharyngeal aspiration	Blood collection	Urine collection
Relief, sample guaranteed	Fear	Pain, crying	Simple, easy
Fear	Relief, sample guaranteed	Difficult	Painless
Pain, discomfort	Pain	Fear	Independence
Trauma	Traumatic, invasive	Easy	Care
Fast	Fast	Fast	Normal

Fear was a key emerging theme from interviews with caregivers concerning gastric aspiration and nasopharyngeal aspiration procedures were fear. For the gastric aspiration, the following quotes capture the fear-related sentiments of the caregivers:

- Pushing tube deep in causes fears and seems traumatic
- Thought pipe would choke [the child]
- The tube scared me a bit as it was being inserted
- Tube insertion is frightening as child seemed to be choking
- I was frightened with the insertion of the tube because it goes a long distance inside
- Frightening as depth of tube seems unusual; even worse with unknown channel
- I was frightened that child might vomit through nostrils & die but trusted in God;
- Initially had fears about the method, tube insertion looks so painful.
- I was frightened by insertion into nostril, thought you would insert into mouth
- It was my first time observing, I thought it would hurt child inside
- Frightening procedure; unknown path of NG tube
- Insertion in nose is scary, child will obviously cry, nose cannot be played with

Similarly, for the nasopharyngeal aspiration, there were nine independent references to fear of death and four to fear of choking. There were concerns raised about the presence of blood in sputum which was understood to indicate damage to internal structures. There were also fears associated with the direction of tube (is it going to the eyes, brain, chest, lungs or heart?) as well as pain to the child and fear of the noise made by the machine noise (six references).

Of interest was evidence emerging of misconceptions related to blood collection, as shown by the following statements:

- Blood came out very little, I think child has less blood
- Blood looked very dark in the syringe; I thought child was so sick
- Blood looked very weak and dirty to me, "blood mixed with marker?"
- Feared that he had minimal blood
- If child has less blood you can finish it all as you draw & child can be weak
- It made me think of so many things like ”will he be able to have children?”
- Child has a problem, blood didn't want to be drawn, didn't come out soon enough
- I think she has less blood in her body or no veins
- Fears that blood may not be obtained if blood is inadequate
- Blood drawn slowly, worried child didn't have enough blood in body
- The blood was slowly draining out, as if child didn't have enough blood in body
- Blood looked so dark, I think child is very sick and am shocked.
- Like you are finishing the blood from child's body

Urine collection was overwhelmingly considered a simple, straightforward procedure. Of note from the data on urine collection is the theme of care by the nurses, with the following issues recognised and appreciated:

- Urine collector bag for infants “child friendly”
- Privacy
- Clear explanations
- Not using a catheter
- Soft, caring, kind attitude of nurse

VI. DISCUSSION

A. Children’s Data

1. Descriptive statistics

As expected, the gender distributions in participants was approximately equal. Unexpectedly, most children were not HIV positive (75%), but most did report a history of TB contacts, (71%). If considered a proxy for exposure, this serves as a reminder of the importance of improving diagnosis.

Almost all participants’ caregivers reported recurrent respiratory tract infection in the child (90%), whilst most (80%) did not report asthma. Only a small proportion (3%) of the children in the study sample was acutely malnourished.

The prevalence of TB (by bacteriological confirmation) was 4%, of which five were gastric aspirate cultures, ten were sputum cultures and four were sputum smears, leaving a total of nineteen confirmed cases. It is interesting to note that the four sputum smear positive samples were not positive on sputum culture. The 4% prevalence (95% CI 2.5%-6.3%) is considerably lower than the expected 30% in a presumptive TB population used for sample calculation.

Of note is the high proportion of TB LAM positive results (13%), three times as many as were bacteriologically confirmed. By contrast Xpert MTB/RIF results were very low, with just three positive results.

Blood cultures revealed no MTB whatsoever, even in the nineteen bacteriologically confirmed cases. The 11% positive when Gram stained were positive for Cocci. This indicates that there is no additional value in using blood samples for TB diagnosis in children.

2. Tests of association

We tested several variables for their associations with the outcome of interest, bacteriologically confirmed TB. As noted previously, no variables selected in this study were found to be statistically associated with a patient having confirmed TB with the exception of asthma. Results showed that the odds of patients with asthma having confirmed TB were 2.9 times higher than in patients without asthma. However, asthma was self-reported by the caregiver and not a medical diagnosis. It is possible that this was detecting respiratory symptoms associated with TB, but understood by the caregiver as being asthma.

Of note is that bacteriological confirmation for TB was not associated with HIV status, age of the child or type of patient (in-patient or out-patient) as might be expected.

3. Diagnostic test validity

When looking at diagnostic value of the LAM and Xpert MTB/RIF tests, sensitivity, specificity, PPV and NPV are considered.

Sensitivity is the proportion of people with bacteriologically confirmed TB disease who have a positive result on the diagnostic tests. A test with a high sensitivity is useful for ‘ruling out’ a disease if a person tests negative. A highly sensitive test is, therefore, most helpful to the clinician when the test result is negative. In this study, the sensitivity of TB LAM among HIV infected patients and Xpert

MTB/RIF in whole sample were found to be 33.3% (95% CI 5.5-88.4 %) and 15.8% (95% CI 3.4%-39.6%) respectively meaning that only 33.3% and 15.8% of patients with bacteriologically confirmed TB tested positive on these tests. These are both very low sensitivities indicating that neither test can be used in ruling out presence of TB in patients. The sensitivity of the urine LAM from this study was within the range found in studies conducted in other countries, which ranges from 28-69%, but the CIs are very wide given that only 75 of all participants were HIV infected and just four were bacteriologically confirmed cases.

Specificity indicates the proportion of people without bacteriologically confirmed TB disease who have a negative diagnostic test result. A test with a high specificity is useful for 'ruling in' a disease if a person tests positive. In this study, the sensitivity of TB LAM among HIV infected patients and Xpert MTB/RIF in whole sample were found to be 88.1% (95% CI 77.8-94.7 %) and 100% (95% CI 99.1%-100.00%) respectively meaning that 88.1% and 100% of patients without bacteriologically confirmed TB tested negative on these tests. The 100% for Xpert MTB/RIF indicates that this is an acceptable diagnostic for ruling in TB (as is already known). The specificity and associated CIs of the urine LAM test tell us that, of 100 patients who test positive with this test, somewhere between 78 and 95 of them will have the disease. This specificity is more in line with evidence from other countries, where specificities ranges from 90-99%.

The major limitation of both sensitivity and specificity is that they are of no practical use when it comes to helping the clinician estimate the probability of disease in individual patients. When a patient has a positive result for a particular diagnostic test, the real question is: what is the chance (probability) of disease given the positive test? Sensitivity and specificity cannot be used to answer such a question. Positive and negative predictive values are more useful in determining the clinical validity of the test as they indicate the chance that a person with a positive test truly has the disease and vice versa, given the actual disease prevalence.

The PPV of TB LAM in HIV infected participants is calculated as the number of TB bacteriologically confirmed children in this group who tested positive divided by the total number of people who tested positive in this group, which is 11.1%. This means that, in this study population with this disease prevalence, just 11.1% of people who test positive with TB LAM are bacteriologically confirmed or put in another way, a person who has a positive test has a 11.1% chance of being a bacteriologically confirmed case. This low PPV predictive value is in contrast to the high specificity. This difference is as a result of the low prevalence in our study sample. If the prevalence of a given disease is low, then the PPV will be low irrespective of the sensitivity and specificity of the test. A higher prevalence will always result in a raised PPV and a lowered NPV.

The NPV of TB LAM in HIV infected participants is 96.7%. This means that 96.7% of people who test negative for TB LAM will not have TB (by bacteriological confirmation), or put in another way, a person who has a negative test has a 96.7% chance of not having bacteriologically confirmed TB. This means that, in this population, a person who tests negative for TB LAM still has a 3.3% chance (or, when considering CIs, between 0.5%-11.4% chance) of having bacteriologically confirmed TB.

A third test of diagnostic validity is the AUROC, which can quantify urine LAM and Xpert MTB/RIF tests' overall ability to differentiate between those individuals with bacteriologically confirmed pulmonary TB, and those without. An area of 0.5 is considered worthless (no better than chance) and that a perfect test with zero false positives and false negatives has an area of 1.00. The AUROC for the TB LAM test in HIV infected patients was 0.61 and that for Xpert MTB/RIF was 0.58 in this study indicating that both tests are very poor at differentiating between those individuals with bacteriologically confirmed pulmonary TB, and those without. Put another way, results from these tests do not provide a very good indication of actual disease (not much better than flipping a coin).

Overall, these findings indicate that the diagnostic value of both the TB LAM and the Xpert MTB/RIF tests are very poor in this study population. Given the relatively high specificity of the TB LAM, it is conceivable that in settings with higher prevalence, its positive predictive value would be higher and that it would be useful as an add-on screening tool to rule in possibility of TB.

4. Culture yield in gastric and sputum samples

Among the 58 participants with both samples, the positivity of both the gastric aspirate and sputum samples is low at 5.5% and 1.8% respectively.

Based on evidence from this study, gastric aspirates yields higher positivity than sputum, which is in line with international evidence. However, given the small sample, it is difficult to say from this data which sample should be recommended nationally. This decision will be informed by the cost-effectiveness and acceptability of the two procedures and the feasibility of their implementation.

5. Cost effectiveness analysis

For efficiency of sample collection by gastric aspiration versus mucus extraction, there was no statistically significant difference in the cost per additional case detected. Mucus extraction was about two-thirds of the price of gastric aspiration. From these results, it appears that efficiency of testing in terms of cost per additional case detected should be the driving force behind the decision of which sample collection method to use.

6. MDR case reports

The high proportion of MDR cases in the small sample of culture positive cases is concerning (3/15=33%). Small though the sample is, the findings indicate that MDR-TB cases are likely to be considerable among paediatric TB cases and it confirms the need to conduct culture and DST on all patients initiated on treatment, as laid out in the guidelines.

Looking at the five cases, one notes that the majority were from Mbabane Government Hospital (4/5) and that the children were mostly below 2 years old (4/5). Only one case reported having had contact with a confirmed TB case, and only one child was known or disclosed to be HIV infected. Caregivers of all five cases reported that the child had had a recurrent respiratory tract infection, which is likely detecting the presence of recurrent cough. However, given that 90 % of all caregivers in the total sample reported this symptom, it is likely not indicative of anything unique to these MDR cases.

The majority (4/5) of the cases had a MUAC reading indicative of being well-nourished (over 13.5 cm), whilst one case's MUAC indicates that a child at risk for acute malnutrition. Two of the cases were identified through gastric aspirate culture whilst three were identified through sputum culture. Of note is that those identified by gastric were negative on sputum culture, whilst those positive by sputum culture did not have a gastric sample (out-patients). AFB smears were negative for all five cases, as were the Xpert MTB/RIF results.

Overall, from this small sample, it appears that MUAC and HIV status are not useful indicators. This is in line with the lack of association seen between these variables and a bacteriological confirmation of TB (see tests of association).

7. Limitations

This study's limitations include the following:

First, we know that the TB LAM is marketed for use in HIV co-infected patients and that only a small number of patients in our sample were HIV-infected (70). The study would have benefitted from enrolling a higher number of HIV infected patients.

Concerning TB LAM, it must also be noted that we used bacteriological confirmation for pulmonary TB as a reference standard, and that extra-pulmonary TB investigations were not conducted in this study (including lymph node and abdominal TB). There is the possibility that the seemingly false positive results of this test are detecting extra-pulmonary cases not diagnosed in this study.

Another study limitation is that we did not collect multiple sputum and gastric samples despite evidence that this improves yield from both culture and Xpert MTB/RIF. This decision was based on cost and practicalities and on the assumption that, in reality, obtaining more than one sample from a given child may be highly unlikely.

B. Caregivers' Data

The value in collecting data on the caregivers' perceptions on the various procedures is to inform healthcare workers practices and ensure that their fears and misconceptions are appropriately addressed. For example, if a caregiver knows what elements of a procedure are considered frightening by a caregiver and the level of trauma associated with these procedures, then the caregiver can be reassured accordingly. Much as the perceptions of the children would be very interesting to know, the rationale for collecting information from caregivers is that they are usually the ones to make a decision on whether or not to seek healthcare for the child. Their experiences in a health care setting may influence this health seeking behaviours in the future, and may influence their decision to recommend the service to someone who may need it.

Quantitative responses are not surprising, with an apparent inverse pattern between procedures found to be easy and procedures found to be inconvenient. Of note from this data, is the fact that caregivers whose children underwent both gastric aspiration and nasopharyngeal aspiration (in-patients) considered the former to be most inconvenient. Whilst caregivers of out-patients who did not witness gastric aspiration considered nasopharyngeal aspiration to be the most inconvenient, the small sample from in-patients suggests that gastric aspiration is considered to be the most inconvenient, followed by nasopharyngeal. This should be considered in any decision to support roll-out either of these two sample collection procedures nationally.

Not surprisingly, the overwhelming majority of caregivers considered urine collection to be the easiest. However, it is interesting to note that a small proportion of caregivers of both out-patients and in-patients selected nasopharyngeal aspiration (9% and 19% respectively). Similarly, 10% of in-patient caregivers selected gastric aspiration as the easiest procedure, an equal number who selected blood collection. These proportions, although not large, might indicate that these more invasive procedures are highly acceptable to a small proportion of caregivers. Perhaps, if they became routine, more widespread acceptability could be achieved if administered with due consideration to caregivers' fears and concerns.

The qualitative data provided interesting findings for each procedure, which should be taken into account by healthcare workers administering them.

With both gastric and nasopharyngeal aspiration, data showed a frequently occurring theme was that of relief that a sample was obtained and that a sample is guaranteed through this method (first most common for gastric aspiration and second most common for nasopharyngeal aspiration). This is indicative of the difficulty associated with obtaining sputum samples from just coughing-out, and was often linked with a frustration at past experiences of being sent home with a sputum bottle for the child to cough into. There appears to be recognition among caregivers of the need for a good sample and the appearance of the relief theme must be balanced against the negative themes associated with these procedures. The other noteworthy positive theme was around the speed of the procedure, with numerous references made to the fast recovery of samples through these methods- a reminder that speed of procedure and not needing to return to the hospital to give a sample may also offset negative associations with these two procedures.

On the negative side, data showed a high frequency of fear, pain and trauma themes for both gastric and nasopharyngeal aspiration. For both, caregivers expressed concerns about the possibility of the child dying or choking, and concern about the unknown direction of the tube. For gastric aspiration, specific reference was made to the length of the tube or depth of insertion. For nasopharyngeal aspiration, the presence of blood in sputum was concerning to many caregivers and the noise from the machine was frightening. This indicates that, much as these procedures may be appreciated for the reasons mentioned before, there is need to ensure caregivers' fears are abated by the healthcare worker as many of them seem very grave.

Much as blood and urine collection are routine procedures, the study revealed interesting findings. Given results from the blood and urine TB tests, these may not have implications for TB screening in children but are interesting for general patient care. Regarding the blood collection, the most notable findings relate to caregivers' misconceptions. Many caregivers expressed concerns about the child

not having enough blood with one caregiver expressing concern about the absence of veins (presumably linked with difficulties associated with finding suitable veins for venipuncture in infants). The colour and consistency of the blood also seemed to be a concern to caregivers. Given the centrality of this procedure to many medical tests, and the regularity with which it is performed, healthcare workers should ensure that that caregivers understand that this procedure is of low risk to the child and that the colour or seeming quantity of blood available are not indicative of child's health status.

Finally, findings from urine collection show that, much as this procedure is easy and painless, there are certain factors that can make the process more pleasant. Healthcare workers should be aware of the value placed on privacy and clear explanations, as well as the use of urine collection bags over catheters for young children.

VII. RECOMMENDATIONS

Based on findings from this research, the following recommendations are offered.

No new diagnostic aids

Overall, from the data collected and analysis conducted, no new tool has been identified through this research study to improve diagnosis of TB in children. Neither the urine LAM test and or blood culturing can be recommended for this population, and it is notable that the Xpert MTB/RIF does not detect many true cases. Of course, if the Xpert MTB/RIF result is positive, one can be sure that the patient has TB and for this reason combined with the rapid turnaround time, it is worth conducting this test. Further research should continue to be conducted to investigate new, novel diagnostic tools.

Collect sample for TB culture for all presumptive cases

What this study tells us most clearly is the need for a culture for all presumptive TB case, much as this has limitations in turnaround time and difficulty in obtaining samples. The imperative is stronger given the high proportion of MDR cases detected in this sample and that these were not detected by Xpert MTB/RIF. The current TB guidelines recommend that culture confirmation be obtained for all patients initiated on treatment based on clinical presentation. Renewed emphasis should be placed on this across all facilities, and resources allocated to training and equipping the hospitals and health centres to collect an adequate sample.

Either gastric aspiration or nasopharyngeal aspiration should be selected as the standard, to ensure consistency in training and improve the likelihood of its uptake. Based on cost effectiveness analysis, neither test is superior to the other so the decision of which to use should be based on other factors. However, bacteriological confirmation should not be a barrier to diagnosis and treatment initiation and guidelines for clinical diagnosis should be improved and tested against bacteriological results.

Active case finding of paediatric MDR TB

The high proportion of drug-resistant (DR) cases in the small sample of bacteriologically confirmed TB cases reinforces what we know that there is transmission in the communities of DR-TB. There is need to intensify active case finding for DR TB in children in the communities, not just in facilities.

The MDR cases also add impetus to the need to obtain bacteriological confirmation from presumptive TB cases, to ensure that patients are initiated on the appropriate treatment.

Train health care workers

Once a decision has been reached concerning which sample collection procedure to use, health care workers will be trained on how to conduct the procedure. As part of this training, evidence from the research on caregivers' attitudes should be incorporated, to ensure that trauma and fear to the caregiver and the child is minimised. This will certainly impact the caregivers' experience in the health facility and may impact their decision to seek health for the child in their care, or recommend the procedure to someone in need. Ideally, gastric aspiration or mucus extraction should be normalised and not deter caregivers from bringing children for TB screening and testing.

Amend laboratory protocols

Currently, the Swaziland Health Laboratory System (SHLS) does not routinely culture and conduct DST on samples that are negative by Xpert MTB/RIF. Given the very poor sensitivity of the Xpert MTB/RIF, it is recommended that these protocols be amended such that, in paediatric cases, culture and DST are done on samples regardless of Xpert MTB/RIF result. Likewise, if there is only sufficient sample collected for one test, priority should be given to the former.

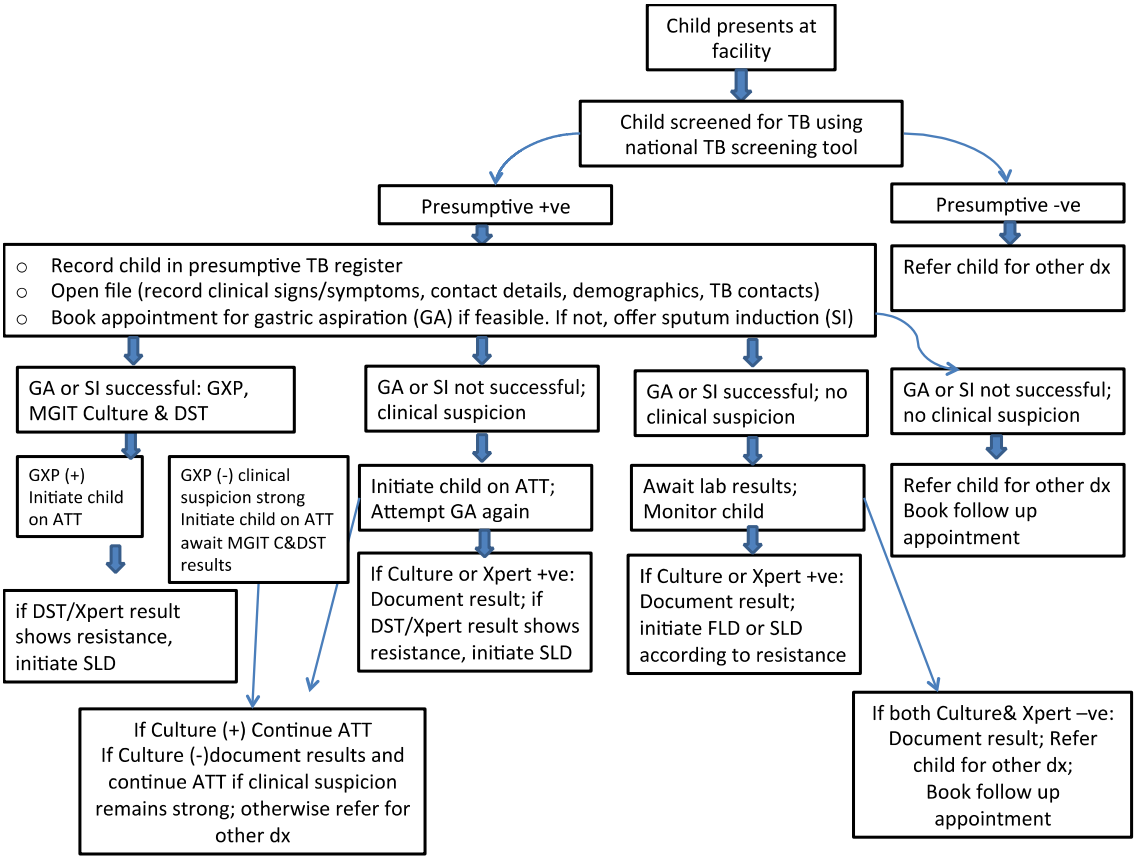
It is strongly recommended that this change happens immediately, as anecdotal evidence from doctors in the Mbabane Government Hospital indicates that this is a real challenge. Given the effort required to collect a sputum or gastric sample, these should not be wasted.

Develop SOPs for linkages to retention and care

Even in the controlled study environment in which nurse research assistants were closely supervised and requested to collect contact details and address for patients, there were instances when the caregiver of a positive case were not contactable and had to be actively traced in the community. This was either due to failure to capture the required information on behalf of the data collector, or provision of faulty contact numbers. It is recommended that nurses be trained on the need to collect accurate and detailed contact information including detailed physical addresses and that, where possible, phone numbers be verified on the spot. Lessons can be drawn from ART (antiretroviral therapy) patient records, and it may be helpful to open case files for all presumptive patients to assist with follow up appointments for gastric aspiration.

Proposed Paediatric TB Diagnostic algorithm

Based on these recommendations we propose the following diagnostic algorithm for diagnosis of TB among children presenting at health facilities. Initially, this should we standardised across hospitals and health centres, and gradually phased into clinics.



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IX. APPENDICES

Appendix 1: Informed Consent Form

Informed Consent Form for Caregivers of Children Presenting for Tuberculosis Diagnostics Testing

Principle Investigators: Samson Haumba, Edward Broughton and Themba Dlamini
Organization: National Tuberculosis and Control Programme and University Research Co., LLC,
Sponsor: USAID / Swaziland

This Informed Consent Form has two parts:

- Information Sheet (to share information about the study with you)
- Certificate of Consent (for signatures if you choose to participate)

You will be given a copy of the full Informed Consent Form.

Part I: Information Sheet

Introduction
My name is ……., working for on this study on behalf of the Ministry of Health, National Tuberculosis Control Programme and University Research Co., LLC. I am doing research on diagnostic tests to tell whether or not children have tuberculosis. I am going to give you information and invite your child, as represented by you, to be a part of this research. Before you decide, you can talk with anyone you feel comfortable with about whether or not to participate. As I go through this information sheet with you, there may be words or ideas that you are not familiar with. Please interrupt me at any time and ask questions. If you have questions later, you can ask them of me or another researcher involved in this study.

Purpose of the research
Tuberculosis or TB is more difficult to diagnose in children than in adults and this is a problem for trying to control the disease for the child and for his or her family and the community generally. This study is investigating the most effective way to get samples to accurately test a child for TB. The study will not involve taking any samples from the child than would normally be taken. The samples will be collected by the regular hospital staff but if necessary to ensure cooperation of your child, the staff will give you instructions on how to collect some of the samples under their supervision– again no different to what would happen in this hospital whether your child was in this study or not. The other part of the study involves me asking the child's carer such as yourself some questions about your experience with the diagnostic sample collection and the experience of the child. We anticipate this questioning will take less than 15 minutes.

Method of data collection
The samples provided by your child will be analyzed by different methods to determine whether or not they are likely to have TB. If the test if positive, your child will receive the same treatment that they would have received whether they were part of this study or not.

Participant Selection
You and have been invited to take part in this study because we are asking all care givers to children 15 years and below if they can participate.

Voluntary Participation
Your participation in this research is entirely voluntary and you will not receive any payment for agreeing to be part of the study. It is your choice whether to participate or not. If you choose not to participate, there will be no negative consequences to you. If you decide to participate, you may change your mind at any time and withdraw with no negative consequences.
Procedures

The samples will be collected by the medical and nursing staff at the hospital as they normally would. We will ask you questions about your experiences during collection of samples from your child. The questions are only about the collection of samples and nothing else.

Duration
The time between when the samples are collected and when the results are available is 4 hours to 6 weeks. Preliminary results will be provided within 24 hours but you might be requested to receive additional results after six weeks after the preliminary results

Risks
There are no additional risks to your child than there would normally be for your child because we are only using samples that are normally collected in these types of cases. There is no risk to you at all.

Benefits
The samples that are obtained from your child will be tested in several ways for TB at no cost to the patient or their family. The number and different types of test are probably higher than a child would normally get. Therefore the family and the medical staff should know with a greater degree of certainty whether or not the child has TB.

Reimbursements
There is no payment for your child's participation in the study.

Confidentiality
The results of the test will be completely anonymous for the researchers and anyone reading about this study once it is completed.

Sharing the Results
The results of this study will be shared widely. But your name and that of the patients or their families will never be associated with the study.

Right to Refuse or Withdraw
Even after you have agreed to participate, you can withdraw from the study at any time with no reason given and no negative consequences to you or your child.

Who to Contact
This proposal has been reviewed and approved by Swaziland Ministry of Health Scientific and Ethics committee, which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find about more about this study; contact Dr Samson Haumba, PO BOX 1404, Telephone 24047156, Dr Eunice Ruhinda, Mbabane Government hospital, Hospital Hill on telephone 24042111 or Dr Malcom Masikati at Piggs peak Hospital on telephone 24373194. This study has also been reviewed by the Institutional Review Board of URC.

You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?
You are also free to ask questions later as well, not only when you are here. Here is the contact information to use if you have any other questions. Like any other information in this study, any questions you ask or anything else you say will be kept confidential.

Part II: Certificate of Consent

I have been invited to participate in this research on TB diagnostic testing.
I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have been asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study

Print Name & Surname of Participant_____

Signature of Participant _____

Date _____
Day/month/year
……

If the study participant is illiterate or unable to sign a document, thumb prints are an acceptable alternative.

Thumb print of participant

A witness is required to observe the consent process and sign below only if written informed consent is not possible and the patient has provided verbal consent. The signature of the witness below means that another person has observed the consenting of the participant. The witness must be impartial and not part of the evaluation staff.

Witness Signature:_____

Date: _____

Appendix 2: Research Assent Form for Children Aged Seven or Older

What is a research study?

Research studies help us learn new things. We can test new ideas. First, we ask a question. Then we try to find the answer.

This paper talks about our research study and the choice that you have to take part in it. We want you to ask us any questions that you have. You can ask questions any time.

Important things to know…

You get to decide if you want to take part.

You can say 'No' or you can say 'Yes'.

No one will be upset if you say 'No'.

If you say 'Yes', you can always say 'No' later.

You can say 'No' at anytime.

We would still take good care of you no matter what you decide.

Why are we doing this research?

We are doing this research to find out more about the best way of testing if children are sick with a disease called Tuberculosis. At the moment it is difficult to test children, so we want to find out if it can be done better so that we can find children who are sick and treat them earlier.

What would happen if I join this research?

If you decide to be in the research, we would ask you to do the following:

Blood sample: You may need a needle poke so we could test some of your blood. If possible, we will try to get blood without a new poke.

Urine sample: I'm going to ask you to pee in a cup for us. Don't worry, you can go to the bathroom to do this.

Sputum collection: I'm going to insert a small pipe through your nose and into your throat, then use a small machine to pull out mucus that is sticking to your throat.

Gastric aspirate: if you're staying overnight in the hospital, I will ask you to not eat anything overnight and then, early in the morning, I will put a tube through your nose. This is to collect mucous that is in your stomach.

Could bad things happen if I join this research?

Some of the tests might make you uncomfortable but we are all very well trained. We will look after you very well and try to make sure that no bad things happen.

The poke to test your blood can hurt. Sometimes the needle can leave a bruise on the skin.

The tube to get mucous from your throat may feel strange and uncomfortable but should not hurt. You may also cough more than usual for the next day, but this is nothing to worry about.

The tube to get mucous from your stomach may also feel strange and uncomfortable, but it is quick and should not hurt.

You can say 'no' to what we ask you to do for the research at any time and we will stop.

Could the research help me?

We think being in this research may help you because we will find out if you are sick, and then we can help make you better.

You are also helping other children as this study will help us find the best way of finding out if other children are sick with Tuberculosis.

What else should I know about this research?

If you don't want to be in the study, you don't have to be.

It is also OK to say yes and change your mind later. You can stop being in the research at any time. If you want to stop, please tell the research doctors.

You would not be paid to be in the study, but we would be very grateful.

You can ask questions any time and ask us any questions you have. Take the time you need to make your choice.

If you do decide to be in the study, we won't tell anyone other than the people working on the study that you have taken part.

Is there anything else?

If you want to be in the research after we talk, we will write your name below. We will write our name too. This shows we talked about the research and that you want to take part.

Name of Participant _____

Printed Name of Researcher _____

Signature of Researcher _____

_____ _____

Date **Time**

Appendix 3: Patient Medical Record

Participant Medical Record

Variable	Description	
Study number	Number assigned to patient for study Eg PP 001, MGH 001	
Patient Number	Unique patient ID number	
Enrollment Day	Date the participant was enrolled in study	
Reason for enrollment	Please list the screening criteria that qualifies this patient for enrollment	
Caregiver Name/Relationship	Please list the caregiver's name and relationship to child	
Caregiver Contact details	Please list the caregiver's phone number	
Caregiver Address	Please list the caregiver's address	
Participant Day of birth	Day of birthday	
Participant Birth month	Month of birthday	
Participant Birth year	Year of birthday	
Region	Region of Swaziland	
Hospital admission day	Day of admission	
Hospital admission month	Month of admission	
Hospital discharge day	Day of discharge	

Variable	Description	
Hospital discharge month	Month of discharge	
Mother alive	Is the child's mother living?	
Father alive	Is the child's father living?	
TB history	Was the child previously positively diagnosed with TB	
HIV +	HIV status	
TB contact	Has child had contact with someone they stay with who has confirmed TB diagnosis?	
Asthma history	Does the child have a history of asthma?	
Recurrent RTI	Has the child has recurrent respiratory tract infections?	
Weight	Weight at admission	
Height	Height / length	
MUAC	MUAC at admission	
CD4 count	CD 4 taken from chart (if applicable)	
CD4 %	CD 4 % taken from chart (if applicable)	
LAM done	Was a sample taken from the patient for LAM analysis?	
LAM results	Result as recorded in the medical chart	
Sputum sample taken for Xpert MTB/RIF ?	Was sputum sample taken for Xpert MTB/RIF analysis? Please list how obtained (spontaneous, induction and cough, nasopharyngeal)	
Xpert MTB/RIF result (Sputum)	Result as recorded in the medical chart	
Sputum sample taken for culture	Was sputum sample taken for culture analysis? Please list how obtained (spontaneous, induction and cough, nasopharyngeal)	
Culture result (Sputum)	Result as recorded in medical chart	
Smear result (Sputum)	A smear will also be done on the sputum samples. Please list result here.	

Variable	Description	
Gastric sample taken for Xpert MTB/RIF ?	Was gastric aspirate sample taken for Xpert MTB/RIF analysis?	
Xpert MTB/RIF result (Gastric aspirate)	Result as recorded in the medical chart	
Gastric sample taken for culture?	Was gastric aspirate sample taken for culture analysis?	
Culture result (Gastric aspirate)	Result as recorded in medical chart	
Smear result (Gastric aspirate)	A smear will also be done on the gastric samples. Please list result here.	
Blood mycobacterium culture done	Was a blood sample taken for culturing?	
Blood culture results	Result as recorded in the medical chart	
Day of result	Day of the first result from any of the diagnostic tests	
Month of result	Month of first result	
Day of initiation of TB Rx	Day that the first medication prescribed for TB was administered	
Month of initiation of TB Rx	Month that the first medication prescribed for TB was administered	

Appendix 4: Caregiver Questionnaire

NOTE: REQUEST PERMISSION TO AUDIO-RECORD THE INTERVIEW

Date of interview	DD	MM	YY
			2014
Care Giver Name & Surname			
Occupation			
Date of Birth or Year of Birth			
Sex			
Study Number			
Address			

Why have you brought the child to the hospital for medical attention today?			
How many times in the last six months have you brought your child in for a respiratory problem?	Number:		
Was a blood sample taken last time you brought your child to the hospital for medical attention for this same problem?	Yes	No	Unknown
Was a urine sample taken last time you brought your child to the hospital for medical attention for this same problem?	Yes	No	Unknown
Was a sputum (phlegm) sample taken last time you brought [child's name] to the hospital for this same problem?	Yes	No	Unknown
If yes, how was the sputum (phlegm) sample obtained from your child? (Can prompted "There can be more than 1 different way to do it"	Spontaneous	Gastric Aspirate	
	Induction & Cough	Nasopharyngeal	
Of today's procedures, what did you like most and least about each sample collection methods and why?			
Most	**Least**		
Urine:	Urine:		

Blood:	Blood:
Induction and Cough:	Induction and Cough :
Nasopharyngeal Aspirate:	Nasopharyngeal Aspirate:
Spontaneous:	Spontaneous:
Gastric Aspirate:	Gastric Aspirate:

Considering just this visit, which among the samples (urine, blood, mucus extraction, sputum induction) do you think was the easiest to collect for both you and the child?	Urine	Blood	Gastric Aspirate
	Induction & Cough	Spontaneous	Nasopharyngeal

Which of these samples do you think was the most inconvenient to collect from your child?	Urine	Blood	Gastric Aspirate
	Induction & Cough	Spontaneous	Nasopharyngeal

How can we improve the current sample collection for TB diagnosis among children
Did your child talk at all about the test after any or all of the tests? If yes, what did they say?
Urine
Blood
Induction and Cough
Spontaneous
Nasopharyngeal
Gastric aspirate

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