NUCLEIC ACID AMPLIFICATION TESTING FOR THE DIAGNOSIS OF OSTEO-ARTICULAR TUBERCULOSIS

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Medicine Orthopaedic Surgery

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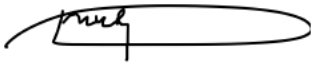
Declaration

I Dharshen Naicker declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Medicine in the branch of Orthopaedic Surgery at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



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(Signature of candidate)

25……day of September……2020…….in Johannesburg……

Dedication

I would like to dedicate this research report to my dear wife and daughter who have supported me in this journey.

Presentations & Publications Arising from this Research

- Presentations arising from research project

Nil

- Publications arising from research project

Nil

Abstract

Background

Osteo-articular tuberculosis constitutes a significant burden of extrapulmonary tuberculosis infection. The identification of *Mycobacterium tuberculosis* (*Mtb*) in osteo-articular specimens by traditional microscopy and culture is difficult, due to the paucibacillary nature of disease. The use of nucleic acid amplification tests (NAATs) may improve the detection of *Mtb* in tissue specimens, allowing for prompt diagnosis and treatment initiation.

Methods

We prospectively evaluated the use of NAATs as compared to traditional tests for the diagnosis of osteo-articular tuberculosis. The study population consisted of adult and paediatric patients at Chris Hani Baragwanath Academic Hospital (CHBAH), suspected of having osteo-articular tuberculosis.

Results

Twenty-nine patients were assessed by using routine histopathology, microscopy and culture, together with two NAAT assays: the Xpert MTB/Rif assay and Line Probe Assay (LPA). In comparison to mycobacterial culture, the Xpert MTB/Rif assay and LPA demonstrated a sensitivity of 90% and 100% respectively; and a specificity of 72% and 79% respectively. In comparison to histopathologic assessment, mycobacterial culture had 69% sensitivity and 100% specificity. Compared to histology, the Xpert MTB/Rif assay demonstrated a sensitivity of 94% and specificity of 100%, whilst the LPA had sensitivity and specificity of 100%. Xpert MTB/Rif assay results were available at three days on average and LPA results were available

at five days, versus 15 days for routine microscopy and culture, and 17 days for histological confirmation.

In HIV-infected patients, both the Xpert MTB/Rif and LPA tests, had sensitivity and specificity of 100% in detecting patients with both culture- and histologically-proven osteo-articular tuberculosis.

One patient had rifampicin-resistant tuberculosis infection. This case was detected by the Xpert MTB/Rif assay by day two post-biopsy, while culture was available on day 11.

Conclusion

The use of NAAT testing in the diagnosis of osteo-articular tuberculosis, demonstrated good sensitivity and specificity for the identification of *Mtb*-associated osteo-articular infection, in both HIV-infected and -uninfected patients. Furthermore, NAAT provides a more rapid time to identification of *Mtb*, and rapid identification of drug resistant infection as compared to traditional diagnostic tests.

Acknowledgements

I would like to sincerely thank my supervisors namely, Dr GB Firth and Dr DP Moore for their guidance, patience and assistance in the design and completion of this study. I will remain eternally grateful for your help.

I would like to thank the patients who agreed to participate in our study.

Thank you to my colleagues who assisted with obtaining biopsy specimens.

I would also like to thank Hain Life Sciences for their donation of Line Probe Assays for use in this study.

Thank you to Dr Jingo for proof reading the report.

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Nomenclature

AFB	Acid-alcohol fast bacillus
CHBAH	Chris Hani Baragwanath Academic Hospital
CI	Confidence interval
CRP	C-reactive protein
EPTB	Extra-pulmonary tuberculosis
ESR	Erythrocyte sedimentation rate
FBC	Full blood count
HIV	Human immunodeficiency virus
INH	Isoniazid
IQR	Interquartile range
LPA	Line probe assay
MDR	Multi-drug resistant
Mtb	*Mycobacterium tuberculosis*
NAAT	Nucleic acid amplification test
NHLS	National Health Laboratory Service
PCR	Polymerase chain reaction
PTB	Pulmonary tuberculosis
SD	Standard deviation
TB	Tuberculosis
WHO	World Health Organisation

CHAPTER 1

Introduction and literature review

1.1 Background

Tuberculosis infection, caused by *Mycobacterium tuberculosis* (*Mtb*), contributes a significant burden of disease in South Africa. It is estimated that 450 000 new cases of active tuberculosis occurred in 2013, with incidence increasing 400% over the last 15 years [(1)]. Extrapulmonary tuberculosis (EPTB) is defined as tuberculosis infection of organs other than the lungs, and accounts for approximately 16% of cases of tuberculosis disease in South Africa [(1)].

Osteo-articular tuberculosis represents 35% of the cases of EPTB in South Africa, and therefore, represents a common differential diagnosis for the pathology encountered in orthopaedic departments in South African public sector hospitals, including Chris Hani Baragwanath Academic Hospital (CHBAH), Soweto, Gauteng Province [(2)]. The diagnosis of osteo-articular tuberculosis is ideally based on the demonstration of *Mtb* in synovial material, or granulomatous inflammation with or without caseous necrosis on histologic examination of the synovium [(2)].

In clinical practice, confirmation of osteo-articular tuberculosis infection via the gold standard of mycobacterial culture, or the alternative comparator of histological identification is difficult, owing to the paucibacillary nature of the specimens, lack of adequate clinical sample volumes and non-uniform distribution of bacteria in those specimens, as well as the disease being localised to sites that are difficult to access [(3)].

These concerns have motivated the proposed study, as we hypothesise that our current diagnostic approach towards suspected cases of osteo-articular tuberculosis, which includes submission of tissue for tuberculosis microscopy, culture and sensitivity testing, together with histological assessment, may have limited sensitivity, leading to potential under detection and under treatment of cases of osteo-articular tuberculosis.

In our study we used nucleic acid amplification tests (NAATs) as part of the diagnostic work-up to assess if they could improve our detection of suspected cases of osteo-articular tuberculosis.

1.2 Literature review

The majority of tuberculous disease in South Africa is pulmonary tuberculosis (PTB), which accounts for approximately 84% of cases [(1)]. The diagnosis of PTB is based on a combination of clinical assessment as well as special investigations such as imaging studies, microscopy and mycobacterial culture [(4)].

The World Health Organisation (WHO) recommends the use of NAATs to aid in the diagnostic assessment of PTB [(5)]. These are molecular-based tests that are designed to identify genetic markers specific to *Mtb*, to facilitate the detection of the organism in sputum samples. Two such tests, which utilise polymerase chain reaction (PCR) for detection of pathogen-specific nucleic acid, are the Xpert MTB/Rif test (Ceiphid, Sunnyvale, CA) and the Line Probe Assay (LPA: Hain Lifescience, Nehren, Germany) [(5)(6)].

The Xpert MTB/Rif test is a rapid automated NAAT based on a PCR providing a detection of *Mtb* complex within two hours. It may also detect mutations in the *rpoB* gene that are associated

with rifampicin resistance [7]. Xpert MTB/Rif, therefore, has the potential to expedite a diagnosis of tuberculosis with or without rifampicin resistance, enabling timeous initiation of appropriate therapy [5].

The WHO also recommends the use of the Xpert MTB/Rif in cases with microscopy negative tuberculosis [6]. This pattern of disease represented approximately 35% of PTB cases in South Africa in 2018 and occurs commonly in patients who have HIV and tuberculosis co-infection, and frequently in children regardless of their HIV infection status [5]. The use and validity of the Xpert MTB/Rif test in the diagnosis of PTB in adults, was demonstrated by Vassal et al., who demonstrated that the test had 92% sensitivity and 99% specificity against the gold standard of mycobacterial culture [7].

The LPA test amplifies mycobacterial DNA from clinical specimens using PCR, followed by reverse hybridization onto nitrocellulose strips, which may identify *Mtb* complex in addition to mutations related to drug resistance. In particular, it can identify *rpoB* gene mutations, which are associated with rifampicin resistance, as well as mutations in the *katG* gene for high-level isoniazid (INH) resistance, and the *inhA* gene for low-level INH resistance [8]. This NAAT is, therefore, able to both detect *Mtb* complex, as well drug resistant and multi-drug resistant tuberculosis infection.

The validity of the use of the LPA in the identification of *Mtb* in adult PTB, was demonstrated by Morgan et al. in their meta-analysis, to have a sensitivity ranging between 82-100% and specificity ranging between 92-100% compared to mycobacterial culture [8].

The use of NAAT was initially indicated only for the detection of *Mtb* with respiratory samples according to WHO recommendations [5]. The increasing body of literature published on the

potential NAAT in EPTB diagnosis has stimulated a further policy review by the WHO, which now advocates NAAT in the diagnosis of EPTB as a conditional recommendation [5].

In countries with a high incidence of tuberculosis and EPTB, the diagnostic confirmation of *Mtb* remains a challenge. Traditional diagnostic methods, such as liquid mycobacterial culture, can detect *Mtb* infection in specimens with 10-100 bacteria/mL of sample [9]. This is regarded as the gold standard for the diagnosis of EPTB and remains the standard against which other diagnostic tests are compared. Histological assessment of specimens searching for evidence of granulomatous infection requires the necessary expertise for histological review and demonstrates a sensitivity of 66.7% and specificity of 33.3% as compared to mycobacterial culture [10].

Even with the submission of specimens for mycobacterial culture and histology, the diagnosis of EPTB remains challenging due to paucibacillary nature of the disease [11]. The difficulty in proving *Mtb* as the causative organism by traditional diagnostic testing often leads to empirical treatment based on clinical grounds. Clinical diagnosis alone is shown to have a sensitivity of 24% and specificity of 94%, compared to mycobacterial culture [5].

In addition to missing potential cases of active EPTB, there is the added burden of morbidity and mortality associated with the delayed diagnosis of mycobacterial infection. Diagnostic delay, which may extend up to 16 to 19 months for osteo-articular tuberculosis, is associated with a poor return to function for patients, as compared to those who have prompt initiation of treatment [12].

A potential solution to challenges associated with traditional diagnostic investigations for osteo-articular tuberculosis, is to use NAATs for the detection of *Mtb* in suspected cases of EPTB. A meta-analysis assessing the Xpert MTB/Rif assay in the diagnosis of EPTB, done by

Denkinger et al., demonstrated 97% sensitivity across various sample types [13]. These results, however, are based only on specimens obtained from pleural fluid, cerebrospinal fluid or lymph nodes; thus not indicating the accuracy of NAAT in suspected osteo-articular disease.

Held et al. looked at the use of NAAT in the assessment of osteo-articular tuberculosis specifically [14][15]. In their study which evaluated the use of the Xpert MTB/Rif assay for suspected cases of spinal tuberculosis, both sensitivity and specificity were 96% compared to mycobacterial culture [14]. Furthermore, the results of the Xpert MTB/Rif test were available within 48 hours, compared to 35 days for mycobacterial culture [14]. Xpert MTB/Rif has been recommended as a first line test in the diagnostic assessment of osteo-articular tuberculosis in children, with a sensitivity of 74% and specificity of 100% compared to mycobacterial culture [15]. Thus the use of NAAT may represent a rapid and reliable diagnostic tool for the identification of *Mtb* in clinical specimens from extrapulmonary sites in both adults and children.

In addition to the potential diagnostic advantage of the use of NAAT, the ability of these investigations to rapidly identify potential drug susceptibility patterns may be highly advantageous in our setting of increasing drug resistant tuberculosis infection. In their review of the prevalence of drug resistant tuberculosis in South Africa, Ismail et al. demonstrated an increase in the prevalence of drug-resistant tuberculosis infection [16]. They identified the current overall prevalence of multi-drug resistant (MDR) tuberculosis to be 2.1% in new cases and 4.6% in retreatment cases, which is the second highest rate globally [16]. An interesting finding in their study was that although the overall prevalence of MDR tuberculosis infection was similar to previous studies, there is a significant increase in the prevalence of rifampicin mono-resistant and isoniazid mono-resistant disease which represents a challenge to empiric anti-tuberculosis therapy [16].

1.3 Study Aim and Objectives

Aim of Study

To assess the diagnostic potential of the Xpert MTB/Rif and LPA assays in the detection of *Mtb* in cases of suspected osteo-articular tuberculosis managed at CHBAH.

The objectives of this study are:

1. To assess the accuracy of the Xpert MTB/Rif and LPA NAATs in the diagnosis of osteo-articular tuberculosis by comparing the sensitivity and specificity of the Xpert MTB/Rif and LPA tests to formal mycobacterial culture and histology.
2. To determine the time taken to identify *Mtb* as a causative pathogen using the Xpert MTB/Rif and LPA, as compared to time taken to diagnosis by formal mycobacterial culture or histology.
3. To determine the accuracy of the Xpert MTB/Rif and LPA tests in identifying drug resistant tuberculosis infection as compared to culture.
4. To assess the impact of HIV infection on the accuracy of NAATs in the diagnosis of osteo-articular tuberculosis.

CHAPTER 2

Methodology

2.1 Research Question

Are nucleic acid amplification tests (NAATs) a useful tool for the diagnosis of osteo-articular tuberculosis?

2.2 Research Design

A prospective cohort study design was used for this study.

2.3 Materials and Methods

The study was conducted at Chris Hani Baragwanath Academic Hospital (CHBAH), Soweto, South Africa. All patients who underwent surgical biopsy of the suspected site of osteo-articular tuberculosis in the Orthopaedic Department at CHBAH from 01 August 2017 through 31 July 2018 were eligible for inclusion in this study.

2.4 Clinical Case Management

Patients were suspected to have osteo-articular tuberculosis based on their clinical presentation and radiological studies. They were broadly grouped into suspected spinal and extra spinal tuberculosis.

Extra spinal osteo-articular tuberculosis was suspected in cases of subacute and chronic monoarticular arthritis with or without associated constitutional symptoms. Radiological

investigations, including plain X-ray, were done in these patients to look for features suggestive of osteo-articular tuberculosis. In terms of suspected spinal tuberculosis, the clinical presentation included back pain associated with constitutional symptoms or the presence of neurological deficits. These patients had imaging studies done demonstrating radiological features of infective spondylitis.

Routine clinical and laboratory evaluations of each patient were done, including baseline laboratory investigations such as full blood count (FBC), erythrocyte sedimentation rate (ESR), and a C-reactive protein (CRP). HIV testing was done once voluntary counselling had been given and consent obtained for such testing.

Spinal biopsy specimens were taken by members of the Orthopaedic Spinal Unit, while other biopsy specimens were taken by Orthopaedic medical officers, registrars or consultants affiliated with the Orthopaedic Department at CHBAH, either on elective lists or emergency lists, depending on available logistics.

2.5 Laboratory Testing

Biopsy specimens included pus, synovial fluid, synovial tissue and/or bone. These specimens were submitted for bacterial microscopy, culture and sensitivity; mycobacterial microscopy, culture and sensitivity; histological assessment; and Xpert MTB/Rif and LPA testing. LPA testing, which is currently done only on acid-alcohol fast bacillus (AFB) smear positive or *Mtb* culture-positive samples under routine conditions, was done routinely on all specimens regardless of AFB smear and culture results as part of the study procedure.

Specimen processing and testing was performed at the National Health Laboratory Service (NHLS) following their routine protocols, which are briefly described below. For the Xpert MTB/Rif assay, lysis buffer was added in 1:3 ratio to prepared tissue biopsy specimens, and

vortexed twice. Two millilitres of the mixture were processed automatically via the Xpert MTB/Rif assay cartridge and the result was recorded. For LPA, specimens were first decontaminated with N-acetyl-L-cysteine-sodium hydroxide. DNA was extracted using a standardised procedure onto nitrocellulose strips. Thereafter, hybridization was performed on these nitrocellulose strips and results interpreted.

2.6 Study Sample

Study participants included paediatric (newborn to 18 years) and adult (> 18 years) patients who, based on clinical or radiological findings, were suspected of having osteo-articular tuberculosis. Patients were classified according to age, sex, site of disease (i.e. limb and joint affected), as well as HIV infection status.

The study period was 12 months, during which time period patients with suspected osteo-articular tuberculosis who required arthrotomy and/or bone drainage with concomitant synovial or tissue biopsy were eligible for inclusion in the study. Consecutive patients admitted to CHBAH with suspected osteo-articular tuberculosis were invited to participate in our study. Those who consented to participate in the study were included. The participant information form, informed consent form and assent form are presented in Appendices C through E.

2.7 Data Collection

Data collection was based on the above-mentioned demographics, as well as on test outcomes with respect to identification of *Mtb* and time taken for results to be available. All data was extracted onto a standardised Data Collection Sheet (see Appendix A) by the primary investigator.

2.8 Data Analysis

All recorded data were entered into an Excel spreadsheet, and analysed using Stata version 13.0 (Stata Corp, College Station, TX) and R statistical software [17]. A confirmed case of EPTB was defined as either the positive identification of *Mtb* on mycobacterial culture, or features of necrotizing granulomatous inflammation on histological assessment. Time (in days) taken to identify *Mtb*, by Xpert MTB/Rif and LPA NAATs, compared to mycobacterial culture and histology, were evaluated by subtracting the date on which the test was resulted from the date of specimen collection.

The study population was analysed using descriptive statistics, including calculation of mean and standard deviation (SD) for normally distributed continuous data, and median and interquartile range (IQR) for non-parametrically distributed continuous data. Categorical data were described in terms of proportions and their 95% confidence intervals (CI).

Statistical analyses were done with the assistance of statisticians based at the University of the Witwatersrand Faculty of Health Sciences. Student's t-test was used for comparison of means, and the Kruskal Wallis test and/or Wilcoxon rank sum test were used for comparison of medians. The Chi-squared test and/or Fisher's exact test (as appropriate) were used for comparison of proportions. Two-sided P-values < 0.05 were considered as being statistically significant.

The diagnostic accuracy of the Xpert MTB/Rif and LPA NAATs were compared against both mycobacterial culture and histology with regards to sensitivity and specificity. Two-by-two contingency tables, comparing mycobacterial culture and NAAT assay performance against our routine indicators (first, microbiologically-confirmed and then histologically-confirmed osteo-articular tuberculosis) were derived using the caret package in R [17].

2.8.1 Interpretation of NAAT assay sensitivity and specificity

Results obtained from both the MTB/Rif assay and LPA were compared against mycobacterial culture results as well as histological assessment, as both of these test are considered as reference standards for the diagnosis of osteo-articular TB. In doing this we aimed to determine the sensitivity, or the ability of the NAAT assays to correctly identify the presence of *Mtb* in culture or histological positive cases; and the specificity, or the ability of the NAAT assay to correctly exclude the presence of *Mtb* in those who have a negative culture or histological result (see Figure 2.1).

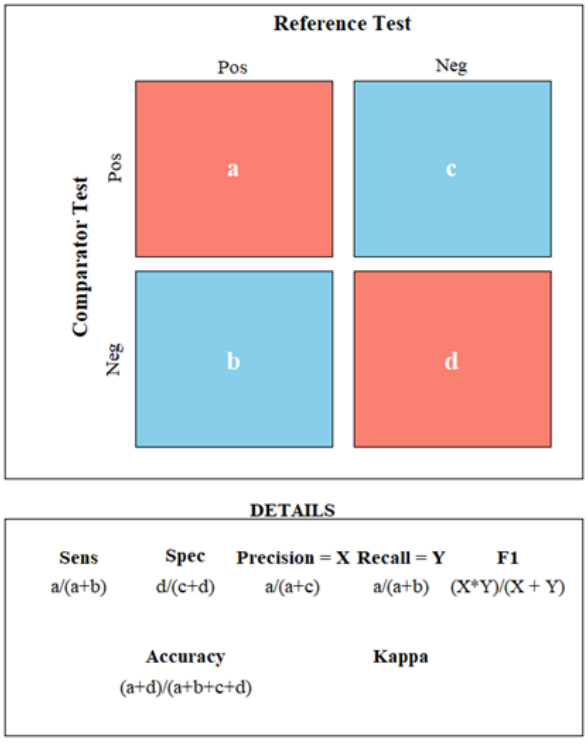

Figure 2.1 Standard Contingency Table for Evaluation of Test Performance against a Gold Standard Indicator

2.9 Ethics

Ethics approval was granted by the University of the Witwatersrand Human Research Ethics Committee (HREC) (Medical) (Clearance Certificate: **M170357**) (see Appendix B).

Written informed consent for inclusion of participants into this prospective investigational study was from patients who were eligible to consent. In the case of minors, consent was obtained from their legal guardians. Assent was obtained from children above 12 years old. The Participant Information Sheet, Informed Consent and Assent Forms are included in the appendices (see Appendices C through E).

CHAPTER 3

Results

A total of 41 cases of suspected osteo-articular tuberculosis were admitted to the CHBAH Orthopaedic Department during the 12-month study period. Of these patients, 29 (70.7%) patients were enrolled into the study (see Figure 3.1). The remaining twelve patients with suspected osteo-articular tuberculosis that were not included in the study, declined to participate in the study or were not consented to participate in the study prior to their surgery.

Of the 29 patients enrolled in the study, 20 patients had suspected extra-spinal osteo-articular tuberculosis. The remainder (9 cases) had suspected spinal tuberculosis. (see Table 3.1 and Figure 3.2).

Table 3.1 Joint Involvement in Suspected Osteo-articular TB Cases Enrolled into the Study

Suspected Osteo-articular TB	Site	Number of cases
Extra Spinal TB (n=20)	Knee	7 cases
	Hip	7 cases
	Wrist	4 cases
	Ankle	1 case
	Elbow	1 case
Spinal TB		9 cases

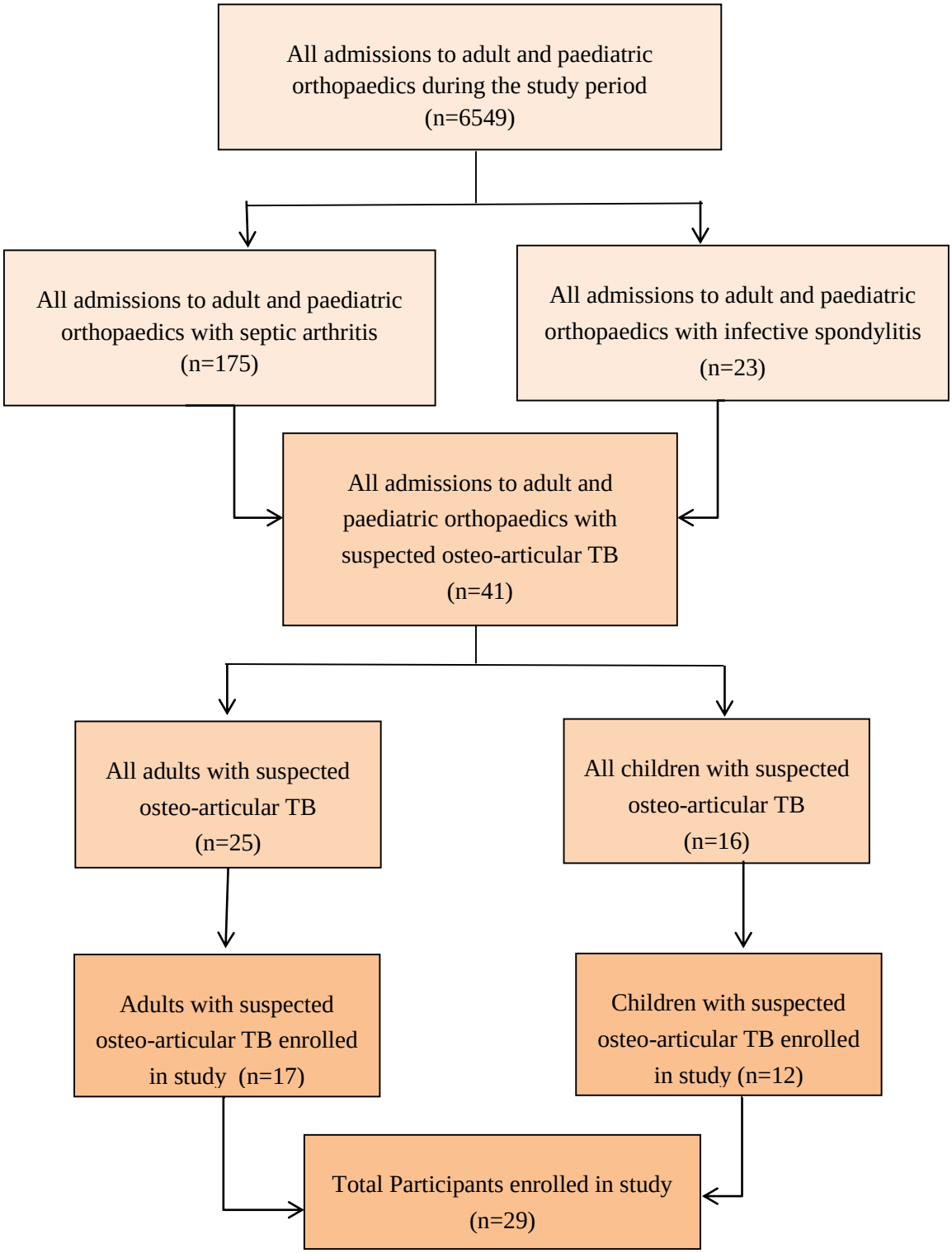

Figure 3.1: Case Flow Diagram

Abbreviation: TB = Tuberculosis.

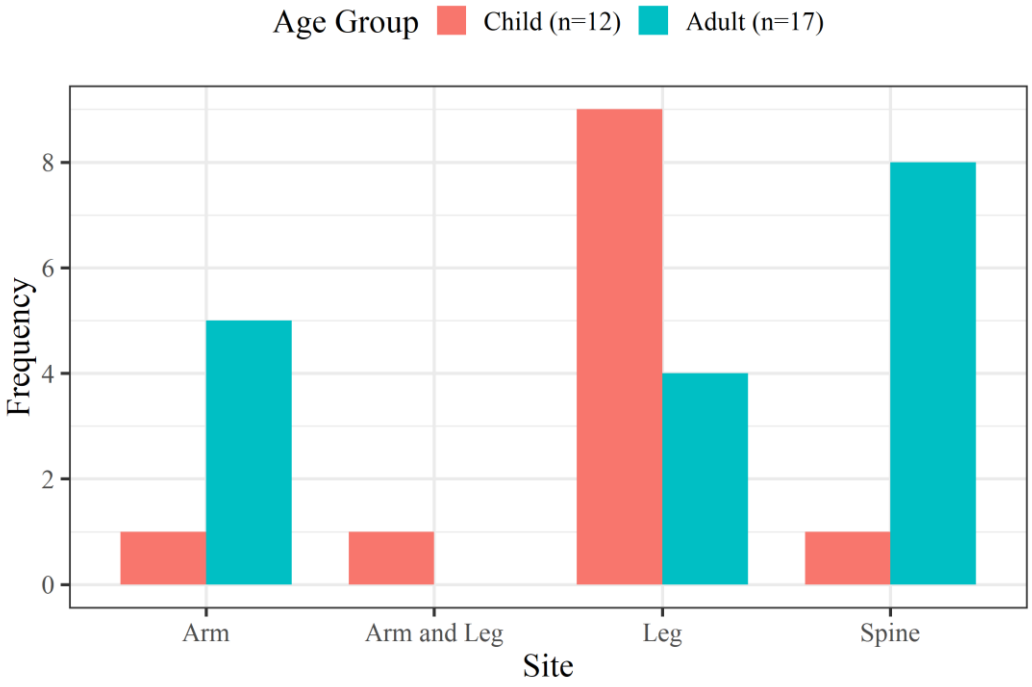

Figure 3.2: Site Distribution of Osteo-articular TB, Stratified by Age Group

3.1 Patient Demographics

Of the 29 patients that were enrolled into the study, 17 (58.6%) were adults and 12 (41.4%) were children. In the adult group, the median age was 43 years (IQR, 35 to 55 years) while in the paediatric group the mean age was 10.5 years (SD, 4.7 years). There was no statistical difference between both groups with respect to gender.

In terms of symptom duration, the overall median duration of symptoms was 5.0 months (IQR, 2.0 to 8.0 months), with the paediatric group having a median duration of symptoms of three months (IQR, 2.0 to 7.5 months) versus six months (IQR, 3.0 to 8.0 months) in the adult group, P = 0.517.

3.2 Pre-operative Assessment

With regards to the pre-operative diagnostic workup, the median haemoglobin and white cell counts were similar across the groups with and without confirmed tuberculosis infection (using histological confirmation of disease as the gold standard indicator) (Table 3.2).

Table 3.2: Pre-operative Haemoglobin and White Cell Count

Variable	Value	Total Study Population (n=29)	Histology not Suggestive of TB (n=13)	Histology Suggestive of TB (n=16)	P-values*
Hb	Mean g/dL (SD)	11.6 ± 2.1	11.9 ± 2.4	11.3 ± 1.9	0.486
WCC	Median cells × 10^9/L (IQR)	7.0 (5.2 to 10.7)	8.3 (5.7 to 10.7)	6.6 (4.9 to 10.0)	0.497

Abbreviations: Hb = Haemoglobin; IQR = Interquartile range; SD = Standard deviation; TB = tuberculosis; WCC = White cell count.

* P-values in the comparison of patients with and without histologically-confirmed osteo-articular tuberculosis. Student t-test for haemoglobin; Wilcoxon rank sum test for white cell count.

ESR was significantly higher in those with histological evidence of osteo-articular tuberculosis (65 mm/hr; IQR, 30-99 mm/h) compared to those without such histological evidence (15 mm/hour; IQR 11-32 mm/hour), P = 0.010 (see Figure 3.3). CRP demonstrated a similar although not statistically significant pattern (see Figure 3.4), with a median CRP of 42.5 mg/L (IQR 28.5 to 87.5 mg/L) in those with histologically-confirmed osteo-articular disease compared to 21.0 mg/L (IQR 2.0 to 79.0 mg/L) in those without histologically evidence of tuberculosis osteo-arthritis (P = 0.219).

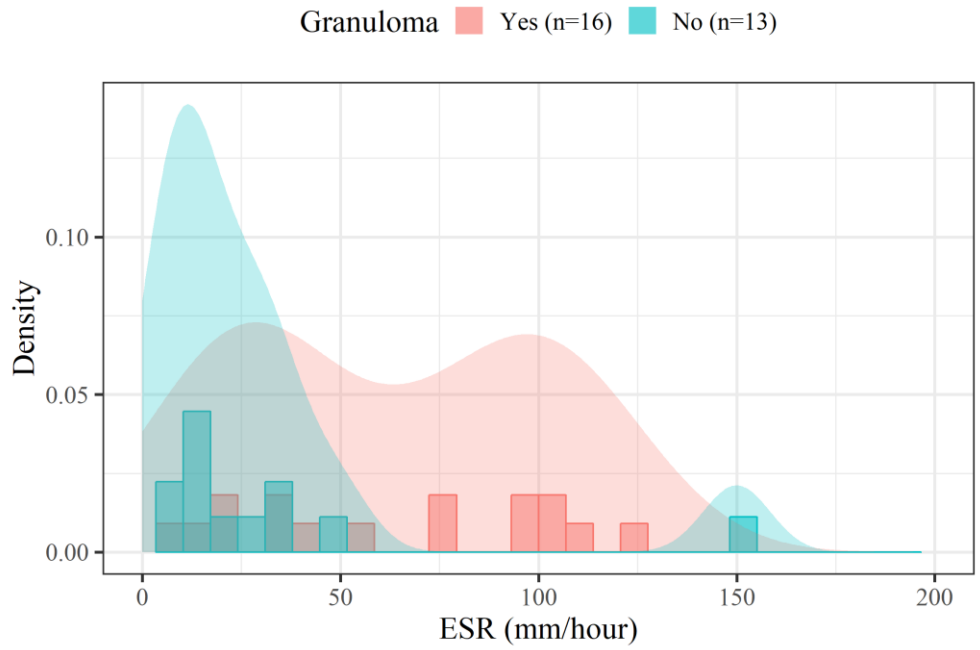

Figure 3.3: Preoperative ESR values

Abbreviation: ESR = Erythrocyte sedimentation rate

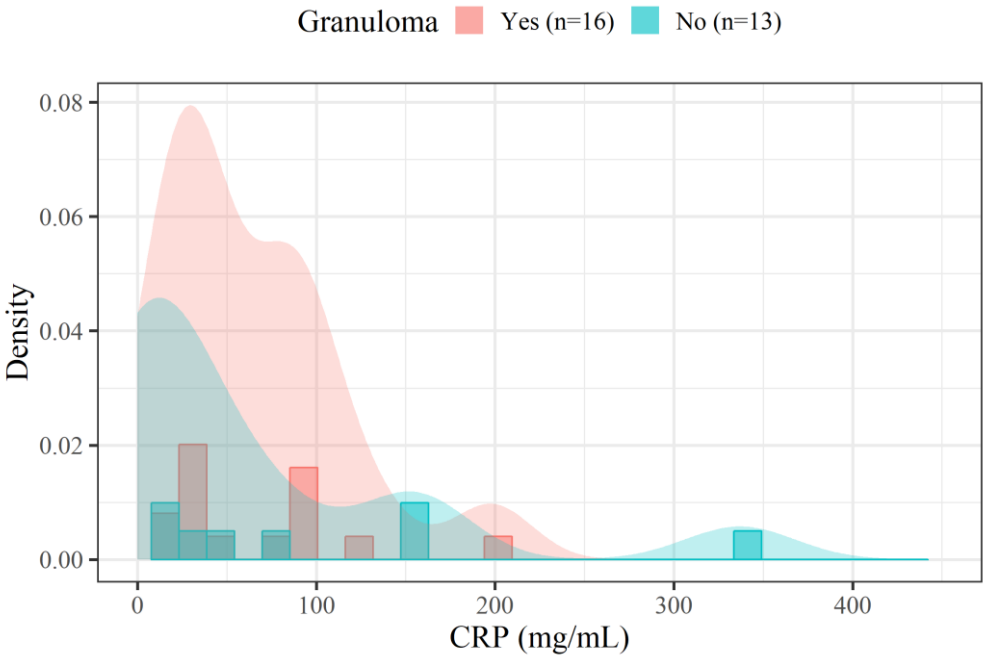

Figure 3.4: Preoperative CRP Values

Abbreviation: CRP = C-reactive protein

3.3 Diagnostic Accuracy of Investigations

Eleven (37.9%) patients had microbiologically-confirmed osteo-articular tuberculosis, whereas 16 (55.2%) of the 29 patients had histologic confirmation of osteo-articular tuberculosis.

3.3.1 Microbiologically-confirmed Tuberculosis as the Gold Standard Comparator

When comparing the NAAT to mycobacterial culture, the Xpert MTB/Rif assay demonstrated a sensitivity of 91% and a specificity of 72% (see Figure 3.5); while the LPA had a sensitivity of 100% and a specificity of 79% (see Figure 3.5).

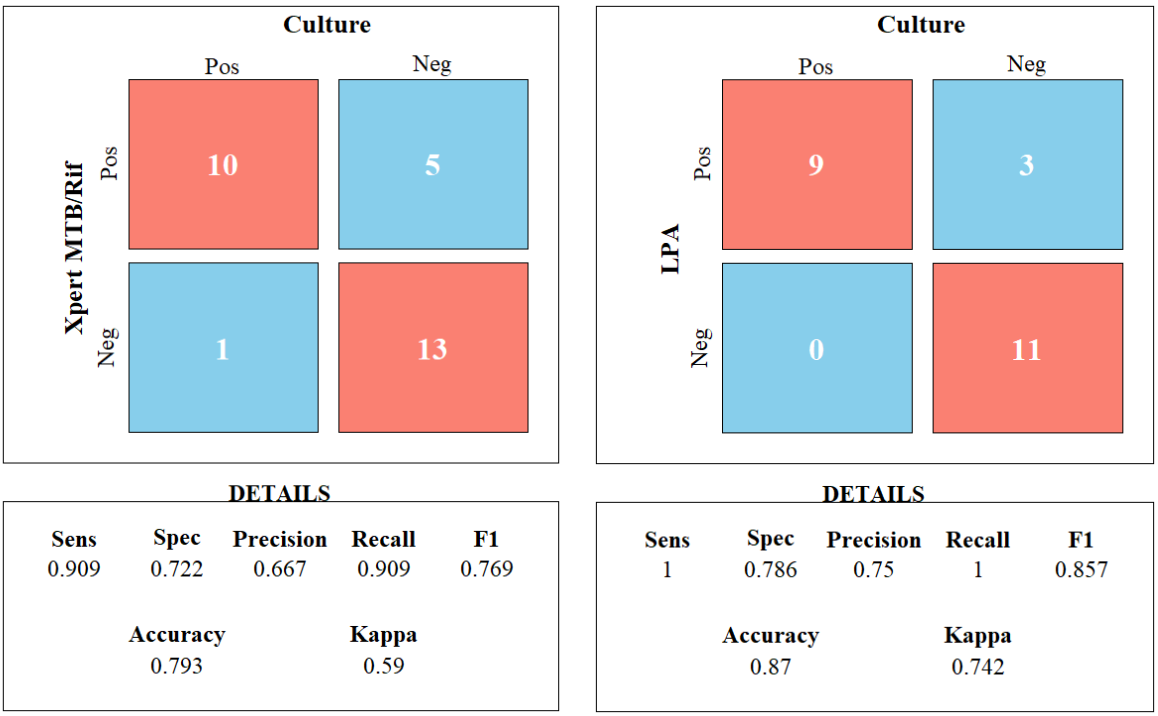

Figure 3.5: Contingency Tables of Culture as the Gold Standard indicator, compared to NAAT Assays

Abbreviations: LPA = Line probe assay; NAAT = Nucleic acid amplification test; Neg = Negative; Pos = Positive; Sens = Sensitivity; Spec = Specificity.

3.3.2 Histologically-suggestive Osteo-articular Tuberculosis as the Gold Standard Comparator

In the 16 patients with histological features of osteo-articular tuberculosis, 11 patients had positive detection of *Mtb* on microscopy and mycobacterial culture, demonstrating a sensitivity of 69% for microscopy and mycobacterial culture in this case series, compared to histologic confirmation of tuberculous osteo-articular disease. The specificity of microscopy and culture compared to histology was 100% (see Figure 3.6).

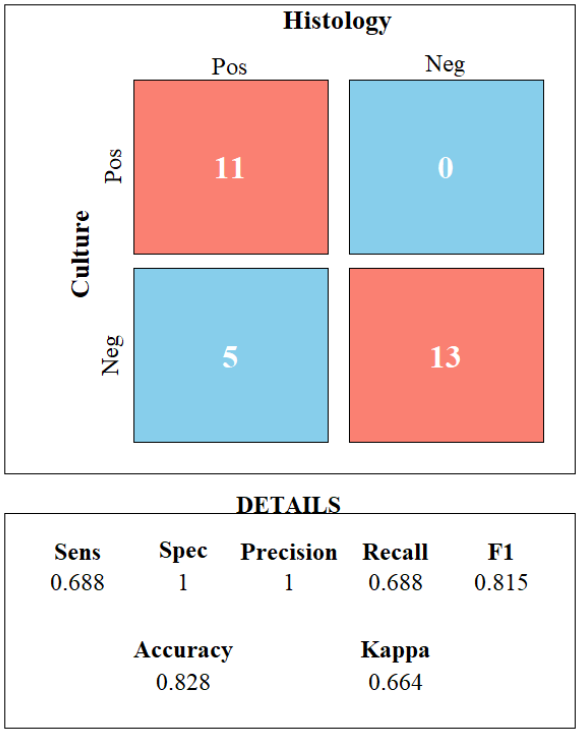

Figure 3.6: Contingency Table of Mycobacterial Culture, compared to Histology as the Gold Standard indicator

Abbreviations: Neg = Negative; Pos = Positive; Sens = Sensitivity; Spec = Specificity.

The results of NAAT assays in patients with histological features of tuberculosis infection showed a positive MTB/Rif assay in 15 of 16 patients, demonstrating a sensitivity of 94%, and a specificity of 100% in our series (see Figure 3.7). The MTB/Rif assay was able to identify four additional cases of tuberculosis (15/29 cases) versus mycobacterial culture (11/29).

The LPA was able to detect *Mtb* in all 12 patients with histological features of tuberculosis infection in whom LPA assays were done, demonstrating a sensitivity of 100% and a specificity of 100% in this case series (see Figure 3.7). Furthermore, the LPA was able to detect an additional case of *Mtb* that was not identified by mycobacterial culture (12/29 versus 11/29). The LPA was also able to detect a case of tuberculosis infection that the Xpert MTB/Rif assay was unable to detect.

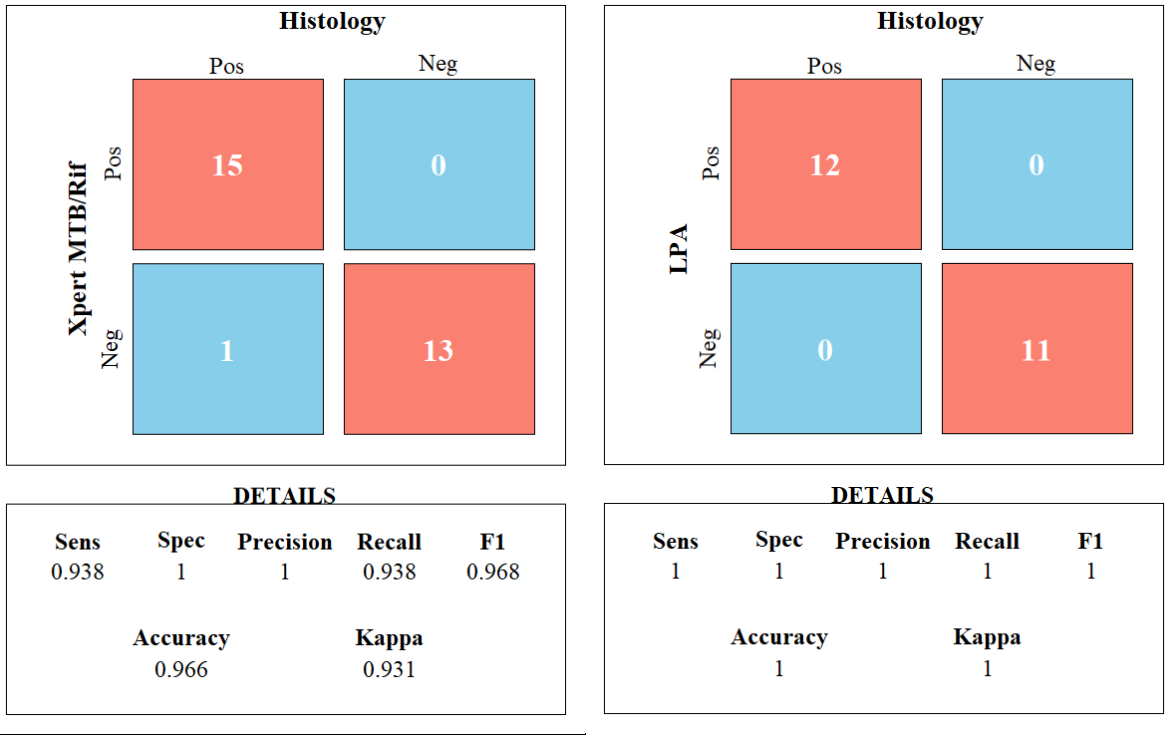

Figure 3.7: Contingency Tables of Histology as the Gold Standard indicator, compared to NAAT Assays

Abbreviations: LPA = Line probe assay; NAAT = Nucleic acid amplification test; Neg = Negative; Pos = Positive; Sens = Sensitivity; Spec = Specificity.

3.4 Time taken to Obtain Results

The median time to obtain a result using the Xpert MTB/Rif assay was three days (Range, 2.0 to 4.0 days) and for the LPA was five days (Range 4.0 to 6.0 days). This was in comparison to a mean of 15 days (SD, 5.9 days) for mycobacterial culture, $P < 0.001$. The median time to obtain a histology result was 17 days (Range, 14 to 19 days).

3.5 Impact of HIV on Diagnostic Accuracy

Nine (31.0%) of the 29 patients were HIV-infected. All HIV-infected patients were on antiretroviral therapy (ART), and had a median CD4 count of 309 cells/mm^3 (IQR, 234 to 360 cells/mm^3). Seven (77.8%) of the 9 HIV-infected patients were virologically suppressed, while two patients had viral loads > 60 000 copies/mL. Two (22.2%) of the 9 HIV-infected patients were children: both were girls, one aged nine and the other 10 years, and both had suppressed viral loads.

Patients who were HIV-infected presented with a median symptom duration of three months (IQR, 2.0 to 8.0 months) compared to 5.5 months (IQR, 2.0 to 8.3 months) in HIV-uninfected group, $P = 0.830$. As only three HIV-infected patients had culture-confirmed osteo-articular tuberculosis, NAAT sensitivity and specificity was derived in comparison to histologic findings. Six (66.7%) of the nine HIV-infected patients had histological features suggestive of tuberculosis infection. Mycobacterial culture had a sensitivity of 50% and specificity of 100% compared to histology in HIV-infected patients (see Figure 3.8).

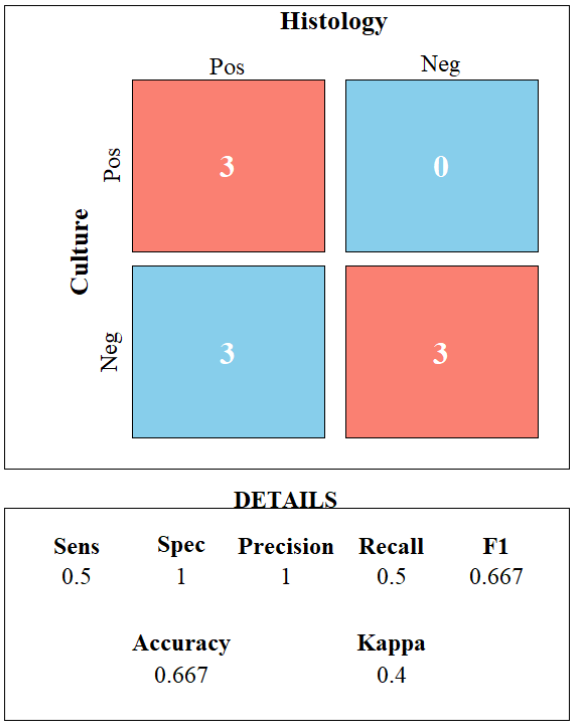

Figure 3.8: Contingency Table of Histology as the Gold Standard indicator, compared to Mycobacterial Culture in HIV-infected Patients

Abbreviations: Neg = negative; Pos = positive; Sens = sensitivity; Spec = specificity.

In assessment of the MTB/Rif assay, all six HIV-infected patients with histological features of tuberculosis infection were identified by the Xpert MTB/Rif assay, giving rise to a test sensitivity of 100% and specificity of 100% in this group (see Figure 3.9). Furthermore, all three of the HIV-infected patients with histological features of tuberculosis whose specimens were subjected to LPA testing were identified by the LPA, which gave rise to a test sensitivity and specificity of 100% (see Figure 3.9).

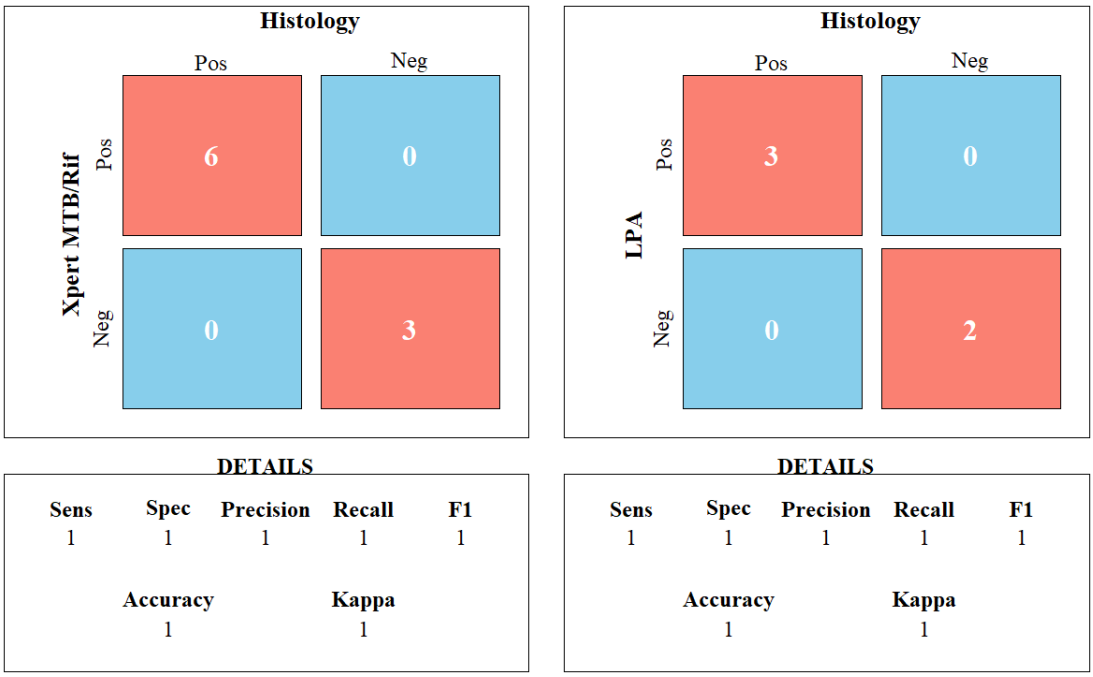

Figure 3.9: Contingency Tables of Histology as the Gold Standard indicator, compared to NAAT Assays in HIV-infected Patients

Abbreviations: LPA = line probe assay; NAAT = Nucleic acid amplification test; Neg = negative; Pos = positive; Sens = sensitivity; Spec = specificity.

3.6 Drug Resistance

One case of rifampicin resistant osteo-articular tuberculosis was identified by the Xpert MTB/Rif assay, and this was subsequently confirmed on mycobacterial culture and sensitivity. This case arose in a 2.9 year old, HIV-uninfected boy with tuberculosis of the thoracic spine. His Xpert MTB/Rif results became available after two days, and LPA result became available on day six; whereas his mycobacterial culture result was positive after 11 days. He was initiated onto drug-resistant tuberculosis therapy six days into his hospitalisation.

CHAPTER 4

Discussion

This study consisted of 29 patients, prospectively enrolled over a 12-month period, with suspected osteo-articular tuberculosis on the basis of clinical or radiological suspicion of tuberculosis infection. The study group consisted of both an adult group (n = 17) and paediatric group (n = 12), and included 71% of all suspected cases of osteo-articular tuberculosis hospitalised in the Orthopaedic Department at CHBAH during the study period.

There were no statistically significant differences in symptom duration between paediatric and adult cases in our cohort. The median duration of symptoms in our series of five months, was in keeping with other study samples which demonstrated a median of 4.5 months history of symptoms [(18)].

The median ESR was significantly higher in patients subsequently diagnosed as having histologically-confirmed osteo-articular tuberculosis, whereas the CRP was not as discriminatory between those with and without histologically-confirmed disease. When assessing the median ESR of 65 mm/h in our series, this value is higher than that expected for PTB which shows a median ESR of 50 mm/hr [(19)]. Aggarwal et al. demonstrated a median ESR of 43 mm/h (IQR 20-58 mm/h) in his series of 32 patients with clinically-suspected osteo-articular tuberculosis in New Delhi [(20)]. The ESR in our series may provide a suitable pre-operative proxy measure when considering the differential diagnosis of osteo-articular tuberculosis in our setting.

In assessing the diagnostic accuracy of the NAAT assays in our series, we compared the results of our findings for the Xpert MTB/Rif and LPA NAATs against the traditional diagnostic investigations of mycobacterial culture and histological assessment. When using mycobacterial culture as the comparative gold standard, the MTB/Rif and LPA assays demonstrated sensitivities of 90% and 100% respectively, and specificities of 72% and 79% respectively (see Figure 3.5). The NAAT sensitivity estimates observed in our study are similar to those demonstrated by Held et al., who in their series demonstrated a sensitivity and specificity of Xpert MTB/Rif of 92% and 99%, respectively, compared to mycobacterial culture [(14)].

When histology was used as the comparator, mycobacterial culture demonstrated a sensitivity of 69%, although specificity was high (100%). This finding is similar to results obtained by Aggaral et al., who in their comparison of mycobacterial culture against histology demonstrated a sensitivity of 36% and specificity of 95% for mycobacterial culture [(20)].

In comparing the results of the NAAT in our series against histology, the Xpert MTB/Rif and LPA tests demonstrated significantly improved sensitivity over mycobacterial culture (94% and 100%, respectively, versus 69%). Specificity of the two NAAT assays were 100%, as was the specificity of mycobacterial culture compared to histology. This improved sensitivity contributed to the Xpert MTB/Rif assay identifying *Mtb* in four additional cases that were missed by culture. The higher test sensitivity of Xpert MTB/Rif and LPA compared to mycobacterial culture highlights the potential benefit of improved case detection with the use of NAAT, as these cases would have been missed if mycobacterial culture alone was used as the diagnostic tool.

The time taken to obtain diagnostic confirmation of *Mtb* was reduced through use of both NAATs in our study, with an average time to result in three days using Xpert MTB/Rif and

five days using LPA, both of which were substantially shorter than the 15 days for microscopy and mycobacterial culture and 17 days for histology. This rapid diagnostic turnaround versus traditional methods facilitates earlier initiation of treatment of tuberculosis.

Another advantage of the Xpert MTB/Rif assay was that it has the capability of rapidly identifying patients with drug resistant *Mtb* disease [(21)]. In our series we identified one case of rifampicin resistant spinal tuberculosis in a child, using both the Xpert MTB/Rif assay and LPA. This result was confirmed on formal culture and sensitivity 11 days post biopsy, by which time the patient had been initiated onto appropriate anti-tuberculous therapy for MDR-TB. The rapid identification of *Mtb* as well as the identification of drug resistant infection allows for the prompt initiation of appropriate chemotherapy, which may improve patient functional outcomes [(12)].

With respect to the use of NAAT assays in the setting of HIV infection, both Xpert MTB/Rif and LPA demonstrated a 100% sensitivity. This suggests that NAAT should be used routinely in the diagnostic work-up of HIV-infected patients with suspected osteo-articular tuberculosis.

The use of NAAT, in the diagnosis of *Mtb* infection has become a popular focus of study in recent literature. The efficacy of NAAT in the rapid identification of *Mtb* and drug resistant infections has resulted in the WHO recommending the conditional use of NAAT for the diagnosis of EPTB [(5)]. This together with the development of improved NAATs such as the Xpert MTB/Rif Ultra, which has been reported to demonstrate improved detection of *Mtb* [(22)], highlights a trend towards the use of NAATs as a routine diagnostic tool in the detection of EPTB *Mtb* infection.

4.2 Limitations

We identified the following limitations in our study:

1. Sample size

Our study sample consisted of only 29 patients representing a small sample size on which our results are based, however the sample represented 71% of all suspected tuberculous osteo-arthritis cases treated at the CHBAH Orthopaedic Department during the study time frame.

2. Biopsy Strategy

Surgical biopsy specimens could not be standardised as they were obtained by different surgeons with different skill levels. Furthermore, there was no way to standardise the actual zone of biopsy as they were taken from various sites at the surgeons' discretion.

3. Time taken from biopsy to specimen processing

The time delay between obtaining biopsy specimens and processing at the laboratory was variable as biopsies were done on both elective and emergency surgical lists, resulting in certain emergency specimens being taken after hours, leading to a potential delay in processing of specimens.

4. Time to initiation of Anti-tuberculous Therapy

In our study we did not record the time taken for the initiation of anti-tuberculosis treatment once a positive identification of *Mtb* was made. Therefore, we could not formally analyse the role which NAAT played in terms of expediting initiation onto appropriate therapy. The single case of multidrug-resistant tuberculosis detected through the study had been initiated onto targeted anti-mycobacterial therapy by day six of hospitalisation, in response to the Xpert MTB/Rif result.

4.3 Recommendations

In our series, 29 of the 41 patients with suspected osteo-articular tuberculosis were successfully enrolled, representing a 71% case enrolment. This represents a smaller sample size than initially anticipated, which may be improved upon on in future studies, by increasing the study time period, as well as widening the study catchment area to include other hospitals in our area. Notwithstanding the small sample size, a number of pertinent recommendations can be made, based on the findings from this study.

In the diagnostic workup of patients with suspected osteo-articular tuberculosis, ESR was shown to be a valuable tool in distinguishing between patients with or without osteo-articular tuberculosis. We support the use of ESR as part of the routine pre-operative work-up of patients with suspected osteo-articular tuberculosis at our facility.

In our series, the operative procedures for biopsy were performed at various sites, by multiple surgeons with varying amount of experience, resulting in a non-standardised sample collection strategy. This could be addressed in further studies by a designated surgical team performing standardised surgical approaches for biopsy, which may result in improved diagnostic accuracy.

NAAT should be included as part of the routine workup of patients with suspected osteo-articular tuberculosis, especially in HIV positive patients, to ensure greater diagnostic accuracy as well as to reduce diagnostic delay. In future studies, the additional assessment of time taken to initiate appropriate anti-tuberculous therapy following positive identification may provide further information on the impact of NAAT diagnostic assays on treatment outcomes in patients with osteo-articular tuberculosis.

CHAPTER 5

Conclusion

NAATs appear to be a valuable tool in the diagnostic assessment of osteo-articular tuberculosis infection in paediatric and adult patients, regardless of HIV infection status. These tests are able to provide identification of *Mtb* DNA as well as potential drug resistance, with rapid turnaround time as compared to mycobacterial culture and histology. In our series, the Xpert MTB/Rif and LPA assays were both demonstrated to be useful and accurate tests for the diagnosis of osteo-articular tuberculosis

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Appendices

Appendix A: Data Collection Sheet

Patient Data			
Patient Code		Age	Gender
Immune Status			
HIV exposed	Exposed	Un-exposed	
HIV status	Reactive	Non-reactive	
If HIV Reactive			
CD4 count			
ART			
Viral Load			
TB History			
Previous TB	Yes	No	
If previous TB			
	Pulmonary	Extra-pulmonary	
Previous TB treatment			
TB regimen			
TB treatment duration			
Clinical presentation			
Presenting symptoms and signs			
Duration of symptoms			
Joint(s) affected			
Clinical findings of affected joint			
Investigations			
Chest X-ray findings			
ultrasound findings			
Limb/joint X-ray findings			
Hb		Lab Sticker/Ref please	
Wcc			
Diff count			
ESR			
CRP			
Blood Culture			
Joint Aspirate	Date specimen taken		
Bacterial MCS	R	Date	
TB MCS	R	Date	
Xpert MTB/RIF	R	Date	
Line Probe Assay	R	Date	
Joint Tissue Biopsy	Date specimen taken		
Bacterial MCS	R	Date	
TB MCS	R	Date	
Histology	R	Date	
Xpert MTB/RIF	R	Date	
Line Probe Assay	R	Date	

Appendix B: Ethics Clearance Certificate

R14/49 Dr Dharshen Naicker and Dr D More

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170357

NAME: (Principal Investigator)	Dr Dharshen Naicker and Dr D More
DEPARTMENT:	Orthopaedic Surgery Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Nucleic Acid Amplification Testing for the Diagnosis of Osteo-articular Tuberculosis
DATE CONSIDERED:	31/03/2017
DECISION:	Approved unconditionally
CONDITIONS:	Title Change (11/08/2017)
SUPERVISOR:	Dr G Firth
APPROVED BY:	Professor P. Cleaton-Jones Chairperson, HREC (Medical)
DATE OF APPROVAL:	21/04/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review March and will therefore be due in the month of March each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 11/08/2017

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix C: Participant Information Leaflet

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT:
NUCLEIC ACID AMPLIFICATION TESTING FOR THE DIAGNOSIS OF OSTEO-ARTICULAR TUBERCULOSIS

RESEARCHERS NAME(S):

DR D NAICKER, DR G FIRTH, DR D MOORE

ADDRESS:

DEPARTMENT OF ORTHOPAEDIC SURGERY, CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL, SOWETO, JOHANNESBURG, GAUTENG, SOUTH AFRICA

CONTACT DETAILS:

DR D NAICKER (RESEARCHER)

0827466099

naickerdharshen@gmail.com

DR G FIRTH (RESEARCH SUPERVISOR)

0119339396

greg.firth@gmail.com

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

UNIVERSITY OF THE WITWATERSRAND

011 717 1252

Greetings Dear Parent/Guardian

Thank you for taking the time to consider our request for your help in taking part in our research project. You and /or your child are being invited to take part in this research project because your child has an infection in their bone and, or joint. As part of the normal treatment of this problem we do tests on the samples of bone and tissue we obtain from surgery to look for what organism is causing the infection. In all bone and joint infections we treat we always look for Tuberculosis Infection so we can ensure we do not miss Tuberculosis infection. Our research project is looking at a new set of test which identifies Tuberculosis by looking for specific materials within the Tuberculosis bacteria. Your help with this study will help us see if these tests will help improve the way we look for Tuberculosis.

Please take some time to read the information presented here, which will explain the details of our intended research project. If you have any questions regarding our project or you would like further

information regarding the study please feel free to speak to us directly or contact us using the contact information provided above.

It is very important that you fully understand what this research project entails so that you can make a well informed decision regarding your participation.

Your participation is **entirely voluntary** and you are free to decide if you would like to participate in our study or not. If you say no, this will not affect you or your child negatively in any way whatsoever, and your child will still receive the best standard of care we can provide.

If you agree to participate in this study, you are also free to change your mind and withdraw from the study at any point without any negative consequences to you or your child.

What is this research study all about?

- This study will be done at Chris Hani Baragwanath Academic Hospital, Soweto, Gauteng, South Africa.
- The study is being done by the Department of Orthopaedic Surgery
- The patients involved in this study will include all adults and children who we suspect have a joint infection caused by Tuberculosis.
- In addition to our routine tests to identify which organisms may be causing this infection, we would like to do additional tests on the samples we obtain to look for tuberculosis infection

Why have you been invited to participate?

- All patients with suspected bone or joint Tuberculosis have been invited to participate in this study. As part of the routine treatment for this problem we try and identify the organisms that are responsible for the infection. One organism that we always look for is Tuberculosis. Our study is looking at new test which looks specifically for the material that makes up the Tuberculosis bacteria. Your help will in study will help us to determine if these tests, the GeneXpert and Line Probe Assay are useful tests to help us look for Tuberculosis.

What will your responsibilities be?

- You and/or your child will have no additional responsibilities, procedures or costs during this study

Are there in risks involved in your taking part in this research?

- There are no added risks to you/your child as there are no extra surgical procedures performed or costs involved.

Who will have access to your medical records?

During the study the researchers will require access to your/ your child's medical records to assess information as well to identify relevant factors that may affect the results of the test.

All efforts will be made to keep personal information confidential and your/your child's, identity and information will be protected by a special identification code that will be assigned to your child.

Please note that absolute confidentiality cannot be guaranteed and medical information may be disclosed if required by law. You will be informed if this is the case before any information is released

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the human research ethics committee and the medicines control council (where appropriate).

If results are published, you or your child's personal information will not be published.

Your participation in the study

You and your child's participation in this study is completely voluntary. If you decline to participate there is no penalty or loss of benefits and your child will still receive the best standard of care possible. You also have the right to withdraw from the study at any time you choose to do so without any negative consequences.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been forced to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

By signing below, I ……..……. agree to take part in/have my child take part in a research study entitled Nucleic Acid Amplification Testing for the diagnosis of Osteo-articular Tuberculosis.

Signed at (*place*) .....…..…….. on (*date*) ……...……..

..... **.....**

Signature of patient/parent/guardian Signature of witness

Declaration by Investigator

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....….....…….. on (*date*) ……...……..

..... **.....**

Signature of investigator Signature of witness

……

Signature of translator (if applicable)

Appendix D: Participant Consent Form

PATIENT CONSENT FORM

TITLE OF THE RESEARCH PROJECT:
NUCLEIC ACID AMPLIFICATION TESTING FOR THE DIAGNOSIS OF OSTEO-ARTICULAR TUBERCULOSIS

RESEARCHERS NAME(S):

DR D NAICKER, DR G FIRTH, DR D MOORE

ADDRESS:

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CONTACT DETAILS:

DR D NAICKER (RESEARCHER)

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DR G FIRTH (RESEARCH SUPERVISOR)

0119339396

greg.firth@gmail.com

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

UNIVERSITY OF THE WITWATERSRAND

011 717 1252

Greetings Dear Patient

Thank you for taking the time to consider our request for your help in taking part in our research project. You are being invited to take part in this research project because you have an infection of the bone and, or joint. As part of the normal treatment of this problem we do tests on the samples of bone and tissue we obtain from surgery to look for what organism is causing the infection. In all bone and joint infections we treat we always look for Tuberculosis Infection so we can ensure we do not miss Tuberculosis infection. Our research project is looking at a new set of test which identifies Tuberculosis by looking for specific materials within the Tuberculosis bacteria. Your help with this study will help us see if these tests will help improve the way we look for Tuberculosis.

Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand.

It is very important that you are fully and clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to take part in or decline to take part in the study. If you say no, this will not affect you negatively in any way whatsoever. If you do agree to take part in this study you can change your decision to participate at any time you choose without affecting you.

What is this research study all about?

- This study will be done at Chris Hani Baragwanath Academic Hospital, Soweto, Gauteng, South Africa.
- The study is being done by the Department of Orthopaedic Surgery
- The patients involved in this study will include all adults and children under that we suspect have a joint infection caused by Tuberculosis
- In addition to our routine tests to identify which organisms may be causing this infection, we would like to do additional tests on the samples we obtain to look for Tuberculosis infection

How will you be involved in the study?

- You will be invited to be included in the study if you have a joint or bone infection that requires surgical treatment.
- A routine step in during the surgical treatment of these bone and joint infections is the taking of fluid and tissue samples from the affected joint or bone to be sent for tests to identify the organism that is causing the infection.
- These tests normally include testing the tissue for the presence of Tuberculosis infection through Mycobacterial Culture Tests (we look for Tuberculosis organism growth from samples taken) and Histological (we look at the tissue under a microscope to look for signs of Tuberculosis) assessment.
- In our study we are using these tests and comparing them to a new type of test that looks for genetic material specific to the organism that causes Tuberculosis. These tests are the Xpert MTB/RIF assay and Line Probe Assay and they work by looking for special building blocks that make up the Tuberculosis bacteria.
- There are no additional procedures that you need to endure and no additional costs that you will be responsible for.
- Tissue samples obtained will not be stored for future testing.
- If there is Tuberculosis infection these tests can also demonstrate if the Tuberculosis organism can be treated with normal Tuberculosis medication.
- We will require access to your medical records in order to more accurately assess the accuracy of the above mentioned tests
- All of your personal information will be kept confidential as you will be allocated a special code for collection and storage of information. This code will be kept secret so that your information will be kept private and safe.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been forced to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.

By signing below, I ……..……. agree to take part in a research study entitled *Nucleic Acid Amplification Testing for the diagnosis of Osteo-articular Tuberculosis*

Signed at (*place*) .....….....…….. On (*date*) ……...…….

..... **.....**

Signature of patient Signature of witness

Appendix E: Participant Assent Form

PARTICIPANT INFORMATION LEAFLET AND ASSENT FORM

TITLE OF THE RESEARCH PROJECT:
NUCLEIC ACID AMPLIFICATION TESTING FOR THE DIAGNOSIS OF OSTEO-ARTICULAR TUBERCULOSIS

RESEARCHERS NAME(S):

DR D NAICKER, DR G FIRTH, DR D MOORE

ADDRESS:

DEPARTMENT OF ORTHOPAEDIC SURGERY, CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL, SOWETO, JOHANNESBURG, GAUTENG, SOUTH AFRICA

CONTACT NUMBER:

Dr D Naicker:

082 746 6099

DR G FIRTH

011 933 9396

What is research*?*

Research is something we do to find new knowledge about the way things (and people) work. We use research projects or studies to help us find out more about disease or illness. Research also helps us to find better ways of helping, or treating children who are sick.

What is this research project all about?

Our research project is based on children who have infections in their joints. Some of these infections may be caused by a special bacterium that causes TB. In our study we are looking to see if using two special tests can help us better identify this TB bacteria. These tests work by looking for special features that only TB bacteria have.

Why have you been invited to take part in this research project?

You have been invited to take part in our study because you have an infection in your joint. This joint infection may or may not be due to TB, but by doing our special test we hope to see if we can improve the way we look for TB.

Will anyone know I am in the study?

You and your parent will have to first agree to let you take part in our study. If you and your parent agree, the doctors looking after you will ask you certain questions and look at your medical file to get basic information about your age, gender and any medical illness you may have or had.

All of this information will be kept confidential which means we will not share it with anyone without obtaining permissions from you and your parent. We will also give you a special code which will be secret identity to allow us to track of your information.

Do you understand this research study and are you willing to take part in it?

YES	NO

Has the researcher answered all your questions?

YES	NO

_____ _____

Signature of Child Date