QUALITY OF TUBERCULOSIS MICROSCOPY IN KWAZULU-NATAL AS DETERMINED BY LABORATORY PROFICIENCY TESTING

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ABSTRACT / SUMMARY

Introduction

Sputum smears stained by the Ziehl-Neelsen method are the least expensive tool for diagnosing patients with infectious tuberculosis. However, false positive and false negative results have serious implications for treatment of patients. Therefore, controlling the quality of sputum microscopy services is important to ensure that the laboratory produce results that are accurate, reliable and reproducible.

Aim

The aim of the study was to determine the quality of tuberculosis smear microscopy in public health laboratories in KwaZulu-Natal between the years 2001 and 2006, and to assess the current knowledge and attitude of laboratory workers and laboratory managers to proficiency testing as a quality assurance technique.

Methods

A secondary analysis of laboratory proficiency testing results, from the KwaZulu-Natal reference laboratory (2001 to 2004) and from the National Health Laboratory Services reference laboratory (2006), was performed. Key informant interviews were conducted to determine the role proficiency testing played as a quality assurance technique.

Results

Overall laboratory performance was 93% from 2001 to 2004 and 98% in 2006. High false negative results were the predominant error. Sensitivity and specificity improved from 91% (for both) in 2001 to 2004 to 97% (for both) in 2006. Overall performance of primary, district and tertiary health care levels were 92%, 93% and 73% respectively in the period 2001 to 2004 and 98%, 98% and 94% respectively in 2006.There was significant ($p<0.01$) improvement in both urban (97%) and rural (98%) laboratory performance in 2006. The overall scores by year ranged from 89% (2002) to 98% (2006), but the annual overall scores (2001 to 2006) only achieved the acceptable level twice.

Key informants indicated that proficiency testing was an essential exercise, however, they reported challenges such as inconsistent feedback, high workload and need for training.

Discussion

Overall performance improved from an unacceptable level of 93% (2001-2004) to a satisfactory level of 98% (2006). Likely reasons include improvement in technical skills of microscopists and improvement in preparation of proficiency testing slides. Proficiency testing is considered an essential exercise to improve laboratory performance, however, participants know that they are being tested and may give 'special attention' to proficiency testing slides resulting in a social desirability bias.

Recommendations

A blinded rechecking programme should be established in conjunction with the use of a standardised checklist during support visits. Feedback, communication and staff training should be improved while the workload should be evaluated.

DECLARATION

I Wayne Ramkrishna, declare that

(i) The research reported in this dissertation, except where otherwise indicated, is my original research.

(ii) The dissertation has not been submitted for any degree or examination at any other university.

(iii) The dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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a) their words have been re-written but the general information attributed to them has been referenced;

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(v) The dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

The dissertation is prepared in partial fulfilment of the requirement of the Master of Public Health degree at the Department of Public Health Medicine, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa.

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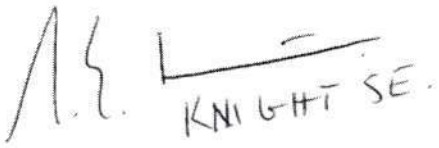
SUPERVISOR SIGNATURE: 

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TABLE OF CONTENTS

TABLE OF TABLES

TABLE OF FIGURES

1 CHAPTER I: INTRODUCTION AND BACKGROUND TO THE STUDY

1.1 INTRODUCTION

Tuberculosis (TB) remains a major health problem for South Africa and indeed, the world. The World Health Organisation (WHO) estimates that eight million new cases and three million deaths are directly attributable to TB each year, making TB the leading cause of death due to a single infectious agent. [1]

The 2007, WHO Global Tuberculosis Control report, estimates the TB incidence risk in South Africa for 2005 to be 600 per 100 000 population. South Africa was ranked seventh in the world by estimated number of incident cases of tuberculosis. In addition, it is estimated that 58% of adult (15 to 49 years) TB cases are co-infected with Human Immunodeficiency Virus (HIV). The incidence risk of tuberculosis has increased hugely since 2002. [2, 3]

The National Department of Health estimates that there were 316 836 TB cases across the whole country in 2006. [4] The incidence risk of TB differs between the provinces in South Africa. [5] Western Cape, Eastern Cape, Northern Cape and KwaZulu-Natal provinces are the most severely affected. The incidence risk for these provinces for 2006 as reported through the electronic TB register were Western Cape 1 031 (actual number: 48 989), Eastern Cape 662 (actual number: 46 716), Northern Cape 922 (actual number: 8 379) and KwaZulu-Natal 911 (actual number: 88 704) per 100 000 population. [5]

Tuberculosis constitutes the leading cause of death in HIV/TB co-infected patients. Stigmatisation of tuberculosis, HIV and Acquired Immune Deficiency Syndrome (AIDS) contributes to sufferers hiding their disease for as long as possible, which allows for further

spread of the tubercle bacillus that could have been prevented. Often TB patients are ill informed about the disease and frequently fail to understand the need to complete at least six months of uninterrupted TB therapy. A treatment interrupter is classified as a patient whose treatment was interrupted for two months or more. [6] This may result in the relapse of the disease and the heightened possibility of developing multidrug-resistant (MDR) forms of tuberculosis. [5]

Patients presenting with symptoms of TB undergo microbiological examination of their sputum to confirm the diagnosis of disease and determine whether they are infectious prior to commencing TB therapy.[7] The examination consists of microscopic examination of sputum, stained by the Ziehl-Neelsen (ZN) method (smear microscopy). If acid-fast bacilli (AFB) are detected by this method, the patient is classified as having smear positive tuberculosis. It is important to conduct smear microscopy because it correctly and efficiently identifies the cases that are infectious and therefore require the highest priority for care in order to break the cycle of infection transmission. [6] Smear negative TB patients although being less infectious than those with positive smears can still transmit *Mycobacterium tuberculosis*. The presence of AFB is not indicative of TB in every case as mycobacteria other than *Mycobacterium tuberculosis* (MOTT) also demonstrate acid fastness. In South Africa, microscopy showing acid-fast bacilli is regarded as being infected with *Mycobacterium tuberculosis* until additional tests prove otherwise.

The acid-fast method for staining sputum smears is the least expensive tool for the rapid identification of potentially infectious tuberculosis patients. [8] Therefore it is most widely used in developing countries. This technique however, lacks sensitivity, detecting only 45 to 60% of culture positive cases.[9] Auramine staining followed by fluorescent microscopy may improve sensitivity of diagnosis but is feasible only in technically advanced laboratories. Sputum culture is the diagnostic 'gold standard' in TB and is even more sensitive but its application is limited by the time required to obtain a positive result, as well as its expense and other technical requirements. None of the commercial serological

antibody tests evaluated for the diagnosis of infectious pulmonary tuberculosis has performed satisfactorily enough to replace sputum smear microscopy. For these reasons, diagnosis of tuberculosis relied on a Ziehl-Neelsen smear of sputum. [10-13]

However, with the recent emergence of extensively drug resistant (XDR) strains of *Mycobacterium tuberculosis*, especially in South Africa, where the rates are among the highest in the world, there is an urgent need to revise the diagnostic algorithms.[14] AFB smear microscopy is even less sensitive in TB-HIV co-infected individuals.[9] Although HIV seropositive patients are likely to test smear negative, they have been shown to still be significantly associated with TB transmission.[7] With more than 30% of over 40 million HIV seropositive patients globally co-infected with TB, and more than 70% of these in sub-Saharan Africa, the low sensitivity of smear microscopy is unacceptable.[9] The difficulty in obtaining suitable sputum specimens in children exacerbates this situation, as does its inability to distinguish between *Mycobacterium tuberculosis* and non-tuberculous mycobacteria.

Despite high specificity and positive predictive values, nucleic acid amplification tests (NAATS), particularly in smear negative cases, are associated with moderate sensitivity that is highly variable.[15] Other disadvantages are the false negative and false positive results and the detection of non-viable bacteria.

A reverse line probe hybridisation assay (MTBDR*plus*) for the diagnosis of multidrug-resistance directly from sputum specimens has recently been shown in South Africa to be highly accurate with high sensitivity and specificity compared to conventional culture and drug susceptibility testing.[14] Performance of the assay among smear positive sputum specimens was equivalent to conventional drug susceptible testing based on Lowenstein–Jensen culture medium. The assay also performed well on smear negative, culture positive specimens. There was 100% correlation in results for detection of rifampicin (16/16 susceptible strains) and isoniazid (INH) resistance (4 resistant and 10 susceptible strains) when compared with conventional drug susceptible testing. Results were produced within 2

days for both smear positive and smear negative, culture positive samples in a high throughput laboratory. The MTBDR*plus* assay targets mutations in the genetic loci associated with isoniazid and rifampicin resistance. Its limitations lie in the potential for lower sensitivity in strains where resistance is coded for by mutations not targeted by the test strip. A similar test for the diagnosis of XDR-TB is available but is yet to be evaluated. On the basis of the results obtained in South Africa, the use of this molecular assay is to be implemented at less than half its cost in 16 under resourced countries around the world through the initiatives of the WHO, Stop TB Partnership, UNITAID[1] and the Foundation for Innovative New Diagnostics [14, 15]

False positive and false negative diagnostic results hold serious implications for a TB control programme. Therefore, quality control of sputum microscopy for TB is of paramount importance, in order to ensure that the microscopy results at the most peripheral level of the health service are both valid[2] and reliable[3]. [10]

The KwaZulu-Natal Provincial Laboratory Services[4] provide laboratory diagnostic services for the public funded health sector in KwaZulu-Natal province. Although the reference laboratory implemented proficiency testing, using unstained slides, in all 79 of the provincial laboratories in 2001, these proficiency test results were not analysed scientifically. The use of unstained slides for proficiency testing assesses several aspects of the laboratory's technical performance including preparation of staining reagents, staining procedure and reading and reporting of results.[11] Since the technical performance

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[1] **UNITAID** is an international facility for the purchase of drugs against HIV/AIDS

[2] 'Validity refers to the extent to which a measure actually measures what it is meant to measure. The measure lacks validity if an observer or instrument measures the characteristic in the same individual or group repeatedly higher or repeatedly lower than the real value'. [10]

[3] 'Reliability refers to the degree of similarity of the information obtained when the measurement is repeated on the same subject or the same group'. [10]

[4] In KwaZulu-Natal, during the study period, 79 laboratories performed TB smear microscopy, two had TB culture facilities and one referral laboratory carried out TB drug susceptibility testing.

impacts on the quality[5], the quality of TB smear microscopy in KwaZulu-Natal laboratories was not known.

1.2 BACKGROUND

1.2.1 What is the Problem?

The quality of TB smear microscopy in KwaZulu-Natal is not known. The knowledge and attitude of laboratory workers and laboratory managers to proficiency testing as a quality assurance tool also needs to be determined.

1.2.2 What is known so far?

Acid-fast staining of sputum in suspected TB infection is the most economical way of identifying potentially infectious TB patients. In low-income countries, the confirmation of the diagnosis of TB is still reliant on detecting the presence of acid-fast bacilli in stained sputum smears. However, TB microscopy services are often a neglected component of the Tuberculosis Control Programme. False positive and false negative microscopy results have serious implications for the diagnosis and subsequent treatment of the patient suffering from tuberculosis as well as providing a cost effective TB management service (Table 1).

Despite acid-fast microscopy being cheap, it is dependent on the quality of the sputum collected from the patient, the staining technique and the ability of the microscopist to detect AFB. A well functioning quality assurance system is therefore essential to ensure that results generated by the laboratory are accurate, reliable and reproducible. [12]

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[5] Quality in this study implies accurate, reliable and reproducible TB smear microscopy results. According to the WHO, quality is accomplished by assessing the quality of specimens, by monitoring performance of microscopy procedures, reagents and equipment against established limits, by reviewing microscopy results and by documenting the validity of microscopy methods.

Table 1: Effects of False Positive and False Negative Results on a Tuberculosis Control Programme [13]

False Positive Results	False Negative Results
• Patients are started on treatment unnecessarily • Anti-tuberculosis drugs are wasted • In follow-up examinations the intensive phase of treatment is continued longer than necessary • Patients may lose confidence in the health services or a particular laboratory	• Patients with tuberculosis are not treated, resulting in suffering, spread of tuberculosis and death • Intensive phase treatment is not extended for the required duration, resulting in inadequate treatment • Patients may lose confidence in the health services or a particular laboratory

1.2.3 What needs to be known?

Clinicians rely heavily on the acid-fast smear laboratory results to diagnose TB in order to decide on patient management and ensuring respiratory isolation of infectious patients. Health authorities also require information from the laboratory to undertake epidemiological investigations, including contact tracing. It is important to establish that the sputum smear microscopy results that are released by the laboratory are accurate, reliable and reproducible so that diagnoses by clinicians can be made with confidence. Therefore, the quality of TB microscopy in KwaZulu-Natal needs to be established.

A well functioning quality assurance programme is essential to determine the quality of sputum microscopy. Proficiency testing is the method used for quality assurance in this study.

The knowledge, attitudes and practices of laboratory personnel towards proficiency testing (PT) could determine the success of the proficiency testing programme. Laboratory personnel who regard proficiency testing as a valuable exercise in assessing quality are likely to approach the programme seriously and ensure the success of the TB diagnostic

programme. Those who see proficiency testing as an additional workload would be less likely to take the proficiency testing exercise seriously. At present, the knowledge, attitudes and practice of laboratory workers involved in the smear microscopy laboratories in KwaZulu-Natal remains unknown.

1.2.4 Why is proficiency testing important?

The advantages of the AFB smear microscopy include that it is a rapid, simple and cheap technique requiring very little in terms of equipment and detecting most infectious TB cases. Despite this the technique retains several disadvantages as well. [16] Valid smear microscopy depends on the quality of the sputum collected from the patient, standardised specimen staining technique employed in the laboratory, quality of stains and the technical ability of the microscopist to read the smear. [12] In order to overcome the disadvantages of the diagnostic method, proficiency testing is recommended to ensure that results generated by the laboratory are accurate, reliable and reproducible.

Proficiency testing would provide an assessment of the status of laboratory performance and detect problems associated with diagnostic performance. [17] This system also possesses the capacity to identify facilities that produce unacceptable levels of false positive and false negative results so that corrective actions can be instituted.

Therefore, to maintain a reliable laboratory service that provides high quality results consistently, a well-organized proficiency testing system is required. [17, 18]

1.2.5 How will the study assess the problem?

In KwaZulu-Natal, proficiency testing of TB microscopy has been conducted since 2001. These results have not been processed and analysed systematically, therefore, the quality of AFB smear microscopy being performed in the laboratories has not been adequately

assessed and monitored. The study will solve this by undertaking quantitative and qualitative analysis and so contribute to an improved TB diagnostic laboratory service.

Quantitative analysis of this existing proficiency testing data will provide a retrospective situational analysis of the TB smear microscopy services in KwaZulu-Natal from 2001 to 2006. It will also identify laboratories that reveal an unacceptable level of performance. Detection of these laboratories through this process will then indicate the need for quality improvement and further intervention to improve the quality of microscopy services for tuberculosis diagnosis in KwaZulu-Natal.

The qualitative information will be obtained by conducting key informant interviews to determine the knowledge, attitudes and practices of laboratory personnel and managers towards proficiency testing. In this way, barriers to an effective quality assurance programme will be identified and corrective measures to improve the TB diagnostic service can then be recommended.

1.3 STATEMENT OF THE PROBLEM

1.3.1 Research Hypothesis

It is hypothesised that, due to the high burden of TB and HIV in KwaZulu-Natal overwhelming the TB laboratory network, the quality of TB microscopy services in KwaZulu-Natal is below the acceptable level of performance.

1.3.2 Research Questions

- What is the quality of TB smear microscopy in public health laboratories in KwaZulu-Natal from 2001 to 2006?
- What is the knowledge and attitude of laboratory workers and laboratory managers to proficiency testing towards development of a quality assurance tool in KwaZulu-Natal?

1.4 PURPOSE OF THE RESEARCH

The purpose of this health systems research was to assess the quality of TB smear microscopy in KwaZulu-Natal from 2001 to 2006 by proficiency testing and to identify possible areas that would require remedying, with the ultimate goal of improving the quality of smear microscopy TB diagnostic services in KwaZulu-Natal.

The success of proficiency testing programmes depends largely on the perception of laboratory staff to this quality assurance intervention. Those who perceive proficiency testing as a means to assess quality and identify and remove barriers to quality smear microscopy results, within the laboratory, will follow the requirements of the programme meticulously in order to achieve its objectives. Laboratory personnel who perceive proficiency testing as an additional workload may not participate in the quality evaluation process as required. Therefore, the purpose of the study also involved assessing the knowledge, attitudes and practices of laboratory personnel towards proficiency testing.

1.5 SPECIFIC OBJECTIVES OF THE RESEARCH

1. To describe and analyse the results of proficiency testing conducted between 2001 and 2006, in the 79 facilities where sputum smear microscopy was undertaken by the KwaZulu-Natal Public Health Laboratory in the province, and to quantify the number and extent of the false results in these laboratories.
2. To identify laboratories that sustain an unacceptable[6]level of performance so that corrective action can be taken.

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[6] The overall aim for laboratories was be to reach 95% agreement (acceptable performance), in reading proficiency testing slides, between the various laboratories and the reference laboratory. Unacceptable level of performance is the failure of laboratories to

3. To compare proficiency testing results obtained by the KwaZulu-Natal reference laboratory and the National Health Laboratory Service reference laboratory.
4. To determine the role laboratory workers and managers consider proficiency testing plays as a quality assurance technique.
5. To make recommendations to decision makers on the key gaps identified from the information obtained in this study.

1.6 ASSUMPTION UNDERLYING THE STUDY

It is assumed that microscopists reading proficiency-testing slides are the same laboratory personnel performing routine TB diagnostic microscopy, and that these laboratory staff processed the test slides in the same manner as they processed routine smear microscopy slides. If proficiency testing slides received more attention than routine microscopy slides the quality of proficiency testing results would be biased, and falsely elevated proficiency testing results would be obtained.

Since secondary data was analysed in the study, it is assumed that the primary data has been accurately transcribed from reports (hard copies) onto electronic format (Excel spreadsheet).

1.7 *OPERATIONAL DEFINITIONS USED IN THE STUDY*

National Tuberculosis Programme (NTP): Countrywide, permanent programme responsible for activities directed at controlling tuberculosis through integrated efforts. It includes implementing the Directly Observed Therapy Short-Course strategy promoted by

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achieve 95% agreement, in reading proficiency testing slides, between themselves and the reference laboratory.

the World Health Organisation and the International Union Against Tuberculosis and Lung Disease (IUATLD).

Directly Observed Treatment Short-Course (DOTS): This the recommended strategy for TB control. DOTS includes (1) government commitment to TB control activities, (2) TB case detection by sputum smear microscopy, (3) directly observed treatment with standardized short-course TB chemotherapy, (4) a regular, uninterrupted supply of anti-TB drugs, and (5) a standardized recording and reporting system.[11]

Peripheral Laboratory: Laboratory located at primary health centre or district hospital.

Reference Laboratory: National reference laboratory or central laboratory. These high level facilities perform an essential role in the organization and maintenance of the network of laboratories, and, *inter- alia,* develop guidelines for standardising smear microscopy, assuring quality testing, and overseeing microscopist training. These facilities also support external quality assessment efforts in collaboration with the National Tuberculosis Programme.

KwaZulu-Natal laboratories were not part of the network of laboratories administered by the National Health Laboratory Services (NHLS) at the time the study was conducted. KwaZulu-Natal laboratories joined the NHLS laboratory network on 1 October 2006. During the study period King George V Hospital's TB laboratory served as the provincial reference laboratory, while the NHLS had another national reference laboratory based in Gauteng, which served the rest of the country.

Health District describes the administrative level at which the National Tuberculosis Control Programme is implemented.

Ziehl-Neelsen Stain (ZN): Acid-fast staining method using carbolfuchsin that is steam heated on the slides, decolourised, then counterstained with methylene blue. AFB appears red against a blue background.

Quality Assurance (QA) is the system designed to continuously improve the reliability and efficiency of laboratory services, and includes internal quality control, external quality assessment and quality improvement.[11]

Quality Control (QC), which is also called Internal Quality Assurance, includes all means whereby the TB smear microscopy laboratory controls operation, including instrument checks and checking new lots of staining solutions.[11]

External Quality Assessment (EQA) is the process that allows participant laboratories to assess their capabilities by comparing their results with those in other laboratories in the TB laboratory network through panel testing, blinded rechecking and on-site evaluation.[11]

Quality Improvement (QI) is a process whereby the components of smear microscopy diagnostic services are analysed with the aim of looking for ways to permanently remove obstacles to success. Data collection, data analysis, and creative problem solving constitutes the key components of this process. It involves continued monitoring and identifying defects, followed by remedial action including retraining of staff when needed, to prevent recurrence of problems. Quality improvement often relies on effective on-site evaluation visits.[11]

Proficiency Testing refers to a system in which 'reference material' (stained and/or unstained TB smears) of known but undisclosed content are forwarded from the reference laboratory to the peripheral laboratories. These smears are then examined by the peripheral laboratory staff using the same procedure as would normally be used to examine patients' specimens of the same type. Smear results are then returned to the reference laboratory

where the results are used to assess the performance of the laboratory that examined the smears.[11]

Major error is a type of error that is considered the most critical since it has the highest potential impact on patient management, and can result in an incorrect diagnosis or improper management of a patient. Major errors may indicate gross technical deficiencies, and include both High False Positive and High False Negative errors.

Correct results are slides that are read without errors or the difference of not more than one grade in reading a positive slide between examinee and controller.

High False Positive (HFP): A negative smear misread as 1+ to 3+ positive (based on IUATLD/WHO recommended grading of sputum smear microscopy results)[7]. HFP is a major error.[11]

High False Negative (HFN): A 1+ to 3+ positive smear (based on IUATLD/WHO recommended grading of sputum smear microscopy results) that is misread as negative at the time of sputum microscopy. HFN is a major error.

Minor error: In clinical practice, minor errors may exert some impact on patient management. However, for evaluating laboratory performance, this type of error is considered less serious, because of inherent limitations in consistently detecting a few AFB that may be unequally distributed within a smear. The frequency of minor errors may indicate technical deficiencies.

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[7] IUATLD/WHO recommended grading of sputum smear microscopy results are:
Negative – No acid-fast bacilli observed (No AFB per 100 fields)
Low Positive: record exact figure (1 to 9 AFB per 100 fields)
1+: 10 to 99 AFB per 100 fields
2+: 1 to 10 AFB per field in 50 fields
3+: more than 10 AFB per field in 20 fields

Quantification Error (QE) is the difference of more than one grade in reading a positive slide between examinee and controller. This minor error generally has no impact on case management.[11]

Low False Positive (LFP) was previously called a scanty false positive. LFP is a negative smear that is misread as a low (1-9 AFB per 100 fields) positive. This type of minor error occurs occasionally even in laboratories that are performing well.

Low False Negative (LFN) was previously termed a scanty false negative. A low (1-9 AFB per 100 fields) positive smear that is misread as negative. This type of minor error occurs occasionally even in laboratories that are performing well.

Low Positive is the term used in this document to describe 1-9 acid-fast bacilli per 100 fields. These results are reported to the physician as the exact number of AFB observed. It remains for the physician and the NTP to decide if this represents an infectious case of tuberculosis or not. A low positive was previously referred to as a 'scanty positive' result.

1.8 SCOPE OF THE STUDY

For the quantitative component, data from 2001 to 2006 was examined. The data set is limited to data collected from the KwaZulu-Natal Laboratory Services and the National Health Laboratory Services. The KwaZulu-Natal laboratory data set contains missing data for quarters of the year where proficiency testing was not conducted.

The qualitative component of the study is limited to interviews with ten key informants involved in the TB Control Programme in KwaZulu-Natal.

1.9 SUMMARY OUTLINE PER CHAPTER

1. Chapter one supplies an introduction, which highlights the extent of the tuberculosis problem globally and in South Africa, the purpose of the research and how the outcome of the research could solve the problem.
2. Chapter two presents a detailed literature review, which highlights the TB control strategy in South Africa, describes the laboratory network in KwaZulu-Natal and the components of a quality assurance programme. Studies conducted both in South Africa and internationally are reported and discussed in this chapter.
3. Chapter three presents the research methods in terms of study design, study population, study area, sampling method, data sources, variables studied, data collection techniques and instruments. Statistical methods are also described in this section.
4. Chapter four contains the results, which are presented in the form of graphs, tables and text.
5. Chapter five discusses the implications of the results of the study and compares the results emanating from the study with those reported in the available literature.
6. Chapter six concludes the report of the study and provides recommendations, which arise out of the findings and also recommend further research required in relation to quality assurance of TB proficiency testing.

2 CHAPTER II: LITERATURE REVIEW

2.1 INTRODUCTION

Tuberculosis represents a major global health problem that accounts for more that eight million new cases and three million deaths each year. The incidence risk of tuberculosis in South Africa for 2005 was estimated to be 600 per 100 000 population, ranking South Africa seventh in the world by estimated number of incident cases. [3]

Direct (unconcentrated) sputum smear microscopy is the primary method for diagnosing pulmonary tuberculosis in low-income and middle-income countries. [19] The acid-fast staining method (Ziehl-Neelsen) employed to stain sputum smears is the least expensive tool for the rapid identification of potentially infectious tuberculosis patients. [8] The technique however lacks sensitivity, detecting only 45 to 60% of culture positive cases. [9] Since false positive and false negative microscopy results hold serious implications for clinical care and public health control measures of tuberculosis, quality control of sputum microscopy for tuberculosis is essential to ensure that sputum smear microscopy results are valid and reliable.

Although KwaZulu-Natal Provincial Laboratory Services implemented proficiency testing in all public funded health laboratories in the province in 2001, these results were not processed, summarised and analysed fully. Therefore, the quality of TB smear microscopy services in these laboratories was not fully evaluated. In addition, the current knowledge and attitude of laboratory workers and laboratory managers to proficiency testing as a quality assurance tool in KwaZulu-Natal has also not been determined.

The literature review highlights the status of knowledge on the role of quality assurance of TB microscopy services and presents evidence of successes and failures of previous studies. Justification for conducting the research is presented in this chapter by identifying

gaps in the National TB Control Programme and explaining the contribution this study would make towards narrowing those gaps.

2.2 *PURPOSE OF THE LITERATURE REVIEW*

The purpose of the literature review was to assess the studies that have already been undertaken nationally and internationally. This was important primarily to ensure that similar studies had not previously been conducted in KwaZulu-Natal resulting in a duplicate research exercise.

The literature review also reviewed completed studies to assess methods used and to identify gaps in this research project.

2.3 *SCOPE OF LITERATURE REVIEW*

2.3.1 Theoretical Application

It was hypothesised that laboratory staff did not perceive proficiency testing as a valuable exercise. If this were true, then the proficiency testing exercise would be conducted rarely in the laboratory leading to substandard proficiency testing results. Poor performance of proficiency testing would result in a failure of the quality assurance system to determine accurately the quality of TB smear microscopy performed by the laboratory. A missed opportunity to identify laboratories performing below the expected standard would have occurred. More importantly, this would result in a missed opportunity to offer assistance of training and mentoring to improve TB smear microscopy services.

2.3.1.1 Concepts and theories

Many countries have conducted quality assurance in one form or another and most TB control programmes have witnessed the benefits of implementing a quality assurance programme. The study reported in Chennai, India in 2003 failed not because the assessment

of quality was not done, but rather because of failure to implement recommendations arising from the process undertaken. [20]

In South Africa, a similar quality assurance study was conducted in 2000 in Limpopo Province (Northern Province) and the benefits of the study were clearly evident. [13] The aim of that health system research was to assess and improve the quality of TB smear microscopy in a systematic way in Limpopo province. The research was also used to pilot the logistics involved in proficiency testing of TB microscopy services for the South African Development Community countries (including South Africa) on behalf of the South African Tuberculosis Control Initiative Laboratory Task Team (in consultation with the National Tuberculosis Control Programme). The purpose of this study was to identify and solve operational problems associated with proficiency testing. Limpopo Province was chosen, as their operational conditions were similar to other South African Development Community countries.[8]

A sample of 19 laboratories out of a total of 36 was included in the study in Limpopo province. Two rounds of proficiency testing were conducted in March and August 2000. The South African Institute for Medical Research prepared the reference slides. They thereafter screened and reported the proficiency testing results after the slides were processed by the participating laboratories. Corrective action was implemented at laboratories that performed poorly in proficiency testing after the first round. Correct results for the first round where obtained from 86% of the prepared slides and this improved significantly to 97% for the second round of proficiency testing after quality improvement was implemented ($p = <0.01$).

It was concluded that the implementation of proficiency testing in resource poor settings was feasible, cost effective and the corrective intervention plan had improved the quality of TB smear microscopy performance. It therefore seemed prudent to extend proficiency

[8] Botswana, Lesotho, Malawi, Namibia, Swaziland, Zambia, and Zimbabwe

testing to KwaZulu-Natal public funded laboratories. TB microscopy services in KwaZulu-Natal province have never been assessed systematically and scientifically.

2.3.2 Conceptual framework

Tuberculosis diagnosis through finding acid-fast bacilli (AFB) on smear microscopy plays a vital role in identifying potentially infectious cases, commencing antibiotic treatment and monitoring therapeutic progress of patients. When TB microscopy services are flawed in terms of producing false positive or false negative results, there are serious implications for the patients; the TB control programme and the economy, as a result of funding unnecessary TB treatment for patients.

It is therefore of paramount importance to be able to trust the validity of TB microscopy investigations carried out by the laboratory. Laboratories producing high quality microscopy could be encouraged to maintain good performance, whereas laboratories providing a service of inferior quality according to proficiency testing reports would be targeted for staff training and other quality improvement activities.

2.4 LITERATURE REVIEWED

2.4.1 Background to study

Directly Observed Treatment Short-Course (DOTS) is the recommended strategy for TB control. DOTS is a comprehensive strategy which primary health care services around the world utilise to detect and cure TB patients. It has 5 key elements, namely (1) government commitment to TB control activities, (2) case detection of infectious TB cases by sputum smear microscopy, (3) direct observation of treatment for TB with standardized short-course chemotherapy, (4) a regular, uninterrupted supply of anti-TB drugs, and (5) a standardized recording and reporting system to monitor and evaluate the effectiveness of the TB Control Programme.

Direct Observation of the treatment being taken is one of the key elements of DOTS. In Direct Observation of treatment, a specific individual (DOTS supporter) supports the patient by actually observing that the pills are swallowed, on a daily (or 3 times a week) basis. DOTS is an effective strategy to assist TB patients to complete their treatment that depends on: [21]

- The National TB Control Programme directing resources towards detecting sick, infectious TB cases, in order that they can be cured.
- Patients being observed when swallowing each dose of their medication by a health care worker or a trained volunteer (DOTS supporter), and being monitored throughout their treatment to ensure cure.
- TB patients having access to the correct anti-TB treatment and the correct combination and dosage of TB drugs being taken for the correct period of time.

DOTS is a solution to the problem of poor adherence and low cure and treatment completion rates. By using DOTS the aim of the National TB Control Programme of reaching a high sputum conversion and cure rate can be achieved. [21]

South Africa supports the DOTS strategy. Directly Observed Treatment is associated with high proportion of TB patients being cured. Up to 95% were reported to be cured in countries with limited resources. Without Directly Observed Treatment, the proportion cured of TB can decline to as low or less than 40%.[21] Directly Observed Treatment prevents new TB infections by stopping transmission through curing infectious patients. An infectious patient can infect, on average, 10-15 family members, friends and co-workers each year. [22]

DOTS prevents MDR TB through uninterrupted treatment, which forms the best way of preventing TB developing resistance. MDR TB is caused by taking anti-TB drugs irregularly which may be a consequence of errors in any of the following:[23]

- Management of drug supply, e.g. frequent or prolonged shortages of anti-tuberculosis drugs due to poor management.
- Patient management, e.g. prescription of inadequate chemotherapy
- Patient adherence, e.g. patient adherence most often becomes a problem when the patient is homeless, has an alcohol or drug problem, when a family member has been unsuccessfully treated previously, or when access to health care is difficult. In-depth discussions with patients at the initiation of treatment can help to decrease these constraints. The discussion can clarify the expectations of both the patient and the health care staff; help the patient try to solve barriers to adherence and assist in building a supportive relationship.

Community based direct observation of therapy is cost effective as it is cheaper than the cost of hospitalising patients for all or some of their course of TB treatment. Ambulant TB therapy can be integrated in an existing primary health care system.[21, 24, 25] Applying the WHO DOTS strategy can improve survival of HIV infected patients by treating and curing TB in these patients.

TB patients can continue working where there are DOTS supporters in the workplace.[26] Since nearly 80% of TB patients are in their most productive years of life, DOTS protects the workforce. Studies in India and Thailand have shown that a small investment in DOTS can save economies huge amounts of money. The DOTS strategy has been identified by the World Bank as one of the most cost-effective health interventions available.[25] It is estimated that implementing a DOTS strategy would cost between US $ 3 and $ 7 for every healthy year of life gained. It allows people to return uninfected to school, work and their families. With DOTS, the health system, the community, as well as the patient is responsible for ensuring that treatment is taken regularly and that treatment is completed.

Globally, more than 26 million patients were managed under the DOTS strategy. By the end of 2006, 199 of 212 countries were implementing the DOTS strategy and 99% of the world's population was living in regions where DOTS was being implemented. [3]

DOTS is also the approach/strategy that is being implemented in an attempt to control the TB epidemic and the emerging multi-drug resistant tuberculosis (MDR TB) epidemic in South Africa. When a patient completes the entire TB short-course chemotherapy treatment, three main aims of the National Tuberculosis Control Programme (NTCP) would have been achieved, namely that the patient is cured, the spread of the disease is arrested and multi-drug resistant tuberculosis will be prevented.

However, the Department of Health Annual Report 2003/2004 reveals that despite the high detection of smear positive cases (86%), the proportion that are cured still remains low (54%), with a high proportion of interruption (13%) and patients transferred (9%). The data indicates that the Directly Observed Treatment[9] programme was failing. Therefore, improvement of the implementation of the Directly Observed Treatment programme was prioritised for the following year (2004/5). The report states that although initially Directly

[9] The implementation of DOTS requires that every patient should have the support of another person to observe and ensure that they swallow their medication daily.

Observed Treatment was successfully implemented in some districts the standard of having every dose of medication seen to be taken, could not be maintained because of insufficient human resources to act as treatment supporters whose activities should be supervised and monitored.[27]

One of the five key elements of the DOTS strategy is to employ sputum smear microscopy to detect the infectious cases of tuberculosis among those people attending health care facilities with symptoms of TB. These symptoms, most importantly include a cough for two weeks or more. These are the infectious group of TB sufferers mainly responsible for feeding the epidemic especially in those co-infected with the HIV. [6, 13]

Tuberculosis laboratory services contribute an essential component of the DOTS strategy of the National Tuberculosis Control Programme by confirming the diagnosis of TB and monitoring of treatment outcomes. However, Tuberculosis laboratory services are often the most neglected component of infectious disease programmes. [28]

2.4.2 Laboratory network

Effective control of tuberculosis is dependent on a network of local laboratories that provide accurate and reliable direct AFB microscopy testing for clinical diagnosis, therapeutic monitoring and epidemiological surveillance. [11] The availability and quality of AFB microscopy relies on programmes that support, train, and monitor the testing performance of individual laboratories and technologists. Serious problems can occur in the laboratory when insufficient attention is given to the quality assurance of the procedures involved in sputum smear preparation and microscope slide reading. False positive and false negative results have been detected in patients' slides in previous studies and these can have serious implications for patient care, further spread of the disease as well as potential economic losses. [12, 16, 29] Therefore, the need to assess laboratory performance has been recognized and many National TB Control Programmes have attempted at one time or another to monitor the quality of microscopy services. Many

countries, however, still do not possess comprehensive laboratory proficiency testing programmes. With the integration of AFB microscopy into general clinical services in many countries an increasing need has arisen to ensure that the AFB smear is performed properly.

Furthermore, the escalation of tuberculosis cases worldwide, driven by the HIV epidemic and aggravated by the emergence of multi-drug resistance and most recently by extreme-drug resistant TB, has resulted in renewed concern about safety and quality assurance in tuberculosis diagnostic laboratories.

2.4.3 Health systems research in Limpopo Province and abroad

In South Africa, many new smear microscopy centres were established during the last decade to facilitate and decentralize sputum smear microscopy services. The TB diagnostic microscopy network was therefore extended but very little attention was devoted to quality assurance of the new service. [13] In the Limpopo Province of South Africa, several activities were introduced in 1997 to improve the quality of TB smear microscopy services, but as the quality of laboratory activities was not assessed in a systematic way, the quality of smear microscopy services remained unknown. [13]

The health systems research conducted in Limpopo Province aimed to assess and improve the quality of TB smear microscopy services in a systematic way. The study revealed that the implementation of proficiency testing in less developed, resource poor settings is feasible, cost-effective and the consequent quality improvement intervention plan did improve the quality of TB smear microscopy services. The overall performance of the laboratories that participated in the study consistently showed that 85% of the test slides were correctly diagnosed. After the laboratories that performed poorly were identified, a number of quality improvement intervention measures were implemented including:

- Guidelines for proficiency testing were developed and used by the evaluator to observe and evaluate the procedures followed by the designated laboratory staff involved with TB diagnosis;
- A standardised demonstration of the Ziehl-Neelsen staining method was conducted; and
- Completed evaluation forms were forwarded to the project manager, who in collaboration with the technical advisor reviewed the evaluation report to identify additional pointers for overall corrective action.

Nineteen out of 36 laboratories participated in the study. The overall performance in all participating laboratories improved from 85% (65 out of 76 slides read correctly) of the smears being correct during the first round to 97% (74 out of 76 slides read correctly) for the second round after the intervention. The improvement in overall performance was statistically significant ($p=<0.01$).

No other reports of TB smear microscopy proficiency testing, or testing methods for the implementation of proficiency testing were found for South Africa. However, there were reports of studies conducted in other countries that assessed their country's TB diagnostic microscopy services. [20, 30-32] Several national TB Control Programmes have significantly improved their microscopy examination of slides by assessing their TB microscopy and implementing corrective measures.

In a study conducted in Mexico, a total of 586 laboratories were inspected and 430 technicians were evaluated by proficiency testing involving microscopy of 10 slides with known numbers of acid-fast bacilli under test conditions. [31] The proficiency test results were compared with ongoing slide re-checking and with repeat proficiency testing performed 2 years later. Of the 430 technicians evaluated by proficiency testing in 1998, 196 (46%) scored less than 80% in the initial proficiency test. After receiving intensive training in 1999 another round of proficiency testing showed a significant improvement in the scores of those who received training from a mean test score of 65% to a test score of

90% (p=<0.01). Microscopists in laboratories whose work was routinely rechecked had a better mean proficiency testing score than those in laboratories that were not (79% and 74% respectively; p=0.002). Some of the limitations of this study included; slide sets produced in 1998 had random numbers of negative, low-positive and 1+ and 2+ slides indicating that the number of low-positive slides in the set was the factor most closely associated with proficiency testing results. A possible sampling bias in rechecking was suggested by the very high agreement in rechecking and the very low number of laboratories with errors found. Furthermore, it could not be established whether the time used for proficiency testing was the same when the slide sets were sent by courier and examined by the technicians as when they were examined in the presence of a supervisor. In spite of the limitations mentioned, this study showed that training technicians proved an essential component in successfully implementing proficiency testing in the national network of TB laboratories in Mexico. [31] The study concluded that external quality assessment and training improves TB diagnostic performance and that rechecking [10] and proficiency testing are both viable measures that can be used in assessing the quality of TB laboratory performance.

A study in Mexico evaluated the results of a 1-year pilot programme involving blinded rechecking of randomly selected AFB slides from TB laboratories in two Mexican states to determine the feasibility of this quality improvement method for future more widespread implementation. [32] These 2 states were selected based on their size, proximity to the reference laboratory, humidity, test volume and estimated prevalence of TB based on smear positivity rates. The process of slide sampling and rechecking was identical. The results revealed that a substantially greater percentage of errors were detected on the randomly

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[10] Rechecking is a process whereby smears are sent from the peripheral laboratory to the reference laboratory for rereading and evaluation. The External Quality Assessment for AFB Smear Microscopy guidelines recommend that rechecking be always blinded, ensuring that the controller does not know the results from the peripheral laboratory.

selected, blinded[11] AFB smears than on the non-randomly selected, non-blinded[12] smears. This implies that the microscopists in the reference laboratory are biased toward agreeing with the peripheral laboratory results when they are known. Rechecking of randomly selected blinded smears gives a more objective diagnostic assessment and therefore reflects the actual performance of the laboratory more accurately.

All errors and error types were recorded for each local laboratory and state. The number of laboratories with no errors detected in State A was 25 (76%) in 1998 and 4 (11%) in 2001. The number of laboratories with no errors detected in State B was 11 (73%) in 1998 and 6 (35%) in 2001. Although the number of laboratories with no errors was substantially lower in 2001 than in 1998, it is unlikely that these results represent a decrease in performance. A more likely explanation is that collection of random samples combined with blinding of the local laboratory results to the reference laboratory technicians rechecking them resulted in a more accurate picture of true laboratory performance in 2001.

On-site evaluations revealed poor quality microscopes in some laboratories and failure of technicians to record and report the exact number of bacilli on low positive smears. The practice of reporting low positives as negative changed during the course of the study.

The study revealed that a smaller random sample of AFB smears could be rechecked to assess the quality of AFB microscopy in Mexico compared to the non-blinded rechecking method used previously and that random blinded rechecking provides more accurate estimates of AFB microscopy results.

In the Accra region in Ghana between 2000 and 2002, the impact of setting up a pilot quality assurance system on the performance of peripheral laboratories performing smear microscopy was evaluated. [12] The results of the study revealed improvement in the average scores for specimen quality, staining ability, smear cleanness, thickness, size and evenness from 64%, 79%, 69%, 46%, 67% and 60% respectively in the last quarter of 2000

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[11] The technician rereading the slide does not know the initial result.

[12] The technician rereading the slide is aware of the initial result. The technician rereading the slide may be biased towards agreeing with the initial result.

to 81%, 90%, 86%, 79%, 80% and 74% respectively at the third quarter of 2002, two years after the establishment of the quality assurance system. Within the same period false-positives and false negatives decreased from 15% and 21%, respectively, to 0% for both outcomes following the intervention. The overall smear positive/negative agreements increased from 85% in the fourth quarter of 2000 to 100% in the third quarter of 2002. The agreement in positive grading also increased from 74% in the fourth quarter of 2000 to 95% in the third quarter of 2002. The study indicated that support visits, which act as motivation for laboratory personnel, on-site and formal training, blinded rechecking of examined slides and timely feedback lead to improvements in TB laboratory services. It was therefore recommended that the system be extended to the rest of the country.

The Tuberculosis Research Centre in Chennai, one of India's National Level Reference Laboratories conducted proficiency testing in 8 regional TB centres at 6-monthly intervals. [20] Five rounds of tests were conducted between 1998 and 2000. In rounds I-IV, each centre received a panel of 100 stained smears comprising 45 negative and 55 AFB positive slides of various grades. In round V the number of slides was reduced to 50 (20 negative and 30 AFB positive slides of various grades). An outcome of this study was that a few good laboratories were identified and were separated from those that performed poorly. The drawbacks in undertaking a proficiency testing programme were also identified. Microscopists in a few centres had not conducted the smear examination independently. It was observed that in at least three centres all the results were identical, including the number of AFB seen in the scanty grades. In such situations it is impossible for more than one reader to examine the same 100 fields and report the exact number of AFB, considering the uneven distribution of bacilli in a smear with less than 10 AFB. In such instances, any error by the first reader will be reflected in the results of subsequent readers. Other drawbacks identified included that microscopists participating in the proficiency testing programme had unlimited time for the slide examination and were aware of being tested. The evaluation system did not address the quality of smear preparation and staining. Acid-fast microscopy technique is not difficult, but it is tedious if large numbers of smears have to be examined on a single day. A heavy workload (>20 smears per day per technician)

may contribute to poor performance. A low workload (<15 smears per week per technician) may not be adequate to maintain proficiency in reading AFB smears.[11] Determining the workload for AFB smear microscopy may be more difficult in laboratories that perform a variety of tests however, this can be achieved during support visits by the district supervisors.

In this study, letters were sent to laboratories, performing poorly, indicating their deficiencies. However, no improvement was observed in subsequent rounds. A copy of the letter was also sent to the Central TB Division for follow-up action. As the Tuberculosis Research Centre had no administrative control over the independent State TB Demonstration and Training Centres (laboratories that participated in the study), the researchers felt that motivation of the technicians concerned and a retraining programme for the technical personnel might result in a better work performance. Implementing these intervention steps were therefore considered.

A study in Argentina analysed registers and records on technical evaluation of AFB smear microscopy from the entire national network laboratories enrolled during 1983-2001. [30] The objectives of the study were to evaluate the technical quality of AFB smear microscopy supervised in 1983-2001 in the Argentine Laboratory TB Network and to analyse the impact of errors made in the various technical steps on smear microscopy results. The Argentine laboratory network consists of all government and some private laboratories with different levels of technical and administrative responsibilities.

As complete information was not available for all smears supervised in the country during the study period, only the registers and records available at the National Institute of Respiratory Disease were analysed. The study took into account the rechecking carried out by the National Reference Laboratory and the Provincial Reference Laboratory. These laboratories commenced sending in their records regularly in 1997. The overall trend of quality could not be analysed as some laboratories stopped submitting their records and results while others were newly enrolled in the system. Instead, the overall quality throughout the study period was considered.

The study provided an indication of the technical quality and allowed the impact of errors in the different technical steps on the quality of the results to be analysed. Results from 26 356 external quality assessed slides were analysed. The number of provinces supervised annually varied between 3 and 22 during the study period, whilst the number of supervised laboratories varied between 17 and 232.

Of the 25 677 sputum specimens rechecked during the study period, 18 926 (74%) were categorised as good (mucopurulent and mucous). The number of AFB positive sputum smears was related to the quality of the sputum specimen. The proportion of 'good'[13] smears was relatively low (65%). The proportion of AFB positives in 'good' smears (16%) was higher than in 'thin' smears (13%, p=0.00008), and the average bacillary count in 'thin' smears was lower than in 'good' smears (p=0.000001).

During the study period, 97% of the slides were qualified as 'good'[14] in terms of staining quality. The average agreement in reading throughout the country was 98%. The study concluded that technical quality and agreement in the laboratory network were satisfactory. However, improvements were needed in the areas of quality of smears, staining and reading, increasing coverage of external quality assessment, decentralisation of supervision, slide selection method and data registration.

The above studies in Mexico and Ghana showed significant improvement in smear microscopy after identification of deficiencies (in sputum smear slide preparation and staining) and implementation of corrective measures such as training. [12, 31] The study conducted in India, however, indicated no improvement in microscopy services of poor performers. [20] Although deficiencies were identified and communicated to the relevant laboratories, corrective measures were not implemented. The study conducted in Argentina

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[13] Smears were classified as good, thin, thick, not homogenous or too short.

[14] Staining was qualified as 'good, when 100 consecutive good microscopic fields could be read without lack of decolouration or presence of fuchsin crystals. If a significant proportion of slides were found to have insufficient decolouration or presence of crystals, this was reported to the supervised laboratory as it may indicate defects in the staining procedure.

evaluated the technical quality of smear microscopy for acid-fast bacilli and analysed the effects of procedural errors on the results. The study also provided recommendations to further improve the quality of sputum microscopy.

A study conducted in India, proved that their current quality control procedure could not find any weakness in their quality assurance procedures under the present level of training/retraining/supervision of laboratory staff in the national TB Control Programme. [33] Therefore quality assurance does not necessarily have to find faults in a laboratory; it could also indicate that a laboratory has satisfactory performance.

2.4.4 Quality Assurance

The World Health Organisation (WHO) described quality assurance relating to TB bacteriology, as a system designed to continuously improve the reliability, efficiency and use of tuberculosis laboratory services. [11, 13, 28] The purpose of a quality assurance programme is to improve the efficiency and reliability of laboratory services.

The components of a quality assurance programme, as defined by the WHO, include quality control, quality improvement and proficiency testing.

Quality control in TB microscopy is the systematic internal monitoring of working practices, technical procedures, equipment and materials, including the quality of stains.

Quality improvement is a process whereby the components of TB smear microscopy diagnostic services are analysed with the aim of looking for ways to permanently remove obstacles to success. Data collection, analysis and creative problem solving constitutes a key component of this process. Quality improvement involves continued monitoring, identifying defects, followed by remedial action including re-training when needed, to prevent recurrence of problems. Quality improvement often relies on effective on-site evaluation or supervisory visits.

The WHO and other institutions use the terms Proficiency Testing and External Quality Assessment (EQA) interchangeably. In this dissertation, proficiency testing (as defined by WHO) is used. Proficiency testing is a system in which 'reference material' of known but undisclosed content are introduced into the laboratory and examined by the staff using the same procedures as would normally be used to examine patients' specimens of the same type. [34] The test material is prepared by a reference laboratory, and sent to lower level laboratories. The laboratories perform the procedures (in this case TB smear microscopy) and then report the results to the reference laboratory, which can then assess their proficiency. Laboratories that produced poor results are identified and are subsequently targeted for quality improvement.

Proficiency testing, as described by the Association of Public Health Laboratories (APHL), has three methods that can and should be combined to evaluate laboratory performance. [11] These include:

- **On-site evaluation** - Trained laboratory personnel from the reference or intermediate laboratory visit peripheral laboratories for the observation of worker performance under actual conditions, including condition of equipment, laboratory safety, adequacy of supplies and the process for smearing, staining, reading, recording and reporting. When problems are identified, solutions can be suggested and potentially implemented immediately.
- **Panel testing** – Is a system for sending stained and/or unstained slides from the central laboratory to the peripheral laboratories, at regular intervals, for reading and interpretation. Smears and results are then sent back to the reference laboratory where the results are used to assess the performance of the laboratory that examined the smears.
- **Blinded re-checking** – This is a process whereby a sample of smears from the peripheral laboratories and the intermediate laboratories are rechecked/reread by

controllers at a higher-level laboratory. This is considered the best method for evaluating performance and providing motivation to staff for improvement.

Each method possesses distinct advantages and disadvantages (table 2), as well as varying levels of resource requirements. The choices as to how to implement proficiency testing depend on both the available resources and the ability to obtain additional resources to support the proficiency testing activities.

The WHO recommends that quality assurance be performed on a regular basis in the microscopy laboratory to ensure reliability and reproducibility of laboratory results. [28] WHO also recommends that, for a quality assurance programme to be of value, it must be both practical and workable.

In KwaZulu-Natal, proficiency testing was chosen to assess the quality of TB microscopy services as this method provides a rapid assessment. It would also form a basis from which an extended quality assurance programme could be developed which would include the implementation of a rechecking programme. Proficiency testing would assist in identifying contributing factors to recognized errors and assist in assessing training programmes for microscopists following the initial assessment of microscopy services. The method selected for the study was influenced by what was available in the province (proficiency testing data) as well as it having been successfully tried and tested in the field. [13, 31]

Table 2: Proficiency Testing Methods –International Union Against Tuberculosis and Lung Disease (IUATLD) Approach [11]

Method	Advantages	Disadvantages	Uses
On-site evaluation	• Direct personal contact • Motivating to staff • Observation of actual work • Identifies causes of errors • Permits verification of equipment and function	• Selective, usually not countrywide if left solely to the reference laboratory • Labour intensive • Costly	• Always during supervisory visits • Implement and monitor quality improvement measures • Data collection and flow of information among laboratory levels • Quarterly by district NTP supervisor • At least annually by the reference laboratory
Panel testing	• Low workload for peripheral centre • Improves laboratory credibility • Rapid response countrywide possible • Use of stained and unstained smears can help to identify source of problem • May lead to identification of faulty equipment	• Does not measure routine performance • High workload for central/reference laboratory • May not be motivating to improve daily performance	• Minimal first step for EQA with limited resources • Rapid assessment of gross deficiencies • Identify factors contributing to errors • Assess training of microscopists
Blinded rechecking	• Low workload for peripheral laboratory • Motivates improved daily performance • Reflects reality of routine performance	• Heavy workload for higher level centre • Unavoidable inaccuracies • Biased if not blinded • Staff must be made available	• Countrywide • Standard for monitoring laboratory performance • Ongoing and permanent

2.5 ROLES OF LABORATORIES IN EFFECTIVE TUBERCULOSIS CONTROL PROGRAMMES

A fundamental aspect of effective tuberculosis control programmes is reliable diagnosis of TB by direct microscopic examination of appropriately stained sputum specimens for tubercle bacilli. The first purpose of TB laboratory services is to detect infectious cases of pulmonary TB, monitor treatment progress and document cure at the end of treatment and the second purpose is to contribute to the diagnosis of cases of pulmonary and extra-pulmonary TB cases. [28] The laboratory therefore plays a critical role in diagnosing TB and monitoring TB treatment.

While developed countries have taken advantage of new technologies that provide rapid detection, identification and drug susceptibility testing of *Mycobacterium tuberculosis*, many developing countries are burdened with high burden of TB and are struggling to provide good-quality microscopy. Access to TB culture and drug susceptibility testing is often scarce or non-existent. [35] Many countries have demonstrated effective TB control using microscopy-based diagnosis and monitoring combined with well-managed treatment programmes. However, inadequate management and support of TB programmes and the laboratory networks are hindering progress against the disease. The HIV/AIDS epidemic and emergence of multidrug-resistant TB (MDR-TB), especially in Africa and Eastern Europe, also limits effective TB control efforts that rely entirely on microscopy-based case detection and management.

2.6 AN EFFECTIVE TB LABORATORY SERVICE INVOLVES:

2.6.1 Microscopy

Rapid TB case detection through sputum smear microscopy remains the mainstay of TB diagnosis, especially for those patients who are most infectious to others. The bacterial load reported on microscopy often reflects the extent of disease requiring immediate treatment. In most countries, especially those with the highest burden of TB, the direct Ziehl–Neelsen smear remains the most common TB diagnostic test. However, its

sensitivity depends on the diligence of the technician and on use of a standard and appropriate technique. The co-epidemics of HIV/AIDS and TB, especially in Africa, and concerns that the Ziehl–Neelsen smear has lower sensitivity in those with HIV infection, have stimulated interest in practical methods to improve microscopy. [35]

External quality assessment programmes are required to ensure that sputum smear microscopy for AFB are performed and interpreted correctly. All microscopy centres should achieve an accepted level of performance. Effective external quality assessment programmes are, however, labour-intensive and complex, requiring dedicated staff for on-site supervisory visits and to recheck results for a relatively large number of smears. International guidelines recommend rechecking a blinded random sample of smears. However, many regions and countries have either not fully implemented rechecking or still use unblinded rechecking, the results of which can be ineffective and misleading. The implementation of external quality assessment for microscopy has the advantage of strengthening laboratory networks and of improving diagnostic quality.

2.6.2 Culture methods and drug susceptibility testing

Although culture methods for TB diagnosis and drug susceptibility testing are practiced routinely in high-resource countries, many low-resource countries continue to struggle to provide culture methods for priority needs such as drug resistance surveillance, extra-pulmonary and childhood TB, and multidrug resistant TB.

Recent outbreaks of extreme drug resistant TB have focused attention on the use of drug susceptibility testing for primary diagnosis as well as for surveillance purposes. Therefore, there is need for TB control programmes to promote appropriate use of culture capacity so that these priority requests are met. TB laboratory services need to be made available throughout the country, and not just in selected urban areas.

2.6.3 Human resources

The management of TB laboratories that provide microscopy, culture and drug susceptibility testing require the input of highly skilled laboratory scientists. However, these personnel are often in low supply or frequently reluctant to work in the lower-paid public funded health service. Many countries are therefore task shifting and training individuals with little or no formal education to perform acid-fast bacilli microscopy and HIV rapid tests. Although these individuals can perform as well as formally trained laboratory technicians, the training programmes must be well structured, with a strong emphasis on effective supervision and need routine external quality assurance to monitor performance.

2.6.4 Laboratory network structure

An effective laboratory network is dependent on the evolving structure of the health-care system. A countrywide, accessible network of TB laboratory services that provides high quality diagnostic services for TB suspects and patients is key to a well functioning TB programme. Many countries with a high burden of TB are struggling to monitor and ensure the quality of testing and reporting in the growing private laboratory sector. [35] Therefore, the national TB programmes and national reference laboratories should develop strategies to enrol private laboratories in external quality assurance programmes and require reporting and referral of TB cases.

2.6.5 Laboratory safety

The process of tuberculosis microscopy, culture, identification and drug susceptibility testing can be an occupational health hazard carrying a risk of causing laboratory-acquired TB infections. These risks present challenges to countries in terms of supporting appropriate facility design and engineering, training and adherence to safety practices, and use and maintenance of biological hazard safety cabinets. As countries are required to expand their culture capacity, there is a need for guidance and decisions on minimum safety standards that are affordable and sustainable. National reference laboratories and national TB control programmes should address such concerns through a combination of

training and education to promote risk assessment and safe practices. Tuberculosis control programme managers should also support reasonable safety improvements with respect to equipment, supplies and facilities.

2.6.6 Quality assurance systems

Quality assurance of TB microscopy, culture and drug susceptibility testing are of paramount importance for an effective TB diagnostic service. Clinicians will forgo existing laboratory testing services and diagnose and treat empirically in situations where there is a lack of trust and credibility concerning the quality of laboratory results. [35] In the presence of HIV infection, many patients will have paucibacillary specimens requiring detection of only a few AFB to obtain the diagnosis of TB. This increases the importance of effective quality assurance systems to improve the sensitivity of TB diagnostic methods.

2.6.7 Turnaround Time

The various health care personnel define turnaround time differently. Some laboratory personnel define turnaround time as the duration of time from specimen receipt in the laboratory to the time the AFB result is released by the laboratory. The South African National TB Control Programme defines turnaround time as the 'duration of time from the taking of a specimen from the patient to the receiving of the result at the health facility'. [23] In South Africa the target for turnaround time is less than 48 hours. For a health facility to be considered as having a turnaround time of within 48 hours, at least 80% of all specimen results must be received by the health facility within 48 hours of the sputum having been collected. [15].

For the third quarter of 2007, the turnaround time for all nine provinces (proportion of facilities within a province with turnaround time within 48 hours) ranged from 19% to

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[15] National Department of Health, personal communication (July, 2008).

75%. None of the provinces achieved the target of 80%. Very few facilities reported a turnaround time of less than 48 hours.

Achieving the targeted turnaround time remains a major challenge. Calculating turnaround times poses a challenge in view of the poor and incomplete reporting of TB data to the National Office[16]. Transportation of specimens from health care facilities to laboratories and delivery of AFB results from the laboratory to the health care facility forms another major challenge especially in remote areas of the country.

Access to laboratory services poses a challenge in remote areas of the country, which lack basic infrastructure such as landline telecommunications, Eskom power supply and adequate roads. The most affected provinces are Eastern Cape, Limpopo, Mpumalanga and KwaZulu-Natal. The turnaround time in these provinces varies from between 2 to 14 days.[27] This is unacceptable for a service that is the cornerstone of the TB control programme. Bacterial coverage has improved in most provinces, which is an indication that most patients were diagnosed using smear microscopy.

The laboratory is often blamed for the prolonged turnaround time. The laboratory argues that the problem (causing prolonged turnaround time) was not the laboratory *per se,* but in transporting specimens to the laboratory and subsequently forwarding the results back to the clinics. The National Health Laboratory Service has tried a number of ways to improve the afferent loop[17] in the Eastern Cape. These include courier services, taxis, ambulances, motorcycles and helicopters. [36].

To improve turnaround times and the speed of communicating TB microscopy results, a 2-year pilot study was carried out in the Port St Johns region of the Eastern Cape from 2001, using a cellphone SMS (Short Message Service: cell phone technology) reporting system. [36] The study showed that when a reliable motorcycle-based transportation

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[16] National office here refers to the National Department of Health, TB Control Unit.

[17] Pre-analytical phase of the laboratory logistic loop, viz. specimen transport in remote areas.

system was provided (for transportation of biological specimens from remote areas to the laboratory), application of the SMS reporting technology was associated with an appreciable increase in the proportion of patients successfully treated for TB according to the directly observed treatment-short course (DOTS) strategy. The National Health Laboratory Service has thus achieved great success with using a motorbike and a local driver to transport specimens to the laboratory. In forwarding the results back to the clinics, the most successful method they have used was sending the laboratory results to the clinic sister via cellphone SMS.

A very innovative project called e-Juba (electronic pigeon) is underway to address the afferent loop.[36] This is a joint operation between the National Health Laboratory Service and Denel Dynamics (unmanned aerial vehicle division) and is based on the principle of the carrier pigeon. Experiments are being conducted to explore the feasibility of using mini-unmanned aerial vehicles designed to transport a payload (biological specimens) of up to 500g over a distance of up to 40 km via multiple Global Positioning System (GPS) to a specified target (testing laboratory).

Efforts to improve the efferent loop[18] also poses challenges as many remote areas of the country in addition to not having suitable roads also lacks access to Telkom landline services and Eskom electric power supply. These areas however are well supplied with wireless communications provided by one or more of the 3 Global System of Mobile (GSM) network service providers[19]. Using these networks enables communication of laboratory results from the testing laboratory to the clinic via the short message service (SMS) and general packet radio service (GPRS) systems. More sophisticated data communication is also possible using interactive SMS, GPRS or Universal Mobile Telecommunication System (UMTS), or 3G.[36] Innovative methods of sending laboratory results from testing laboratories to the clinics were pioneered by the South African Institute of Medical Research and National Health Laboratory Service in 2000.

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[18] Post-analytical communication of laboratory results.

[19] MTN, Vodacom and Cell-C

The latest equipment released was a custom-designed SMS printer capable of printing a hardcopy report from a wireless GSM signal at any remote clinic in South Africa or in much of the African continent.

In KwaZulu-Natal, the eThekwini Municipality acquired three TB laboratory park-homes, which will function as decentralised TB laboratories. The park-homes were situated at three strategic locations (clinics) within the municipality and are expected to reduce turnaround times, which are as high as one week in some areas in the municipality.

2.7 SUMMARY

Many countries, including South Africa, are regarding External Quality Assurance seriously and have implemented quality assurance in one form or another. The province of KwaZulu-Natal, although implementing an external quality assurance programme in 2001, remains with the question of, 'what is the quality of sputum smear microscopy in KwaZulu-Natal?'

Implementation of an external quality assurance programme in settings where overworked laboratory staff regards it as an additional responsibility will simply not achieve the desired outcome. Assessment of the perception of laboratory staff and managers towards proficiency testing would therefore be valuable in structuring an acceptable proficiency testing exercise.

The literature review reinforces the need for TB control programmes to conduct quality assurance to determine the quality of sputum smear microscopy. A gap exists in KwaZulu-Natal, as the quality of sputum smear microscopy is not known. The literature review highlights the benefits of conducting quality assurance in TB control programmes and provides motivation for conducting the study in KwaZulu-Natal.

3 CHAPTER III: METHODS

3.1 INTRODUCTION

Chapter 3 presents the methods used in the study. The study design, study population, study area, sampling, variables studied, data collection techniques and instruments as well as statistical analysis are described in this chapter.

Proficiency testing is one method of external quality assessment that can be used to determine whether a laboratory technician can adequately detect acid-fast bacilli using smear microscopy to diagnose tuberculosis. This method was used to assess the quality of TB microscopy in KwaZulu-Natal. Errors observed using this method may be linked to possible causes including problems with microscopes, problems with stains and other reagents, ability of technicians to identify acid-fast bacilli, administrative errors and negligence (refer to table 17: investigation of errors). [11]

3.2 TYPE OF RESEARCH

The study is health systems research.

3.3 STUDY DESIGN

The study utilises an observational study design that had both a descriptive as well as an analytic cross sectional component.

The analytic component involved repeated cross sectional analysis of the TB proficiency testing results. The repeat cross sectional analysis component of the study contributed towards the achievement of objectives 1-3, namely[20]:

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[20] To achieve these specific objectives proficiency testing data was analysed to determine the following variables:

Correct: No errors

- The analysis of proficiency testing results (2001 to 2006) and quantification of the number and extent of the false results in these laboratories;
- Identification of laboratories that sustained an unacceptable level of performance; and
- Comparison of proficiency testing results obtained by the KwaZulu-Natal reference laboratory and the National Health Laboratory Service reference laboratory.

The descriptive component provided an assessment of the TB diagnostic smear microscopy services in the KwaZulu-Natal public funded health sector. The descriptive component of the study contributed towards the achievement of objectives 5 and 6, namely:

- The perception of laboratory workers and managers towards proficiency testing as a quality assurance technique[21]; and

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QE	Quantification error	Minor error
LFN	Low False Negative	Minor error
LFP	Low False Positive	Minor error
HFN	High False Negative	Major error
HFP	High False Positive	Major error

Sensitivity

Specificity

Positive predictive value (PPV)

Negative predictive value (NPV)

Summary of results by level of health care facility

Summary of results by urban and rural facilities

Summary of results by region

Summary of results by quarter

Trend of overall performance by year

[21] Key informant interviews were conducted to determine the role proficiency testing played as a quality assurance technique.

- In making recommendations to decision makers on the key gaps identified from the information obtained in this study.

3.4 TARGET POPULATION

Health systems research is generally only applicable to the province in which the study was conducted. In this case, it applies to all 79 public health laboratories that provide TB diagnostic smear microscopy services in KwaZulu-Natal. However, it is hoped that all laboratories, performing TB smear microscopy in South Africa, would benefit from the lessons learnt from this study.

3.5 STUDY POPULATION

Proficiency testing

The study population for the proficiency testing programme were all public health laboratories performing TB smear microscopy in KwaZulu-Natal from 2001 to 2006.

Key informant interviews

The study population for the key informant interviews were all mid-level or senior laboratory personnel involved in some way in managing the TB smear microscopy and proficiency testing in public funded laboratories in KwaZulu-Natal.

3.5.1 Inclusion / Exclusion

Inclusion criteria:

The inclusion criteria were all public health laboratories in KwaZulu-Natal that provided TB microscopy services between the years 2001 and 2006.

Exclusion criteria:

All private funded laboratories and personnel were not part of the study populations.

3.5.2 Sampling

3.5.2.1 Method of selecting sample

Proficiency testing

For objectives 1 to 3, a sampling method was not applicable as all public health laboratories that provide TB microscopy services in KwaZulu-Natal (N=79) were included in the sample.

Selection of key informants

For objective 4 (key informant interviews), non-probability/convenience sampling of a range of key informants involved directly or indirectly with the TB diagnostic service from national to district level was used.

Selection of key informants was very focused, as the key informants had to have personal knowledge and experience in laboratory diagnosis of tuberculosis and proficiency testing. This was important to ensure that information obtained from key informants accurately reflected operational conditions in TB laboratories in KwaZulu-Natal.

3.5.2.2 Size of sample

Proficiency testing

All 79 public health laboratories performing TB microscopy testing were included in the study. However, the number of laboratories that participated in the study varied in each quarter as some laboratories were newly enrolled in the proficiency testing programme while others failed to submit results for the proficiency testing programme every quarter.

Key Informant Interviews

Ten key informants were interviewed in the study.

3.6 DATA SOURCES

3.6.1 KwaZulu-Natal reference laboratory

Quantitative data was sourced from the KwaZulu-Natal reference laboratory's (King George V Hospital's TB laboratory) existing database. The KwaZulu-Natal reference laboratory collected this data routinely as part of their province-wide quality assurance programme. Although the data was collected, it was not processed to establish the quality of TB smear microscopy in KwaZulu-Natal. No information for management had been developed from the collection of this data.

3.6.2 Telephonic interviews

Primary qualitative data was collected by interviewing key informants telephonically, to determine the role they believed proficiency testing played as a quality assessment technique.

3.7 VARIABLES

3.7.1 Measurement instruments

Data was transcribed into an EXCEL spreadsheet format suitable for analysis (Annexure 01: Data collection tool).

3.7.2 Measures to ensure reliability and validity

The entire quantitative data set was entered by two data capturers independently and the two data entries were compared to identify any discrepancies. Discrepancies were followed-up and corrected by returning to the original data.

3.7.2.1 Internal validity

3.7.2.1.1 Reduction of bias

Selection bias

The sample is a census of all public health laboratories that provide TB microscopy services in KwaZulu-Natal. Therefore, internal validity is ensured as selection bias is ruled out.

Three proficiency testing slides were sent to participating laboratories for processing each quarter. Each slide set contained slides negative and positive for AFB of varying degree. Proficiency testing slides with AFB grading of 2+ and 3+ positive would be easier to examine microscopically whereas slides with AFB grading of 1+ positive and slides negative for AFB may pose a greater challenge to some microscopists. It is possible that microscopists would report on the 'easy' slides and not forward a report for the more challenging slides. Therefore, laboratories not reporting on all three slides were removed from the study for that round to reduce the bias of laboratories reporting on only the less challenging slides.

Information bias

The quality control slides are accompanied by instructions on how the slides should be read by the microscopist who normally reads slides to diagnose TB. It was also recommended that these slides be treated as routine patient slides. However, it is not known whether the instruction to process the slides by the usual microscopist in the usual way had actually been followed.

It is also not known whether, the best microscopist read the quality control slides. The slides could have been read by several microscopists and the results debated before entry; or a longer time could have been spent on reading the proficiency testing slides.

The technologists/technicians participating in the proficiency testing programme had unlimited time for the slide examination and they were aware that they were being tested. The method followed, does not allow a true assessment to be made of the quality of slide

reading under routine conditions. Therefore, proficiency testing assesses the quality of results produced by the laboratory and not by each individual.

It cannot be established whether the proficiency testing slides were read by the same people reading the patient slides. Information bias, was evaluated and commented on through the key informant interviews. Interviewees were encouraged to respond honestly and openly by assuring them of anonymity and confidentiality of interview material.

Interviewer bias

Interviewer bias was minimized by using an interview guide to maintain consistency when conducting the interview.

Interviewee bias

Interviewee bias was minimized through the following:

- Interviewees were ensured that their identity was kept anonymous;
- All information generated from the interview remained confidential. It was only made available to those directly involved[22] in the research; and
- All tapes of the interviews were destroyed after being transcribed.

3.7.2.2 External validity

During the study period, the study laboratories were administered by KwaZulu-Natal Provincial Laboratory Services whereas other public health laboratories in South Africa were administered by the National Health Laboratory Service. The unique organisation of laboratory services in KwaZulu Natal could limit the generalisability of findings to other provinces in South Africa. However, quality of results produced by KwaZulu-Natal laboratories could be similar to other laboratories in South Africa that are similar.

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[22] The people directly involved in the study are the researcher and the research supervisor.

3.8 LIST OF VARIABLES

Proficiency testing

1. Correct
2. Quantification error
3. Low false negative
4. Low false positive
5. High false negative
6. High false positive
7. Sensitivity
8. Specificity
9. Positive predictive value (PPV) and
10. Negative predictive value (NPV)
11. Summary of results by level of health care facility
12. Summary of results by urban and rural facilities
13. Summary of results by region
14. Summary of results by quarter
15. Trend of overall performance by year

Key informant interviews

Knowledge, attitudes and practices: These variables were not known before the study commenced.

3.9 PILOT STUDY

3.9.1 Proficiency testing data

The study involved a secondary analysis of existing data; therefore, a pilot study would not have improved validity of the data.

3.9.2 Key informant interviews

A pilot study was conducted by first interviewing two of the 10 key informants and extracting comments on relevance, balance and adequacy of the interview guide in

relation to the interview objectives. The interview guide was reviewed and amended where necessary.

3.10 DATA COLLECTION

3.10.1 Proficiency testing

Hard copies of proficiency testing results were obtained from the reference laboratory and transcribed into EXCEL format for analysis (Annexure 01: Data Collection Tool).

3.10.2 Key informant interviews

Open-ended questions were asked and probes were used to encourage conversation without influencing the response. An interview guide (Annexure 02: Interview Guide) was used to guide the interview to achieve the broad objectives of the interview as stated below.

Broad objectives of the interview:

A. To ascertain the knowledge that laboratory personnel have about proficiency testing, attitudes towards proficiency testing as a quality assurance technique, and practices when processing proficiency testing slides.
B. To ascertain what laboratory staff thought about proficiency testing.
C. To identify problems regarding proficiency testing.
D. To identify possible solutions to problems highlighted.

Qualitative data from key informant interviews were recorded on audiotape. Where approval to record on audiotape was denied by the interviewee, then the interview was recorded by taking notes during the interview.

3.11 DATA HANDLING

3.11.1 The Measures taken to ensure safe storage of data:

Proficiency testing data was entered on a Microsoft EXCEL spreadsheet. Key informant interviews were transcribed electronically as a Microsoft Word document. All data was

stored electronically as well as on hard copies. Access to this data was limited to only those people directly involved in the research.

Names of participant laboratories were coded to ensure anonymity of the laboratories. However, a list of the laboratories will be forwarded to the reference laboratory manager.

3.12 STATISTICAL PROCESSING AND ANALYSIS

3.12.1 Proficiency testing

a) Results were tabulated (Table 3) and analysed. Microsoft EXCEL (Microsoft Excel, Palisade Corp, Newfield, NY, USA) was used to quantify the variables listed in chapter 3.8.

b) Significance of differences between variables (proportions) was determined by calculating p-values using the Mantel-Haenzel Chi squared test (X^2-Test).

3.12.2 Classification of errors

Proficiency testing results were classified as indicated in table 3 below.

Table 3: Cross classification and tabulation of proficiency testing errors

Expected result	**Result of microscopy centre**				
	Negative	**Scanty**	**1+**	**2+**	**3+**
Negative	Correct	LFN	HFN	HFN	HFN
Scanty	LFP	Correct	QE	QE	QE
1+	HFP	QE	Correct	QE	QE
2+	HFP	QE	QE	Correct	QE
3+	HFP	QE	QE	QE	Correct

LFP = Low false positive; HFP = High false positive; HFN = High false negative;
QE = Quantification error; LFN = Low false negative

3.12.3 Scoring system

Laboratories reporting correct results were awarded a maximum possible score of 10 points per slide. Incorrect results i.e. high false positive and high false negative were awarded a score of zero. Low false positive and low false negative slides had five points deducted, giving these slides a score of five points. Slides with quantification errors were awarded a score of ten points.

Scoring was done as indicated in table 4 below.

Table 4: Point allocation according to classification of error

Error	Points
Correct	10
QE	10
LFN	5
LFP	5
HFN	0
HFP	0

3.12.4 Criteria for assessment for performance

Criteria for assessment of performance in this study was adapted from recommendations by the American Public Health Laboratory and Centre for Disease Control and from the study conducted in Limpopo, South Africa. [11, 13]

- **Errors**: The target for optimal performance was for laboratories not to have any errors of any type.
- **Major Errors:** Any major error (HFP or HFN) would indicate unacceptable performance and should trigger an evaluation and corrective action if needed. It is

possible that no significant problems in laboratory practice would be identified, and performance trends should be monitored over time.

- **Minor errors**: Minor errors (LFP or LFN) would require further evaluation if they exceed the average number seen in all TB microscopy centres in the province or if the number of minor errors over time demonstrates a trend.
- **Quantification errors**: Disagreement on bacillary concentration is less serious and is not usually calculated as a percentage error. Quantification errors are not that important, as they do not drastically influence the decision making on patient management. This type of error only distinguishes the good from the very good microscopist.

Overall Disagreement on false positivity and false negativity should be less than 5%.

3.12.5 Assessment of overall performance of the province

To calculate overall performance for the province, all the scores for all the laboratories were combined and divided by the number of participating laboratories and calculated as percentage correctness in microscopy diagnosis for the province. The overall aim was to reach 95% agreement, between all the participating laboratories and the reference standard, for the province as a whole.

3.12.6 Assessment of overall performance of individual laboratories

To calculate overall performance for individual laboratories, all the scores for all the slides processed by each laboratory were combined and calculated as percentage correctness in microscopy diagnosis for the laboratory. The overall aim was be to reach 95% agreement (acceptable performance), in reading proficiency testing slides, between the various laboratories and the reference standard.

It was recommended that should the overall disagreement (error) exceed the accepted critical value of 5% (proportion discordant among negative and positive slides, or overall discordance), then the entire procedure, including quality of smear preparation, reagents,

staining method and reading should be reviewed by the supervisor and the technologist/technician concerned. [13]

3.12.7 Calculation of sensitivity, specificity, positive predictive value and negative predictive value

Results were analysed on 2 x 2 tables as indicated in Table 5 below.

Table 5: Analysis of Results on 2 x2 Tables

		Reference Material		
		Positive	**Negative**	**Total**
Laboratory	**Positive**	a	b	(a+b)
Result	**Negative**	c	d	(c+d)
	Total	(a+c)	(b+d)	a+b+c+d

The formulas for calculating sensitivity[23], specificity[24], positive predictive value[25] and negative predictive[26] value are as follows:

- Sensitivity = [a/(a+c)] x 100
- Specificity = [d/(b+d)] x 100
- Positive Predictive Value = [a/(a+b) x 100]
- Negative Predictive Value = [d/(c+d) x 100]

Overall sensitivity, specificity, positive predictive value and negative predictive value for the province were calculated using the reference laboratory as a comparator.
The targets for sensitivity, specificity, positive predictive value and negative predictive value were as follows:

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[23] Percentage of positive test result out of all true positives

[24] Percentage of negative test results out of all true negatives

[25] Percentage of positive test result that are truly positive

[26] Percentage of negative test results which are truly negative

- **Sensitivity**: Since the low positive slides were removed from the study all other positive slides should be read correctly. However, 100% sensitivity is very difficult to achieve even by the controller, therefore sensitivity was set at 95%.[11]
- **Specificity**: Any false negative results would indicate unacceptable performance and should trigger corrective action. However, Minor errors, do occur occasionally even in laboratories that are performing well. Therefore the target for specificity was set at 95%. [11]
- **Positive predictive value**: Any false positive results would indicate unacceptable performance and should trigger corrective action. However, Minor errors, do occur occasionally even in laboratories that are performing well, therefore the target for positive predictive value was set at 95%
- **Negative predictive value:** Any false negative results would indicate unacceptable performance; However, Minor errors, do occur occasionally even in laboratories that are performing well. Therefore the target for negative predictive value was set at 95%.

The above recommendations were adopted to assess the proficiency of TB smear microscopy in public health TB microscopy laboratories in KwaZulu-Natal.
A comparison was made with the National Health Laboratory Service proficiency testing results, as they were conducting proficiency testing for the year 2006 and would therefore provide additional knowledge to the status of TB microscopy services in KwaZulu-Natal. Analysis of the National Health Laboratory Service data would also form the basis for comparison with the other provinces in the country.

3.12.8 Key informant interviews

Each issue discussed in the interview was categorized, grouped and summarized.

3.13 ETHICS

3.13.1 Institutional Review Board

Ethics approval (ref: BE003/07) for the project was obtained from the University of KwaZulu-Natal, College of Health Sciences, Biomedical Research Ethics Committee (Annexure 03: Ethics Approval Form).

3.13.2 Permissions

Permission to use proficiency test data was obtained from the Director: KwaZulu-Natal Laboratory Services and Chief Executive Officer of the National Health Laboratory Services.

Consent for the qualitative assessment (interview) was obtained from key informants. Consent forms (Annexure 04: Consent Document) and information sheets (Annexure 05: Information Document) were faxed to the key informants, following telephone discussions and their agreement to participate in the study. Key informants were requested to fax the consent form back to the researcher indicating the following:

- Willingness to participate in the research
- Date and time when interview could be conducted
- Approval to record the interview on audiotape

3.14 REFERENCE SLIDE PREPARATION PROCEDURE

Real patient sputa[27] were used to prepare proficiency testing slides used in this study[28]. These sputa were processed for AFB examination using the sodium hypochlorite ("JIK") method (Annexure 06: Processing of specimens for microscopy).

Slides were prepared (Annexure 07: making of smear for microscopy only) and stained using the Ziehl-Neelsen staining method (Annexure 08: Staining of slides with Ziehl-

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[27] These sputa were collected for tuberculosis diagnosis, from patients suspected of tuberculosis, in health care facilities.

[28] Proficiency testing slides were prepared at the King George V Hospital TB laboratory. Slide preparation and staining procedures varied in other laboratories according to laboratory specific protocol.

Neelsen stain). The slides were then thoroughly examined (Annexure 9: Examination of slides using a light microscope).
Since these were real patient samples the routine procedure of recording and reporting of AFB results was followed.

Appropriate samples that were found to be negative, scanty, 1+, 2+, and 3+ were used to make serial slides that were used as reference slides for this study.

3.14.1 Distribution of reference slides

A batch of three unstained slides that were negative to positive with various concentrations of AFB were sent to each participating laboratory via the KwaZulu-Natal Department of Health transport unit. The AFB results of the reference slides were known to the reference laboratory but not to the laboratories being evaluated.
The reference slides were accompanied by instructions to process the slides and forward the results together with the slides back to the reference laboratory.
The returned results were recorded on the laboratory computer in Microsoft Excel format.

3.15 SUMMARY

An observational study that had both a descriptive component and an analytic cross sectional component was conducted.
The analytic study was used to conduct repeat cross sectional analysis of the TB proficiency testing results. This component of the study contributed towards the achievement of objectives 1-3.

The descriptive study was used to provide an assessment/situational analysis of the TB microscopy service in KwaZulu-Natal. This component of the study contributed towards the achievement of objectives 4 and 5.
Objective 5 is addressed in chapter 6 (Recommendations and conclusions) of this study.

4 CHAPTER IV: RESULTS

4.1 INTRODUCTION

The purpose of this health systems research was to assess the quality of TB smear microscopy in KwaZulu-Natal from 2001 to 2006 by proficiency testing of unstained sputum smears and to assess the knowledge, attitudes and practices of laboratory personnel towards proficiency testing. The ultimate goal of the study was to improve TB diagnosis using smear microscopy in KwaZulu-Natal.

4.1.1 Removal of round two from the study

The second round of proficiency testing was conducted in the third quarter of 2001 and comprised of 3 slides in the panel, with one slide having 1+, one being negative and the third side having 3+ AFB. Results for this round are illustrated in table 6.

Due to the high number of discrepant results in this panel, which indicated a problem with slide preparation in the reference laboratory it was decided, in consultation with the reference laboratory, to remove this round of proficiency testing from the study. [29]

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[29] The set of three slides were forwarded to each of the 61 participating laboratories in that quarter. Response for the first slide (1+) indicated a high number of discrepant results. The reference laboratory indicated that the target result was '1+'. Of the 61 slides forwarded to the participating laboratories, results from 8 laboratories were not submitted to the reference laboratory, and smear material from one slide was washed off. Of the remaining 52 slides, only 6 were correctly read as 1+, 36 slides were read as negative and 10 were read as low positives.

The target result for the second slide was 'Negative'. Of the 61 slides forwarded to the participating laboratories, results from 8 laboratories were not submitted to the reference laboratory, smear material from one slide was washed off and one slide broke. Of the remaining 51 slides, 34 were correctly read as 'Negative', 11 were read as 'scanty', four were read as 1+ and two were read as 2+.

Table 6: Summary of TB Microscopy Proficiency Testing Results for Round Two, Third Quarter of 2001, KwaZulu-Natal Public Health Laboratories.

Result of Microscopy Centre	Expected Result		
	1+ (N=61)	Negative (N=61)	3+ (N=61)
Negative	36	34	1
Scanty	10	11	2
1+	6	4	1
2+	0	2	6
3+	0	0	43
No result submitted	8	8	8
Smear material washed off	1	1	0
Slide broke	0	1	0

4.1.2 Presentation of data

The results are organised and presented according to the specific objectives mentioned in chapter 1.

Data from the KwaZulu-Natal proficiency testing programme was used to assess laboratory performance from 2001 to 2004 (KZN panel). Results from the National Health Laboratory Service proficiency testing programme was used to assess laboratory performance for year 2006 (NHLS panel). No proficiency testing data is available for year 2005, as proficiency testing was not conducted for this period.

The target result for the third slide was 3+. Of the 61 slides forwarded to the participating laboratories, results from 8 laboratories were not submitted to the reference laboratory. Of the remaining 53 slides, results for 49 were correct, with 43 achieving the target results of 3+. One slide was read as 'Negative', 2 were read as 'scanty' and one was read as 1+.

4.2 Quantitative analysis

4.2.1 Specific objective 1: Description and analysis of proficiency testing data

In this section, results for specific objective 1 are presented. Specific objective 1 was to describe and analyse the proficiency testing results carried out between 2001 and 2006 in the 79 facilities where sputum smear microscopy was carried out by the KwaZulu-Natal public health laboratories in the province and to quantify the size of the false results in these laboratories.

Since the results in this study are presented from two different reference laboratories, results are presented separately as the KZN panel (2001-2004) and the NHLS panel (2006). The variables discussed in chapter 3.8 were used to assess the participating laboratories in both the KwaZulu-Natal proficiency testing programme and the National Health Laboratory Service proficiency testing programme.

Overall performance was calculated for each of the participating laboratories and for the province as a whole for both the KwaZulu-Natal proficiency testing programme and the National Health Laboratory Service proficiency testing programme, as described in chapter 3.

Summary of results

Total slides reviewed

A total of 1684 proficiency testing slides were processed for the KZN panel during 2001 to 2004. During 2006, 1279 slides were processed for the NHLS panel.

Total non-returns

A total of 94 slides were classified as non-returns for the KZN panel and 36 were classified as non-returns for the NHLS panel.

Broken slides (excluding scanty positive slides)

Eight slides were reported as broken for the KZN panel whereas none were reported as broken for the NHLS panel.

Slides not evaluated

Twelve slides were not evaluated for the KZN panel whereas all slides were evaluated for the NHLS panel.

Agreement in reading positive and negative slides

The overall agreement in slide reading by the reference laboratory and the laboratories being evaluated are presented in Tables 7 and 8 for 2001-2004 and 2006 respectively.

Table 7: KZN data (2001-2004): Sensitivity, specificity, positive predictive value, negative predictive value

		Reference Material		
		Positive	**Negative**	**Total**
Laboratory Result	**Positive**	944	60	1004
	Negative	95	585	680
	Total	1039	645	1684
Sensitivity		**90.9%**		
Specificity		**90.7%**		
Positive Predictive Value		**94.0%**		
Negative Predictive Value		**86.0%**		

Table 8: NHLS Data (2006): Sensitivity, specificity, positive predictive value, negative predictive value

		Reference Material		
		Positive	**Negative**	**Total**
Laboratory Result	**Positive**	935	9	944
	Negative	26	309	335
	Total	961	318	1279
Sensitivity		**97.3%**		
Specificity		**97.2%**		
Positive Predictive Value		**99.0%**		
Negative Predictive Value		**92.2%**		

Overall summary of results is presented in Tables 9 and 10.

Table 9: Summary of Results of Proficiency Testing Conducted in KwaZulu Natal Public Health Laboratories for the Period 2001 to 2004 (KZN Panel) and for 2006 (NHLS Panel)

Classification of results	KZN data excluding scanty positive slides 2001-2004	NHLS data 2006	
	% (N)	% (N)	p- value
Total non returns	94	36	
Total Slides reviewed	1684	1279	
Correct	82.5% (1390)	93.3% (1193)	<0.01
Quantitation Errors	8.3% (139)	4.2% (54)	<0.01
False positives	3.6% (60)	0.5%(6)	<0.01
False negatives	5.6% (95)	2.0% (26)	<0.01
High false positive	0.7% (11)	0.1% (1)	0.02
Low false positive	2.9% (49)	0.4% (5)	<0.01
High false negative	5.6% (95)	2.0% (26)	<0.01
Low false negative	0*	0*	
Score (overall agreement)	15570 (92.5)	12495 (97.7)	<0.01
Broken slides	8	0	
Slides not evaluated	12	0	

* **Slides classified as 'low positive' (scanty) were removed from the KZN panel to maintain consistency with the NHLS panel.**

Table 10: Quality of Readings of Proficiency Testing Conducted in KwaZulu-Natal Public Health Laboratories for the Period 2001 to 2004 (KwaZulu Natal-Panel) and for 2006 (National Health Laboratory Service Panel)

Results	KZN data % (N)	NHLS data % (N)	p-value
Sensitivity	90.9%	97.3%	<0.01
Specificity	90.7%	97.2%	<0.01
False-positives	3.6% (60)	0.5% (6)	<0.01
False-negatives	5.6 (95)	2.0% (26)	<0.01
Positive predictive value	94.0%	99.0%	<0.01
Negative predictive value	86.0%	92.2%	<0.01
Total disagreement	7.5%	2.3%	<0.01
Total agreement	92.5%	97.7%	<0.01

4.2.1.1 Correct

Correct results are slides that are read without errors or the difference of not more than one grade in reading a positive slide between examinee and controller. This was the target for reading all proficiency testing slides.

Of the 1684 slides processed, 1390 slides (83%) were read as 'correct' for the KZN panel (2001-2004). In 2006, of the total of 1279 slides, 1193 (93%) were read as 'correct'. There was significant improvement (p=<0.01) in the reading of proficiency testing slides from the 2001-2004 period to 2006.

4.2.1.2 Quantification errors

Quantification Error (QE) is the difference of more than one grade in reading a positive slide between examinee and controller. This minor error generally has no impact on case management, therefore laboratories producing quantification errors were not penalised and were awarded full score (10 points) for slides read as quantification errors.

A total of 139 (8%) proficiency testing slides were read as 'quantification errors' for the KZN panel whereas 54 (4%) were read as 'quantification errors' for the NHLS panel. There was significant reduction in quantification errors (p=<0.01) from 2001-2004 to 2006.

4.2.1.3 Low false negative results

Low positive slides

Two sets of low positive slides were included in the proficiency testing panel slides prepared by the KwaZulu-Natal Reference Laboratory. The first slide was slide-B in round 6 and the second slide was slide-B in round 7. The National Health Laboratory Service reference laboratory did not include low positive slides in their proficiency testing panel.

Therefore, in order to achieve a more accurate comparison between the KZN data set and the NHLS data set, results for the 'low positive' slides in the KZN data set was removed. Results from these slides were however quantified and commented on (see low false negatives below).

Low false negatives

The proportion of low false negative results for period 2001-2004 (KZN panel) was 1.4% (n=26). The number of Low false negative results ranged from 1-2 in some laboratories. It was observed that for the period 2001-2004, twenty-four laboratories reported at least one low false negative result. For the same period, two laboratories reported two low false negative results each. The remaining laboratories (n=48) did not report a single low false negative result.

4.2.1.4 Low false positives

The proportion of low false positive results for period 2001-2004 (KZN panel) was 3% (n=49) and for 2006 (NHLS panel) was 0%[30] (n=5). The number of low false positive results ranged from 1-3 in some laboratories. It was observed that for the period 2001-2004, thirty-five laboratories reported at least one low false positive result. For the same period, 4 laboratories reported 3 and 5 reported 2 low false positive results. The remaining laboratories (n=37) did not report a single low false positive result.
The number of low false positive results reported during 2006 was 5. Five laboratories reported 1 low false-positive result each. The remaining laboratories (n=51) did not report a single low false positive result.

4.2.1.5 High false negative results

The proportion of high false negative results for period 2001-2004 (KZN panel) was 6% (n=95) and for 2006 (NHLS panel) was 2% (n=26). The number of high false negative results ranged from 1-7 in some laboratories. It was observed that for the period 2001-2004, 42 laboratories reported at least 1 high false negative result. For the same period, 1 laboratory reported 6 and another reported 7 high false negative results. The remaining laboratories (n=30) did not report a single high false negative result.

The number of high false negative results during 2006 ranged from 1-2. Twenty-one laboratories reported at least 1 high false negative result. Five laboratories reported 2 high false negative results each. The remaining laboratories (n=45) did not report a single high false negative result.

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[30] Although proportion of low false positive results is zero, the actual number of low false positive results was 5.

4.2.1.6 High false positive results

The proportion of high false positive results for the period 2001-2004 (KZN panel) was 1% (n=11) and for 2006 was 0%[31] (n=1). The number of high false positive results reported for period 2001-2004 ranged from 1-2 in some laboratories. It was observed that 10 laboratories reported at least 1 high false positive result each whereas 1 laboratory reported 2 high false positive results. The remaining laboratories (n=62) did not report a single high false positive result. Only 1 laboratory reported a single high false positive result during 2006 (NHLS panel).

4.2.1.7 Sensitivity (Positive consistency)

The proportion of positive slides read correctly as positive during the period 2001-2004 was 91% (Table 10). The proportion of positive slides read correctly as positive during the period 2006 was 97%. There was significant improvement (p=<0.01) in sensitivity from 2001-2004 to 2006.

4.2.1.8 Specificity (Negative consistency)

The proportion of negative slides read correctly as negative during the period 2001-2004 was 91% (Table 10). The proportion of negative slides read correctly as negative during the period 2006 was 97%. There was significant improvement (p=<0.01) in specificity from 2001-2004 to 2006.

4.2.1.9 Positive predictive value

The proportion of positive test results that are truly positive for period 2001-2004 and for period 2006 was 94% and 99% respectively (Table10).

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[31] Although proportion of high false positive results is zero, the actual number of high false positive results was one.

4.2.1.10 Negative predictive value

The proportion of negative test results that are truly negative for period 2001-2004 and for period 2006 was 86% and 92% respectively (Table 10).

Overall agreement

The total number of slides reviewed by all participating laboratories for the period 2001 to 2004 was 1684.The proportion of slides read as correct was 83 % (n=1390). The proportion of slides with quantification errors was 8% (n=139). Overall performance of all participating laboratories for period 2001 to 2004 was 93%. The overall range of agreement for all participating laboratories was between 67% and 100%. Twelve out of 72 participating laboratories achieved 100% consistency for all the rounds that they participated in.

The total number of slides reviewed by all participating laboratories for year 2006 was 1279. The proportion of slides read correctly for this period was 93 % (n=1193). The proportion of slides with quantification errors was 4% (n=54). Overall performance of all participating laboratories for period 2006 was 98%. Thirty-nine (n=66) laboratories achieved 100% consistency for all the rounds that they participated in.

False negatives:

A total of 6% (95) of proficiency testing slides were read as false negative for the KZN panel whereas 2% (26) were read as false negative for the NHLS panel.

False positives:

A total of 60 (3%) of proficiency testing slides were read as false positive for the KZN panel whereas 6 (0.5%) were read as false positive for the NHLS panel.

4.2.1.11 Summary of results by level of health care facility

During the study period, 79 TB microscopy centres were operational in KwaZulu-Natal; 26 were classified as primary health care level (situated at clinics), 51 were classified as district level (situated at district hospitals) and 2 were classified as tertiary level (situated at tertiary hospitals). Overall summary of results by level of health care facility is presented in table 11.

Table 11: Summary (Number & Percent) of TB Microscopy Proficiency Test Results by Level of Health Care Facility Combining Data for the Period 2001 to 2004 and 2006

	Primary Health Care level			District Level			Tertiary Level		
	KZN (%)	**NHLS (%)**	**p**	**KZN (%)**	**NHLS (%)**	**p**	**KZN (%)**	**NHLS (%)**	**p**
Total non returns	28 (6.2%)	15 (5.2%)		59 (4.5%)	18 (1.8%)		7 (38.9%)	3 (15.8%)	
Total Slides reviewed	426	272		1247	991		11	16*	
Correct	351 (82.4)	264 (97.1)	<0.01	1034 (82.9)	914 (92.2)	<0.01	5 (45.5)	15 (93.8)	0.01
Quantification Errors	35 (8.2)	2 (0.74)	<0.01	101 (8.1)	52 (5.2)	0.01	3 (27.3)	0	0.03
False positives	16 (3.8)	1 (0.4)	0.01	44 (3.5)	5 (0.5)	<0.01	0	0	
False negatives	24 (5.6)	5 (1.8)	0.01	68 (5.5)	20 (2)	<0.01	3 (27.3)	1 (6.3)	0.14
High false positive	5 (1.2)	0	0.07	6(0.5)	1 (0.1)	0.12	0	0	
Low false positive	11 (2.6)	1 (0.4)	0.03	38 (3.0)	4 (0.4)	<0.01	0	0	
High false negative	24 (5.6)	5 (1.84)	0.01	68 (5.5)	20 (2.0)	<0.01	3 (27.3)	1 (6.3)	0.14
Low false negative	0	0		0	0		0	0	
Overall Score	3930 (92.3)	2665 (98.0)	<0.01	11560 (92.7)	9680 (97.7)	<0.01	80 (72.7)	150 (93.8)	<0.01

* All 16 slides were processed by only 1 of the 2 tertiary level facilities.

The overall score obtained for proficiency testing for all the health care levels in KwaZulu-Natal for the period 2001 to 2004 (KZN panel) was below 95%. However, all results improved significantly (p=<0.01) in 2006 (NHLS panel).

The primary health care level and the district level obtained similar results for the KZN panel (92% and 93% respectively) and for the NHLS panel (98% for both).
The tertiary level facilities performed the worst for both the KZN panel and the NHLS panel (overall scores of 73% and 94% respectively).

All three levels of health care facilities performed below the acceptable level of performance (i.e. overall scores of <95%) for the KZN panel. Significant improvement (p=<0.001) was observed for all levels of health care facilities in 2006 (NHLS panel) with the primary health care level facilities and the district level facilities exceeding 95% in overall scores. The tertiary level facilities failed to achieve overall scores of 95% or more (94%).

A total of 1684 slides were reviewed for the KZN panel and 1279 slides for the NHLS panel. The tertiary level facilities processed fewer slides than the other two levels, namely 11 (1%) slides for the KZN panel and 16 (1%) slides for the NHLS panel. In addition only 1 out of the 2 tertiary level facilities participated in the 2006 proficiency testing programme (NHLS panel). The primary health care level processed 426 (25%) slides for the KZN panel and 272 (21%) slides for the NHLS panel. The District level processed 1247 (74%) of the slides for the KZN panel and 991 (78%) of the slides for the NHLS panel.

The tertiary level had a much lower proportion of correct results (46%) for the KZN panel than the primary health care level (82%) and district level (83%). However, results improved significantly (p=<0.01) in 2006, as indicated by the NHLS panel, for all 3 levels with the primary health care level achieving 97%, the district level achieving 92% and the tertiary level achieving 94%. The tertiary level had a much higher proportion of quantification errors (27%) than the primary health care level (8%) and the district level (8%) for the KZN panel. However the tertiary level had zero quantification errors for the NHLS panel, whereas both the primary health care level and the district level had 8% quantification errors. The primary health care level and the district level had 4% false positives whereas the tertiary level had zero false positives for the KZN panel. There was

significant improvement (p=<0.05) in 2006 (NHLS panel) as the primary health care level and district level achieved <1% false positives with actual numbers being 1 and 5 respectively. The tertiary level again achieved zero false positives in 2006.

The primary health care level and district level each had 6% false negative results with actual numbers being 24 and 68 respectively for the KZN panel. Both these levels had significant improvement (p=<0.01) in 2006 (NHLS panel) as the proportion of false negatives dropped to 2% for both levels. The tertiary level had a much higher proportion of false-negatives (27% for the KZN panel and 6% for the NHLS panel) than the other 2 levels. There was no significant improvement (p=0.14) in reading of false negatives in 2006 as compared to 2001-2004.The tertiary level produced 1 high false positive result in 2006 but, since this level processed only 16 slides, it resulted in a higher proportion of false results.

In the study, all false negative results could be classified as either low false negatives or high false negatives. Since low positive slides were removed from the study there was no longer a possibility of reporting low false negative results. Therefore, all false negative results were high false negatives.

A total of 94 slides were classified as non-returns for the KZN panel and 36 for the NHLS panel. Of all the slides classified as non-returns, the primary health care level was responsible for 28 (30%) for the KZN panel and 15 (42%) of the slides for the NHLS panel. The district level was responsible for 59(63%) for the KZN panel and 18(50%) for the NHLS panel. The tertiary level was responsible for 7(19%) for the KZN panel and 3(8%) for the NHLS panel. Since all 3 levels of health care facilities did not process the same number of slides, the slides classified as non-returns are presented as a percentage of slides read as well. This would give a better understanding of the levels of health care facilities that are responsible for the greater proportion of the non-returns.

The number of non-returns as a proportion of the number of slides processed at primary health care level was 7% for the KZN panel and 6% for the NHLS panel. At district level

it was 5% for the KZN panel and 2% for the NHLS panel. At tertiary level it was 64% for the KZN panel and 19% for the NHLS panel. It is therefore evident that the tertiary level was responsible for the highest proportion of non-returns for both the KZN and the NHLS panels. The reason for the substantial non-returns recorded by the tertiary level cannot be confirmed. However possible reasons could be problems related to communication between the reference laboratory and the tertiary level laboratories, transport of slides and results between the reference laboratory and the tertiary level laboratories, the tertiary level laboratories were too occupied with routine work and did not have time to process the proficiency testing slides or there was a lack of confidence in the proficiency testing programme and therefore the tertiary level laboratories neglected to participate. The high number of non-returns recorded by the tertiary level laboratories need to be explored further. The reason/s for the non-returns must be identified and rectified as they pose an obstacle to determining the true quality of smear microscopy in these laboratories.

4.2.1.12 Summary of results by urban and rural facilities

Overall summary of results by urban and rural facilities is presented in Table 12.

Table 12: Summary (Number & Percent) of TB Microscopy Proficiency Test Results for Urban and Rural Laboratories Combining Data for the Period 2001 to 2004 and 2006

	Urban laboratories			Rural Laboratories		
	KZN data	NHLS data	p value	KZN data	NHLS data	p value
Total non returns	44 (5.7%)	18 (3.3%)		50 (4.8%)	18 (2.3%)	
Total Slides reviewed	712	535		972	744	
Correct	607 (85.3)	506 (94.6)	<0.01	783 (80.6)	687 (92.3)	<0.01
Quantitation Errors	50 (7.0)	13 (2.4)	<0.01	89 (9.2)	41 (5.5)	0.01
False positives	20 (2.8)	2 (0.4)	<0.01	40 (4.1)	4 (0.5)	<0.01
False negatives	35 (4.9)	14 (2.6)	0.04	60 (6.2)	12 (1.6)	<0.01
High false positive	4 (0.6)	0	0.08	7 (0.7)	1 (0.1)	0.08
Low false positive	16 (2.3)	2 (0.4)	0.01	33 (3.4)	3 (0.4)	<0.01
High false negative	35 (4.9)	14 (2.6)	0.04	60 (6.2)	12 (1.6)	<0.01
Low false negative	0	0		0	0	
Score	6660 (93.5)	5200 (97.2)	<0.01	8905 (91.6)	7295 (98.1)	<0.01
Total possible score	7120	5350		9720	7440	
Slides broke	6	0		13	0	
Slides not evaluated	13	0		14	0	

Urban laboratories had a higher proportion of 'correct' results for both the KZN panel and the NHLS panel They also had lower proportions of quantification errors[32] for both the KZN panel and the NHLS panel (Annexure 11: Summary of proficiency testing scores by Urban/Rural laboratories in KwaZulu-Natal, 2001-2006).

[32] Quantification Error (QE) is the difference of more than one grade in reading a positive slide between examinee and controller. This minor error generally has no impact on case management.

Rural laboratories had twice as many low false positives than urban laboratories, i.e. 33 (3%) compared to 16 (2%) for urban laboratories for the KZN panel. However, the proportions of low false positives for the KZN panel and the NHLS panel were the same (0.4%) in 2006.

Rural laboratories also had a much higher number of high false negative results than urban laboratories, i.e. 60 (6%) compared to 35 (5%) for urban laboratories for the KZN panel, although the proportions are similar. Since high false negative results are major errors and should not occur, both rural and urban laboratories had unacceptably high levels of high false negative results. Significant reduction ($p=<0.05$) in high false-negative results was observed for both levels in 2006 (NHLS panel).

Urban laboratories achieved higher overall scores that rural laboratories for the KZN panel. However, both urban and rural laboratories failed to achieve the acceptable overall score of 95%. There was a significant improvement ($p=<0.01$) in 2006 (NHLS panel) with both urban and rural laboratories producing overall scores in excess of 95% i.e. 97% and 98% respectively.

There was significant improvement in processing of proficiency testing slides from 2001-2004 (KZN panel) to 2006 (NHLS panel) for both urban laboratories and rural laboratories. There was statistical improvement ($p=<0.05$) in processing slides classified as correct[33], quantification errors, false positives, false negatives, low false positive[34], high false negative[35] and low false negative[36].

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[33] Correct results are slides that are read without errors or the difference of not more than one grade in reading a positive slide between examinee and controller.

[34] Low False Positive (LFP) was previously called a scanty false positive. LFP is a negative smear that is misread as a low (1-9 AFB per 100 fields) positive. This type of minor error occurs occasionally even in laboratories that are performing well.

4.2.1.13 Summary of results by region

Although KwaZulu-Natal has 11 health districts, the laboratory services are provided by 9 regions (Annexure 12: Classification of laboratories into regions and Annexure 13: Map of Districts and NHLS Laboratories in KZN)). Therefore, it was more appropriate to determine the performance of TB microscopy services by regions. Overall summary of results by the 9 regions is presented in table 13.

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[35] High False Negative (HFN): A 1+ to 3+ positive smear (based on International Union against TB and Lung Disease (IUATLD)/WHO recommended grading of sputum smear microscopy results) that is misread as negative. HFN is a major error.

[36] Low False Negative (LFN) was previously termed a scanty false negative. A low (1-9 AFB per 100 fields) positive smear that is misread as negative. This type of minor error occurs occasionally even in laboratories that are performing well.

Table 13: Summary (Number & Percent) of TB Microscopy Proficiency Test Results by 9 Regions in KwaZulu-Natal Combining Data for the Period 2001 to 2004 and 2006

	Region														
	1			2			3			4			5		
	KZN	NHLS	p	KZN	NHLS		KZN	NHLS	p	KZN	NHLS	p	KZN	NHLS	p
Total non returns	16	6		8	0		2	6		23	2		3	2	
Total Slides reviewed	245	192		218	180		148	131		142	152		86	48	
Correct	204 (83.3)	181(94.3)	<0.01	186(85.3)	167 (92.8)	0.02	129 (87.2)	126 (96.2)	0.01	115 (81.0)	135(88.8)	0.06	69 (80.2)	45 (93.8)	0.04
Quantitation Errors	23 (9.4)	6 (3.1)	0.01	11 (5.0)	9 (5)	0.98	9 (6.1)	3 (2.3)	0.12	7 (4.9	10 (6.6)	0.55	10 (11.6)	2 (4.2)	0.15
False positives	7 (2.9)	0	0.02	9 (4.1)	0	0.01	3 (2.0)	1 (0.8)	0.38	6 (4.2)	1 (0.7)	0.05	5 (5.8)	0	0.09
False negatives	11 (4.5)	5 (2.6)	0.30	12 (5.5)	4 (2.2)	0.10	7 (4.7)	1 (0.8)	0.05	14 (9.9)	6 (3.9)	0.05	2 (2.3)	1 (2.1)	0.93
High false positive	1 (0.4)	0	0.38	2 (0.9)	0	0.20	0	0		2 (1.4)	0	0.14	1 (1.2)	0	0.46
Low false positive	6 (2.4)	0	0.03	7 (3.2)	0	0.02	3 (2.0)	1 (0.8)	0.38	4 (2.8)	1 (0.7)	0.15	4 (4.7)	0	0.13
High false negative	11 (4.5)	5 (2.6)	0.30	12 (5.5)	4 (2.2)	0.10	7 (4.7)	1 (0.8)	0.05	14 (9.9)	6 (3.9)	0.05	2 (2.3)	1 (2.1)	0.93
Low false negative	0	0		0	0		0	0		0	0		0	0	
Regional Score	2310	1870	<0.01	2015	1760	<0.01	1395	1295	<0.01	1240	1455	<0.01	810	470	<0.01
Total possible score	2450	1920		2180	1800		1480	1310		1420	1520		860	480	
Regional score (%)	94.3	97.4		92.4	97.8		94.3	98.9		87.3	95.7		94.2	97.9	

Table 13 continued...: Summary (Number & Percent) of TB Microscopy Proficiency Test Results by 9 Regions in KwaZulu-Natal Combining Data for the Period 2001 to 2004 and 2006

	Region											
	6			7			8			9		
	KZN	NHLS	p	KZN	NHLS	p	KZN	NHLS	p	KZN	NHLS	p
Total non returns	6	1		1	3		7	1		28	15	
Total Slides reviewed	163	136		90	40		166	128		426	272	
Correct	134 (82.2)	128 (94.1)	<0.01	63 (70.0)	29 (72.5)	0.77	139 (83.7)	118 (92.2)	0.03	351 (82.4)	264 (97.1)	<0.01
Quantitation Errors	18 (11.0)	5 (3.7)	0.02	14(15.6)	10 (25.0)	0.20	12 (7.2)	7 (5.5)	0.54	35 (8.2)	2 (0.7)	<0.01
False positives	5 (3.1))	1 (0.7)	0.15	4 (4.4)	0	0.18	5 (3.6)	2 (1.6)	0.42	16 (3.8)	1 (0.4)	0.01
False negatives	6 (3.7)	2 (1.5)	0.24	9 (10.0)	1 (2.5)	0.14	10 (6.0)	1 (0.8)	0.02	24 (5.6)	5 (1.8)	0.01
High false positive	0	1 (0.7)	0.27	0	0		0	0		5 (1.2)	0	0.07
Low false positive	5 (3.1)	0	0.04	4 (4.4)	0	0.18	5 (3.0)	2 (1.6)	0.42	11 (2.6)	1 (0.4)	0.03
High false negative	6 (3.7)	2 (1.5)	0.24	9 (10.0)	1 (2.5)	0.14	10 (6.0)	1 (0.8)	0.02	24 (5.6)	5 (1.8)	0.01
Low false negative	0	0		0	0		0	0		0	0	
Regional Score	1545	1330	<0.01	780	390	<0.01	1545	1260	<0.01	3930	2665	<0.01
Total possible score	1630	1360		900	400		1660	1280		4260	2720	
Regional score (%)	94.8	97.8		86.7	97.5		93.1	98.4		92.3	98	

None of the nine regions had overall scores equal to or above 95% for the KZN panel whereas all nine regions had overall scores above 95% for the NHLS panel (Table 13). Region 4 had low proportions of correct results i.e. 78% (n=119) and 89% (n=135) for the KZN panel and the NHLS panel respectively. Region 4 also had high proportions of false negative results for both the KZN panel and the NHLS panel. This was mainly due to the high proportion of high false negative results i.e. 10% (n=14) for the KZN panel and 4% (n=6) for the NHLS panel. This region had a low overall score of 87% in 2001-2004 but significant improvement was observed in 2006 (96%).

Region 5 had low proportion of correct results (80%) for the KZN panel. This Region also had 6% (n=5) false-positive results. The overall score for this region for the KZN panel was 94% but significant improvement (p=<0,01) was observed for the year 2006 (98%).

Region 7 had a low proportion of 'correct' results for both the KZN panel and the NHLS panel i.e. 70% (n=63) and 73% (n=29) respectively. This region also had high proportions of quantification errors, for both the KZN panel and the NHLS panel, of 16% (n=14) and 25% (n=10) respectively. Region 7 had an overall score of 87% for the KZN panel, which is well below the acceptable level of performance, but significant improvement (98%) is noted for the year 2006 (NHLS panel).

Regions 1, 2, 3, 4, 7, 8 and 9 had high proportions of high false-negative results of 5%, 6%, 5%, 10%, 10%, 6% and 6% respectively for the KZN panel.

4.2.1.14 Summary of TB microscopy proficiency testing results in KwaZulu-Natal by quarter: 2001-2004

Overall summary of results by quarter is presented in Table 14.

Laboratory performance by quarter varied from 87% (survey 5, quarter 2 of 2002) to 96% (survey 1, quarter 2 of 2001). Of the eleven quarters assessed by the KZN laboratory, the overall scores were equal to or exceeded 95% (the acceptable level of performance) only for four quarters (Fig 1).

Table 14: Summary of TB Microscopy Proficiency Testing Results in KZN by Quarter: 2001-2004

Survey	Score per survey	Total possible score per survey	Total score per survey (%)
1 Quarter 2 2001	1375	1430	96.2
3 Quarter 4 2001	1425	1530	93.1
4 Quarter 1 2002	1450	1640	88.4
5 Quarter 2 2002	1385	1590	87.1
6 Quarter 3 2002	1450	1670	86.8
7 Quarter 4 2002	1690	1780	94.9
8 Quarter 1 2003	1505	1620	92.9
9 Quarter 2 2003	1585	1650	96.1
10 Quarter 3 2003	1630	1730	94.2
11 Quarter 1 2004	1555	1620	96.0
12 Quarter 2 2004	1545	1740	88.8

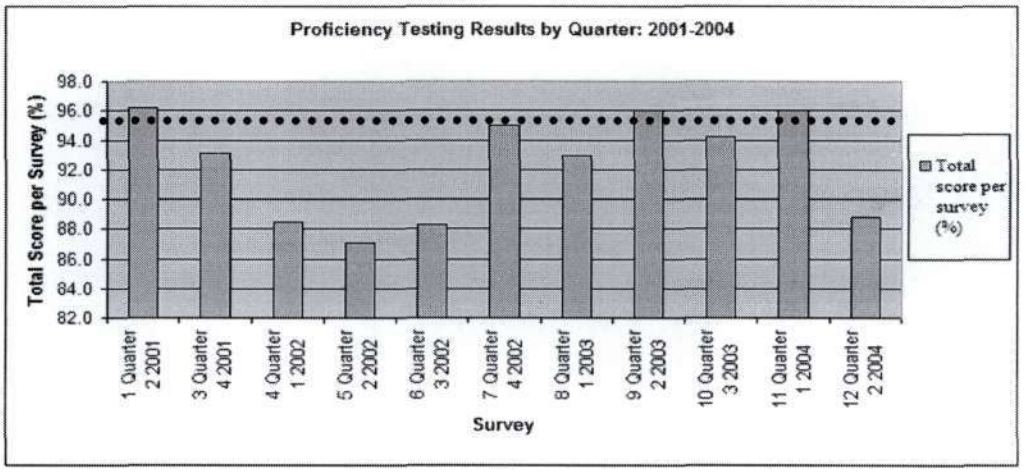

Figure 1: TB Microscopy Proficiency Testing Results in KZN by Quarter: 2001-2004

..... = 95 % LEVEL

4.2.1.15 Summary of TB microscopy proficiency testing results by year: 2001 to 2006

Overall summary of results by year is presented in Figure 2.

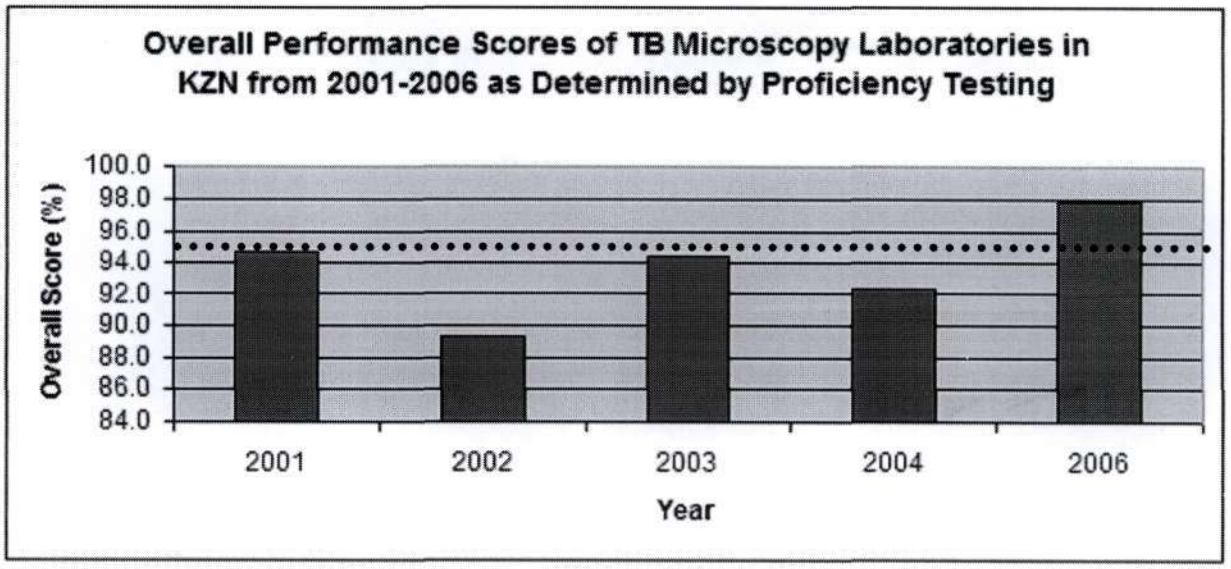

Figure 2: Overall Performance of TB Microscopy Laboratories by Year: 2001 to 2006 as Determined by Proficiency Testing.

••••• = 95 % LEVEL

The KwaZulu-Natal reference laboratory conducted proficiency testing from year 2001 to 2004. Proficiency testing in year 2005 was not conducted. The NHLS conducted proficiency testing for year 2006.

The overall scores ranged from 89% in 2002 to 98% in 2006. For the five years assessed, the overall scores have exceeded 95%, the acceptable level, only twice (years 2001 and 2006).

4.2.2 Specific objective 2: Identification of laboratories that have unacceptable levels of performance

Overall scores were used to identify laboratories with unacceptable levels of performance. Overall scores of 95% and above were deemed to be the measure of acceptable performance.

The overall scores of all laboratories ranged from 67% to 100% for the KZN panel and 88% to 100% for the NHLS panel (Table 15). Thirty-three laboratories scored 95% or more while 39 laboratories scored less than 95% on the overall scores for the KZN panel. Twelve laboratories scored 100% for the KZN panel.

Fifty-one laboratories scored 95% or more while 14 laboratories scored less than 95% on the overall scores for the NHLS panel. Thirty-nine laboratories scored 100% for the NHLS panel.

Nine laboratories scored less than 95% for both the KZN panel and the NHLS panel (Fig 3).

Table 15: Overall Performance of Laboratories (Number & Percent) for TB Microscopy Proficiency Test Results for the Period 2001 to 2004 (KZN panel) and 2006 (NHLS panel).

	KZN Panel	NHLS Panel
Number of participating laboratories	72	66
Minimum score from all participating laboratories	66.7%	87.5%
Maximum score from all participating laboratories	100.0%	100.0%
Number of laboratories with overall scores of 95% or more	33	51
Number of laboratories with overall scores of less than 95%	39	14
Number of laboratories with a score of 100%	12	39

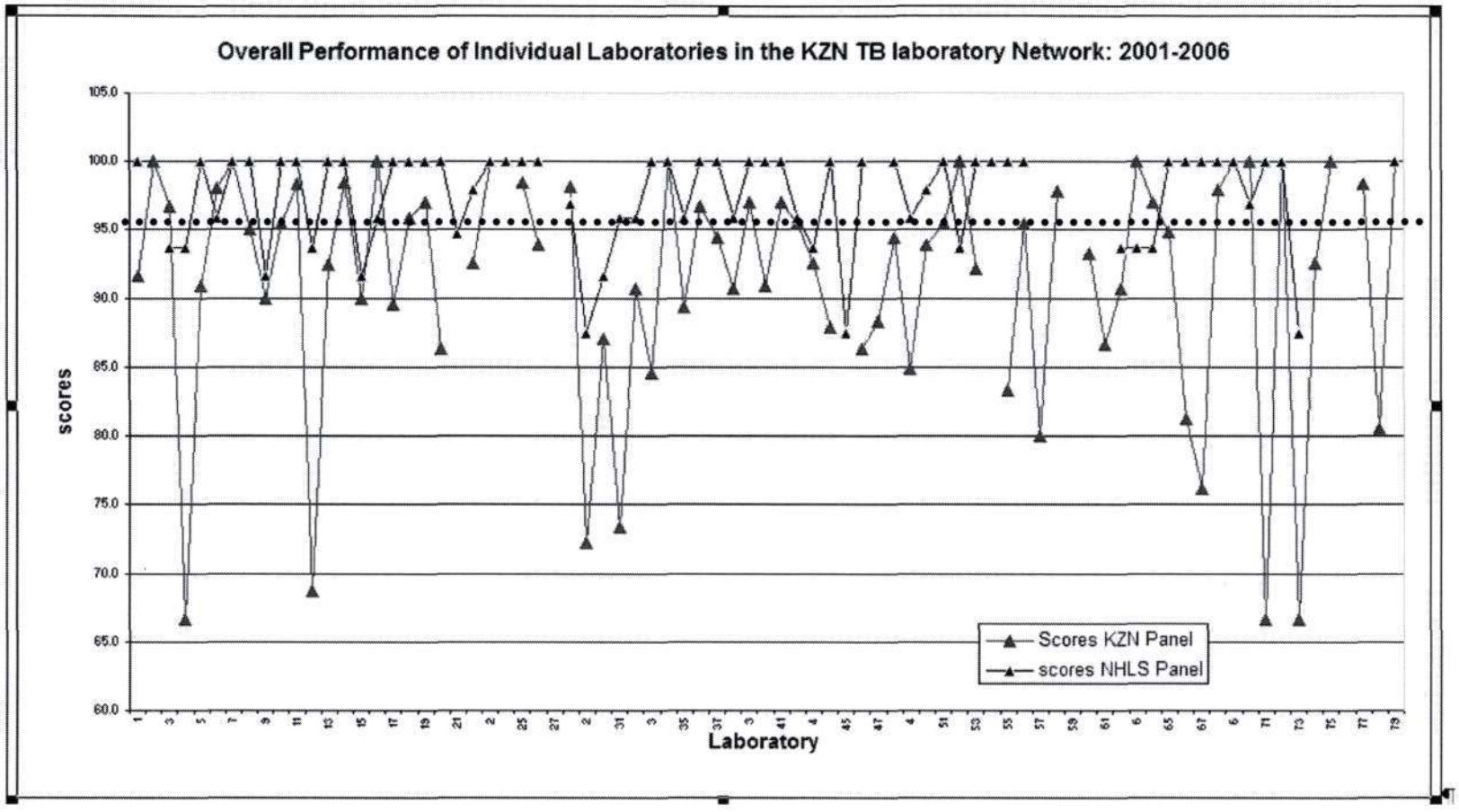

Figure 3: Overall TB Microscopy Proficiency Testing Scores (Percent) of Individual Laboratories in KwaZulu-Natal combining data for the Period 2001 to 2004 and 2006.

..... = 95 % LEVEL

Poor performers

Five laboratories scored 100% for both the KZN and the NHLS panel (Fig 3).
Thirty-two laboratories scored 100% for the 2006 panel after scoring less than 100% in 2001-2004 panels (Annexure: 10).
Of these 32 laboratories, 13 scored between 95-100% for the KZN panel. Nine laboratories scored between 90-95% for the KZN panels but improved to 100% for the NHLS panel.
Of the remaining 10 laboratories that scored 100% for the NHLS panel; 2 did not participate in previous proficiency testing exercises (KZN024 and KZN054), 6 laboratories scored between 80-90% and 2 laboratories scored below 80% (KZN067: 76% and KZN071: 67%) in the KZN panel.

Two laboratories scored 100% in the KZN panel but did not participate in the NHLS panel (KZN002 and KZN075).
Of the 4 laboratories that scored 100% for the KZN panel but less than 100% for the NHLS panel; 2 laboratories had 1 high false-negative result each and 2 had 1 low false positive result each.

Fourteen laboratories scored less than 95% for the NHLS (2006) panel (Table 16). Of these 14 laboratories, 13 recorded at least 1 high false negative result and 1 laboratory recorded 1 low false positive result. Of the 13 laboratories recording high false negative results, 5 laboratories reported 2 high false negative results each and 8 reported 1 high false negative result each. Nine laboratories had less than 95% for both the KZN and the NHLS panels.

High false negative results were the predominant error observed for the laboratories performing below acceptable standard.

Overall performance of individual laboratories

Annexure 10 illustrates the overall performance scores of individual laboratories.

Table 16: KwaZulu-Natal Public Health Laboratories Performing Below the Acceptable Level of Proficiency Performance (overall scores < 95%) for the NHLS panel (2006).

	KZN Panel (2001-2004)															NHLS Panel (2006)											
CODE	Total non returns	Total slides reviewed	Correct	Quantification Errors	LFN	LFP	HFN	HFP	False positives	False negatives	Slide broke	Not evaluated	Score	Total possible score	Final score %	Total non returns	Total Slides reviewed	Correct	Quantification Errors	False positives	False negatives	High false positive	Low false positive	High false negative	Low false negative	Score	Total possible score	Total score (%)
KZN029	8	9	6	0	1	0	1	1	1	2	0	0	65	90	72.2	1	16	14	0	0	2	0	0	2	0	140	160	87.5
KZN045	0	0	0	0	0	0	0	0	0	0	0	0	0	0		1	8	5	2	0	1	0	0	1	0	70	80	87.5
KZN073	0	3	2	0	0	0	1	0	0	1	0	0	20	30	66.7	1	16	14	0	0	2	0	0	2	0	140	160	87.5
KZN009	1	30	21	5	1	1	2	0	1	3	0	0	270	300	90.0	0	24	20	2	0	2	0	0	2	0	220	240	91.7
KZN015	1	30	26	0	0	2	1	1	3	1	0	0	270	300	90.0	0	24	21	1	0	2	0	0	2	0	220	240	91.7
KZN030	0	27	22	1	0	1	2	1	2	2	7	0	235	270	87.0	0	24	18	4	0	2	0	0	2	0	220	240	91.7
KZN003	0	30	26	3	0	0	1	0	0	1	0	0	290	300	96.7	1	16	15	0	0	1	0	0	1	0	150	160	93.8
KZN004	6	9	5	1	0	0	3	0	0	3	0	0	60	90	66.7	1	16	15	0	0	1	0	0	1	0	150	160	93.8
KZN012	2	24	14	2	1	0	6	1	1	7	0	0	165	240	68.8	0	16	14	1	0	1	0	0	1	0	150	160	93.8
KZN043	2	27	22	3	0	0	2	0	0	2	0	0	250	270	92.6	1	16	13	2	0	1	0	0	1	0	150	160	93.8
KZN052	5	18	17	1	0	0	0	0	0	0	0	0	180	180	100.0	1	8	7	0	1	0	0	1	0	0	75	80	93.8
KZN062	1	27	24	0	0	1	2	0	1	2	0	0	245	270	90.7	0	16	15	0	0	1	0	0	1	0	150	160	93.8
KZN063	1	30	30	0	0	0	0	0	0	0	0	0	300	300	100.0	1	16	15	0	0	1	0	0	1	0	150	160	93.8
KZN064	0	33	29	2	1	1	0	0	1	1	0	0	320	330	97.0	1	16	13	2	0	1	0	0	1	0	150	160	93.8

4.2.3 Specific objective 3: Comparison of proficiency testing results obtained by the KZN reference laboratory and the NHLS reference laboratory

The KwaZulu-Natal reference laboratory conducted eleven rounds of proficiency testing whereas the NHLS reference laboratory conducted only three. The total non-returns were much higher for the KZN panel (n=94) compared to the NHLS panel (n=36); see Table 9. KwaZulu-Natal laboratories performed much better in the proficiency testing programme conducted by the NHLS reference laboratory than by the KZN reference laboratory (figure 4). This is evidenced by the significantly higher proportions of 'correct' results and lower proportions of 'quantification errors', 'false positives', 'false negatives', 'high false positives', 'low false positives' and 'high false negative' results. Higher overall scores also indicate better performance on the NHLS panel (98%) than on the KZN panel (93%).

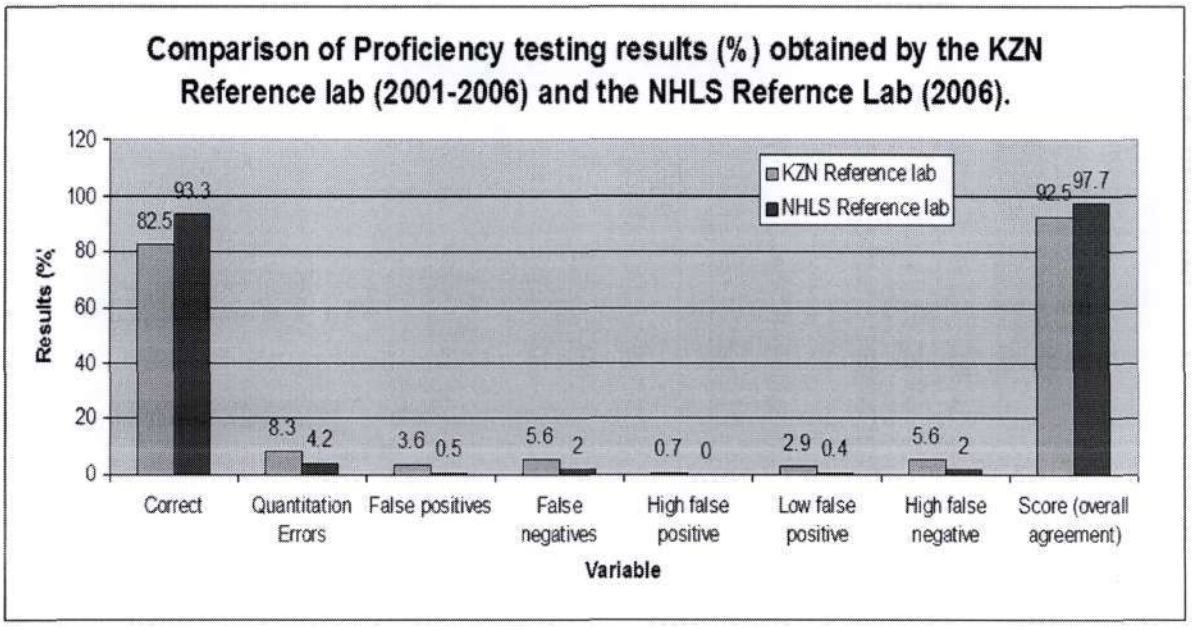

Figure 4: Comparison of Proficiency Testing results obtained by the KZN Reference Laboratory (2001-2004) and the NHLS Reference Laboratory (2006)

Comparison of overall performance

The better overall performance in the NHLS panel is also indicated by the much higher proportion of laboratories scoring over 95% for the NHLS panel (77%) than the KZN panel (46%). The maximum score reported for both panels were the same (100%). The minimum score reported for the KZN panel was 68% whereas the minimum score reported for the NHLS panel was 88% (figure 5).

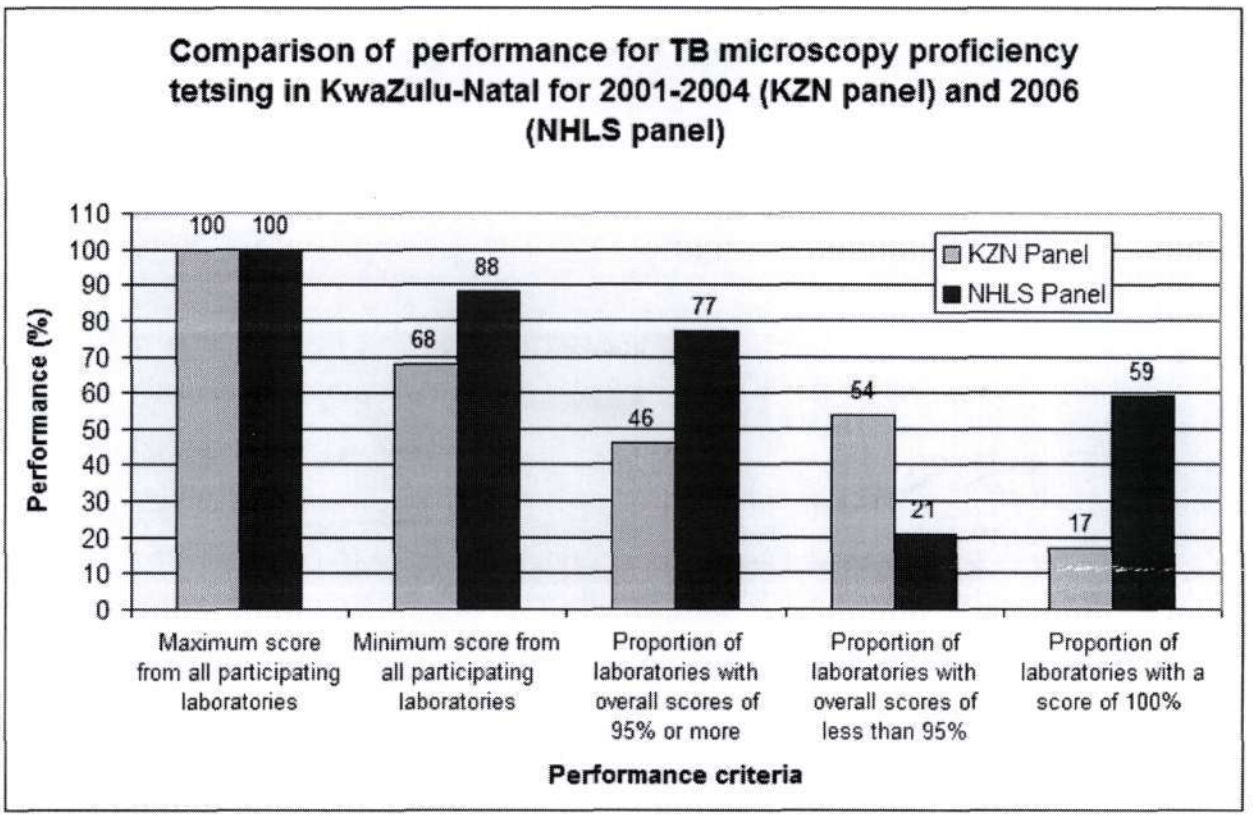

Figure 5: Comparison of performance for TB microscopy proficiency testing in KwaZulu-Natal for 2001-2004 (KZN panel) and 2008 (NHLS panel)

4.3 Qualitative analysis

4.3.1 Specific objective 4: Determination of the role laboratory workers and managers think proficiency testing plays as a quality assessment technique.

For this component of the study a standardised questionnaire was used to interview key informants telephonically. All respondents were involved with TB proficiency testing. This component of the study was mainly to ascertain the following:

- Knowledge, attitudes, and practices regarding proficiency testing.
- The perception of laboratory staff towards proficiency testing.
- Problems experienced with proficiency testing.
- Identification of possible solutions to problems highlighted

For this component of the assessment, the key finding that emanated from the discussions was the following:

3.3.1.1 Knowledge Attitudes and practices of laboratory staff towards proficiency testing

All responders agreed that proficiency testing was a worthwhile exercise. Several reasons for saying that it was a worthwhile exercise were noted. One laboratory supervisor mentioned that 'faults such as not filtering stains, not observing expiry dates, debris, not recognising the differences between artefacts and TB bacilli were picked-up. Microscopists were kept on their toes knowing that they were being tested'. Another laboratory manager felt that it was a good exercise to see how laboratories performed and how good the programme was in the province. Proficiency testing was also used to test the technologists'/technicians' degree of accuracy. One microscopist felt that it was a good exercise 'to get practice, exercise and work with confidence'.

4.3.1.2 Perception of laboratory staff towards proficiency testing

Quality assurance/Quality control programmes in laboratories

All respondents except one agreed that standard operating procedures were available in all TB laboratories. All responders except one mentioned that quality assurance/quality control was performed regularly in their laboratory.

Most responders (80%) indicated that proficiency testing slides were processed by the same people processing routine TB specimens. However, one laboratory encourages all microscopists to read proficiency testing slides and record their results. The proficiency testing slides are then checked by a senior medical technologist. It can be assumed from this that the results were discussed or even debated before they were sent back to the reference laboratory. The advantage of this is that all microscopists were given the opportunity to read the slides and was mentored. In reality routine patient specimens are not treated in such a manner and patients do not have the luxury of having their sputum examined by several microscopists. The disadvantage is that the proficiency testing results sent to the reference laboratory would be biased.

One respondent mentioned that proficiency testing slides were not always processed by the same person performing TB microscopy routinely. He felt that there was a perception among laboratory staff that proficiency testing was a punitive measure and that if someone read slides incorrectly then they would be punished. Therefore a small number may have treated proficiency testing slides in a 'special way'.

All respondents except one felt that proficiency testing was a valuable exercise and not a waste of time. One microscopists at facility level mentioned that she felt that proficiency testing was 'good practice and not waste of time, it gives us an idea of how we are working and it encourages us to read slides properly; I like QC'. One laboratory manager mentioned that 'it is very valuable and ensures that correct, reliable and accurate results are released'.

One respondent felt that blinded re-reading would be more valuable than proficiency testing. He felt that people knew that they were being tested and therefore asked friends to assist or spent more time reading proficiency testing slides.

Processing of proficiency testing slides

Five respondents agreed to some extent that some laboratory managers get the best person to process proficiency testing slides or even process it themselves. However this was not a widespread phenomenon as one laboratory manager mentioned 'this could be true but not in most labs'. This would also depend on the size of the laboratory as some laboratories have only one staff. Proficiency testing results would be biased in laboratories where laboratory mangers punish staff for producing poor results. In these laboratories staff would be pressured to produce correct results and therefore would resort to seeking assistance when reading slides.

Six (60%) of the respondents felt that proficiency testing was effective enough to detect errors in microscopy technique. One respondent felt that it was effective to a certain extent, while another felt that it was effective provided that slides were read in duplicate. This might be to confirm results of the first reading by the second reader. However, this would also bias the proficiency testing results.

Two respondents felt that proficiency testing was not effective enough to detect errors in microscopy technique. One respondent mentioned 'they don't tell us anything about over-decolourising or staining. Hence we introduced rechecking to supplement proficiency testing. Proficiency testing is the minimum requirement by WHO'. It is a concern that some laboratory staff did not regard the proficiency testing exercise as effective enough. However, it is encouraging to note that another method of quality assurance (slide re-checking) was explored. Blinded rechecking is considered the best method for evaluating performance and providing motivation to staff for improvement.[11]

The time spent reading slides varies between laboratories and depend, to a large extent, on workload of the laboratory and on the individual. Five respondents mentioned that about five minutes are spent on reading each slide, one respondent mentioned 5- 10 minutes and another mentioned about 30 seconds for positive slides. Three respondents were doubtful and implied that much less than the recommended five minutes are spent reading each slide due to the high workload. One microscopist mentioned that she would seek assistance when she encountered problems reading slides. Two respondents expressed that low positives are missed when microscopists do not spend adequate time reading slides.

Feedback

Respondents indicated that feedback was inconsistent from both the KwaZulu-Natal reference laboratory as well as the National Health Laboratory Service reference laboratory. This could be due to several reasons. Some of the reasons mentioned were: proficiency testing results could be going to unit business managers and was not filtering through to the microscopists, communication between reference laboratories and the peripheral laboratories were poor and all TB coordinators except for one was removed from the programme.

Standard operating procedures

Nine (90%) of the respondents were convinced that standard operating procedures were available in the, laboratories. However one respondent felt that standard operating procedures might not be available in all smaller laboratories in KwaZulu-Natal, whereas all National Health Laboratory Service laboratories had standard operating procedures.

Training

Many respondents (60%) felt that microscopists are adequately trained but many (40%) of them also expressed doubt. The level of training depended on whether microscopists attended a formal training course or whether they were trained at a laboratory. There is a perception that medical technologists and microscopists that attended a formal training

course was better trained than microscopists that trained at a laboratory with a high workload.

Training was provided in 2006 for TB microscopists in all districts. In addition 46 new TB microscopists were employed in almost all districts in the province. Other categories of staff were also employed in the laboratories.

Problems experienced with proficiency testing.

Quality assurance/Quality control programmes in laboratories

- Some laboratory managers get the best people to process proficiency testing slides or even process it themselves
- Proficiency testing does not assess quality of stains or the staining process

Processing of proficiency testing slides

- Some laboratory managers punish staff for producing substandard proficiency testing results.
- High workload prevented people from spending the recommended amount of time on each slide.
- Low positives are missed when less than the recommended amount of time is spent on reading slides. However, failure to correctly diagnose low positives (low false negative results) may be due to other causes as well (see table 17).

Feedback

- Feedback from the reference laboratories was inconsistent
- Poor communication between the reference laboratory and the peripheral laboratory.
- Poor communication between the laboratory management and staff.

Standard operating procedures

- Standard operating procedures may not be available in all laboratories

Training

- There was doubt as to whether all TB microscopists were adequately trained.
- Refresher training was conducted in 2006 in all districts.

4.3.1.4 Identification of possible solutions to problems highlighted

Respondents highlighted numerous means by which the quality assurance system could be improved. The most frequently mentioned measures to improve the quality assurance system were:

Quality assurance/Quality control programmes in laboratories

- Introduce a blinded rechecking programme
- When reviewing slides at the reference laboratory assess slide preparation in terms of adequacy of material, quality of staining etc. The slide processing technique can be assessed in detail during the support visits using a standardised checklist.

Processing of proficiency testing slides

- Improve communication between laboratory managers and staff to clear misperceptions regarding proficiency testing and reinforce the aim of the proficiency testing programme.
- Review workload of microscopists

Feedback

- Improve feedback from the reference laboratory through regular and timeous return of proficiency testing results.
- Consider the re-instatement of TB coordinators to improve feedback and provide support visits to peripheral laboratories.

Standard operating procedures

Reference laboratories should ensure that the relevant standard operating procedures are displayed in strategic points in the laboratory for easy reference and application. This would improve adherence to standard operating procedures and the TB diagnostic technique.

Training

- Improve staff training and address the issue of staff shortage

4.3 Specific objective 5: To make recommendations to decision makers on the key gaps identified from the information obtained in this study.

Recommendations to decision makers on the key gaps identified from the data analysis in this study is addressed under chapter 6, recommendations and conclusion.

4.4 SUMMARY

The overall performance of participating laboratories and the province was significantly higher in 2006 than in 2001-2004 (p=<0.01).

All three levels of health care facilities performed below the acceptable level of performance during 2001-2004. Significant improvement (p=<0.001) was observed for all levels of health care facilities in 2006 with the primary health care level facilities and the district level facilities exceeding 95% in overall scores. The tertiary level facilities failed to achieve overall scores of 95% or more (94%).

Urban laboratories achieved higher overall scores that rural laboratories for the period 2001-2004. However, both urban and rural laboratories failed to achieve the acceptable overall score of 95%. There was a significant improvement (p=<0.01) in 2006 with both urban and rural laboratories producing overall scores in excess of 95%.

None of the nine regions had overall scores equal to or above 95% for the period 2001-2004 whereas all nine regions had overall scores above 95% in 2006.

Laboratory performance by quarter varied from 87% to 96%. Of the eleven quarters assessed during 2001-2004, the overall scores were equal to or exceeded 95% only for four quarters. The overall scores by year ranged from 89% in 2002 to 98% in 2006. For the five years assessed, the overall scores have exceeded 95% only twice (years 2001 and 2006).

All respondents agreed that proficiency testing was a worthwhile exercise as it enabled the detection of 'faults' and also tested the technical ability of technologists/technicians. However, the process can be flawed when the correct procedure is not followed. Therefore, acceptance of the proficiency testing programme by laboratory staff is imperative for success of the programme.

5 CHAPTER 5: DISCUSSION

5.1 INTRODUCTION

Results of the study were presented in chapter 4. In this chapter the results that were presented in chapter 4 are discussed. The discussion is presented according to each specific objective of the study with recommendations reported in chapter 6.

5.2 Quantitative analysis

5.2.1 Specific objective 1: Analysis of proficiency testing results (2001–2006)

Correct results

There was significant improvement (p=<0.01) in the reading of proficiency testing slides classified as 'correct' and those classified as 'quantification error' in 2006 as compared to 2001-2004.

Since quantification errors generally have no impact on the scoring system, these results were combined with the results classified as 'correct' to observe the change in the results and its implication on the overall score (performance).

Combination of 'correct' results and 'quantification errors' gave a value of 91% (83% + 8%) and 97% (93% + 4%) for 2001-2004 and 2006 respectively. The level of 91% for the period 2001-2004 indicates sub-standard laboratory performance for the province. The value of 97% in 2006 indicates laboratory performance above the minimum acceptable level (95%) for the province and also indicates that 97% of reported AFB results are reliable.

Low false negatives

Low Positive is the term used in this document to describe 1-9 acid-fast bacilli per 100 fields. These results are reported to the physician as the exact number of AFB observed. It remains for the physician and the National TB Control Programme to decide if this represents a case or not. Failure of the microscopist to detect any acid-fast bacilli on low positive slides would give rise to a low false negative result.

Low False Negative (LFN) is a minor error that occurs occasionally even in laboratories that are performing well. However, minor errors would require further evaluation if they exceed the average number seen in all TB microscopy centres in the province or if the number of minor errors over time demonstrates a trend.

Low false negative results may be an indication that microscopists are unable to detect low AFB counts. This could be due to microscopists not reading all fields, or due to the high workload. To detect acid-fast bacilli on light microscopy, 5000-10000 bacilli/ml must be present in sputum while the infecting dose of *Mycobacterium tuberculosis* is estimated to be less than 10 organisms.[7] Patients with paucibacillary tuberculosis have very few AFB in their sputum although they may still be infectious. Therefore, it is important that microscopists are technically skilled to read low positive slides correctly. Failure of the laboratory to detect low positive slides would result in delayed treatment, prolonged transmission and unnecessary patient suffering.

Programmatic use of 'low positives'

Van Deun *et al.* concluded from a study that scanty results (IUATLD/WHO scale, <10/100 high power fields) are not rare and should not be ignored. [37]They proposed that adoption of a considerably lower positivity threshold would significantly increase the sensitivity of the test, and consequently the number of smear-positive cases detected, without losing much in specificity. This would be appropriate in control programmes where basic

conditions for reliable AFB microscopy, including regular quality assessment, are present. [37, 38]

In view of the fact that KwaZulu-Natal is an endemic area for TB with a very high incidence rate, 'low positive' results are often evaluated in light of the patient's clinical presentation. Ideally a repeat specimen should be requested for microscopy and culture, but for practical reasons patients are started on TB treatment if clinically suspected of tuberculosis. Some clinicians fear that patients may not return for additional diagnostic tests or fear the spread of TB in the community. Therefore, patients clinically suspected of tuberculosis with a 'low positive' AFB result are started on treatment empirically, while additional diagnostic tests are conducted. A TB microscopy result of 'low positive' is therefore seen as significant in the high endemic province of KwaZulu-Natal. Two microbiologists consulted, confirmed the above and added that 'low positive' slides should be included as part of a proficiency testing panel of slides.

Since only two 'low positive' slides were included in this study the error of 'low false negatives' could not be well assessed. Low positive slides should be included in future rounds provided that the proficiency testing slides are well prepared and assessed before distribution to peripheral laboratories and assessed again in the reference laboratory if discrepant results were received.

Low False Positives

This type of minor error occurs occasionally even in laboratories that are performing well. However, minor errors would require further evaluation if they exceed the average number seen in all TB microscopy centres in the province or if the number of minor errors over time demonstrates a trend. [11] In KwaZulu-Natal, the average number of low false positive results per laboratory was 1. Therefore, all laboratories reporting more than 1 low false positive result should be investigated.

Major Errors

A major error is considered the most critical since it has the highest potential impact on patient management. A major error results in an incorrect diagnosis of tuberculosis, improper management of a patient, prolonged transmission of tuberculosis and patient suffering. Major errors may indicate gross technical deficiencies, and include both high false positive and high false negative errors. Any major error would indicate unacceptable performance and should trigger an evaluation and corrective action.

High False Negative (HFN)

High false negative results would result in delayed/non treatment of patients, further community spread and patient suffering. Therefore, provided that the proficiency testing slides are well prepared, any high false negative results are unacceptable and should trigger an evaluation and corrective action.

High false negative results may be an indication that the microscopists are overworked and additional staff may be needed to resolve the problem. High false negative results may also be due to technical problems such as poor stains, insufficient staining time or heating, microscopes in poor condition or inadequate training. High numbers of high false negative results may indicate gross neglect and an overall lack of motivation.

Although there was a significant reduction ($p<0.01$) in high false negative results in 2006 (2.0%) as compared to period 2001-2004 (6%), high false negative results are still being reported. Therefore, all laboratories that produced high false negative results should be evaluated to determine its contributing factors.

High False Positive (HFP)

High false positives result in people being treated for tuberculosis when they do not have the disease. Any high false positive result is an indication of a problem and should warrant prompt investigation into its cause. An isolated high false positive may be due to a clerical error at the peripheral laboratory. More frequent high false positive results may be due to microscopes in poor condition, untrained or inexperienced microscopists or neglect. [11]

There was a reduction in reporting of high false positive results. The proportion of high false positive results for the period 2001-2004 (KZN panel) was 1% (n=11) and for 2006 was 0%[37] (n=1). The reporting of high false positive results are not a problem in KwaZulu-Natal public health laboratories as indicated by the 2006 figures (n=1).

Sensitivity, Specificity, Positive predictive value, Negative predictive value

There was significant improvement (p=<0.01) in sensitivity, specificity, positive predictive value, and negative predictive value from 2001-2004 to 2006 (using the reference laboratory as comparator). Sensitivity, specificity, positive predictive value, and negative predictive value improved from 91%, 91%, 94% and 86% respectively in 2001-2004 to 97%, 97%, 99% and 92% respectively in 2006. All these measurements, except negative predictive value, reached the target value[38]. The main indicators are sensitivity and specificity. The sensitivity and specificity was above the target level in 2006 indicating that the laboratory diagnosis of positive and negative slides are acceptable. The sensitivity and specificity found in this study is similar to a study reported in Malawi.[39]

Summary of results by level of health care facility

Both primary health care level and district health care level had similar overall scores for the 2001-2004 and the 2006 proficiency testing exercises. Therefore, the quality of TB microscopy services in these two levels of health care facilities are similar. The performance of these 2 health care levels are satisfactory.

Tertiary level health care facilities had overall scores below the acceptable level of performance for both the 2001-2004 and the 2006 proficiency testing exercises. In this study tertiary level health care facilities comprised of two laboratories at tertiary level hospitals (KZN002 and KZN004). It should be noted that these two laboratories processed

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[37] Although the proportion of high false positive results is zero, the actual number of high false positive results was one.

[38] Sensitivity – 95%, specificity – 95%, positive predictive value – 95%, negative predictive value – 95%

much fewer slides than most other laboratories. The first laboratory (KZN002) scored 100% for the KZN panel (3 slides reviewed) but did not participate in the NHLS proficiency testing programme. The second laboratory (KZN004) scored 80% for the KZN panel (8 slides reviewed) and 94% for the NHLS panel (16 slides reviewed). This laboratory had 3 high false negative results for the KZN panel and 1 high false negative result for the NHLS panel. It appears from this study that this laboratory is producing high false negative results.

Even though the number of slides processed by the tertiary level laboratories was considerably low, an assessment of the results could still be made since all the errors recorded were high false negative results. For the tertiary level laboratories, 3 high false negative results contributed to the substandard performance for the period 2001-2004, whereas 1 high false negative result contributed to the substandard performance for 2006. Since tertiary level laboratories were situated in academic institutions it was expected that this level of laboratories would have better access to academic activities and would be better equipped and therefore should produce better proficiency testing results. However, in this study other factors need to be considered as well, such as;

- Staff turnover:

  This level of laboratory has reported loss of experienced staff on a regular basis. Experienced staff is often replaced by less experienced staff.

- Workload:

  workload was reported to be very high in tertiary level laboratories with TB microscopists examining more that 50-60 slides per day (personal communication).

- Staff motivation

  Staff motivation was reported to be low as evidenced by high absenteeism. It was also reported from both tertiary level laboratories that slides obtained from the KZN reference laboratory were of a very poor quality, 'staff battled to read slides as there were no background material and inoculums were not marked'. Therefore the entire slide had to be viewed. It was reported that senior medical technologists had also examined some of these slides and did not observe any AFB, but feedback from the

reference laboratory indicated that the slides were AFB positive. The perception that proficiency testing slides were not of high quality was evident. A resultant negative attitude towards proficiency testing was also evident and could have influenced the laboratory's motivation in conducting or continuing the proficiency testing exercise. This would also provide a possible the reason for the high number of non-returns recorded for the tertiary level laboratories (i.e. 38.9% during 2001-2004 and 15.8% for 2006)

The above factors were discussed and confirmed with tertiary level staff and are likely to have influenced the proficiency test scores and resulted in the substandard performance during 2001-2004. In 2006, since only one tertiary level laboratory participated in the proficiency testing exercise, the results should be interpreted with caution. The laboratory (KZN 004) only recorded one error (high false negative) out of 16 slides read. Both tertiary level laboratories are encouraged to participate in all future rounds of proficiency testing. All future results should be carefully analysed and monitored to establish the true performance and trend and to identify and remove any obstacles.

Summary of results by urban and rural facilities

Both urban and rural laboratories scored below the acceptable level of performance (94% and 92% respectively) during 2001-2004. However, significant improvement ($p=<0.01$) was observed in both the urban and rural laboratories (97% and 98% respectively) in 2006. As of 2006 both these categories of laboratories are producing similar results, which are within the acceptable levels of performance.

Summary of results by region

All regions performed below the acceptable level of performance (overall scores <95%) during 2001-2004 (87%-94%). However, significant improvement ($p=<0.01$) was observed in 2006 where overall scores ranged from 96% to 99% indicating satisfactory performance at regional levels.

Summary of results by quarter

Overall laboratory performance varied during the quarters of the year assessed (2001-2004). Since only a single proficiency testing exercise was conducted per quarter, each proficiency testing exercise also represents the quarters of the year. A possible explanation for the inconsistent overall performances could be attributed to the composition of the proficiency test panel of slides. The proficiency testing panel consisted of random numbers of negative, low positive, 1+, 2+ and 3+ slides. Another study conducted in Mexico, published in 2003, showed that the number of low positive slides in the panel was the factor most closely associated with proficiency testing results and therefore influenced the laboratory performance. Although low positive slides were removed from the study, the variation in the composition of the slide panels is likely to have contributed to the variation in the overall performance of the laboratories per quarter. This would therefore stress the importance of following approved guidelines when selecting proficiency testing slide panels. Other factors such as workload, poor reagents and equipment, staff training and motivation could have contributed to the inconsistent performance as well.

Trend of overall performance by year

Trend analysis using overall proficiency testing performance is important to monitor the diagnostic capability of the TB microscopy laboratories. The study revealed that the annual overall performance of TB microscopy laboratories in KwaZulu-Natal was inconsistent with fluctuations ranging from 89% in 2002 to 98% in 2006. The substandard overall performance for years 2002, 2003 and 2004 cannot be attributed to the composition of the proficiency testing slide panel alone and other factors could have played a part, such as: workload, poor reagents and equipment, staff training and motivation.

The study indicates significant improvement in overall performance from 93% in 2001-2004 to 98% in 2006. The majority of errors experienced in this study were high false negative results although low false positive results were experienced to a lesser extent. Major errors such as high false negatives should not occur. This is a concern as detection of these errors in a proficiency testing exercise (where people are aware that they are being tested) indicates a much more serious situation in TB diagnostic laboratories.

Overall laboratory performance should be monitored closely in future rounds of proficiency testing to ensure that the current level of performance (2006) does not decline.

5.2.2 Specific objective 2: Identification of laboratories that sustained an unacceptable level of performance

For the period 2001-2004, 39 laboratories performed below the accepted level of performance. In 2006, 14 laboratories fell into this category. Of these 14 laboratories, 13 had high false negative results i.e. 8 laboratories had 1 high false negative result and 5 laboratories had 2 high false negative results. The fourteenth laboratory in this category had only 1 low false positive result and an overall score of 94%. This laboratory only processed 8 proficiency testing slides therefore the effect of only 1 low false positive reading, resulted in this laboratory being categorised as a laboratory performing below the acceptable level of proficiency.

The proficiency testing conducted in 2006 should be focussed on, as this is the most recent period of assessment and should reflect the current situation. Therefore to improve performance of laboratories producing substandard performance, high false negative results should be eliminated (refer to table 17 for possible causes of high false negative results and suggested steps for investigation).

5.2.3 Specific objective 3: Comparison of proficiency testing results obtained by the KwaZulu-Natal reference laboratory and the National Health Laboratory Service reference laboratory.

The quality of smear microscopy as determined by the NHLS data set (overall performance 98%) is far superior to the quality as determined by the KZN data set (overall performance (93%).

Some of the likely reasons are:

- Improvement in the technical skills of the microscopists as a result of the proficiency testing programme. This was also observed in a study conducted in Malawi. [39]
- Operational conditions[39] could have improved since the KwaZulu-Natal reference laboratory last conducted proficiency testing. The lower number of 'non-returns'[40], 'broken slides'[41] and 'slides not evaluated[42]' in 2006 is an indication of improved communication and a better transport system.
- Quality of proficiency testing slides produced by the KwaZulu-Natal reference laboratory was not as good as the NHLS reference laboratory. This is evident from the higher proportion of quantification errors reported for the KZN panel (8%) as compared to the NHLS panel (4%).
- Low positive slides were included in the KZN data set but not in the NHLS data set. However, not much difference was discernible when the low positives were removed from the KZN data set.
- Bias of reporting to NHLS and fear of producing inferior proficiency testing results. It would be important to assess the sustainability of the improvement in the coming rounds. Implementation of onsite supervisory visits and using a standardised

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[39] Improved staff training, better equipment and reagents and improved workload.

[40] Proficiency testing slides and results not sent back to the reference laboratory

[41] Slides that were broken during transport or during the slide processing procedure (staining, reading etc.)

[42] Slides that were not evaluated due to laboratories not sending results for all slides in the panel.

checklist to determine problems and implementing corrective measures would contribute greatly towards keeping laboratory staff motivated and sustaining good performance.

Although quantification errors do not influence case management of patients, they do, however, distinguish the good technician from the very good technician (producing fewer quantification errors). Many more quantification errors were observed for the KZN data set as compared to the NHLS data set. This could be an indication of the quality of slides prepared by the KwaZulu-Natal reference laboratory. A high number of quantification errors indicates that the number of AFB on the slides was not consistent among slides with the same grading.

Prior 2006 the workload in the laboratory was very high (some microscopists were examining 60-80 slides per day) and laboratory staff were under tremendous pressure (personal communication). In 2006, training workshops were held and a total of 46 microscopists were employed in almost all districts in the province. Other categories of laboratory staff were also employed during this period. The province attributes the improvement in proficiency testing to the training conducted in 2006 and additional staff employed in TB microscopy laboratories.

In view of the quantitative results, poor slide preparation at the KZN reference laboratory would have had a more pronounced effect on the overall scores. The fact that 46% of the laboratories scored 95% and above with 14% scoring 100%, points to other factors as contributing to the poor performance. In addition overall performance exceeded 93% for 7 of the 11 quarters assessed by the KZN reference laboratory.

Qualitative results indicated that prior to 2006, workload in the laboratories were high and training, communication and feedback was poor. Attitude of laboratory staff towards proficiency testing also points to factors contributing to substandard performance. Activities in 2006 that could have improved laboratory performance are:

- Provision of training in all districts
- Employment of additional microscopists and other staff in TB laboratories
- NHLS becoming the administrator of the KwaZulu-Natal laboratory services. Proficiency testing conducted by the NHLS in 2006 could have been perceived as an assessment by the new employer, therefore employees would have felt the urge to perform well. The lower number of non-returns, broken slides and slides classified as not evaluated indicates that the NHLS was more efficient in conducting the proficiency testing exercise.

The above factors therefore provides the reasons for the improvement in smear microscopy in 2006.

5.3 Qualitative analysis

5.3.1 Specific objective 4: Perception of laboratory workers and managers towards proficiency testing as a quality assurance technique.

Knowledge, attitudes and perceptions of laboratory staff towards proficiency testing

The key informants had a good understanding of the basic theoretical aspects of proficiency testing as a quality assurance technique.

The value of an external proficiency testing programme depends largely on the attitudes and perceptions of laboratory staff towards the programme. Laboratory personnel who regard proficiency testing as a valuable exercise to assess quality and as a means to identify problems within the laboratory will take the programme seriously and ensure success of the programme. Taking the proficiency testing programme seriously would mean adhering to all the principles of the programme and following the instructions/recommendations accompanying the proficiency testing slides. Some of these principle/instructions include; treating the proficiency testing slides as routine patient slides and not award them 'special treatment', the slides should be read by the same person reading routine patient slides and proficiency testing results and slides should be sent back to the reference laboratory within the specified time.

If more than one technician performs AFB microscopy in the laboratory, the proficiency testing slides should be examined by the microscopist conducting smear microscopy for the day. The results should be entered on the form and signed by the same technician before forwarding it to the reference laboratory. This would allow discrepancies in slide reading to be followed-up with the microscopist concerned and corrective action can be taken. Results should not be discussed with anyone before entry onto forms and sending to the reference laboratory. This would ensure that results of the proficiency testing slides truly reflect the quality of results produced for routine patient slides. In this way, errors or deficiencies in the laboratory would be identified and corrective action can be instituted.

Those who consider proficiency testing as an additional workload will not take the proficiency testing exercise seriously. Some of these staff would consult other colleagues for assistance or discuss results before sending them to the reference laboratory. Some staff may spend additional time reading proficiency testing slides for the fear of sending incorrect results to the reference laboratory.

Laboratory managers also contribute to the success or failure of the proficiency testing programme. Such individuals contribute to the success of the programme by ensuring that all the principles and instructions of the programme are followed meticulously. They also sometimes contribute to the failure of the programme by assigning staff other than those involved with routine patient samples to process proficiency testing slides or even process them himself/herself. The quality of results produced in this way is falsely elevated, clouding the true situation in the laboratory. Opportunities for addressing problems and for quality improvement would be missed. Incorrect results in reading patient slides and all the consequences that follow (e.g. TB patients are not treated or patients are treated for TB when they do not have the disease) will continue.

The general feeling among laboratory personnel is that proficiency testing is an essential exercise to ensure correct, reliable and accurate results. However, there is a small number of staff that may give proficiency testing slides more attention than they would give routine

patient slides. There was a perception among some people that proficiency testing was a punitive measure and that if they read proficiency testing slides incorrectly then they would be punished.

Technicians reading proficiency testing slides know that they are being tested. Key informants have indicated that technicians allocate more time to reading proficiency testing slides than they do reading routine patient slides. This is not widespread but they do bias/influence the proficiency testing results. These challenges were also observed in a study conducted in Chennai, India where it was observed that identical results in 3 laboratories indicated that microscopists did not conduct the proficiency testing smear examinations independently. [20] Other drawbacks observed in the study was that microscopists had unlimited time for slide examination and were aware of being tested.

Communication is very important for a successful proficiency testing programme. It is important to consider the communication between the reference laboratory and the laboratory being assessed as well as the communication between the laboratory manager and laboratory staff.
Key informants indicated that proficiency testing results are influenced by laboratory managers. When laboratory managers are too strict and punish staff for performing poorly in a proficiency testing exercise, staff would be tempted to 'cheat' to escape punishment. Therefore, it is essential that laboratory managers explain to staff the purpose of proficiency testing and the importance of 'honest' results. Indifference to poor laboratory performance in quality assurance programmes will perpetuate poor performance as was observed in the Indian study. [20]

Laboratory managers themselves must bear in mind the importance of following the procedures of proficiency testing strictly. They must ensure that the appropriate person processes the proficiency testing slides in the same manner, as they would process routine patient slides. They must also use the proficiency testing results to implement the necessary changes for quality improvement.

Laboratory managers must create an environment where staff are not afraid to release 'honest' proficiency testing results. Staff must know that quality assurance is not a punitive measure to identify and punish workers that produce incorrect results. Good communication between laboratory managers and staff is crucial to eliminating misperceptions of proficiency testing.

The reference laboratory should consider monitoring the practices in the laboratory, regarding proficiency testing, through regular surveys, key informant interviews and during support visits.

Workload and Processing of proficiency testing slides

Quality of sputum smear examination also depends on the workload of the laboratory. Acid-fast microscopy becomes tedious when large numbers of slides have to be examined. There may be a loss of technical accuracy if large numbers of routine patient slides and proficiency testing slides are processed together on one day. Hence, there remains a need to obtain the smallest sample size and to specify the maximum number of slides to be processed each day.

The number of slides in a proficiency testing panel should be sufficient to validate the exercise as a quality assessment indicator and yet not add an unnecessary burden to the workload of the technician in the laboratory being evaluated. [11]Laboratories with a low turnover of specimens (<500 slides annually) may have difficulty in maintaining a high quality of standard. [40]. This study did not assess workload but several key informants indicated that microscopists are under pressure to complete their daily tasks and therefore cannot afford to spend the recommended amount of time reading/examining each slide. Key informants reported that some microscopists examine 60-80 slides per day.

Wilkinson *et al*, discussed in his study that a single positive smear is diagnostic of tuberculosis when accompanied by an abnormal chest X-ray. [41] Most patients (84%) in

the study were TB positive on the first smear and almost all were positive after 2 smears. It was felt that further testing of specimens from patients who have already produced a single positive smear might increase laboratory workload unnecessarily. A study conducted in Vietnam estimated that 186 smears needed to be examined to detect one additional TB case by a third smear examination. [42] As the third specimen has limited impact on case finding omitting it will reduce the workload of the laboratory. However, a better laboratory performance cannot be guaranteed in the absence of a quality assurance programme that includes specimen evaluation, staining, assessment of equipment etc. The advantages should thus be carefully weighed against the disadvantage of detecting fewer cases. In South Africa, at least two sputum samples are collected for the bacteriological diagnosis of TB. [6]

Feedback

Similar to a study conducted in the Democratic Republic of Congo, key informant interviews indicated that feedback from reference laboratories to peripheral laboratories was inconsistent.[22] People who are expected to perform additional duties like processing proficiency testing slides should see the benefits of their labour. They should see that proficiency testing is used in a positive way to improve staff skills, motivate for more staff or equipment etc. Therefore, timely feedback to laboratory staff would be motivating and would contribute to the success of the programme. Studies conducted in Uganda and the Democratic Republic of Congo found feedback and on-site assessments to be a valuable tool in quality assurance and was key in motivating laboratory technicians. [22, 43]

Standard operating procedures

Standard operating procedures are present in most laboratories however; it is likely that a few laboratories still do not possess them. The reference laboratory should ensure that all laboratories have the essential guidelines and protocols available.

Training

Not all microscopists in the province are adequately trained and there is a need for refresher courses on TB microscopy diagnosis. This is in keeping with what studies conducted in Mexico and the Democratic Republic of Congo reported, which showed improvement in laboratory performance following training.[22, 31] All microscopes should be examined and all faulty ones should be either repaired or replaced immediately. Training cannot compensate for poorly functioning microscopes. As observed in the study conducted in Democratic Republic of Congo, staff training in conjunction with microscope distribution resulted in marked improvement in smear microscopy.[22] A study conducted in Malawi showed that with basic training and support it is possible for laboratory staff to incorporate simple procedures into everyday practices for assessing quality of their own work.[39]

Slide rechecking

Although proficiency testing can be used to determine whether a laboratory technician can adequately perform AFB smear microscopy, blinded rechecking is considered more effective in assessing the reality of routine performance resulting in improved diagnosis and monitoring of treatment response.[11, 22, 31, 32] Studies conducted in India, the Philippines, Mexico and Democratic republic of Congo found blinded rechecking and using the LQAS[43] strategy to be operationally feasible. [22, 32, 40, 44, 45] Blinded rechecking identified laboratories that produced errors during smear microscopy and identified laboratories with unacceptable performance.

Rechecking a sample of routine smears from peripheral laboratories by the reference laboratory is considered the best method for evaluating performance and providing motivation to staff for improvement. [11] Rechecking however, requires more resources and are more expensive than the other quality assurance techniques (i.e. proficiency testing and on-site evaluation. When implementing a blinded rechecking programme, the following resources need to be considered:

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[43] Lot Quality Assurance System: a technique using the smallest possible sample size that allows solid conclusions to be made about the performance of a laboratory.

- Available financial support.
- Capacity of peripheral laboratories to store smears for rechecking.
- Availability of properly trained personnel to collect appropriate samples of slides from peripheral sites.
- Capacity of the reference laboratory staff to reread smears from peripheral sites, including second rereading to resolve discrepancies as needed.
- Capacity of the reference laboratory to provide results of rechecking as well as feedback to implement effective corrective action.

Blinded rechecking of slides at regular intervals (at least quarterly) should be the goal for optimal quality assurance.

Improve support visits

On-site supervisory support visits by experienced personnel offer the best opportunity to review proficiency testing results, identify potential sources of error (such as smear preparation, staining and reading) and implement corrective action.[11, 22] These support visits should be conducted at least once a year and more frequently, if significant problems are identified. Use of a standardised checklist is recommended as a tool for monitoring the performance of TB microscopy centres during support visits. The checklist described in the guidelines on external quality assessment for AFB smear microscopy can be used as a tool during support visits. [11, 22]

The study conducted in Limpopo reported that an evaluation guideline (checklist) was one of the implementation measures that contributed to improved laboratory performance. [13]The other implementation measures included review of the evaluation forms to identify additional areas for corrective action and a standardised demonstration of the Ziehl-Neelsen staining method. Elements for monitoring and evaluation on the checklist should include availability of standard operating procedures in the laboratory, laboratory reagents, laboratory materials and equipment, safety measures and waste disposal, smearing and staining, workload, major challenges encountered and training requirements.[43] However,

implementation of the checklists should not make the on-site evaluation process too lengthy and time consuming.[22]

When conducting investigations, all possible sources of error should be examined, including: quality of stains, quality of microscopes and administrative procedures that may contribute to recording errors. All possible causes of error must be resolved. Possible causes of errors and suggested evaluation steps are illustrated in table 17.[11]

Turnaround time

Turnaround time was not assessed in the study but the literature review revealed challenges in obtaining TB smear microscopy results within the target time of less than 48 hours. Turnaround time in some provinces could be as high as 14 days. The areas of the country that are most affected are those that lack basic infrastructure such as landline telecommunications, Escom power supply and adequate roads.[36] In addition, laboratories and/or TB microscopy centres manned by a single person is at times non-functional when the staff member is unable to report for duty when ill. In such instances the facility may be unmanned for hours or even days before alternative measures are taken to transport specimens to other laboratories or before replacement staff is deployed, resulting in turnaround times far beyond the target time of 48 hours. Although bacterial coverage using smear microscopy is improving in most provinces the high turnaround time in certain areas is unacceptable.

The laboratories argue that the delay is not within the laboratory but rather the delay in transportation of specimens from the clinics to the laboratory. However, the problem remains, positive TB cases go untreated in the community while they pose a risk of transmitting the disease. Some may lose faith in the health system and may not return to the health facility if previous attempts to access TB results failed. The transport costs of visiting health care facilities may be discouraging to patients who are often indigent. Therefore reduction of the turnaround time to within the target time (less than 48 hours) is crucial for the success of the TB control programme.

While problems relating to the efferent[44] loop may be solved by automatically sending TB results to the clinic via short message system (SMS – cellphone technology) as soon as they are entered on the laboratory database, problems with the afferent loop[45] still exists. Possible solutions are to use innovative means of transport such the unmanned aerial vehicles described by Professor Barry Mendelow. [36] With the advances in molecular diagnostics requiring smaller sample sizes and novel dried spot format to eliminate biological hazard and to bypass the cold chain coupled with advances in engineering and electronic technology, unmanned aerial vehicles may be a reality in the near future. These technologies make elimination of services such as microscopy centres a possibility. The advantage is that if efficient and affordable transport was available to transport biological specimens from surrounding health facilities to a centralised laboratory for TB diagnosis then there wouldn't be a need for establishing and maintaining microscopy centres. The challenge with microscopy centres is that they require well-trained staff who need regular supervision and monitoring, well functioning equipment such as microscopes and since smear microscopy examinations are tedious and repetitive a great deal of motivation is required to obtain acceptable results. At present many microscopy centres are staffed by high school graduates with basic training on TB diagnosis using smear microscopy. Centralising microscopy services would eliminate the need for microscopy centres that are inadequately staffed and equipped and is therefore supported.

The 2002 EQA guidelines state that effective TB control depends on a network of local laboratories that provide accurate and reliable direct sputum smear microscopy. [11] Therefore maintenance of microscopy centres that constantly produce substandard performance is debatable especially considering the effects false results have on patient management and control of spread of the disease. Centralising TB microscopy services would require policy changes at a national level as South Africa is promoting the decentralisation of TB microscopy services and primary health care. Therefore,

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[44] Post analytical communication of laboratory results

[45] Pre-analytical phase of the laboratory logistic loop, viz. specimen transport in remote areas

centralisation of TB microscopy services is really not an option. What is now required is for the NHLS to commit to employing more staff in laboratories that are under-staffed and also creating a service where 'buffer staff' is available to be redeployed to areas at short notice (within hours). Redeploying staff at short notice may be a challenge but it can be implemented e.g. by using a call system[46] or a roster and providing incentives such as an allowance. While innovative solutions such as the unmanned aerial vehicles may address challenges related to transportation of sputum specimens is remote areas and is eagerly awaited, it may be years before they are implemented. Therefore additional resources must be committed to improving the system for transportation of sputum and other laboratory specimens to the relevant diagnostic laboratories.

Implementation of new technology to detect multidrug-resistant (MDR) and extensively drug resistant (XDR) TB

The recent emergence of MDR and XDR strains of *Mycobacterium tuberculosis* warrants an urgent need for alternate methods to identify MDR and XDR tuberculosis.[14, 46, 47] In KwaZulu-Natal, many patients with MDR TB and HIV died soon after diagnosis of the disease (median survival time of 16 days from the date of diagnosis).[14] Therefore, with early diagnosis of MDR TB and rapid initiation of appropriate therapy, many lives would be saved.[47]

Barnard and colleagues described a commercially available molecular assay (GenoType MTBDRplus) that is capable of detecting MDR TB organisms in sputum specimens within 24 hours. [14] This technique performed better than the acid-fast bacilli stained smears and the broth culture methods (which is considered the gold standard). Although MTBDRplus was originally designed for detection of drug resistance in isolates, the sensitivity (approximately 80%) in acid-fast bacilli smear-negative specimens is quite favourable as well.[47] The high degree of accuracy, the substantial reduction in turnaround time (less

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[46] This is where a staff member can be assigned a specified period to be available/on standby to be redeployed at short notice (within hours), when required.

than 7 days versus several weeks to months with conventional methods) and the substantial cost saving of the technique makes it a favourable alternative to the more costly conventional culture and drug susceptibility techniques.[14] The obstacles to implementation of this technique are that it requires well-qualified staff, a substantial increase in laboratory space and an efficient sputum collection and transport system. There is a high TB drug resistance and TB-HIV co-infection in KwaZulu-Natal, therefore the NHLS and the Department of Health should urgently eliminate the obstacles to implementation of the more efficient methods, such as molecular assays, for detecting *Mycobacterium tuberculosis* drug resistance.

Need for further studies

In this study, proficiency testing was used as the method of external quality assessment to determine whether a laboratory technician can adequately detect acid-fast bacilli using smear microscopy to diagnose tuberculosis. Errors observed using this method can be linked to possible causes including problems with microscopes, problems with stains and other reagents, ability of technicians to identify acid-fast bacilli, administrative errors and negligence (refer to table 17: investigation of errors). [11] There are other factors that influence the quality of sputum smear microscopy namely; quality of sputum specimens, sputum collection and transport, smearing and staining technique, laboratory safety and infection control measures, assessment of laboratory equipment and workload. Evaluation of these factors was beyond the scope of this study and was therefore not assessed. Further studies of these factors are therefore needed.

5.4 LIMITATIONS

Proficiency testing

This study assessed laboratories and not microscopists as it cannot be ascertained whether the same microscopist had read all the slides or whether results were discussed before being forwarded to the reference laboratory. It could not be established whether the time spent on reading proficiency testing slides were longer than recommended. Similar limitations were experienced in a study conducted in Mexico. [31]

This study is limited to pre-existing data. The National Health Laboratory Service is now the administrator of the public health laboratory services in KwaZulu-Natal. Therefore, laboratory processes and conditions may have changed. The analysis of the 2006 data (proficiency testing conducted by the National Health Laboratory Service) however, does provide a more accurate picture of the present situation in terms of laboratory performance.

Key informant interviews

Due to financial constraints interviews were conducted telephonically and was limited to ten key informants. Therefore the face-to-face interaction during the interview as well as the assessment of the interviewee's body language was lacking. The interviewer could have probed much more if the interviews were face-to-face. However, the interviews were very informative as the interviewees expressed their views quite freely. Valuable information on the operational issues in the laboratory, such as human resources and capacity, workload, communication and feedback was collected. The key informants also suggested measures that should be implemented to improve the quality assurance system and smear microscopy in the province.

Despite the limitations, the study provides a retrospective analysis of TB microscopy for 2001-2006. Constant monitoring of all future proficiency testing is recommended.

5.5 EFFECT MODIFICATIONS

5.5.1 Low positive slides

Low false negative and low false positive results are considered to be errors of a less serious nature and should be expected. [20] Aid-fast bacilli (AFB) are not homogenously distributed in sputum. A few AFB (less than 10) identified by one technician may not be identified by another technician reading the same slide as they may not read the same fields. In addition, preparation of 'low positive' slides for the purpose of proficiency testing requires a meticulous technique to ensure consistency of the concentration of AFB (i.e. 1-9/100 high power fields). It is for these reasons the National Health Laboratory Service excluded 'low positive' slides from the NHLS panel. The KZN panel however, included 'low positive' slides. To compare the two data sets (time periods) the proficiency testing slides had to be similar. Therefore, 'low positive' slides were removed from the KZN panel. Incorrect reading of 'low positive' slides would result in low false negatives results. Results obtained from these 'low positive' slides were analysed and are presented below.

The proportion of low false negative results for period 2001-2004 (KZN panel) was 1.4% (n=26). The number of low false negative results ranged from 1-2 in some laboratories. It was observed that for the period 2001-2004, twenty-four laboratories reported at least one low false-negative result. For the same period, 2 laboratories reported 2 low false-negative results each. The remaining laboratories (n=48) did not report a single low false-negative result.

Summary

There was an unacceptably low level of overall performance in 2001-2004, however, overall performance improved to a satisfactory level in 2006. Reporting of high false negative results remain a concern, although there was a reduction in reporting of this error from 6% (2001-2004) to 2% (2006). Reporting of high false positive results is not a problem in KwaZulu-Natal public health laboratories. The sensitivity and specificity of

reading proficiency testing slides (using the reference laboratory as comparator) improved from unacceptable levels in 2001-2004 to satisfactory levels in 2006.
For the period 2001-2004, 39 laboratories performed below the accepted level of performance. This level reduced to 14 laboratories in 2006. The quality of TB microscopy services in the primary health care level and the district health care level are similar, however, tertiary level health care facilities are performing below the acceptable level of performance. As of 2006 both urban and rural laboratories are producing similar results, which are within the acceptable levels of performance. All regions have a satisfactory performance. Of the 11 quarters assessed (2001-2004), satisfactory overall performance was reported for only 4 quarters. The quality of smear microscopy as determined by the NHLS data set was far superior to the quality as determined by the KZN data set.

Qualitative analysis

Respondents had a good understanding of the basic theoretical aspects of proficiency testing. Laboratory personnel expressed that proficiency testing is an essential exercise to ensure good quality results. Some of the major problems experienced by laboratory staff were the inherent problems with proficiency testing (e.g. it cannot be ascertained whether the correct person reads the proficiency testing slides), high workload, poor feedback and communication from the reference laboratories, and need for in-service training. The key informants suggested possible solutions, which were incorporated into the overall recommendations of the study (see Chapter 6).

6 CHAPTER VI: RECOMMENDATIONS AND CONCLUSIONS

6.1 INTRODUCTION

In this chapter, objective 5, which is the recommendations to decision makers on key gaps identified from the information obtained in the study is addressed. This chapter also presents conclusions based on the findings made in each important issue explored in the study.

6.2 RECOMMENDATIONS

6.2.1 Quantitative analysis

As correct results are the goal of the proficiency testing programme, all other results should be reviewed. The high number of quantification errors in the study may be indicative of the quality of the proficiency testing slides and that concentrations of AFB may not be consistent with the designated grading. Therefore it is crucial that the National Health Laboratory Services ensure that proficiency testing slides are prepared meticulously and reviewed before sending to peripheral laboratories. Discrepant results should be reviewed again in the reference laboratory before the allocation of scores.

Low positive slides should be included in future rounds provided that the proficiency testing slides are well prepared and assessed before distribution to peripheral laboratories and assessed again in the reference laboratory if there are discrepancies between the peripheral laboratory and the reference laboratory.

Approved guidelines should be followed when selecting slide panels. The number and variation in grades of positive slides should be maintained as recommended. [11]

Laboratory performance through external quality assurance should be monitored quarterly so that corrective measures can be implemented timeously.

Concentrating on laboratories performing poorly

Similar to the study conducted in Limpopo Province, the use of proficiency testing results in this study achieved the objective of identifying laboratories that performed below the acceptable level of performance.[13] Investigations should be conducted to identify the reason/s for poor performance. These investigations should include:

- Evaluating overall performance of all participating laboratories to determine whether the problem was poor slide preparation at the reference laboratory or due to errors in the peripheral laboratory.
- On-site evaluations should be conducted for individual laboratories to determine the source of the problem.

6.2.2 Qualitative analysis

Introduce slide rechecking

Although proficiency testing is recommended as the most efficient means of making the first broad assessment of sputum smear microscopy in KwaZulu-Natal, rechecking a sample of routine smears from peripheral laboratories by the reference laboratory is considered the best method for evaluating performance and providing motivation to staff for improvement. [11, 48] Therefore blinded rechecking, of slides at regular intervals (at least quarterly), should be introduced as the next step towards optimal quality assurance.

Improve support visits

It is recommended that support visits be conducted at least once a year and more frequently, if significant problems are identified. Use of a standardised checklist is recommended as a tool for monitoring the performance of TB microscopy centres during support visits. The checklist described in the guidelines on external quality assessment for AFB smear microscopy can be used as a tool during support visits. [11, 22] All possible sources of

errors should be examined, including: quality of stains, quality of microscopes and administrative procedures that contribute to recording errors. All possible causes of errors must be resolved. Possible causes of errors and suggested evaluation steps are illustrated in table 17.[11]

Improve communication

The reference laboratory should clearly communicate to the peripheral laboratories all the requirements expected from them. Issues such as who should process the slides, recording and reporting process as well as time frames should be clearly stipulated. A contact person in the reference laboratory should be appointed to address queries from the peripheral laboratories regarding issues of quality assurance.

The reference laboratory should clear any misperceptions regarding proficiency testing during training sessions and through newsletters. Managers should also be encouraged during meetings and through newsletters to improve communication between themselves and laboratory personnel reporting to them. TB coordinators conducting support visits should emphasise the principles of proficiency testing to laboratory managers and staff and encourage them to follow the rules/instructions of the programme.

Staff training

Several studies have shown that training improved laboratory performance, therefore remedial training is recommended for all laboratory technologists/technicians in laboratories that reported major errors.[13, 22, 31]Training should include smear preparation and staining as well as various ways to identify faulty equipment, reagents and stains. Training should include maintenance of microscopes and other equipment as well as

infection control[47]. It is recommended that quality assurance be included in TB training programmes for technologists and technicians as part of their in-service training.

Reassess staff allocations

Even the best technicians make mistakes when overloaded with work.[29] Therefore, an audit of staff providing TB diagnostic services should be conducted to establish their workload. Staffing at TB diagnostic centres should be optimised to ensure that adequate time is available to perform the various steps in TB diagnosis.

Improve feedback

Provide feedback to all participating laboratories as soon as possible after assessments are available. This is important to identify errors and implement corrective action immediatcly. This would also keep participating laboratories/staff motivated and eager to continue, knowing that their efforts are making a difference.

Introduction of new technology to detect drug resistance

This recommendation is not based on the results, but on the literature review and the context of where we are in terms of the burden of TB and drug resistance in the province. Due to the high TB drug resistance and TB-HIV co-infection in KwaZulu-Natal, it is recommended that the National Health Laboratory Services and the Department of Health fast track the implementation of the more rapid molecular assay techniques for rapid detection of drug resistance in isolates.

Feedback on findings of this study

Feedback on findings of this study will be provided to key role players including:

- National, Provincial and District TB Programme Managers

[47] Infection control in this document refers to a comprehensive programme that encompasses all aspects of infection prevention and control including education and training, surveillance, environmental management, waste management, cleaning disinfection and sterilisation, and employee health.

- NHLS TB Quality Assurance Manager and all NHLS TB laboratory Managers and Staff.

Feedback will be in the form of a summary report highlighting the findings and recommendations. An academic paper/peer reviewed journal will also be drafted for publication and wider dissemination of the study results.

Table 17: Investigation of errors

Pattern of errors	Possible cause	Suggested investigation steps
HFP and HFN	1. Unusable microscope	1. Examine a 3+using that microscope
	2. Staining problems	2. Check stains and staining procedure
	3. Technician cannot recognize AFB	3. Test with clear-cut pos / neg and good microscope
	4. Gross neglect	4. Exclude other cause
A single HFP	1. Administrative error	1. Compare lab-register with QC-listing: correct slide number and result?
	2. As for more frequent HFP	2. Exclude causes of more frequent HFP
Regularly a HFP or without LFP	1. Poor registration routine	1. Check accuracy of lab-register and other record keeping
	2. Staining problems/fading	2. Check stains and staining procedure, consider re-staining for rechecking
	3. Technician unclear on AFB appearance	3. Look for inconsistent results of suspects (regularly single pos./low positive) in lab register
Rare LFP	To be expected	No investigation unless numbers increase
Many LFP, with or without occasional HFP	1. Problem with controllers	1. Evaluate controllers
	2. Technician unclear on AFB appearance	2. Recheck special sample of LFP from laboratory register
	3. Contaminated stain reagents	3. Test stain with known negative smears
Single high false negative	1. Administrative error	1. Compare lab-register with QC listing : correct slide number and results?
	2. Very thick smears and/or poor light	2. Evaluate quality of smear preparation, check microscope
	3. Gross neglect	3. Exclude other causes
Frequent HFN and/or Many LFN	1. Staining problems/Fading	1. Check stains and staining procedures, consider re-staining for rechecking
	2. Poor smearing-technique	2. As above, single HFN
	3. Problem with microscope	3. Check microscope with positive slide
	4. Careless microscopy	4. Exclude other causes
	5. Contaminated stain reagents/water	5. Test stain with known negative smears
Very high proportion LFN	Contaminated methylene blue or rinse water	As above
Many QE (too low gradings)	1. Poor staining	As above
	2. Problem with microscope	As above

Source: Aziz, M.A., et al., *External quality assessment for AFB smear microscopy*. Association of public health laboratories. 2002, Washington DC.

6.3 CONCLUSIONS

In the absence of newer technology that is accessible to resource poor settings, the Ziehl-Neelsen test will remain the cornerstone of TB diagnosis. Therefore quality assurance is vital to ensure high quality TB microscopy results. The recommendations made in this study are consistent with those of another study conducted in Ghana, which indicated that support visits, which act as motivation for laboratory personnel, on-site and formal training, blinded rechecking of examined slides and timely feedback lead to improvements in TB laboratory services.

Quantitative analysis

This study demonstrated that, although there was an unacceptably low level of overall performance in 2001-2004 (0verall agreement = 93%), overall performance improved to a satisfactory level in 2006 (0verall agreement = 98%). Reporting of high false negative results remain a concern, although there was a reduction in reporting of this error from 6% (2001-2004) to 2% (2006). Of the 19 errors detected in 2006, 18 were high false negative results. Reporting of high false positive results is not a problem in KwaZulu-Natal public health laboratories. The sensitivity and specificity of reading proficiency testing slides (using the reference laboratory as comparator) improved from unacceptable levels in 2001-2004 to satisfactory levels in 2006. Although the overall agreement of 98% is pleasing, several laboratories performed below the acceptable level in 2006. Immediate attention should therefore be focussed on these laboratories first.

For the period 2001-2004, 39 laboratories performed below the accepted level of performance. In 2006, 14 laboratories fell into this category.

The quality of TB microscopy services in the primary health care level and the district health care level are similar (overall performance of 98% for both levels) and are satisfactory. Tertiary level health care facilities are performing below the acceptable level of performance however results should be interpreted with caution as only one tertiary level

laboratory participated in the 2006 proficiency testing exercise and one error (HFN) was reported.

As of 2006 both urban and rural laboratories are producing similar results (97% and 98% respectively), which are within the acceptable levels of performance. At a regional level, all regions have a satisfactory performance ranging from 96% to 99%. The overall laboratory quarterly performance was inconsistent, ranging from 87% to 96%. Therefore a trend was not observed. The inconsistent performance in 2001-2004 is likely to be due to the high workload and insufficient training. The quality of the proficiency testing would have affected performance to a lesser extent.

The quality of smear microscopy as determined by the NHLS data set (overall performance 98%) is far superior to the quality as determined by the KZN data set (overall performance (93%).

Qualitative analysis

The key informants had a good understanding of the basic theoretical aspects of proficiency testing as a quality assurance technique. The general feeling among laboratory personnel is that proficiency testing is an essential exercise to ensure correct, reliable and accurate results. There was a perception among laboratory staff that proficiency testing was a punitive measure and that staff producing substandard performance would be punished. Some of the major problems experienced by laboratory staff were the inherent problems with proficiency testing (e.g. it cannot be ascertained whether the correct person reads the proficiency testing slides), high workload, poor feedback and communication from the reference laboratories and need for in-service training.

The key informants suggested possible solutions, which were incorporated into the overall recommendations of the study. Some of the major recommendations of the study are:

Improvement in the method of quality assurance such as the introduction of a blinded re-checking programme is essential. Support visits by reference laboratory personnel to the peripheral laboratories should be strengthened as the true assessment of the quality of sputum smear microscopy at the field level can best be monitored by supervisory field visits. These visits should be used to motivate laboratory personnel to express their concerns and problems and also to listen and take note of any solutions they might have.

Before a new proficiency testing programme is implemented, clear interpretation guidelines must be agreed upon by the reference laboratory. Systems must be established to provide feedback and technical support to identify and correct problems identified during proficiency testing and support visits.

An External Quality Assurance process that is limited to the assessment of the current level of performance possesses little value unless the data is used to implement improvement strategies and measure ongoing performance improvement.[11] Therefore every effort must be made to assess proficiency testing results and provide feedback to participating laboratories timeously so that corrective measures could be implemented without delay.

This study described the quality of TB smear microscopy in KwaZulu-Natal from 2001 to 2006. It identified laboratories that performed below the acceptable level of performance and also highlighted some of the drawbacks of proficiency testing as a quality assurance technique.

6.4 RECOMMENDATION FOR FURTHER STUDY

This study was a rapid assessment of the quality of TB microscopy services in KwaZulu-Natal. Existing proficiency testing results was used to determine whether a laboratory technician could adequately detect acid-fast bacilli using smear microscopy to diagnose tuberculosis. Errors observed using this method can be linked to possible causes including problems with microscopes, problems with stains and other reagents, ability of technicians

to identify acid-fast bacilli, administrative errors and negligence (refer to table 17: investigation of errors). [11] There are other factors that influence the quality of sputum smear microscopy namely; quality of sputum specimens, sputum collection and transport, smearing and staining technique, laboratory safety and infection control measures, assessment of laboratory equipment and workload. Evaluation of these factors was beyond the scope of this study and was therefore not assessed. Therefore a broader study to investigate fully all factors affecting laboratory diagnosis of TB is recommended.

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8 ANNEXURES

ANNEXURE 01: DATA COLLECTION TOOL

Data collection tool

SURVEY	LAB	LAB_NAME	REGION	REGION NAME	NON - RETURN	A	TARGET A	ERROR_A	SCORE_A	B	TARGET B	ERROR B	SCORE B	C	TARGET C	ERROR C	SCORE C

ANNEXURE 02: INTERVIEW GUIDE

Broad objectives

- To Ascertain Knowledge, Attitudes and Practices about proficiency testing
- To determine what people think about proficiency testing.
- To identify problems in the proficiency testing programme
- To recommend possible solutions

List of key informants to interviewed

Interviewee	Title	Level of facility	Urban/rural
1.	Manager	Provincial Dept of Health	
2.	TB Control Coordinator	Provincial Level	
3.	Manager	NHLS Quality assurance	
4.	Lab Supervisor	Tertiary	Urban
5.	Medical technologist	Tertiary	Urban
6.	Microscopist	Clinic	Urban
7.	Microscopist	Hospital	Urban
8.	Medical technologist	Hospital	Rural
9.	Microscopist	Hospital	Rural
10.	Microscopist	Clinic	Rural

Proficiency testing

1. Have you been involved in a proficiency testing programme?
2. Do you think it was a worthwhile exercise?
3. Does your laboratory have internal QA/QC programme Yes No
4. Is internal QA/QC performed regularly seldom not at all
5. Who processes proficiency testing slides (bearing in mind that reports have to written on corrective measures, if results are unsatisfactory)? ----------
6. Some people say that proficiency testing is a waste of time. What do laboratory staff/managers think about proficiency testing?

7. It has been said that some laboratory managers get the best person to process proficiency testing slides or even process it themselves. What do you think?
8. In your opinion, is the proficiency testing programme effective enough to detect errors in microscopy technique?
9. What are the problems experienced?
10. Does the same person process routine TB specimens? ----------
11. How much time is spent reading each slide (average)? ----------
12. Did you get feedback after submitting proficiency testing results?
13. Are standard operating procedures available in the laboratory?----------
14. Do you think that microscopists are adequately trained to perform their duties?
15. How can the QA system be improved?

ANNEXURE 03: ETHICS APPROVAL FORM

UNIVERSITY OF
KWAZULU-NATAL

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office
Room N40 - Govan Mbeki Building
University Road, **WESTVILLE CAMPUS**
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: ngwenyap@ukzn.ac.za - Website: www.ukzn.ac.za

04 June 2008

Mr W Ramkrishna
Department of Public Health Medicine
Nelson R Mandela School of Medicine
Faculty of Health Sciences
University of KwaZulu-Natal

PROTOCOL: Quality of TB Microscopy in KwaZulu-Natal as determined by Proficiency Testing. Dept. of Public Health Medicine. Wayne Ramkrishna.

Dear Mr Ramkrishna

RECERTIFICATION APPLICATION APPROVAL NOTICE

Reference Number:	**BE003/07**
Approved:	**04 June 2008**
Expiration of Ethical Approval:	**04 June 2009**

I wish to advise you that your application for Recertification received by us on **26 May 2008** for the above protocol has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse effects occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received a BREC approval letter for the change.

The approval will be ratified by a full sitting of the Committee at a meeting to be held on **08 July 2008.**

Yours sincerely

Pp:

Prof. D Wassenaar
Chair: Biomedical Research Ethics Committee
DW/pn

UNIVERSITY OF
KWAZULU-NATAL

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
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14 July 2008

Mr W Ramkrishna
Department of Public Health Medicine
Nelson R Mandela School of Medicine
Faculty of Health Sciences
University of KwaZulu-Natal

PROTOCOL: Quality of TB Microscopy in KwaZulu-Natal as determined by Proficiency Testing. Dept. of Public Health Medicine. Wayne Ramkrishna. Ref: BE003/07.

Dear Mr Ramkrishna

PROTOCOL RECERTIFICATION RATIFICATION

Further to our letter to you dated 04 June 2008, this letter serves to notify you that at a full sitting of the Biomedical Research Ethics Committee Meeting held on **08 July 2008**, the Committee RATIFIED the sub-committee's decision to approve the Recertification of the above protocol received by us on the 26 May 2008.

Yours sincerely

Prof. D Wassenaar
Chair: Biomedical Research Ethics Committee

ANNEXURE 04: CONSENT DOCUMENT

CONSENT DOCUMENT

Consent to Participate in Research

I have read this form and voluntarily agree to participate in this research study called 'Quality of tuberculosis microscopy in KwaZulu-Natal as determined by proficiency testing'. The purpose of the study, the procedures, and the risks and benefits has been explained to my satisfaction. My signature indicates that I consent to participation in the research study (interview).

Do you consent to having the interview recorded on audiotape?

Please indicate with a tick | YES | NO |

_____ _____

Signature of Participant Date

_____ _____

Signature of Witness Date
(Where applicable)

_____ _____

Signature of Translator Date
(Where applicable)

ANNEXURE 05: INFORMATION DOCUMENT FOR STUDY PARTICIPANTS

INFORMATION DOCUMENT FOR STUDY PARTICIPANTS

STUDY TITLE: The quality of tuberculosis microscopy in KwaZulu-Natal as determined by proficiency testing

GREETING: Good Day

INTRODUCTION

My name is Wayne Ramkrishna. I am a Masters in Public Health student at the University of KwaZulu-Natal. . I am conducting a study for the Masters Degree in Public Health

The purpose of the study is to determine the quality of tuberculosis microscopy in KwaZulu-Natal as determined by proficiency testing

The objectives of the study are to:

1. To describe and analyse the proficiency testing results carried out between 2001 and 2006 in the 72 facilities where sputum smear microscopy is carried out by the KZN PHL in the province, in order to determine quality and trend by year and district.
2. To identify laboratories that have an unacceptable level of performance so that corrective action can be taken.
3. To quantify the size of the false results in these laboratories.
4. To compare proficiency testing results obtained by the KZN reference laboratory and the NHLS reference laboratory.
5. To determine the role of proficiency testing as a quality assessment technique
6. To make recommendations to decision makers on the key gaps identified from the data analysis in this study.

This interview is intended to ascertain knowledge, attitudes, and practices towards proficiency testing. The study will identify areas where problems exist in terms of proficiency testing. A suitable plan of action will be recommended, to key decision makers

on how the problems identified could be rectified. The study will be beneficial to all TB laboratory staff, particularly to KZN. The study will also be indirectly beneficial to all citizens of the country as improved TB diagnosis would improve patient management and reduce our risk of contracting the disease.

I would like to use information from this interview for research purposes. I am therefore requesting you to take part in the research project.

What is involved in the study

As a participant you are expected to avail yourself for approximately 30 minutes for the interview. You are expected to answer the questions honestly and openly so that a realistic assessment can be made.

Number of people that will take part in the interview

Approximately 10 people who are involved in TB diagnosis and proficiency testing will participate in the study.

Risk of being involved in the study:

The are no risks being involved in the study

Benefits of being in the study -

It is an opportunity for you as a participant to freely express your views during the interview. These views will be analysed and used to identify problems that you experience and to recommend possible solutions. Should the results indicate problems such as inadequate training or resources, the study will then emphasise remedial measures and advise on improving these areas. This would improve your facility's TB diagnostic capabilities and efficiency. This would ultimately improve TB case detection, treatment follow-up of patients and reduce the risk of TB to the community.

Should the results of the study indicate acceptable levels of performance, this information may be motivating to staff to know that they are doing a good job.

Participation is voluntary

The study will be conducted in partial fulfilment of the requirements for a Masters Degree in Public Health. Participation in the research however, is voluntary, you will not be penalized if you refuse to participate and you may discontinue participation at any time.

Confidentiality

For the purpose of this research all personal information will be kept confidential and any access by any person to obtain such details from the researcher or other participants will be forbidden.

You may contact any of the following persons if you have questions or problems:

Researcher

Mr Wayne Ramkrishna

Address: National Department of Health

Room 1404, Hallmark Building

Pretoria

0001

Phone Number: 012 3123186

Fax Number: 0123123113

Cell Number: 0823174687

ANNEXURE 06: PROCESSING OF SPECIMENS FOR MICROSCOPY

NUMBERING OF SLIDES FOR MICROSCOPY.

Slides are numbered using a diamond pencil. Only one number per slide. The complete number is scored on the end of the slide. The number must be written neatly and clearly. (AA123/06)

PROCESSING OF SPECIMENS FOR MICROSCOPY ONLY.

1. Ensure that sample details correspond with request form.
2. Ensure that only AFB or Direct microscopy has been requested.
3. Heat decontaminate the numbered specimen by placing the sample in the hot air oven at 85^0 C for 20 minutes.
4. Remove sample from hot air oven and allow to cool.
5. Working in a safety cabinet, add equal volume of 5% Sodium Hypochlorite (Jik) to the sample and shake briefly.
6. Allow to stand for 15 minutes at room temperature.
   NB: Timing is critical as Sodium hypochlorite may destroy the TB bacilli if allowed to act too long.
7. Add Sterile Distilled water to the full mark of the specimen container and mix gently.
8. Pour into appropriately labelled centrifuge tube.
9. Centrifuge at 3000 rpm for a minimum of 20 minutes.
10. Decant supernatant and resuspend deposit.
11. Make a smear. (see making of smear for microscopy)
11. Prepare a positive control (H37), a negative control (E. coli) from the stock cultures .(See quality controls)

ANNEXURE 07: MAKING OF SMEARS FOR MICROSCOPY

MAKING OF SMEARS FOR MICROSCOPY ONLY.

1. Label a new clean unscratched slide at one end with the appropriate lab number using a diamond pencil. (Slides are cleaned by flaming over a Bunsen burner prior to use). Make a circle 1.5 cm in diameter on centre of the slide with a diamond pencil.

2. Vigorously vortex the sediment from the concentrated sample.

3. Using a sterile 3 mm loop transfer a representative portion of the concentrated sample to the appropriate slide. Spread the loopful in the circle previously scored.
   NB. Only one smear per slide. Smear size should be a minimum of 1.5 by 1.5.

4. Fix smears by placing on an electric slide warmer at 65^0 C . When smears are dry place in the hot air oven for at least 1 hour at 85^0 C

5. Positive (H37), negative (E.coli)

6. Stain slides. Refer to staining of slides.

7. Read slides. Refer to reading of slides.

QUALITY CONTROL

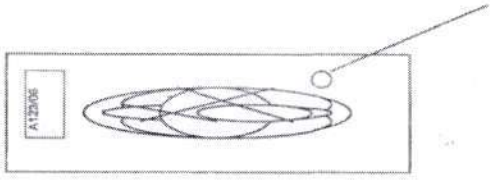
1. **NEGATIVE CONTROL.** Prepare a No. 1 McFarland turbidity suspension from a fresh culture of E coli in 3 ml sterile saline in a 15 ml tube
2. **POSITIVE CONTROL.** Prepare a No. 1 McFarland turbidity suspension of H37 Middlebrook culture in 2 ml sterile Tween in a 15 ml tube.

3. The positive and negative controls are used to prepare slides for the "microscopy only" process.

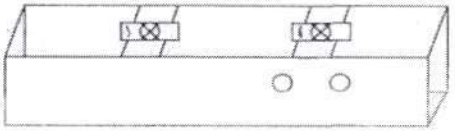
ANNEXURE 08: STAINING OF SLIDES – ZIEHL-NEELSEN STAIN

ZIEHL-NEELSEN STAIN

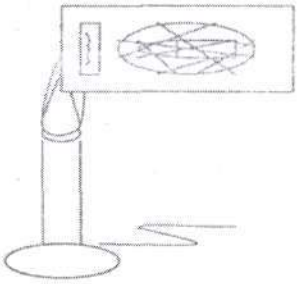
1. Smear decontaminated specimen Using a 3 mm wire loop onto a Glass slide.



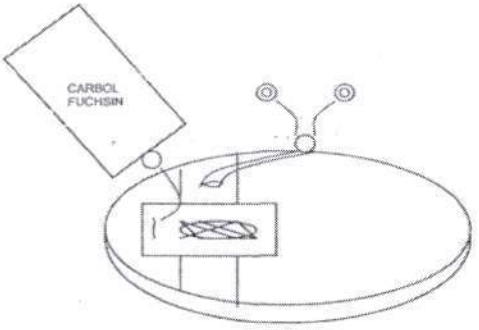
2. Dry slide on a heating block (Slide warmer)



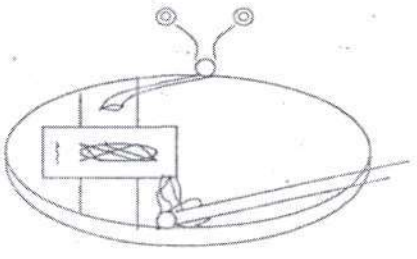
3. Pass the slide 3 times through a bunsen burner to fix the smear



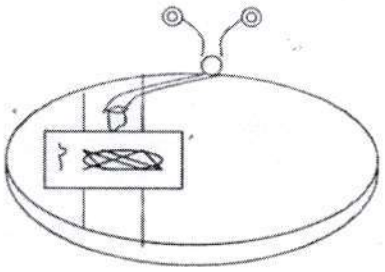
4. Flood the slide with carbol fuchsin



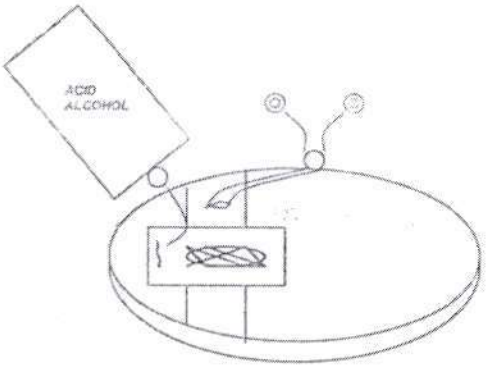
5. Heat the slide with a flame until it steams for 5 minutes



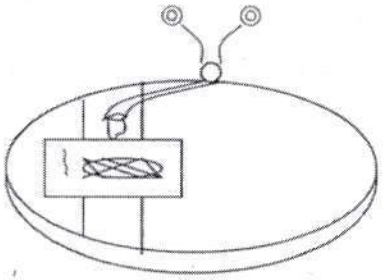
6. Rinse the slide with water



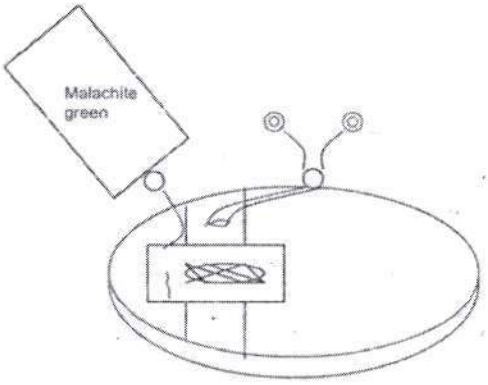
7. Add 3% Acid alcohol until no more colour runs.



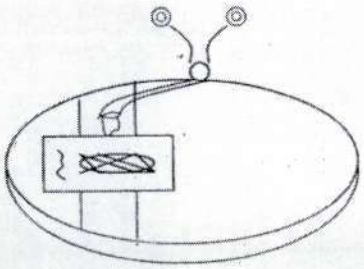
8. Rinse the slide with water



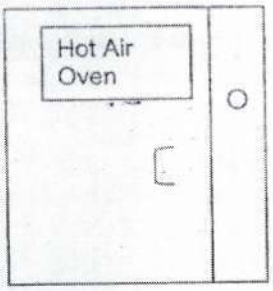
9. Add Malachite green (counter stain) for 30 seconds



10. Rinse the slide with water



11. Air dry or use a hot air oven 80^{0}C to dry the slide.
(do not blot the slide)



ANNEXURE 09: EXAMINATION OF SLIDES USING LIGHT MICROSCOPY

EXAMINATION OF SLIDES USING A LIGHT MICROSCOPE.

1. Examine carbolfuchsin stained smears with a 100x oil immersion objective.

2. Thoroughly examine each slide for the presence of acid fast bacilli. A minimum of 100 fields must be examined before a smear is reported as negative.

3. Adopt a procedure that ensures that a representative of the smear is viewed. The reading must be systematic and standardized. For instance, begin the reading of a slide in the center of the left end of the smear. After examining a microscopic field, move the slide longitudinally so that the neighbouring field to the right can be examined. In this manner, all the microscopic fields from beginning to end of the central length of the slide should be examined. The number of microscopic fields in one length of the slide correspond to at least 100.

4. When no acid fast bacilli(AFB), (TB bacilli are stained red and the background is light green), are found in 100 fields, a more thorough search should be made in 50 –100 new fields.

5. A positive microscopy is reported as follows:

   Scanty - (1-9 AFB seen in the entire slide)
   \+ - (10- 100 AFB seen in the entire slide)
   ++ - (1-10 AFB seen in every field)
   +++ - (> 10 AFB seen in every field)

6. Examine positive and negative controls before commencing with the specimen smears. If any of the controls are not acceptable report immediately to a Senior Medical Technologist.

7. Read specimen smears and record results in the appropriate book or register. Check and recheck lab numbers on both smears and in the book to ensure that the correct result is recorded for the corresponding lab number.

8. All slides are stored in slide boxes for a minimum of 6 months.

ANNEXURE 10: SUMMARY OF PROFICIENCY TESTING SCORES (2001-2006)

Laboratory Code	KZN panel (2001-2004)															NHLS panel (2006)												
	Total non returns	Total slides reviewed	Correct	Quantification Errors	LFN	LFP	HFN	HFP	False positives	False negatives	Slide broke	Not evaluated	SCORE	Total Possible Score	FINAL SCORE (%)	Total non returns	Total Slides reviewed	Correct	Quantification Errors	False positives	False negatives	High false positive	Low false positive	High false negative	Low false negative	Score	Total possible score	Total score (%)
KZN001	3	18	14	2	1	0	1	0	0	2	0	0	165	180	91.7	0	24	23	1	0	0	0	0	0	0	240	240	100
KZN002	1	3	1	2	0	0	0	0	0	0	0	0	30	30	100.0	2	0	0	0	0	0	0	0	0	0	0	0	
KZN003	0	30	26	3	0	0	1	0	0	1	0	0	290	300	96.7	1	16	15	0	0	1	0	0	1	0	150	160	93.75
KZN004	6	9	5	1	0	0	3	0	0	3	0	0	60	90	66.7	1	16	15	0	0	1	0	0	1	0	150	160	93.75
KZN005	0	33	29	1	0	0	2	1	1	2	0	0	300	330	90.9	0	8	8	0	0	0	0	0	0	0	80	80	100
KZN006	1	26	23	2	0	1	0	0	1	0	2	0	255	260	98.1	0	24	20	3	0	1	0	0	1	0	230	240	95.8
KZN007	4	21	21	0	0	0	0	0	0	0	0	0	210	210	100.0	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN008	0	30	25	1	0	3	1	0	3	1	0	0	285	300	95.0	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN009	1	30	21	5	1	1	2	0	1	3	0	0	270	300	90.0	0	24	20	2	0	2	0	0	2	0	220	240	91.7
KZN010	0	33	27	4	0	1	1	0	1	1	0	0	315	330	95.5	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN011	0	30	27	2	1	0	0	0	0	1	0	0	295	300	98.3	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN012	2	24	14	2	1	0	6	1	1	7	0	0	165	240	68.8	0	16	14	1	0	1	0	0	1	0	150	160	93.75
KZN013	0	33	28	1	0	3	1	0	3	1	0	0	305	330	92.4	0	20	18	2	0	0	0	0	0	0	200	200	100
KZN014	0	33	30	2	1	0	0	0	0	1	0	0	325	330	98.5	0	24	23	1	0	0	0	0	0	0	240	240	100
KZN015	1	30	26	0	0	2	1	1	3	1	0	0	270	300	90.0	0	24	21	1	0	2	0	0	2	0	220	240	91.7

Laboratory Code	KZN panel (2001-2004)															NHLS panel (2006)												
	Total non returns	Total slides reviewed	Correct	Quantification Errors	LFN	LFP	HFN	HFP	False positives	False negatives	Slide broke	Not evaluated	SCORE	TOTAL POSSIBLE SCORE	FINAL SCORE (%)	Total non returns	Total Slides reviewed	Correct	Quantification Errors	False positives	False negatives	High false positive	Low false positive	High false negative	Low false negative	Score	Total possible score	Total score (%)
KZN016	0	33	29	4	0	0	0	0	0	0	0	0	330	330	100.0	0	24	22	1	0	1	0	0	1	0	230	240	95.8
KZN017	3	24	18	1	1	2	2	0	2	3	0	0	215	240	89.6	0	24	23	1	0	0	0	0	0	0	240	240	100
KZN018	2	24	23	0	0	0	1	0	0	1	0	0	230	240	95.8	0	24	22	2	0	0	0	0	0	0	240	240	100
KZN019	0	33	30	2	0	0	1	0	0	1	0	0	320	330	97.0	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN020	0	33	25	3	0	1	4	0	1	4	0	0	285	330	86.4	2	8	8	0	0	0	0	0	0	0	80	80	100
KZN021	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	19	18	0	0	1	0	0	1	0	180	190	94.7
KZN022	2	27	22	3	0	0	2	0	0	2	0	0	250	270	92.6	0	24	22	1	1	0	0	1	0	0	235	240	97.9
KZN023	0	32	31	1	0	0	0	0	0	0	2	0	320	320	100.0	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN024	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	24	24	0	0	0	0	0	0	0	240	240	100
KZN025	0	33	31	1	1	0	0	0	0	1	0	0	325	330	98.5	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN026	0	33	28	2	0	2	1	0	2	1	0	0	310	330	93.9	0	24	22	2	0	0	0	0	0	0	240	240	100
KZN027	0	0	0	0	0	0	0	0	0	0	0	0	0	0		2		O	O	O	O	0	0	0	0	0	0	
KZN028	2	27	24	2	1	0	0	0	0	1	0	0	265	270	98.1	1	16	15	0	1	0	0	1	0	0	155	160	96.875
KZN029	8	9	6	0	1	0	1	1	1	2	0	0	65	90	72.2	1	16	14	0	0	2	0	0	2	0	140	160	87.5
KZN030	0	27	22	1	0	1	2	1	2	2	7	0	235	270	87.0	0	24	18	4	0	2	0	0	2	0	220	240	91.7
KZN031	1	30	20	1	1	1	7	0	1	8	0	0	220	300	73.3	0	24	21	2	0	1	0	0	1	0	230	240	95.8
KZN032	2	27	22	1	2	1	1	0	1	3	0	0	245	270	90.7	0	24	22	1	0	1	0	0	1	0	230	240	95.8
KZN033	2	26	20	1	1	1	3	0	1	4	2	0	220	260	84.6	0	24	22	2	0	0	0	0	0	0	240	240	100
KZN034	8	6	5	1	0	0	0	0	0	0	0	7	60	60	100.0	0	24	23	1	0	0	0	0	0	0	240	240	100
KZN035	0	33	25	3	1	2	1	1	3	2	0	0	295	330	89.4	0	24	21	2	0	1	0	0	1	0	230	240	95.8
KZN036	1	30	23	5	1	1	0	0	1	1	0	0	290	300	96.7	1	8	8	0	0	0	0	0	0	0	80	80	100

Laboratory Code	KZN panel (2001-2004)															NHLS panel (2006)												
	Total non returns	Total slides reviewed	Correct	Quantification Errors	LFN	LFP	HFN	HFP	False positives	False negatives	Slide broke	Not evaluated	SCORE	Total Possible Score	Final Score (%)	Total non returns	Total Slides reviewed	Correct	Quantification Errors	False positives	False negatives	High false positive	Low false positive	High false negative	Low false negative	Score	Total possible score	Total score (%)
KZN037	2	27	23	2	0	1	1	0	1	1	0	0	255	270	94.4	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN038	1	27	22	2	1	0	2	0	0	3	0	0	245	270	90.7	0	24	23	0	1	0	1	0	0	0	230	240	95.8
KZN039	0	33	27	4	0	2	0	0	2	0	0	0	320	330	97.0	0	24	23	1	0	0	0	0	0	0	240	240	100
KZN040	3	22	16	3	1	1	1	0	1	2	4	0	200	220	90.9	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN041	0	33	27	4	1	1	0	0	1	1	0	0	320	330	97.0	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN042	0	33	29	2	0	1	1	0	1	1	0	0	315	330	95.5	0	24	21	2	0	1	0	0	1	0	230	240	95.8
KZN043	2	27	22	3	0	0	2	0	0	2	0	0	250	270	92.6	1	16	13	2	0	1	0	0	1	0	150	160	93.75
KZN044	0	33	27	3	0	0	3	0	0	3	0	0	290	330	87.9	1	8	7	1	0	0	0	0	0	0	80	80	100
KZN045	0	0	0	0	0	0	0	0	0	0	0	0	0	0		1	8	5	2	0	1	0	0	1	0	70	80	87.5
KZN046	0	33	17	10	0	3	3	0	3	3	0	0	285	330	86.4	0	24	17	7	0	0	0	0	0	0	240	240	100
KZN047	1	30	24	2	0	1	3	0	1	3	0	0	265	300	88.3	1	0	0	0	0	0	0	0	0	0	0	0	
KZN048	2	27	24	1	0	1	1	0	1	1	0	0	255	270	94.4	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN049	0	33	24	3	1	1	4	0	1	5	0	0	280	330	84.8	0	24	20	3	0	1	0	0	1	0	230	240	95.8
KZN050	0	33	28	2	0	2	1	0	2	1	0	0	310	330	93.9	0	24	23	0	1	0	0	1	0	0	235	240	97.9
KZN051	0	33	28	3	0	1	1	0	1	1	0	0	315	330	95.5	0	24	22	2	0	0	0	0	0	0	240	240	100
KZN052	5	18	17	1	0	0	0	0	0	0	0	0	180	180	100.0	1	8	7	0	1	0	0	1	0	0	75	80	93.8
KZN053	0	32	26	2	1	0	3	0	0	4	0	0	295	320	92.2	0	24	22	2	0	0	0	0	0	0	240	240	100
KZN054	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	24	24	0	0	0	0	0	0	0	240	240	100
KZN055	5	18	13	2	0	0	3	0	0	3	0	0	150	180	83.3	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN056	0	33	28	3	1	0	0	1	1	1	0	0	315	330	95.5	0	24	24	0	0	0	0	0	0	0	240	240	100

Laboratory Code	KZN panel (2001-2004)																NHLS panel (2006)												
	Total non returns	**Total slides reviewed**	**Correct**	**Quantification Errors**	**LFN**	**LFP**	**HFN**	**HFP**	**False positives**	**False negatives**	**Slide broke**	**Not evaluated**	Score	**Total Possible Score**	**Final Score (%)**	**Total non returns**	**Total Slides reviewed**	**Correct**	**Quantification Errors**	False positives	**False negatives**	**High false positive**	**Low false positive**	**High false negative**	**Low false negative**	**Score**	**Total possible score**	**Total score (%)**
KZN057	2	20	14	2	0	0	4	0	0	4	0	15	160	200	80.0	2	0	0	0	0	0	0	0	0	0	0	0	
KZN058	3	23	20	2	1	0	0	0	0	1	2	0	225	230	97.8	1	0	0	0	0	0	0	0	0	0	0	0	
KZN059	3	0	0	0	0	0	0	0	0	0	0	0	0	0		2	0	0	0	0	0	0	0	0	0	0	0	
KZN060	1	30	26	2	0	0	2	0	0	2	0	0	280	300	93.3	1	0	0	0	0	0	0	0	0	0	0	0	
KZN061	2	15	13	0	0	0	2	0	0	2	0	0	130	150	86.7	1	0	0	0	0	0	0	0	0	0	0	0	
KZN062	1	27	24	0	0	1	2	0	1	2	0	0	245	270	90.7	0	16	15	0	0	1	0	0	1	0	150	160	93.8
KZN063	1	30	30	0	0	0	0	0	0	0	0	0	300	300	100.0	1	16	15	0	0	1	0	0	1	0	150	160	93.8
KZN064	0	33	29	2	1	1	0	0	1	1	0	0	320	330	97.0	1	16	13	2	0	1	0	0	1	0	150	160	93.8
KZN065	1	29	24	2	0	3	0	0	3	0	2	0	275	290	94.8	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN066	2	24	16	2	2	1	3	0	1	5	0	0	195	240	81.3	0	8	8	0	0	0	0	0	0	0	80	80	100
KZN067	1	21	14	1	1	1	3	1	2	4	0	0	160	210	76.2	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN068	0	24	23	0	0	0	0	1	1	0	0	0	235	240	97.9	0	8	8	0	0	0	0	0	0	0	80	80	100
KZN069	0	6	6	0	0	0	0	0	0	0	0	0	60	60	100.0	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN070	0	6	5	1	0	0	0	0	0	0	0	0	60	60	100.0	0	16	15	0	1	0	0	1	0	0	155	160	96.9
KZN071	0	3	1	0	0	1	1	0	1	1	0	0	20	30	66.7	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN072	0	3	2	0	0	1	0	0	1	0	0	0	25	30	83.3	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN073	0	3	2	0	0	0	1	0	0	1	0	0	20	30	66.7	1	16	14	0	0	2	0	0	2	0	140	160	87.5
KZN074	1	27	21	4	0	0	2	0	0	2	0	7	250	270	92.6	0	0	0	0	0	0	0	0	0	0	0	0	
KZN075	1	30	24	6	0	0	0	0	0	0	0	0	300	300	100.0	0	0	0	0	0	0	0	0	0	0	0	0	
KZN076	1	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0		

Laboratory Code	KZN panel (2001-2004)															NHLS panel (2006)												
	Total non returns	Total slides reviewed	Correct	Quantification Errors	LFN	LFP	HFN	HFP	False positives	False negatives	Slide broke	Not evaluated	Score	Total Possible Score	Final Score (%)	Total non returns	Total Slides reviewed	Correct	Quantification Errors	False positives	False negatives	High false positive	Low false positive	High false negative	Low false negative	Score	Total possible score	Total score (%)
KZN077	1	30	27	2	0	1	0	0	1	0	0	0	295	300	98.3	0	0	0	0	0	0	0	0	0	0	0	0	
KZN078	2	18	10	4	0	1	1	2	3	1	0	0	145	180	80.6	1	0	0	0	0	0	0	0	0	0	0	0	
KZN079	0	3	2	1	0	0	0	0	0	0	0	0	30	30	100.0	1	8	8	0	0	0	0	0	0	0	80	80	100
Total	**94**	**1800**	**1476**	**143**	**26**	**49**	**95**	**11**	**60**	**121**	**21**	**29**	**16595**	**18000**		**36**	**1279**	**1193**	**54**	**6**	**26**	**1**	**5**	**26**	**0**	**12495**	**12790**	
Total (%)			82	7.9	1.4	2.7	5.3	0.6	3.3	6.7	1.2				92.2			93.3	4.2	0.5	2.0	0.1	0.4	2.0	0			97.7

ANNEXURE 11: SUMMARY OF PROFICIENCY TESTING SCORES BY URBAN/RURAL LABORATORIES IN KWAZULU-NATAL (2001-2006)

	KZN Panel (2001-2004)																	NHLS Panel (2006)												
Laboratory Code	Urban (U) / rural (R)	Total non returns	Total slides reviewed	Correct	Quantification Errors	LFN	LFP	HFN	HFP	False positives	False negatives	Slide broke	Not evaluated	Score	Total possile score	Final score (%)	Total non returns	Total Slides reviewed	Correct	Quantitation Errors	False positives	False negatives	High false positive	Low false positive	High false negative	low false negative	Score	Total possile score	Final score (%)
KZN001	U	3	17	14	2	0	0	1	0	0	1	0	0	160	170	94.1	0	24	23	1	0	0	0	0	0	0	240	240	100
KZN002	U	1	3	1	2	0	0	0	0	0	0	0	0	30	30	100	2	0	0	0	0	0	0	0	0	0	0	0	
KZN003	U	0	28	24	3	0	0	1	0	0	1	0	0	270	280	96.4	1	16	15	0	0	1	0	0	1	0	150	160	93.8
KZN004	U	6	8	4	1	0	0	3	0	0	3	0	0	50	80	62.5	1	16	15	0	0	1	0	0	1	0	150	160	93.8
KZN005	U	0	31	27	1	0	0	2	1	1	2	0	0	280	310	90.3	0	8	8	0	0	0	0	0	0	0	80	80	100
KZN006	U	1	24	21	2	0	1	0	0	1	0	2	0	235	240	97.9	0	24	20	3	0	1	0	0	1	0	230	240	95.8
KZN007	U	4	19	19	0	0	0	0	0	0	0	0	0	190	190	100	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN009	U	1	28	20	5	0	1	2	0	1	2	0	0	255	280	91.1	0	24	20	2	0	2	0	0	2	0	220	240	91.7
KZN010	U	0	31	25	4	0	1	1	0	1	1	0	0	295	310	95.2	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN011	U	0	28	26	2	0	0	0	0	0	0	0	0	280	280	100	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN013	U	0	31	27	0	0	3	1	0	3	1	0	0	285	310	91.9	0	20	18	2	0	0	0	0	0	0	200	200	100
KZN015	U	1	28	24	0	0	2	1	1	3	1	0	0	250	280	89.3	0	24	21	1	0	2	0	0	2	0	220	240	91.7
KZN016	U	0	31	27	4	0	0	0	0	0	0	0	0	310	310	100	0	24	22	1	0	1	0	0	1	0	230	240	95.8
KZN017	U	3	22	17	1	0	2	2	0	2	2	0	0	200	220	90.9	0	24	23	1	0	0	0	0	0	0	240	240	100
KZN019	U	0	31	28	2	0	0	1	0	0	1	0	0	300	310	96.8	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN021	U	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	19	18	0	0	1	0	0	1	0	180	190	94.7

KZN023	U	0	30	29	1	0	0	0	0	0	0	2	0	300	300	100	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN052	U	5	17	16	1	0	0	0	0	0	0	0	0	170	170	100	1	8	7	0	1	0	0	1	0	0	75	80	93.8
KZN055	U	5	17	12	2	0	0	3	0	0	3	0	0	140	170	82.4	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN056	U	0	31	27	3	0	0	0	1	1	0	0	0	300	310	96.8	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN057	U	2	19	13	2	0	0	4	0	0	4	0	13	150	190	78.9	2	0	0	0	0	0	0	0	0	0	0	0	
KZN058	U	3	21	19	2	0	0	0	0	0	0	2	0	210	210	100	1	0	0	0	0	0	0	0	0	0	0	0	
KZN060	U	1	29	25	2	0	0	2	0	0	2	0	0	270	290	93.1	1	0	0	0	0	0	0	0	0	0	0	0	
KZN061	U	2	13	11	0	0	0	2	0	0	2	0	0	110	130	84.6	1	0	0	0	0	0	0	0	0	0	0	0	
KZN062	U	1	25	22	0	0	1	2	0	1	2	0	0	225	250	90	0	16	15	0	0	1	0	0	1	0	150	160	93.8
KZN063	U	1	28	28	0	0	0	0	0	0	0	0	0	280	280	100	1	16	15	0	0	1	0	0	1	0	150	160	93.8
KZN064	U	0	31	28	2	0	1	0	0	1	0	0	0	305	310	98.4	1	16	13	2	0	1	0	0	1	0	150	160	93.8
KZN066	U	2	22	16	2	0	1	3	0	1	3	0	0	185	220	84.1	0	8	8	0	0	0	0	0	0	0	80	80	100
KZN067	U	1	20	14	1	0	1	3	1	2	3	0	0	155	200	77.5	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN069	U	0	6	6	0	0	0	0	0	0	0	0	0	60	60	100	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN070	U	0	6	5	1	0	0	0	0	0	0	0	0	60	60	100	0	16	15	0	1	0	0	1	0	0	155	160	96.9
KZN072	U	0	3	2	0	0	1	0	0	1	0	0	0	25	30	100	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN073	U	0	3	2	0	0	0	1	0	0	1	0	0	20	30	66.7	1	16	14	0	0	2	0	0	2	0	140	160	87.5
KZN079	U	0	3	2	1	0	0	0	0	0	0	0	0	30	30	100	1	8	8	0	0	0	0	0	0	0	80	80	100
KZN077	U	1	28	26	1	0	1	0	0	1	0	0	0	275	280	98.2	0	0	0	0	0	0	0	0	0	0	0	0	
Total (%)		44	712	607	50	0	16	35	4	20	35	6	13	6660	7120	93.5	18	535	506	13	2	14	0	2	14	0	5200	5350	97.2

Laboratory Code	KZN Panel (2001-2004)																NHLS Panel (2006)											
	Urban (U) / rural (R)	Total non returns	Total slides reveiwed	Correct	Quantification Errors	LFN	LFP	HFN	HFP	False positives	False negatives	Slide broke	Not evaluated	Score	Total possile score	Final score (%)	Total non returns	Total Slides reviewed	Correct	Quantitation Errors	False positives	False negatives	High false positive	Low false positive	High false negative	low false negative	Score	Total possile score	Final score (%)
KZN008	R	0	28	23	1	0	3	1	0	3	1	0	0	265	280	94.6	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN012	R	2	22	13	2	0	0	6	1	1	6	0	0	150	220	68.2	0	16	14	1	0	1	0	0	1	0	150	160	93.8
KZN014	R	0	31	29	2	0	0	0	0	0	0	0	0	310	310	100	0	24	23	1	0	0	0	0	0	0	240	240	100
KZN018	R	2	22	21	0	0	0	1	0	0	1	0	0	210	220	95.5	0	24	22	2	0	0	0	0	0	0	240	240	100
KZN020	R	0	31	23	3	0	1	4	0	1	4	0	0	265	310	85.5	2	8	8	0	0	0	0	0	0	0	80	80	100
KZN022	R	2	25	20	3	0	0	2	0	0	2	0	0	230	250	92	0	24	22	1	1	0	0	1	0	0	235	240	97.9
KZN024	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	24	24	0	0	0	0	0	0	0	240	240	100
KZN025	R	0	31	30	1	0	0	0	0	0	0	0	0	310	310	100	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN026	R	0	31	27	1	0	2	1	0	2	1	0	0	290	310	93.5	0	24	22	2	0	0	0	0	0	0	240	240	100
KZN027	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0		2		O	O	O	O	0	0	0	0	0	0	
KZN028	R	2	25	23	2	0	0	0	0	0	0	0	0	250	250	100	1	16	15	0	1	0	0	1	0	0	155	160	96.9
KZN029	R	8	8	6	0	0	0	1	1	1	1	0	0	60	80	75	1	16	14	0	0	2	0	0	2	0	140	160	87.5
KZN030	R	0	26	21	1	0	1	2	1	2	2	5	0	225	260	86.5	0	24	18	4	0	2	0	0	2	0	220	240	91.7
KZN031	R	1	28	19	1	0	1	7	0	1	7	0	0	205	280	73.2	0	24	21	2	0	1	0	0	1	0	230	240	95.8
KZN032	R	2	25	22	1	0	1	1	0	1	1	0	0	235	250	94	0	24	22	1	0	1	0	0	1	0	230	240	95.8
KZN033	R	2	24	19	1	0	1	3	0	1	3	2	0	205	240	85.4	0	24	22	2	0	0	0	0	0	0	240	240	100
KZN034	R	8	6	5	1	0	0	0	0	0	0	0	7	60	60	100	0	24	23	1	0	0	0	0	0	0	240	240	100
KZN035	R	0	31	24	3	0	2	1	1	3	1	0	0	280	310	90.3	0	24	21	2	0	1	0	0	1	0	230	240	95.8
KZN036	R	1	29	23	5	0	1	0	0	1	0	0	0	285	290	98.3	1	8	8	0	0	0	0	0	0	0	80	80	100
KZN037	R	2	26	22	2	0	1	1	0	1	1	0	0	245	260	94.2	1	16	16	0	0	0	0	0	0	0	160	160	100

KZN038	R	1	25	21	2	0	0	2	0	0	2	0	0	230	250	92	0	24	23	0	1	0	1	0	0	0	230	240	95.8
KZN039	R	0	31	25	4	0	2	0	0	2	0	0	0	300	310	96.8	0	24	23	1	0	0	0	0	0	0	240	240	100
KZN040	R	3	20	15	3	0	1	1	0	1	1	4	0	185	200	92.5	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN041	R	0	31	26	4	0	1	0	0	1	0	0	0	305	310	98.4	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN042	R	0	31	27	2	0	1	1	0	1	1	0	0	295	310	95.2	0	24	21	2	0	1	0	0	1	0	230	240	95.8
KZN043	R	2	25	20	3	0	0	2	0	0	2	0	0	230	250	92	1	16	13	2	0	1	0	0	1	0	150	160	93.75
KZN044	R	0	31	26	2	0	0	3	0	0	3	0	0	270	310	87.1	1	8	7	1	0	0	0	0	0	0	80	80	100
KZN045	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0		1	8	5	2	0	1	0	0	1	0	70	80	87.5
KZN046	R	0	31	15	10	0	3	3	0	3	3	0	0	265	310	85.5	0	24	17	7	0	0	0	0	0	0	240	240	100
KZN047	R	1	28	22	2	0	1	3	0	1	3	0	0	245	280	87.5	1	0	0	0	0	0	0	0	0	0	0	0	
KZN048	R	2	26	23	1	0	1	1	0	1	1	0	0	245	260	94.2	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN049	R	0	31	23	3	0	1	4	0	1	4	0	0	265	310	85.5	0	24	20	3	0	1	0	0	1	0	230	240	95.8
KZN050	R	0	31	26	2	0	2	1	0	2	1	0	0	290	310	93.5	0	24	23	0	1	0	0	1	0	0	235	240	97.9
KZN051	R	0	31	26	3	0	1	1	0	1	1	0	0	295	310	95.2	0	24	22	2	0	0	0	0	0	0	240	240	100
KZN053	R	0	30	25	2	0	0	3	0	0	3	0	0	280	300	93.3	0	24	22	2	0	0	0	0	0	0	240	240	100
KZN054	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	24	24	0	0	0	0	0	0	0	240	240	100
KZN059	R	3	0	0	0	0	0	0	0	0	0	0	0	0	0		2	0	0	0	0	0	0	0	0	0	0	0	
KZN065	R	1	27	22	2	0	3	0	0	3	0	2	0	255	270	94.4	1	16	16	0	0	0	0	0	0	0	160	160	100
KZN068	R	0	22	21	0	0	0	0	1	1	0	0	0	215	220	97.7	0	8	8	0	0	0	0	0	0	0	80	80	100
KZN071	R	0	3	1	0	0	1	1	0	1	1	0	0	20	30	66.7	0	24	24	0	0	0	0	0	0	0	240	240	100
KZN074	R	1	25	19	4	0	0	2	0	0	2	0	7	230	250	92	0	0	0	0	0	0	0	0	0	0	0	0	
KZN075	R	1	28	22	6	0	0	0	0	0	0	0	0	280	280	100	0	0	0	0	0	0	0	0	0	0	0	0	
KZN076	R	1	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0		
KZN078	R	2	16	8	4	0	1	1	2	3	1	0	0	125	160	78.1	1	0	0	0	0	0	0	0	0	0	0	0	
Total (%)		50	972	783	89	0	33	60	7	40	60	13	14	8905	9720	91.6	18	744	687	41	4	12	1	3	12	0	7295	7440	98.1

ANNEXURE 12: CLASIFICATION OF TB MICROSCOPY LABORATORIES INTO THE NINE REGIONS IN KWAZULU-NATAL

REGION 1	REGION 4	REGION 8
ADDINGTON	CATHERINE BOOTH	CHURCH OF SCOTLAND
ALBERT LUTHULI	EKOMBE	DUNDEE
CLAIRWOOD	EMPANGENI	GREYTOWN
KING EDWARD	ESHOWE	MADADENI
KING GEORGE V	MBONGOLWANE	NEWCASTLE
KWAMASHU POLY	NGWELEZANA	CHARLES JOHNSON
MAHATMA GHANDI	NKANDLA	
OSINDISWENI		
PRINCE MSHIYENI		
R K KHAN	**REGION 5**	**REGION 9**
WENTWORTH	STANGER	EZAKHENI
	UMPHUMULO	DURBAN CHEST CLINIC
REGION 2	UNTUNJAMBILI	DON MCKENZIE HOSPITAL
APPELBOSCH		ST MARY'S MARRIANHILL
EDENDALE		NEW GERMANY/BHEKIMPILO TRUST
EMMAUS	**REGION 6**	ST MARY'S MELMOTH
ESTCOURT	BENEDICTINE	MC CORDS HOSPITAL
GREYS	BETHESDA	LAMONTVILLE CLINIC
LADYSMITH	HLABISA	PINETOWN CHEST CLINIC
MONTEBELLO	MANGUZI	TONGAAT CHEST CLINIC
NORTHDALE	MOSVOLD	KHOTSONG
	MSELENI	DUNSTAN FARREL
REGION 3		CHURCH STREET CLINIC
CHRIST THE KING		IMBALENHLE CLINIC
KOKSTAD	**REGION 7**	PHOLELA COMMUNITY HEALTH CENTRE
MURCHISON	CEZA	FOSA TB LAB
PORT SHEPSTONE	ITSHELEJUBA	INANDA CLINIC
SCOTTBURGH	NKONJENI	NDWEDWE HEALTH CLINIC
ST ANDREWS	VRYHEID	VERULUM HEALTH CENTRE
ST APOLLINARIS		RICHMOND CLINIC
TAYLER BEQUEST		E.G. & USHER MEMORIAL
		G.J.CROOKS
		LOWER UMFOLOZI
		MRC
		SUNDUMBILI CLINIC
		MPOPHOMENI CLINIC

Annexure 13: Map of Districts and NHLS Laboratories in KZN

Map of NHLS Laboratories in KZN

Map of Health Districts in KZN