

*Full Length Research Paper*

# Comparison of acid-fast bacilli (AFB) positivity rate obtained by applicator stick and the plastic loop in a high TB incidence population of Zambia

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The discovery of the causative organism of Tuberculosis (TB) dates back to 1882 by Robert Koch and described the isolation of the tubercle bacilli. Since then, a large number of *Mycobacterium* species responsible for causing pulmonary and extra pulmonary infections have been identified. Tuberculosis (TB) is an airborne disease caused by a pathogen belonging to the species *Mycobacterium tuberculosis*. Infection is spread through aerosols released in the air when a contagious person speaks, coughs, sneezes or sings. TB and poverty come together to perpetuate a vicious cycle and contributes to its spread as people are forced to share crowded living quarters and are often in overall poor health. Simultaneously, costs associated with diagnosis and treatment creates further hardship, both for patients and their families including their children. Worldwide, TB creates hundreds of thousands of orphans, increases child malnutrition and forces many children to leave school in order to work and care for the family. Tuberculosis presents a major threat to the health of the population of Zambia. It is one of the leading causes of morbidity, accounting for 13% of all adult hospital deaths and being one of the top ten leading causes of hospital admissions. Tb is curable if detected early but delayed diagnosis result in disease progression and spreads rapidly from person to person. Failure to identify cases early by health providers and also inability to adhere to treatment results into problems and untold misery.

**Key words:** *Mycobacterium tuberculosis*, tubercle bacilli, aerosols, Zambia.

## INTRODUCTION

*Mycobacterium tuberculosis* (MTB) diagnosis using smear microscopy is the appropriate technology due to its easy procedure, cheap nature, may be performed with or without electricity, availability of reagents and skilled personnel. The disposable plastic loop is standardized (3 mm diameter) than the applicator stick which is not standardised, and it adequately picks bacilli equivalent to 10 mm per drop of sputum. Sputum is mucous and may be slippery so with the applicator stick it is difficult to pick the desired portion of the sputum sample than the loop.

Methods for making smears before, used to be standardized loop, but this was switched to applicator stick without scientific evidence. Therefore, this study aimed at making comparison between the two methods and thus, determined the factors that led to this switch and to assess the performance of the applicator stick.

## Objective

To compare the results of acid-fast bacilli (AFB) smear positivity rate obtained by the use of standard plastic loop and applicator stick in a high TB incidence population of Zambia.

## Specific objectives

1. To examine sputum smears to confirm TB diagnosis;
2. To compare the positivity, agreement, smear positive and false-negative rates between standardized plastic loop and applicator stick;
3. To investigate effects of the quality of sputum on the two methods compared;

4 To determine the recommendations for the TB diagnosis and control.

## MATERIALS AND METHODS

This was a prospective comparison study by using the Ziehl-Neelsen (ZN) staining technique. The sputum was from patients who never and patients who had been on treatment, specimen quality was well maintained and no specimen was older than 3 days. Proper sample collection procedures were followed and three consecutive day sample (spot, morning, spot) were collected and sent to the laboratory, the samples were processed on the same day. Sputum smear examination request form indicating whether the examination was for diagnosis or bacteriological follow-up of treatments, were recorded in the log book. Sample receipted was 2 to 10 ml, and less than that was rejected as part of quality control. The slides were numbered, sputum smeared by applicator stick and plastic loop for each slide separately, air dried and heat fixed. Safety measures while processing the specimens were applied according the safety manual.

### Internal quality control

Internal quality control included monitoring quality of stain, staining procedure, by using known positive slide for acid-fast bacilli was included on staining. Distilled water was used for rinsing. The WHO standard grading scale was used during reading of the smears. And smear reading of the applicator stick was blinded to that of the plastic loop.

### Type of sampling method

Probability sampling method was used. The variation of 20 and 50% smear positivity between health facilities in the country was adopted in calculating sample size and the sample size was 426 smears.

## RESULTS

426 samples examined and 253 were AFB positive by at least one method then 231 of 426 results were in agreement (173 negative, 58 positive) and 16 of 58 samples positive by both methods were in agreement by AFB quantity. 187 samples positive by the standard loop were negative by the applicator stick. 8 samples positive by the applicator stick were negative by the standard loop. The smear positivity rate of the applicator stick was only 15.49% (66/426) 95% (CI), 80.2 to 89.7 whereas the positivity rate of the standardized loop was 57.5% (245/426) 95% (CI), 41.7 to 54.0. The plastic loop and applicator stick were both disposable after decontamination with 5% phenol, but the applicator stick posed danger to cause injury when breaking during smear making.

## DISCUSSION

The sputum was both from patients who never or have

been on treatment. Specimen quality was well maintained for no specimen was older than 3 days as stated on inclusion criteria. The agreement rate of smear result of the applicator stick and the plastic loop was 59.38% (253/426)

The positivity rate of the applicator stick was at 15.49% (66/426); the positivity rate of the standard plastic loop was at 57.5% (245/426). This showed double improvement in TB diagnosis. 187 smear results of the applicator stick indicated negative but with the plastic loop as actual (1 to 9 AFB). This was false negative on the applicator stick method; this was attributed to many factors, for example, breaking of the applicator stick causing the edges to become rough and some bacilli sticks in the rough edges, and lack of specified volume.

The 8 smear results of the applicator stick on the other hand indicated actual (1 to 9 AFB) while plastic loop was negative. This was false positive, false positives which may be due to acid-fast particles other than tubercle bacilli includes; food particles, precipitated stains, fibres and pollens, scratches on the slides and contamination through the transfer of bacilli from one smear to another. TB medications are wasted, in follow up cases the intensive phase of treatment is continued longer than necessary and patients may lose confidence (Management of Tuberculosis, 2000). For false negative result, the patients with TB are not treated, resulting in more suffering, spread of TB and eventually premature death. Intensive phase treatment is not extended for the required duration, resulting in inadequate treatment (Management of Tuberculosis, 2000). 37 smears indicated 1+ on the plastic loop while it was negative by the applicator stick. This was a major error and diagnosis was gravely missed. The amount of sputum on a slide may approximately be 0.01 ml delivered by a loop with an internal diameter of 3 mm, this sputum once spread over an area of 600 mm<sup>2</sup> (20 × 30 mm) and the area observed in the microscopes is about 0.02 mm<sup>2</sup> 10 000 such fields need to be screened in order to examine the whole smear. If 100 oil-immersion fields are examined, only 1% of the smear is screened. Thus, if a sputum specimen contains about 500 bacilli per ml the whole smear may contain about 50 bacilli and if these 50 bacilli were evenly distributed over the 10,000 fields of the smear, there would be 1 bacillus in 100 fields, therefore by examining 100 fields there would be a 50% chance of finding this bacillus. By examining 300 fields there is an approximately 50% chance of finding 3 bacilli (recommended as the minimum number for a smear to be reported as positive (Toman, 1979). Table 1 shows the number of bacilli observed in smears, concentration of culturable bacilli in sputum specimens and the probability of positive results

The applicator stick is only able to pick AFB in a high yielded sputum sample. The quality of specimen, staining, cleanness, as assessed showed 85% satisfactory.

**Table 1.** The number of bacilli observed in smears, concentration of culturable bacilli in sputum specimens and the probability of positive results.

Number of bacilli observed	Estimated concentration of bacilli per ml of specimen	Probability of a positive result (%)
0 in 100 fields	less than 1000	less than 10
1 to 2 in 100 fields	5,000 -10,000	50
1 to 9 in 100 fields	about 30,000	80
1 in 10 fields	about 50,000	90
1 to 9 per field	about 100,000	96.2
10 or more	about 500,000	99.95

## Conclusion

The applicator stick is not an ideal tool to use for smear making .The loop, which is 3 mm diameter, is ideal to use in order to capture many smear positives in a high TB Incidence population.

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