

## ***Short Research Article***

### **Feasibility for Same Day Tuberculosis Diagnosis Using the Smear Microscopy Approach in Rural South Western Uganda**

#### **ABSTRACT**

##### **Background:**

Current WHO guidelines require that TB suspects submit 2 sputum samples on 2 different days which is time consuming, needs multiple visits leading to a high dropout. We studied the feasibility of same day sputum smear microscopy in rural refugee settlement in South Western Uganda.

##### **Methods:**

Sputum specimens were collected from tuberculosis suspects at two health centers in Nakivale refugee settlement in South Western Uganda. Patients submitted two spot samples one hour apart and an early morning sputum sample. All samples were stained by Ziehl-Nielsen stain. The first two samples were examined on the first day and the morning sample was examined on day 2. Results for the two spot samples were given on day one and the morning sample results were given on day 2. Patients found to have TB were referred for treatment on reception of their results.

##### **Results:**

Of the 316 TB suspects, 190 (60.1%) were males and the mean age of 40 years. Overall smear positivity rate was 46/ 316 (15.0%). Of the 40 smear positive TB cases, 38 (95%) were positive on the spot1, 35 (92.5%) on spot 2 while 31 (85%) were positive on the early morning specimen. Only one TB suspect had negative sport1 sample that was positive with spot2 and early morning. Three TB suspects who had positive spot1 result did not return with spot2 sample. Six (15%) patients with a positive spot 1 did not bring the early morning sample. Though the bacillary load differed on the spot2 and morning samples the difference was not significant  $P > 0.05$ , all samples that were positive on morning sample were also positive on the spot 2. There was no association between HIV and TB infection.

##### **Conclusion:**

Same day smear microscopy for diagnosing tuberculosis is feasible in a rural setup by examining two spot samples.

**Keywords:** Same day microscopy, Tuberculosis, ZN,

#### **1. INTRODUCTION**

In most TB high burden countries, like Uganda, the infrastructure for the diagnosis of infectious diseases is not adequately resourced. Despite numerous technical advances, microscopy remains the cornerstone of TB diagnosis, particularly in developing countries.[1, 2] Owing to low sensitivity of sputum smear microscopy, the diagnosis of TB requires repeated sputum examinations on several days [3]. The only diagnostic technique for TB, suitable to peripheral levels of health services, is serial sputum smear microscopy with

Ziehl Neelsen (ZN) staining. Patients submit sputum samples over multiple days incur considerable costs. Direct sputum smear examination by Ziehl Neelsen (ZN) stain is a simple, economical tool that is widely used not only for diagnosis of tuberculosis but also for monitoring treatment of tuberculosis in several countries. It is also a key component of the DOTs and DOTS plus strategies for management of tuberculosis. With the current World Health Organization (WHO) policy on PTB diagnosis (spot-morning)[4], there is a need for a patient to make a minimum of two visits to the health facility in a bid to submit all required sputum samples, and then acquire laboratory results and treatment. This protracts the diagnostic process. The cost incurred to complete the ~~diagnostic pathway~~ is prohibitively high even though the TB services offered in Nakivale refugee settlement are free of charge. There is a likelihood that patient dropout will be high. Failing to complete the diagnosis, therefore, is a major obstacle to accessing treatment in these settings.

The WHO has recently recommended that it is sufficient to examine two specimens per patient in locations where smear microscopy is quality assured, and that these specimens can be collected in an accelerated scheme (called front-loaded or same-day microscopy). This study was carried out to establish the feasibility of same day tuberculosis diagnosis using sputum smear microscopy in a Refugee settlement in rural South Western Uganda

## 2. MATERIAL AND METHODS

The study was conducted at Nakivale Health Centre III and Kibengo Health Centre II in Nakivale refugee settlement located in Isingiro district, South Western Uganda. The settlement hosts refugees mainly from the Democratic Republic of Congo, Rwanda, Somalia, Burundi, Ethiopia and Eritrea. Sputum specimens were collected from 316 randomly selected tuberculosis suspects who attended at the Health units. **The patient was explained the importance of submitting sputum rather than saliva.** ~~Patients submitted a first spot sample and then a second spot sample after one hour and home collected morning sputum sample[5].~~ All the samples received were given different numbers and stained by acid fast Ziehl-Neelsen (ZN) stain. The first two samples were examined on the first day and the morning sample was examined on the second day. ZN staining technique and smear grading were done as per the Uganda national guidelines. For quality control, all the positive slides and randomly, 5% of negative slides were screened by another technician at Mbarara University of Science and Technology. Patients were referred for treatment as soon they received their results. We performed data analysis using Stata Version 12.1 (Stata Corp., College station Texas). Percentages and proportions were calculated and a **Pvalue** < 0.05 was regarded as significant. ~~This study received ethical clearance from the faculty research and ethics committee of the faculty of medicine of Mbarara University of Science and Technology and the institutional review board of Mbarara University of Science and Technology~~

## 3. RESULTS

Out of the 316 patients recruited in the study, 190 (60.1%) were males and 127 (39.9%) were females with a mean age of 40years. ~~As shown in table 1, 316 submitted the first sample, 262 submitted the second spot sample and 253 submitted the morning sample. Of the 316 TB suspects, 54 (17%) and 63 (20%) did not submit the spot2 and early morning samples respectively~~

62 The overall smear positivity rate was 46/ 316 (15.0%. Out of the 40 smear positives TB  
 63 cases, 38(95%) were positive on the spot1, 35 (92.5%) were positive on the spot 2 while  
 64 31(85%) were positive on the early morning specimen. (See table 1 below)

65 Only one TB suspect had negative sport1 sample that was positive with spot2 and early  
 66 morning. Of the 40 Smear positive TB cases on spot1, 6(15%) patients did not bring the  
 67 early morning sample. There were 3 TB suspects who had spot1 results but did not return  
 68 with spot2 sample (See table 1 below)

69 **Table 1: ~~Positivity rate versus~~ timing of specimen collection**

	Spot1 N (%)	Spot2 N (%)	Early morning N (%)
Negative	271 (85.02)	225(85.66)	219(86.95)
Positive	45(14.98)	42(14.34)	35(13.08)
Total	316	262	253

70

71 ~~Table 2 below shows that,~~ though the bacillary load ~~differed on~~ the spot2 and morning  
 72 samples the difference was not significant  $P > 0.05$ , all samples that were positive on  
 73 morning sample were also positive on the spot 2. One sample was negative on spot 1 but  
 74 positive on spot2 and morning and one sample was positive on spot1 (1-9/100) but negative  
 75 on spot2 and morning. The patient who had a scanty positive ZN result on spot1 was also  
 76 HIV positive.

77 **Table 2: Bacillary load of the samples collected at the different times**

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<del>Timing</del> of sample	Bacilli load				
	1-9/100	1+	2+	3+	Total
spot1	4	7	7	27	45
spot2	2	6	7	27	42
Morning	0	4	7	24	35

79 Of the 316 TB suspects who submitted the first sample 9.2 % ( 29/ 316) did not submit the  
 80 ~~second and~~ second spot and 19% (60/316) failed to submit the morning sample. Three Tb  
 81 suspects that had a positive ZN smear for ~~the~~ spot1 did not submit the second spot and four  
 82 TB suspects that had a positive ZN smear for spot2 did not submit the morning sputum  
 83 sample.

84 HIV and ZN Positivity among those who were sputum positive 24 were HIV negative, 21  
 85 were HIV positive and for the HIV status was unknown (see table 3).  
 86 There was no association between HIV and TB infection. ~~50% of TB infected people had HIV~~  
 87 ~~as well as those negative for HIV, with chi square test of 0.49 and P= 0.48~~

88 **Table 3: HIV status and ZN results**

ZN Results	HIV status		Total n(%)
	Neg n(%)	Pos n(%)	
<b>Neg</b>	131 (56.73)	107(43.27)	238(100.00)
<b>Pos</b>	25(54.35.)	21(45.65)	46 (100.00)
<b>Total</b>	156(55.67)	128(44.33)	284 (100.00)

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90 **4. DISCUSSION**

91 Many patients screened for TB abandon the diagnostic process and 20% of the participants  
 92 in the present study failed to bring the second day specimens.

93 As patients screened with spot-morning and spot-morning-spot specimens receive all test  
 94 results together, when all specimens have been examined, patients not returning the second  
 95 day fail to start treatment even if their first smear was positive. Under routine conditions, this  
 96 number is much more[6-8]. Examination of two spot smears enables us to identify most  
 97 smear-positive cases on the first day they consult which is beneficial in our setting and would  
 98 lead to more TB suspects completing the diagnosis process and be able to start treatment  
 99 thereby limiting transmission.

100 Most smear-positive patients were identified by the first smear. This study shows that the  
 101 spot-spot and spot-morning schemes have similar yields. This indicates that front-loaded TB  
 102 diagnostic services are feasible and would not be associated with significantly less yield than  
 103 the equivalent standard approach.

104 Although it was widely accepted that overnight specimens were more likely to contain more  
 105 bacilli, it was also acknowledged that in less favorable circumstances, it was more practical  
 106 to obtain specimens at the time the patient was attending the service. Though sputum  
 107 sample collected in the morning is 10% more sensitive compared to spot sample because of  
 108 higher bacillary load[9]. In our study there was no big difference in the yields of the spot2  
 109 sample and morning sample. Therefore as per our study, if two spot samples are properly  
 110 collected on the same day, we can do away with morning samples as the difference is not  
 111 statistically significant.

112 Further, this same day diagnostic approach for PTB can help to initiate therapy on the same  
 113 day and can save time as well as resources of the patients.

114 **5. CONCLUSION**

115  
 116 According to the findings of this study there is sufficient generalizable evidence that same-  
 117 day diagnosis using the spot spot alogarithm (microscopy of two consecutive spot-spot  
 118 sputum specimens) is equivalent, in terms of diagnostic accuracy, to conventional case-  
 119 finding strategies by microscopy that is to say spot – morning sputum specimens. Same day  
 120 smear microscopy for diagnosing tuberculosis is feasibly in a rural setup as the two spot  
 121 samples on the same day yielded comparable results and there was no much gain from  
 122 early morning sample.  
 123

## COMPETING INTERESTS

The authors declare that they have no competing interests

## CONSENT

"All authors declare that 'informed consent was obtained from all the participants.

## ETHICAL APPROVAL

This study was approved by the Institutional Ethics review committee of Mbarara University of Science and technology and the Uganda National Council for Science and Technology

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