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Impact of Sputum Sampling Instructional Video on Ziehl-Neelsen Positivity in Tuberculosis Diagnostic Centers at BOMA: Multicenter Randomized Controlled Trial

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Abstract

Background and Purpose: Tuberculosis is a global scourge. In DRC, Ziehl-Neelsen (ZN) is the most widely used reference test for the detection of Mycobacterium tuberculosis. Its performance can be improved by certain strategies before sputum collection, particularly the instructional video. The purpose of this study is to evaluate the effectiveness of the instructional video showing and explaining appropriate sputum collection techniques for the diagnosis of tuberculosis. **Methods**: Multicenter, single-blind, randomized controlled trial conducted in Boma. Two hundred suspected TB assigned at random, either to the intervention group (n = 100) before sputum collection, or to the control group (n = 100). Sputum quality was assessed by appearance and volume before ZN staining by laboratory technicians blind to study groups. **Results**: The mean age was 38.1 ± 16.0 years. In the two samples (Contact and morning), the positive smear rate was very significant ($p < 0.0001$) in the intervention group vs control group (46% vs 19% contact smear and 48% vs 18% morning smear), the smear bacillary density rate was very significant ($p < 0.0001$) in the video group vs non-video group (e.g. 20% vs 4% more density) and, the rates of the mucous appearance purulent and volume ≥ 3 ml were very significant ($p < 0.0001$) in the intervention group vs control group (91% vs 66% aspect and 54% vs 21% volume). **Conclusion**: Instructional video is a strategy that improves ZN yield and sputum quality.

Subject Areas

Internal Medicine, Public Health

Keywords

Tuberculosis, Video, Ziehl-Neelsen, Boma

1. Introduction

Tuberculosis (TB) is a plague worldwide. The World Health Organization (WHO) estimates its incidence at 10 million cases, its mortality at 1.6 million deaths as well as 558,000 cases of multidrug-resistant TB (MDR-TB) [1]. The Democratic Republic of Congo (DRC) ranks 3rd in Africa and 9th in the world with an estimate of 270,000 new cases in 2018 (or 321 new cases per 100,000 inhabitants) [2]. TB is the 8th leading cause of death worldwide, the 3rd leading cause of death from infectious disease and the 1st leading cause of death due to a single bacterium [3] [4]. To contain TB, several strategies have been put in place, among others: the DOTS strategy (1993) [5] [6] [7], the Stop TB strategy (2006) [8] [9] and the End TB strategy (2015) [10]. All of these strategies have decreased: the incidence rate of 2% per year; the TB death rate of about 3% per year; the case fatality rate fell from 20% in most countries of the African region to 16% in 2017 [1]. From a diagnostic standpoint, WHO has recommended Xpert MTB/RIF as the only Rapid Screening Test (RDT) since 2010 [11]. Established in the DRC in 2012, this test has the advantage of screening both for Mycobacterium Tuberculosis and resistance to Rifampicin [8] [11] [12] [13]. Some countries have already adopted it as the initial diagnostic test for anyone with signs and symptoms of pulmonary TB [12]. However, in the DRC due to geographic and financial obstacles, this test is more used as a first-line test for eligible people such as PLHIV, children under 15, relapse and retreatment cases, patients with smears in the second month treatment (F2) and third (F3) are always positive, cases contact MDR-TB and in active case finding (ACR). Also its national coverage is weak, being limited to the big cities and to the Tuberculosis Diagnostic Centers (CSDT) with a high volume of work [8]. Thus, the Ziehl-Neelsen (ZN) is the test mainly used in the DRC with around 1 million smears in 2018, including 3089 in Boma (source: Boma urban health zone). This diagnostic tool is rapid, the cost is low, generalizable to all and ensures optimal national coverage. Although its sensitivity is low (60% to 70% compared to the culture considered as gold standard), ZN is used brilliantly in countries with high TB endemicity because of its high specificity for TM [14]. However, regardless of the method used, the optimal performance of a test depends on the quality of the sputum samples. A test performed on good quality sputum, that is, sputum coughed up from the bottom of the lungs, which are the sites of active disease, maximizes the chances of getting an accurate diagnosis [15] [16] [17] [18]. Instructions on how to produce a good sputum sample are included in the

National Tuberculosis Control Program (PNLT) manuals, but it is extremely difficult to formulate them in a way that patients can understand [16] [19]. A randomized controlled trial conducted in Tanzania (2015) showed that instructional video on sputum submission leads to increased detection of TB cases [16]. In the DRC, to our knowledge, no study has examined the impact of an instructional video on sputum collection for microscopy. This absence justifies the initiation of this study. The overall objective of this study is to assess the effectiveness of the instructional video showing and explaining appropriate sputum collection techniques for the diagnosis of tuberculosis.

2. Patients and Methods

This study was a single-blind, multicenter randomized controlled trial, conducted in the town of Boma (**Figure 1**), simultaneously at the General Reference Hospital (HGR) in the West of Boma, at the Center Mères et Enfants Kiveve in the North of Boma and in the Center of Health Oracle (formerly Forabola/Soforma) east of Boma, which covered a period from October 29, 2018 to February 18, 2019. These three CSDTs are specialized in TB screening.

The inclusion criteria were to be 15 years of age and over, to have symptoms or signs suggestive of TB and a notion of contagion, to have submitted two sputum samples (contact sample and morning sample) and to have consented in writing or verbally to participate in the study. We did not include bedridden patients who did not submit two sputum samples and did not consent to participate in the study.

To get our sample size, we should first know the number of participants per study group. This number was calculated by considering a baseline smear-positive prevalence of 50% in the DRC and a power of 80% to detect a difference in prevalence of 20% between the two groups with a significance level of 0.05 according to the formula:

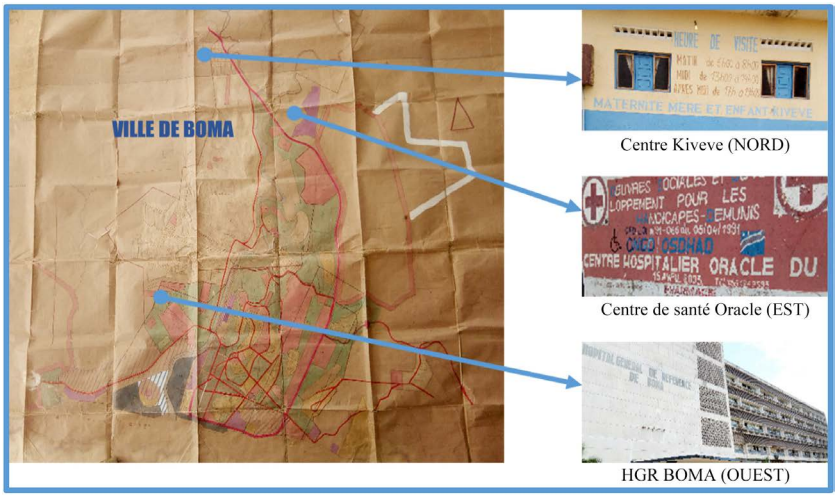


Figure 1. The map of the town of Boma. Source: Mr ETAFE (retired from town planning and housing). The target population consisted of suspected tuberculosis patients referred for screening by healthcare staff (doctor or nurse).

$n \geq 2\left(Z_{\alpha} + Z_{1-\beta}\right)^2 \times p(1-p) \Big/ \left(p_0 - p_1\right)^2$ [20] n = number of participants, α = risk of error, $(1 - \beta)$ = the desired power and β the corresponding risk, p_0 = the expected proportion of subjects in the control group, p_1 = the expected proportion of subjects in the intervention group, Z_{α} = this is the value of $Z_{1-\alpha}$ for a one-sided test, $Z_{1-\beta}$ = this is the value of Z for a test power of $1 - \beta$ for both a one-sided and two-sided test. The result was 74.4 participants per group which we rounded to 100 per group. However, each participant should give two samples, which gives us a total of 200 samples per group. Since we have two groups, this brings to 400 sputum samples.

In the present study, we used the material listed below: recruitment register comprising on the left the consecutive numbers of the study and on the right the codes of the slides examined, eligibility form, consent form and/or free assent and lighted, data collection sheet, small opaque plastic bucket for randomization, graduated and transparent plastic spittoons 3 cm high, 4 cm in diameter at the base and 5 cm in diameter at the top. The graduation was done using an indelible ink marker. The lines were placed at the level of 2 ml, 3 ml and 5 ml so as to obtain 4 slices of volume: <2 ml; 2.0 - 2.9 ml; 3.0 - 4.9 ml; ≥5 ml.

The plastic jar for transporting samples; PNLT register; the HIV serology kit centrifuge; the video viewed from the laptop in the Kikongo language; the optical microscope; slides and reagents for ZN staining.

The methods used were:

On day 1 to constitute our two study groups, we: recruited, excluded ineligible and non-consenting participants and then randomized. Of the 210 participants recruited, 1 was bedridden and 9 did not consent to the study. To randomize: we prepared 200 pieces of paper (100 with the letter "A" and 100 others with the letter "B". These pieces of paper were distributed in the 3 centers in an opaque plastic bucket with lid; we introduced these double-folded pieces of paper in each bucket. Due to their capacity, General hospital reference and the Kiveve mother and child center received more pieces of paper in their buckets.; participants were ordered to draw a piece of paper at random from the bucket (either A or B); those who pulled A were assigned to the "control group" that is to say the group of people who will follow the routine instructions from a medical or paramedical staff from the center before the sputum collection and B to the "intervention group" that is to say the group of people who will watch the video before the sputum collection. intervention group (B) were notified (a briefing one minute) before viewing the video; we gave them a first empty graduated spittoon for collecting the contact sample outside; they handed in the first sample; they were questioned to complete our form (socio-demographic characteristics, concept of contagion, symptoms and answered the questionnaire on the evaluation of the video); we identified the first sample (study sample code) and a slide code (PNLT register number); then, we noted the appearance and the volume in our cards; we gave them a second graduated spittoon empty from the home sample (morning sample); as HIV serology is compulsory according to the national protocol for any TB investigation, we collected 5 to 10 ml of blood from the vo-

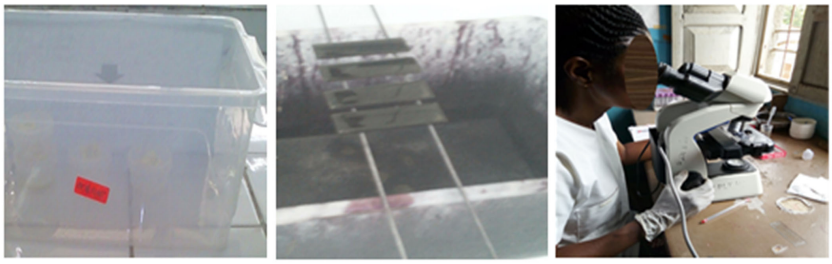
lunteers for these tests (Determine, Unigold and Vikia); we took this sample when the 2nd empty graduated spittoon was handed over; this blood was centrifuged to obtain a serum (tube without anticoagulant) or a plasma (tube with anticoagulant) allowing the following tests to be carried out on site (according to the national protocol: source PATI-5): Determine and Unigold and Vikia (if Determine positive).

The results of the serology were noted in our cards. The participants in the control group (A) received the 1st empty graduated spittoon from the contact sample and went to a medical or paramedical staff of their choice to follow the routine instructions; we gave them a first empty graduated spittoon for collecting the contact sample outside; they handed in the first sample; they were questioned for the completion of our file (socio-demographic characteristics, notion of contagion and symptoms); we identified the first sample (study sample code) and a slide code (PNLT register number), then, we noted the appearance and the volume in our cards, we gave them a second empty graduated spittoon for the home sample (morning sample); as HIV serology is compulsory according to the national protocol for any TB investigation, we collected 5 to 10 ml of blood from the volunteers for these tests (Determine, Unigold and Vikia); we took this sample when the 2nd empty graduated spittoon was handed over; this blood was centrifuged to obtain a serum (tube without anticoagulant) or a plasma (tube with anticoagulant) which made it possible to carry out the following tests on site (according to the national protocol): Determine and Unigold and Vikia (if Determine positive). The results of the serology were noted in our cards.

Early afternoon:

These sputum samples were collected in a plastic jar to be deposited in the laboratory for microscopy (ZN according to PATI-5); the laboratory technician trained and regularly retrained by the PNLT was blind to belonging to the group of participants, that is to say did not know who was in group A or in group B (single blind); samples deposited in the laboratory were subjected to a ZN stain followed by an immersion reading by the laboratory technician (**Figure 2**). Scoring of results was done as recommended by the Integrated Tuberculosis Program in Primary Health Care (5th Edition, PATI-5) as presented in **Table 1**.

Using a data collection sheet with a structured and standardized questionnaire, information was obtained according to socio-demographic, clinical, biological data and related to the evaluation of the video. Sociodemographic data included sex, age; the marital status, level of education and occupation of the suspected TB. Clinical data included concept of contagion, HIV status and ARV status. Laboratory data included the appearance (quality) of the sputum produced (Salivary, Purulent, Mucoid or Blood Tinted). The volume of sputum produced (less than 2 ml; between 2.0 and 2.9 ml; between 3.0 and 4.9 ml; greater than or equal to 5 ml). The types of specimen (sample) sample taken on contact (1st sample) and sample taken early in the morning (2nd sample). ZN positivity microscopy results and sputum positivity level (the AFB density on the smear).



Transport jar | Coloration of ZN | Microscopic reading
Source: PATI-5.

Figure 2. Transport jar, ZN staining and Microscopic reading.

Table 1. Scoring of the results by the Ziehl-Neelsen technique (PATI-5) [5].

Nombre des BAAR	Nombre de champ examines	Notation
Absence of BAAR	for 100 fields	0
1 à 9 BAAR	for 100 fields = 1 length	exact Number
10 à 99 BAAR	for 100 fields	+
1 à 10 BAAR/fields	50 fields	++
More than 10 BAAR/fields	20 fields	+++

For the intervention group, questions about the video instructions were asked to elicit commentary on how they viewed the video. The parameters of interest retained were: Video help; Video comprehension; Appropriate and usable video in our CSDT and Video and confidence in the result.

Operational definitions

- Suspected tuberculosis cases (presumed TB) = any patient who presents symptoms or signs suggestive of TB and a notion of contagion.
- The saliva sputum sample = transparent and watery sample with bubbles (foams).
- Purulent sputum specimen = specimen containing dead tissue, usually in large quantities with a foul odor, yellow or green in color.
- The mucoid sample from the sputum = sample containing mucus, thicker and yellow or green in color.
- The blood-stained sample (hemoptoic) = sample containing streaks of blood.

Statistical analyzes.

Statistical analyzes of the data were carried out according to standards. The data was encoded and coded using Microsoft Excel 2013 with a microcomputer; in order to obtain a database previously cleaned by means of completeness and consistency tests capable of obtaining harmonization of reliable data. These data were imported to be organized, analyzed and then processed on the IBM Statistical Package for the Social Sciences (SPSS) version 22 software of Microsoft Windows 7 Professional. Statistical calculations made it possible to obtain the means with standard deviations (SD), absolute and relative frequencies. The results have been presented in the form of tables and figures. Statistical inference

was applied for an analytical approach through comparisons and finding associations. Analysis of variance (ANOVA) was used to compare the means of the qualitative variables of interest. Descriptive statistics presented quantitative variables as means ± standard deviations and qualitative variables as numbers (n) and proportions (%). The p-value < 0.05 was considered to be the level of statistical significance. The study was approved by the ethics committee of the School of Public Health at the University of Kinshasa, Approval No.: ESP/CE/092/2019. Informed consent and/or assent were obtained from all study participants. The confidentiality of patient information was guaranteed at all times and the data was anonymized during coding.

3. Results

The present study recruited a total of 200 suspected TB of which 105 (52.5%), 76 (38.0%), and 19 (9.5%) were respectively from HGR, Center Mothers and Children of Kiveve and Center Oracle.

General characteristics of the population by group.

Table 2 shows that the 16 - 27 age group was predominant in both groups. There was a predominance of males in both groups. Married people predominated in the control group with 52 (52.0%) cases, yet single people predominated in the intervention group with 50 (50.0%). There was a predominance of secondary education in both groups. The unemployed occupation predominated in both groups followed by the informal private occupation. The number of participants with concept of TB contagion was 10 (10.0%) in the intervention group against 8 (8.0%) in the control group. The number of participants with HIV status was 10 (10.0%) in the control group versus 4 (4.0%) in the intervention group. The number of participants taking ARVs was 5 (5.0%) in the control group versus 2 (2.0%) in the intervention group. There was no statistically significant difference between the control and intervention groups in overall general characteristics of the study population.

Comparison of AFB density on the smear between the control group and the intervention group.

For the contact samples, there was 20% with a three crosses density in the intervention group against 4% in the control group, 9% with a two crosses density in the intervention group against 6% in the control group, 17% with a one cross density in the intervention group against 7% in the control group and 0% of paucibacillary in the intervention against 2% of paucibacillary in the control group; with a statistically very significant difference (p < 0.0001) (Figure 3).

For the morning samples, there were 27% with a thee crosses density in the intervention group against 10% in the control group, 10% with a two crosses-density in the intervention group against 4% in the control group, 9% with a one cross density in the intervention group against 4% in the control group and 2% of paucibacillary in the intervention against 0% of paucibacillary in the control group; with a statistically very significant difference (p < 0.0001) (Figure 4).

Table 2. General characteristics of the study population by intervention group and control group (Control).

Variables	Over all n (%)	Control n (%)	Case n (%)	p
Age (years)				0.166
16 - 27	67 (33.5)	28 (28.0)	39 (39.0)	
28 - 37	40 (20.0)	19 (19.0)	21 (21.0)	
38 - 47	36 (18.0)	17 (17.0)	19 (19.0)	
48 - 57	26 (13.0)	15 (15.0)	11 (11.0)	
≥58	31 (15.5)	21 (21.0)	10 (10.0)	
Sex				0.444
Male	104 (52.0)	53 (53.0)	51 (51.0)	
Female	96 (48.0)	47 (47.0)	49 (49.0)	
Marital status				0.321
Married	95 (47.5)	52 (52.0)	43 (43.0)	
Single	90 (45.0)	40 (40.0)	50 (50.0)	
Divorced	6 (3.0)	2 (2.0)	4 (4.0)	
Widower	9 (4.5)	6 (6.0)	3 (3.0)	
Educational level				0.718
No	31 (15.5)	18 (18.0)	13 (13.0)	
Primary	65 (32.5)	31 (31.0)	34 (34.0)	
Secondary	88 (44.0)	42 (42.0)	46 (46.0)	
University	16 (8.0)	9 (9.0)	7 (7.0)	
Profession				0.959
Unemployed	89 (44.5)	44 (44.0)	45 (45.0)	
Student/pupil	24 (12.0)	11 (11.0)	13 (13.0)	
Informal private	66 (33.0)	33 (33.0)	33 (33.0)	
Public	18 (9.0)	10 (10.0)	8 (8.0)	
Formal private	3 (1.5)	2 (2.0)	1 (1.0)	
Contage concept	18 (9.0)	8 (8.0)	10 (10.0)	0.403
HIV status	14 (7.0)	10 (10.0)	4 (4.0)	0.202
ARV status	7 (3.5)	5 (5.0)	2 (2.0)	0.222

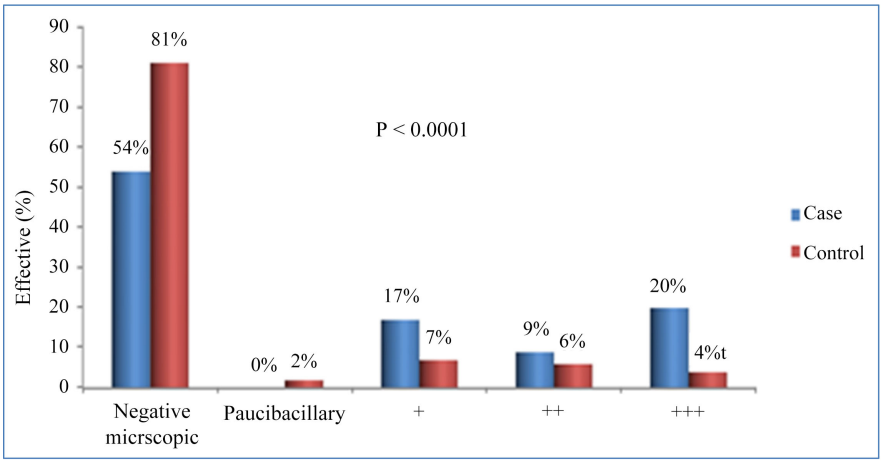


Figure 3. The density of AFB on the smear of contact samples.

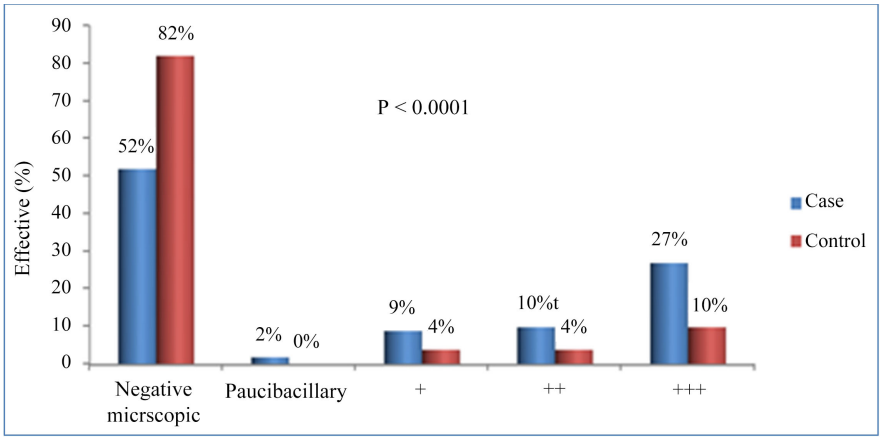


Figure 4. The density of AFB on the smear of the morning samples.

Comparison of the appearance and volume of sputum between the intervention group and the control group.

For the contact samples as for the morning samples, the saliva aspect was more important in the control group with 34 (34.0%) samples against 9 (9.0%) samples in the intervention group; on the other hand, the mucopurulent aspect predominated in the intervention group with 91 (91.0%) samples against 66 (66.0%) samples in the control group ($p < 0.0001$) (**Table 3**).

For the contact samples as for the morning samples, the volume < 3 ml was greater in the control group with 79 (79.0%) samples against 46 (46.0%) samples in the intervention group; on the other hand, the volume ≥ 3 ml predominated in the intervention group with 54 (54.0%) samples against 21 (21.0%) samples in the control group ($p < 0.0001$) (**Table 3**).

The fact that identical results are obtained 24 hours apart shows an excellent degree of reproducibility of the procedure and on the part of participants and investigators.

Evaluation of the video by the participants of the intervention group.

Table 4 shows that 89% of participants benefited from the help of the video, 97% understood the message of the video, 97% testified that the video was appropriate and could be used in our CSDTs and 97% would trust to the results of ZN.

4. Discussion

ZN is the most widely used standard test for detecting TM in low- and middle-income countries [12] [14]. Its performance can be improved by certain interventions or strategies, particularly video before sputum collection [15] [16]. This study set out to test the hypothesis that instructional (educational) video improves sputum quality for optimal microscopic yield (ZN).

The yield of microscopy.

ZN positive or AFB detection.

Our results corroborate those found by:

Table 3. Appearance and volume of sputum in the two study groups.

Variables	Contact sputum sample			Morning sputum sample		
	Control n (%)	Case n (%)	p	Control n (%)	Case n (%)	p
Aspect			**<0.0001**			**<0.0001**
Salivary	34 (34.0)	9 (9.0)		34 (34.0)	9 (9.0)	
Mucopurulent	66 (66.0)	91 (91.0)		66 (66.0)	91 (91.0)	
Volume			**<0.0001**			**<0.0001**
<3 ml	79 (79.0)	46 (46.0)		79 (79.0)	46 (46.0)	
≥3 ml	21 (21.0)	54 (54.0)		21 (21.0)	54 (54.0)	

Table 4. Evaluation of the video by the participants of the intervention group.

Variables	n (%)
Video help	
Yes	89 (89.0)
No	11 (11.0)
Video comprehension	
Yes, very well understood	83 (83.0)
Yes, quite well understood	14 (14.0)
Do not know	3 (3.0)
Appropriate video that can be used in our CSDTs	
Yes	97 (97.0)
Do not know	3 (3.0)
Video and confidence in the result	
Yes	97 (97.0)
No	3 (3.0)

Mhalu G *et al.*, who only analyzed the morning specimen and used the instructional video as an intervention before sputum collection, found 56% of smears positive for the intervention group against 23% for the control group [21]. This difference is explained by the fact that they used fluorescence microscopy, which is more sensitive than the optical microscope [15] [21] [22].

Alisjanhbana B *et al.*, who analyzed the specimens on-site-morning-on-site, and used the verbal instructions with educational image limited to sputum quality as an intervention before sputum collection, found 46% of smears positive for the intervention group against 26% for the control group in the first samples on site and 40% of positive smears for the intervention group against 31% for the control group in the morning samples [23]. This strategy used is close to ours, with the difference that the visual signal was limited to the quality of sputum and not to the sampling techniques [23].

Khan MS *et al.*, who analyzed two specimens, punctual and early in the morning, and used verbal instructions as an intervention before the collection of sputum, found in the women 17% of smears positive for the intervention group

against 11% for the control group in the point specimen, and 17% smears positive for the intervention group against 13% for the control group in the early morning specimen [24]. This proves that the visual effect associated with an explanation is better understood and less faded in memory than a simple explanation [24].

Hirooka T *et al.*, who analyzed one sample per patient and used respiratory rehabilitation (respiratory physiotherapy) as an intervention before sputum collection, found 21.1% of positive smears for the physiotherapist group against 10.4% for the non-physiotherapy group [25]. This strategy is used more for patients who have difficulty expectorating spontaneously [25].

Our results contrast with those found by:

Mariette KUPA *et al.*, who analyzed three specimens, on-site-morning-on-site, and used an instruction leaflet as an intervention before sputum collection, did not find the statistically significant difference between the two groups [26]. Indeed, they randomized the centers instead of the participants [26].

YJ Lee *et al.*, who analyzed three consecutive morning samples, and used a leaflet on instructions for sputum collection as an intervention, did not find a statistically significant difference between the groups [26]. Indeed, these authors gave a verbal explanation to all the participants including all the important points of the sample before giving the participants of the intervention group the brochures before randomization; suggesting that instruction without the brochure was sufficient [27].

Nelson Kalema *et al.*, who analyzed only one specimen per participant, and used oral rinsing with chlorhexidine and nystatin as an intervention, did not find a statistically significant difference between the groups [28]. This strategy is more used for the oral decontamination of patients, particularly PLWHIV who must submit sputum intended for culture [28].

AFB density or positivity level.

Our results corroborate those found by Mhalu G *et al.*, who found for the early morning specimen an overall increase in bacillary density at all levels in the intervention group compared to the control group [16].

On the other hand, our results are close to those of Alisjanhbana B *et al.* who found in the set of samples an increase in bacillary density at all levels, except at the level of the positivity of 1+ in the intervention group by compared to the control group [26].

Sputum quality.

Appearance of sputum.

Our results corroborate those found by:

Mhalu G *et al.* who found that more high-quality sputum (purulent or mucoid) was more frequent in the intervention group compared to the control group as well as saliva samples were less frequent in the intervention group ($p < 0.0001$) [20].

Hirooka T *et al.* who found a decrease in M1 saliva samples (Miller & Jones classification) in the intervention group than in the control group ($p < 0.0001$)

[27].

Khan MS *et al.* who found a decrease in saliva samples in the intervention group than in the control group in women (p = 0.003) [24].

5. Sputum Volume

Our results corroborate those found by Mhalu G *et al.* who found for the single morning samples that they were able to process, that the recommended volume of 3 ml or more was obtained more frequently in the intervention group compared to the control group (p < 0.0001) [16].

Video rating.

Most of our intervention group participants (97%) understood the message of the video; this opinion was the same for the participants of the study by Mhalu G *et al.* [16].

We found that 97% of the participants in the intervention group testified that the video was appropriate and could be used in our CSDTs, while Mhalu G *et al.* found that 92% thought that the future use of the video would be useful [16]. Most of our intervention group participants (89%) said they had benefited from using video for sputum collection; Mhalu G *et al.* found that 79% said the knowledge gained from the video could help other patients produce good quality specimens [16]. The present study notes a main limitation linked by its experimental nature, which we have carried out in a hospital setting, which can lead to selection bias. Nevertheless, the strength of this study is that it has the merit of being a pilot study in the DRC.

6. Conclusion

Video is a simple, yet important intervention that helps educate the patient in the diagnosis of TB. Its contribution showed that the sensitivity of ZN is improved both for the contact samples and for the morning samples. Faced with the lack of resources to have an Xpert MTB/RIF in all CSDTs in the country since 2007 to date, the contribution of the video should improve the positivity of the microscopy.

Authors' Contributions

AKS and CMN designed and analyzed the statistical data for the study. LB contributed to the data collection. OLM, AMS and BLM supervised the study. All authors have read and approved the final and revised version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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