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Original Article

Evaluation of Slit Skin Smear (SSS) Microscopy for Leprosy in Various Districts of Tamil Nadu

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Diagnosis of leprosy is primarily based on clinical criteria. However, quality Slit Skin Smear (SSS) microscopy has a role in correct disease classification and patients' selection for Drug resistance surveillance. This study has been carried out to understand the adequacy of SSS services and its quality among participating laboratories, and to find out the level of concordance and error types among the participating laboratories in comparison with the reference lab. The evaluation of SSS microscopy labs was carried out by performing on-site evaluation (OSE) and panel testing involving 13 laboratories from various centres in five districts of Tamil Nadu. Overall performance was based on scoring the key components – infrastructure, availability of trained manpower, reagents, technique of specimen collection, slide preparation, microscopic examination and reporting of results. Scores more than 70% were considered satisfactory. Among 13 participating laboratories, performance of six (50%) was satisfactory during on-site evaluation; ten had a satisfactory performance in panel testing wherein the participating lab technicians (LTs) read a set of slides prepared, stained and read in advance by the expert LTs of CLTRI. The panel test results revealed a concordance of 55.4% between the participating laboratories and reference laboratories along with an overall error of 30 among 65 smears (46.2%) of which 6 (9.2%) and 24 (36.9%) were major and minor errors respectively. SSS Microscopy services need to be strengthened in terms of infrastructure, logistics and trained manpower.

Key words : Slit Skin Smear Microscopy, Leprosy, Quality Assurance, SSS

Introduction

Leprosy is a chronic infectious disease caused by *M. leprae* and it remains a major public health problem since centuries. There are several states which are presently having a prevalence of >1/10,000 population (CLD - NLEP annual report 2016-17). Demonstration of *M. leprae* in slit skin smear (SSS) was an essential component of NLEP for several years. World Health Organization in 1988 introduced the operational classification based on clinical criteria. However, diagnosis of leprosy merely based on clinical ground may lead

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to misclassification of the disease in a proportion of cases (CLD 2019).

In India, National Leprosy Eradication Programme is a centrally sponsored health scheme under Ministry of Health and Family Welfare. The programme formulation occurs centrally while its implementation is carried out by states/ Union territories. Leprosy was declared as eliminated at the national level in 2005 and currently has a cumulative prevalence rate of 0.63 / 10,000 population, Annual New Case Detection Rate (ANCDR) of 8.90 per 100,000 population and Grade 2 Deformity rate of 3.04% among new cases. Post 2005 declaration, several strategies were changed in the program, such as integration into general health services and doing away with skin smears for diagnosis. This change has led to the drastic reduction in the number of laboratories performing SSS examination for leprosy diagnosis. Currently, only tertiary referral centres, medical colleges and few NGO laboratories perform this as a part of routine diagnosis.

SSS helps in classification of leprosy into paucibacillary (PB) and multibacillary (MB), thereby has a role in deciding the treatment regimen, monitoring response to treatment, differentiation of relapse from reaction and selection of cases for surveillance /testing for antimicrobial resistance (Mahajan 2013). Ensuring the quality of SSS microscopy is important, due to the following reasons - False negative SSS result in a new case will lead to either ignoring a true case of leprosy as not a case of leprosy, or misclassification of MB into PB leprosy, False positive SSS result in a new case may result in either unnecessary treatment for a non-leprosy patient or misclassification of PB into MB leprosy thereby unnecessarily prolonging the duration of treatment, False positive SSS result during the follow-up case may raise the suspicion of drug resistance and correct grading (BI) is essential for selection of cases for surveillance of antimicrobial resistance (AMR).

There is no External quality assurance (EQA) program for slit skin smear microscopy functioning currently. EQA program is a systematic assessment of the quality of laboratory services for corrective actions. Besides, the basic infrastructure and logistics are necessary prerequisites for quality laboratory services. Hence, this study was carried out to assess the availability of necessary infrastructure, the quality of SSS microscopy technique and its reporting, in selected districts of Tamil Nadu.

The objectives of this study were to assess the adequacy of SSS services and its quality among participating laboratories and to find out the level of concordance and types of errors when compared to the reference readers.

Methodology

Institute Ethics committee approval was obtained at CLTRI before commencement of the study. Administrative approval from State level authorities were also obtained.

Study setting:

The study was conducted from June 2019 to March 2020 in laboratories located in 13 health institutions in five purposively selected districts (Chennai, Villupuram, Madurai, Tirunelveli & Vellore) of Tamil Nadu State.

Data collection instrument and procedure:

Data were collected using on site evaluation checklist and panel testing using five blinded smears. In each laboratory, on site evaluation and panel testing were performed on the same day of the visit.

On site evaluation:

The on-site evaluation was conducted using a predesigned and pre-tested checklist (Annexure 1) in order to perform the comprehensive assessment of infrastructure, manpower, Biomedical

waste (BMW) management (DGHS & CPCB - BMW rules, 2016), SSS procedure - selection of sites for obtaining specimen from patients, preparation of the site, specimen collection, smear preparation, staining technique, microscopic examination, reporting and storage of slides (WHO 1986, CLD 2019). To assess the performance of SSS procedure, the participating centers were asked to schedule a patient for undergoing SSS procedure and staining technique on the day of assessment. The performance was assessed on 55 parameters comprising of: infrastructure-9, sample collection-18, staining technique-11, microscopic examination-9, reporting and storage of slides-8. Each parameter was given a score of one. The performance was considered satisfactory, if the participating laboratory obtained a total score of $\geq$ 39 (70 %).

Panel testing:

A standard set of stained positive and negative SSS stained slides from the reference lab were collected. In each laboratory the key staff, preferably the lab Technician entrusted to perform with SSS microscopy regularly was given a set of five blinded SSS stained smears for microscopy examination and calculation of BI. The results were analyzed based on the scores obtained by participants by comparing their reports with that of reference laboratories. Cumulative scores are calculated as follows: each correctly identified smear is given a score of 10 points, maximum possible score was 50, smear with major error - score 0, smear with minor error and quantification error - score 5. Laboratories with 70% and above score (score $\geq$ 35) were considered satisfactory. Error types were interpreted as shown in Table 1 (CTD 2005).

Assessment of participating laboratories:

The performance of the participating laboratories was considered satisfactory: if the center scored 70% in on-site evaluation and panel testing each individually; good performers: if obtained score of 80% in on-site evaluation and had no major error in panel testing.

Also, the constraints faced in performing slit skin smear (SSS) were ascertained using the interview guide containing open ended questions and hand recorded. For this purpose, one of the key staff preferably lab technician at each participating lab, totally 12 technicians, were enrolled into the study after obtaining his/her informed written consent.

Data management and statistical analysis:

Data collected was digitised in Microsoft Excel, checked for its completeness and analysed using SPSS version 22. The results were summarized as

Table 1 : Classification and interpretation of error types

Error type	Bacteriological Index (BI) report		Categorization of
	Assessing centre	Participating lab	the error
High False Negative (HFN)	3+ to 6+	Negative	Major error
High False Positive(HFP)	Negative	3+ to 6+	
Low False Positive(LFP)	Negative	1+ to 2+	
Low False Negative(LFN)	1+ to 2+	Negative	Minor error
Quantification Error(QE)	difference of more than two grades in reading a positive slide between two centres		

proportions for discrete variables and mean / median for continuous variables. Chi-square was used for comparing proportions and t-test / f-test for means. Agreement in reading between participating diagnostic centres and the reference laboratory readings were interpreted using *kappa* statistic.

For Qualitative data (interview), the responses of individual participants were read repeatedly and jointly by all the investigators and co-investigators of the study. The most common constraints as elicited during the interviews and the suggestions for quality improvement were synthesized in text format.

Results

A total of 13 laboratories in the centres from five districts of Tamil Nadu participated in panel testing and 12 laboratories participated in on - site evaluation (one centre could not participate due to non-availability of patient during the visit). Among these 13 labs, six were part of various medical colleges, four were part of district hospitals and three were part of Non-governmental organizations. The number of SSS procedure done in each laboratory varied from 1 to 70 per month. Number of smears performed was higher in laboratories located in medical colleges than other labs.

On-site evaluation results:

Fig. 1 depicts the overall scores obtained by each lab during on-site evaluation under various aspects.

Infrastructure and manpower:

A functional microscope, either Binocular or monocular, in working condition was available in 10 labs. All the twelve labs had Laboratory technicians to perform SSS procedure, five labs had technicians who have been trained in the procedure, and among them two were trained within last two years and three in the remote past.

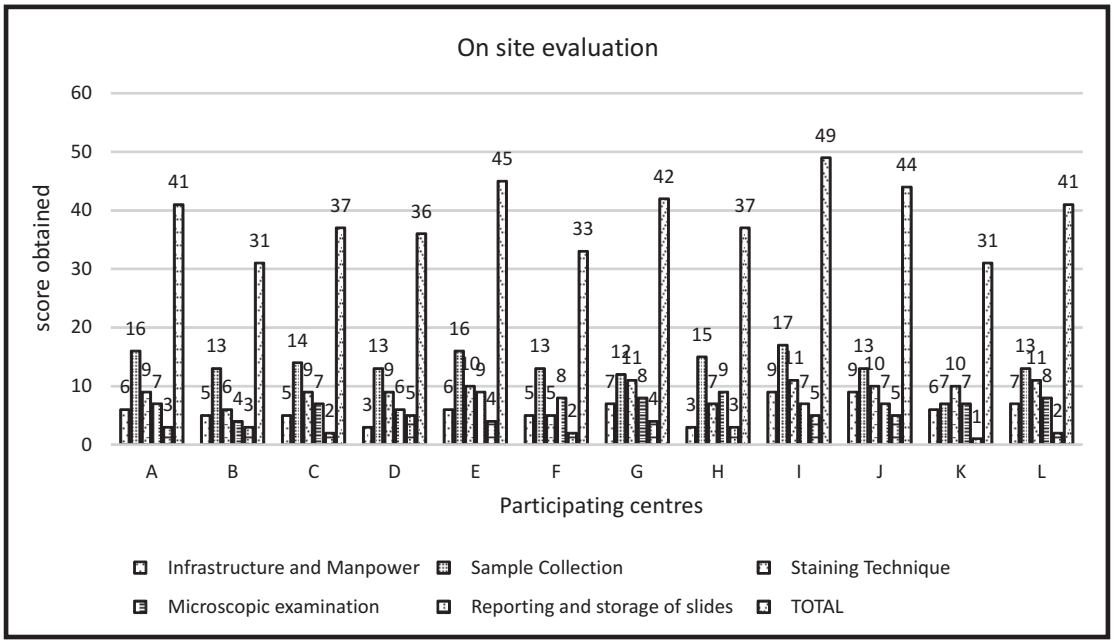

Fig. 1 : On site evaluation results

Table 2 : Infrastructure and manpower

Characteristics	No of laboratories (n=12)
LT trained in SSS procedure	05
Microscope in good working condition	10
Staff knowledge on BMW	06
Availability of all color-coded bins for BMW management	05

Table 3 : Overall performance of labs on onsite evaluation

Participating lab	Score obtained n=55 (%)	Interpretation
A	41 **(74.55%)**	**Satisfactory**
B	31 (56.36%)	Not Satisfactory
C	37 (67.27%)	Not Satisfactory
D	36 (65.45%)	Not Satisfactory
E	45 **(81.82%)**	**Good performer**
F	33 (60.00%)	Not Satisfactory
G	42 **(76.36%)**	**Satisfactory**
H	37 (67.27%)	Not Satisfactory
I	49 **(89.09%)**	**Good performer**
J	44 **(80.00%)**	**Satisfactory**
K	31 **(56.36%)**	**Not Satisfactory**
L	41 **(74.55%)**	**Satisfactory**

Five of the twelve labs were equipped with all the color-coded bins for biomedical waste management; staffs in six laboratories were either unaware or partly aware of infection control practices. Except for one lab, none was equipped with separate work room for leprosy diagnostics. (Table 2)

Sample collection for SSS and smear preparation: While assessing the procedure during on-site evaluation, it was observed that none of the laboratories had laid down Standard operating procedure (SOP) for SSS procedure. The personal protective equipment like gloves and masks were used by laboratory technicians while performing the procedure in 11 laboratories (91.6%). Eight out of twelve laboratories (67%) were routinely collecting SSS specimen from at least four sites, among which two laboratories were collecting specimen from six sites. Ten laboratories (83%) achieved adequate length and depth during SSS procedure. However, presence of blood in collected material was noted in five labs (41.6%). Appropriate size and evenness of the smear was noted in smears performed in 11 labs (91.6%).

Staining technique and microscopic examination: Reagents for acid fast staining, sink facility and water were available in all 12 (100%) labs evaluated. Appropriate timings for staining, counter-staining and decolonization for each step

of staining procedure were followed by 10 labs. None of the labs had displayed charts for grading bacteriological index. On examination of the stained slides, good background was observed in smears performed by nine (75%) labs.

Reporting and storage of slides: While reporting, two laboratories reported only the presence of acid fast bacilli (AFB) but did not report the bacteriological grading and none of the laboratories were regularly storing the slides for cross check. The reports were counter checked by medical officers in six (50%) laboratories. Also, 50% of the laboratories spent sufficient time of 15 minutes on smear reading.

Overall, there was four laboratories which scored more than 70% (score > 39) showing satisfactory result during onsite evaluation, two laboratories scoring more than 80% were considered as good performers and the performance of remaining six was evaluated to be unsatisfactory (Table 3).

Panel testing results:

A concordance of 55.4% was observed between the participating laboratories and reference laboratories in the panel testing results. From the total of 65 panel test smears (five smears for each lab) examined, a total of 30 (46.2%) errors were reported, of which 06 (9.2%) were major and 24 (36.9%) were minor errors. Twelve labs (92.3%) committed at least one microscopy error. Four labs committed one major error each, and one lab had reported 2 major errors (Table 4). Among the thirteen labs, the performance of 10 labs were considered satisfactory based on their scores obtained in panel testing [06 labs scored $\geq$ 40 (80%), 04 labs obtained scores $\geq$ 35 (70%)] and the average panel testing score was 36.1 (72.3%).

Agreement in the smear results on panel testing:

The sensitivity, specificity, positive and negative predictive values of participating labs in reading of SSS were 75%, 69.23%, 90.7% and 40.91%, respectively with a p value of <0.01 (Table 5). The overall agreement between participating laboratories and the assessing laboratory in SSS microscopy was given with a kappa value of 0.48 (Kappa with quadratic weighting).

Constraints elicited during the interview laboratory technician:

Six labs reported shortage in manpower. No constraint was observed in laboratory consumables for SSS, there was a continuous supply of all items required in medical college labs and NGO labs. The district hospital labs were provided with

Table 4 : Microscopy errors observed on panel testing in participating laboratories

Error type	Number of smears (n=65)
Major error	
High false positive	01
High false negative	05
Total	06 (9.2%)
Minor error	
Low false positive	04
Low false negative	07
Quantification error	13
Total	24 (36.9%)
Total errors reported	30 (46.2%)

Table 5 : Agreement in smear results between participating laboratories and assessing centre

Participating laboratories	Assessing centre		Total	P value
	Positive	Negative		
Positive	39	04	43	0.0065
Negative	13	09	22	(Fisher's
Total smears	52	13	65	exact test)

all required consumables and reagents by the district leprosy officer, as and when needed. While considering the SSS procedure, reading and reporting of slides, the technicians in five labs stated that, they can perform better if regular refresher trainings were provided. With regards to biomedical waste management, seven labs stated that they require training on BMW management. Also, four labs were in need of a good quality microscope and regular supply of consumables.

Discussion

Following the declaration of leprosy elimination at the national level in India, the technicians trained in SSS procedures were diverted to general health laboratories. Currently most of the senior technicians trained in SSS microscopy have retired and the new technicians were not been trained in this procedure. This led to lack of trained LTs. Further, the introduction of WHO's clinical classification in leprosy into PB and MB has alleviated the absolute necessity of performing SSS microscopy for diagnosis. As a result, majority of the centres are not performing this procedure, except for medical colleges and tertiary care centres.

This study carried out in five districts of Tamil Nadu was able to document existence of only 13 SSS laboratories altogether in five districts which is a serious constraint for leprosy related services. This is in contrast with the number of labs performing sputum smear examination for Tuberculosis, where there is either a designated microscopy centre (DMC) or an additional microscopy center for every 65000 population on an average.

Even among these 13 laboratories, only four (medical colleges) were noted to be currently referring cases for performing SSS microscopy to their respective laboratories for all suspected cases on regular basis. Whereas other laboratories receive patients for SSS microscopy only for doubtful cases as most of the physicians consider clinical diagnosis alone for patient management. This has resulted in wide range of variation in number of smears performed in different laboratories ranging from 1 to 70 per month.

In this study, On-site evaluation was conducted in 12 laboratories using a structured questionnaire. The labs were assessed for infrastructure and manpower, specimen collection, staining, smear microscopy including reading, reporting and storage of slides. Majority of centres (11/12) did not have a separate work room for leprosy diagnostic activities and they were combined with general laboratory services. This could result in unfavorable working environment leading to lack of quality in smear collection, staining and reporting procedures. Only 50% (6 centres) of the centres scored >70 %, which indicates that 50% of the centres lack adequate facilities needed to perform the procedure. This could be due to the fact that SSS microscopy is not recommended as a

mandate for leprosy diagnosis in the current NLEP guidelines and therefore adequate attention was not paid for retaining the previously existing infrastructure and manpower or further strengthening of the same.

With regards to performance of SSS procedure, lack of uniformity was observed in most of the laboratories, like number of sites included for SSS collection in each patient ranging from 3 to 6 sites, lack of BI grading in 2 labs, presence of blood stain in smears prepared in 5 labs and variations in time spent for smear examination. All these could have occurred due to the lack of a SOP for this procedure in their laboratories. In the study done in Nigeria by Wofeso (1993), only 12% smears were found to be of good quality due to less lymph material with too much of blood and only 1% of the SSS slides were properly stained. According to Vettom & Pritze (1989), smear quality, staining and reading was unsatisfactory in 26%, 22% and 36% respectively. A similar study by De Rijk et al (1985), showed that 20% of smears had poor quality of staining.

In our study, a total of 30 (46.2%) errors were reported in the panel testing results of which 9.2 % were major error with 1.5% as HFP and 7.6% as HFN. This could be due to lack of adequate training and not spending adequate time for examining each smear. The same should also have led to the sensitivity and specificity of SSS reporting as low as 75% and 69.23%, respectively. In addition, none of these labs had the reports countersigned or verified by the medical officers. None of the labs were storing the slides on a regular basis for performing internal/external quality control, due to lack of established quality assurance mechanism. Only few among them stored minimum number of positive slides for the purpose of academic demonstrations.

Shortage in the manpower and lack of regular training in the procedure were the major constraints faced by lab technicians in most of the labs. However, four laboratories had problem with the availability of good quality binocular microscope and regular supply of consumables and reagents.

Conclusions and Future perspective

The overall performance in SSS microscopy was satisfactory in half of the laboratories currently performing this technique in the five districts of Tamil Nadu. It is to be noted that equal number of labs were not up to the mark and therefore these labs need to be improved in terms of infrastructure, manpower and training.

The study was limited in that the evaluation was done only in selected five districts of Tamil Nadu. Similar evaluations need to be conducted in other parts of the country and required steps should be taken to strengthen the required infrastructure for SSS microscopy especially for selection of cases for the upcoming National antimicrobial resistance surveillance programme. It is also recommended to have a continuous EQA program in place to minimize the reporting errors and to sustain the quality of this technique.

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References

1. Central Leprosy Division (2016-17). Directorate General of Health Services, Ministry of Health and

Family Welfare. NLEP Annual reports 2016-17 [Accessed on 2018 Oct 22]. Available from http://nlep.nic.in/data.html.

2. Central Leprosy Division (2019). National leprosy Eradication Programme-Training manual for laboratory technicians, 2019. Directorate General of Health Services, Min of Health and family Welfare, New Delhi, India. URL- https://cltri.gov.in / LTTrainingManual.pdf.
3. Central TB Division (2005). Directorate General of Health Services, Ministry of Health and Family Welfare. RNTCP laboratory network guidelines for quality assurance of smear microscopy for diagnosing tuberculosis. New Delhi, India: CTD, 2005. URL-https://tbcindia.gov.in/WriteReadData/l892s/4234099618RNTCPLabNetworkGuidelines.pdf.
4. De Rijk A, Nilsson T, Chonde M (1985). Quality control of skin smear services in leprosy programmes: preliminary experience with inter-observer comparison in routine services. *Lepr Rev*. **56**: 177-191.
5. Diagnosis and classification of Leprosy (2002). In: Report of the International Leprosy Technical Forum 2002. *Lep Rev*. **73S**: 17-26.
6. Directorate General of Health Services and Central Pollution Control Board (2016). Guidelines for Management of Healthcare Waste as per Bio-medical Waste Management Rules, 2016. Ministry of Health & Family Welfare & Ministry of Environment, Forest & Climate Change, Government of India. URL-http://www.hp.gov.in/dhsrhp/Guidelines_healthcare_June_2018.pdf.
7. Mahajan VK (2013). Slit-skin smear in leprosy: lest we forget it! *Indian J Lepr*. **85**: 177-83.
8. Wofeso NA (1993). Inventory of skin smear practices in 6 leprosy control programmes in Nigeria. *Lepr Rev.* **64**: 50-56.
9. World Health Organization. Leprosy Unit (1986) Laboratory techniques for Leprosy. WHO, Geneva, WHO/CDS/LEP/86.4.https://apps.who.int/iris/handle/10665/61778.
10. Vettom L, Pritze S (1989). Reliability of skin smear results: experience with quality control of skin smears in different routine services in leprosy control programmes. *Lepr Rev*. **60**: 187-196.

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ANNEXURE - I

Assessment form for On-site evaluation

Date of Assessment : **Participating centre :**

No. of SSS/month :

I. Infrastructure and Manpower:

S. No	Check points	Response		Comments
		Yes	No	
1	Whether lab technician available for performing SSS technique?			
2	Whether lab technician has undergone training in SSS microscopy?			
3	Procedure room available for performing SSS technique?			
4	Microscope in working condition?			
5	Yellow colour bin available for disposal of used cotton?			
6	White colour bin available for disposal of blade?			
7	Red colour bin available for disposal of gloves?			
8	Blue colour bin available for disposal of broken slides?			
9	Staff aware about use of different color bins?			

II. Sample collection:

S. No	Check points	Response		Comments
		Yes	No	
1	Sample collection chart/SOP available in procedure room?			
2	Seating facility available for patient in procedure room?			
3	Light present in the procedure room?			
4	Disinfectant available in procedure room?			
5	Gloves available in procedure room?			
6	Surgical blade and scalpel available in the procedure room?			
7	Spirit lamp/ bunsen burner/match box available in the procedure room?			
8	Tincture iodine available in procedure room?			
9	New slides available in procedure room?			
10	Diamond pencil / sticker / marker available in procedure room?			
11	Whether 4-6 sites smeared for each patient?			
12	5x2mm length and depth of slit achieved during SSS procedure?			
13	Whether slide was marked with patient number and sites to be smeared?			
14	Whether pulpy material obtained?			
15	Whether material is not blood stained?			
16	Sample material evenly distributed in the smear?			
17	Whether able to read numbers of wrist watch through 80% of smear?			
18	Whether smear is of 5-7mm diameter?			

III. Staining technique:

S. No	Check points	Response		Comments
		Yes	No	
1	Sink available in the staining area?			
2	Staining rod/rack available in staining area?			
3	Water supply available in staining area?			
4	Spirit lamp/lighter available in the staining area?			
5	Whether strong carbol fuchsin available?			
6	Timing for carbol fuchsin (5 min) is followed?			
7	Whether 3% acid alcohol available?			
8	Timing for decolouriser (5-10 sec for 3% acid alcohol) is followed?			
9	Whether methylene blue available?			
10	Timing for methylene blue (1 min) is followed?			
11	Blotting paper available?			

IV. Microscopic examination:

S. No	Check points	Response		Comments
		Yes	No	
1	Cedar wood oil / immersion oil available?			
2	Grading chart displayed near microscope area?			
3	Lens cleaning tissue available?			
4	Whether microscope objective is cleaned after use?			
5	Whether sufficient background material is present?			
6	Whether smear background is stained blue?			
7	Whether smear is free of stain deposits and artefacts?			
8	Whether adequate time spent for examining each smear (15 mins)?			
9	Whether adequate number of oil immersion fields examined (100)?			

V. Reporting and storage of slides:

S. No	Check points	Response		Comments
		Yes	No	
1	Laboratory register maintained for SSS microscopy?			
2	Printed request form available for SSS microscopy?			
3	Printed report form available for SSS microscopy?			
4	Whether grading is given for Bacteriological index?			
5	Reports are counter checked and signed by medical officers?			
6	Slide storage box available?			
7	Whether slides are stored month wise?			
8	Whether slides are checked for inter-observer reliability?			

Scoring:

S. No	Study variables	No. of questions (Each question-one mark)	Marks scored	% scored
I	Infrastructure	9		
II	Sample collection	18		
III	Staining technique	11		
IV	Microscopic examination	9		
V	Reporting and storage of slides	8		
	Total	55		

Satisfactory: ≥ 39 marks (70%)

Not satisfactory: < 39 marks (70%)