Report on AFB smear Panel testing of District Hospital Laboratories 2008

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ACRONYMS

AFB … … … Acid Fast Bacilli

EQA … … … External Quality Assessment

HFN … … … High False Negative

HFP … … … High False Positive

LFN … … … Low False Negative

LFP … … … Low False Positive

QE … … … Quantification Errors

NTP … … … National Tuberculosis Programme

NaOH … … … Sodium hydroxide

TB … … … Tuberculosis

PHL … … … Public Health Laboratory

WHO … … … World Health Organization

IUATLD .. … … International Union Against Tuberculosis and Lung Diseases

Z-N … … … Ziehl-Neelsen

NTI … … … National Tuberculosis Institute

NTRL … … … National Tuberculosis Reference Laboratory

NEQAS … … … National External Quality Assurance Scheme

DoPH … … … Department of Public Health

Executive summary

PHL has implemented NEQAS in sputum microscopy to improve the standard of sputum microscopy in the country**.** Panel testing is one of the methods of NEQAS conducted twice a year (8^{th} and 9^{th} round in 2008). A panel for every round consists of 10 slides (5 stained and 5 unstained smears) prepared by PHL according to WHO/IUATLD standard procedures and sent to district labs.

In 8^{th} and 9^{th} rounds, four and six labs proficiency level in microscopic examination were found unacceptable respectively. Many labs continue to report lot of errors; both high and low false positive and negative. Few hospital labs never participate in the NEQAS.

1. Background

Direct sputum smear microscopy still remains gold standard and the most cost effective tool for diagnosing patients with infectious tuberculosis and monitoring the progress of treatment. The World Health Organization global strategy (DOTS) to fight against tuberculosis relies on a network of laboratories that provide quality sputum microscopy service. Since both diagnosis and treatment monitoring depends on sputum microscopy, providing reliable smear result is pivotal. The reliable results can only be assured through implementation of quality assurance program; National External Quality Assessment Scheme (NEQAS) which aims to assess the quality standard and implement improvement measures. NEQAS on sputum microscopy was started in 2005. Proficiency testing is one of the NEQAS components. PHL has conducted two round (8^{th} and 9^{th}) proficiency testing in 2008.

2. Objective

To improve and maintaining proficiency in AFB smear examination in all district laboratories that provide sputum microscopy service.

3. Methods and materials

3.1 Panel slide preparation

Sputum specimen used for panel slide preparation was not more than 2 days old. Panel slides prepared are of negative and positive of different grade. Preparation of different grades of slides is shown in **Annex I**.

3.2 Characterization of panel slides

Each panel set consists of 10 known smears; some stained (Ziehl-Neelsen staining as described in **Annex II**) and some unstained. Both stained and unstained comprises of negative and positive slides of different grades with code numbers.

3.3. Dispatch of panel slides

Panel slides are packed in slide boxes and sent to districts through ambulances. Labs are given one month deadline for reporting the result to PHL from the date of panel slides receipt.

3.4 Reporting Criteria

Reporting criteria was applied according to WHO/IUATLD guidelines.

Table 1: Grading of AFB smears by Z-N microscopy WHO/IUATLD criteria

No. of Acid Fast Bacilli (AFB)	Fields	Report
No AFB	In 100 oil immersion fields	Negative
1-9 AFB	In 100 oil immersion fields	Record exact figure
10-99 AFB	In 100 oil immersion fields	1+
1-10 AFB	Per field (examine 50 fields)	2+
More than 10 AFB	Per field (examine 20 fields)	3+

3.5 Evaluation table

Table 2: Standard result analyzing table

Result of district Laboratories	Result of Controlling Laboratory (PHL)				
	Negative	1-9 AFB/100 F	1+	2+	3+
Negative	Correct	LFN	HFN	HFN	HFN
1-9 AFB/100 F	LFP	Correct	Correct	QE	QE
1+	HFP	Correct	Correct	Correct	
2+	HFP	QE	Correct	Correct	Correct
3+	HFP	QE	QE	Correct	Correct

Correct	*No error*	
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*

Overall Agreement Rate (OAR): Number of (-) & (+) consistencies within range by grading **x 100**= __%
Grand Total ___

[***Rating of OAR**: □ >95% (Excellent), □ >90% (Satisfactory), □ <90% (Poor performance)*]

3.6 Performance rating

Each slide carries 10 points, total possible score= 100 (for 10 slides)

a. Score of Major Error= 10
b. Score of Minor Error= 5
c. Acceptable score= 90

4. Results of Proficiency Testing:

4.1 Result of 8th round proficiency testing

In 8th round panel test, performance of four of 19 laboratories, who have reported back the results, were found unsatisfactory (below acceptable score < 90%) (Figure 1, Table 3). However, fourteen district labs reported six major errors (5 HFN & 1 HFP) and twenty one minor errors (17 LFN, 2 LFP & 2 QE) (Figure 2, Table 3). Nine district laboratories did not send their reports.

Figure 1. Performance rating of labs for 8th round

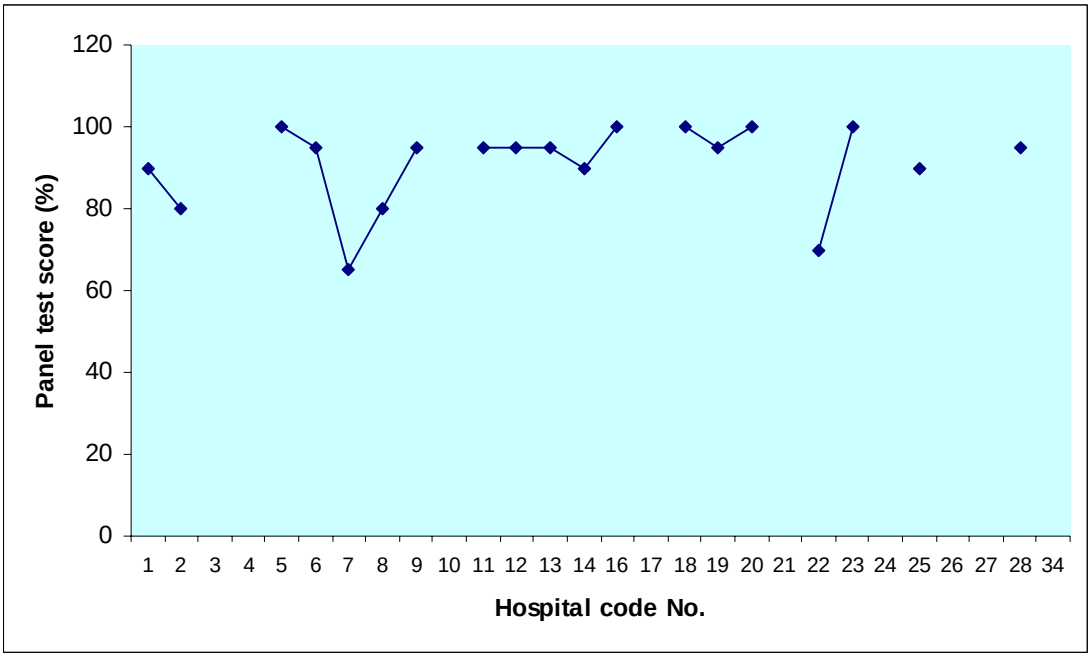

Figure 2: Types of errors reported in 8th round

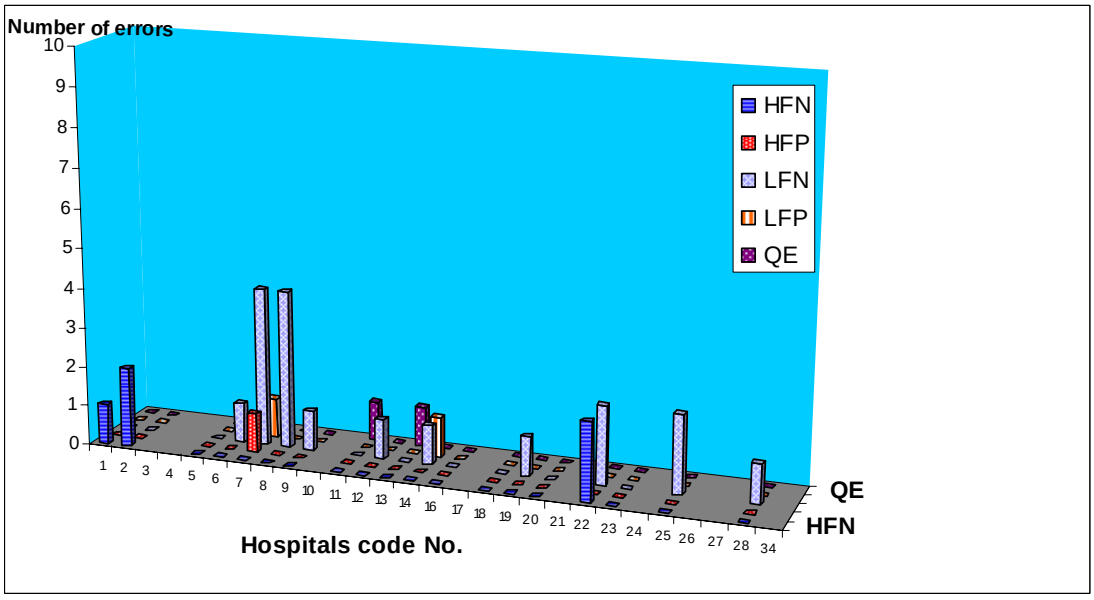

Table 3: Results (8th Round Proficiency Testing)

Lab code No.	HFN	HFP	LFN	LFP	QE	Total Errors	Total Score
LAB-01	1	0	0	0	0	1	90
LAB-02	2	0	0	0	0	2	80
LAB-03*	-	-	-	-	-	-	-
LAB-04*	-	-	-	-	-	-	-
LAB-05	0	0	0	0	0	0	100
LAB-06	0	0	1	0	0	1	95
LAB-07	0	1	4	1	0	6	65
LAB-08	0	0	4	0	0	4	80
LAB-09	0	0	1	0	0	1	95
LAB-10*	-	-	-	-	-	-	-
LAB-11	0	0	0	0	1	1	95
LAB-12	0	0	1	0	0	1	95
LAB-13	0	0	0	0	1	1	95
LAB-14	0	0	1	1	0	2	90
LAB-16	0	0	0	0	0	0	100
LAB-17*	-	-	-	-	-	-	-
LAB-18	0	0	0	0	0	0	100
LAB-19	0	0	1	0	0	1	95
LAB-20	0	0	0	0	0	0	100
LAB-21*	-	-	-	-	-	-	-
LAB-22	2	0	2	0	0	4	70
LAB-23	0	0	0	0	0	0	100
LAB-24*	-	-	-	-	-	-	-
LAB-25	0	0	2	0	0	2	90
LAB-26*	-	-	-	-	-	-	-
LAB-27*	-	-	-	-	-	-	-
LAB-28	0	0	1	0	0	1	95
LAB-34*	-	-	-	-	-	-	-

** Report not received*

4.2 Results of 9th round proficiency testing

In 9th round panel test, performance of six of 26 laboratories, who have reported back the results, were found unsatisfactory (below acceptable score < 90%) (Figure 3, Table 4). However, thirteen district laboratories have reported five major errors (5 HFN) and twenty one minor errors (18 LFN & 3QE) (Figure 4, Table 4). Two district laboratories did not send their reports.

Figure 3. Performance rating of labs for 9th round

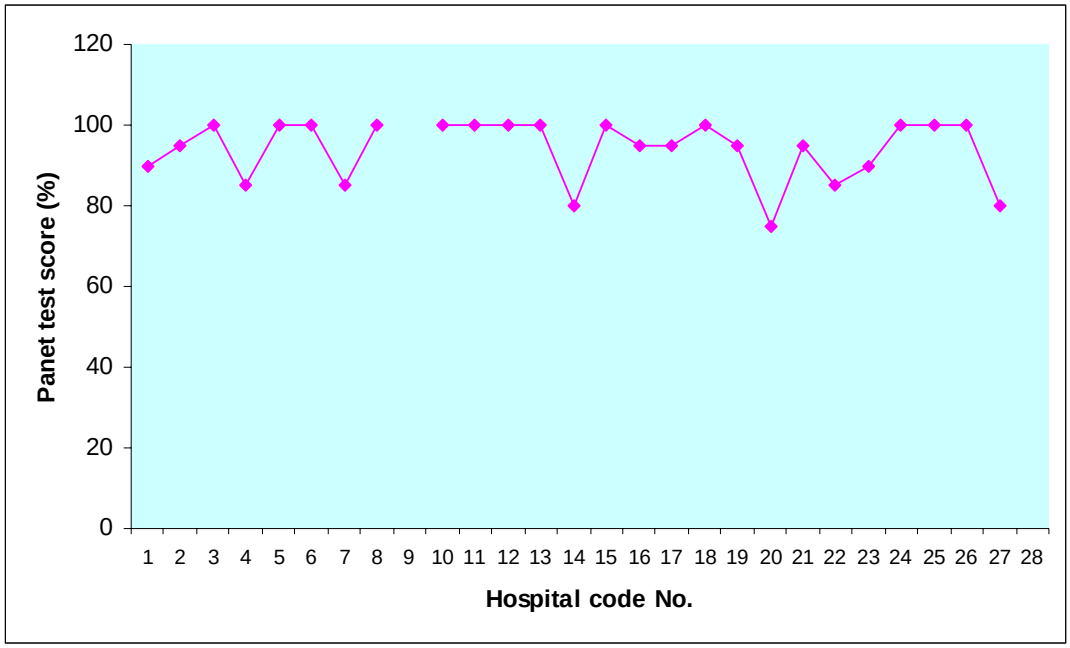

Figure 4: Types of errors reported in 9th round

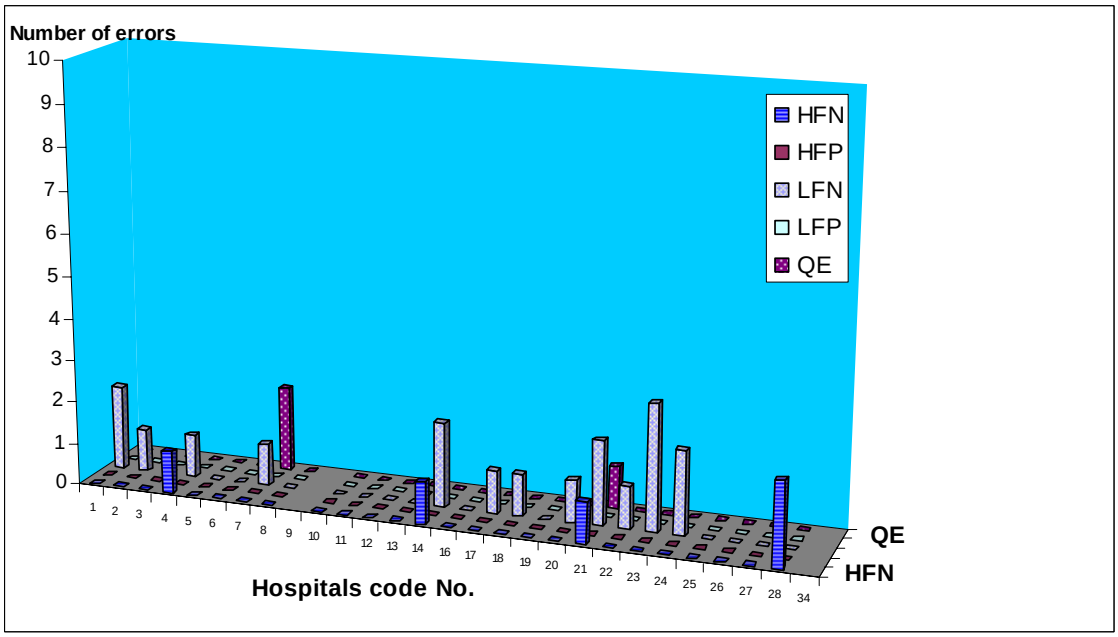

Table 4: Analysis of Results (9th Round Proficiency Testing)

Lab code No.	HFN	HFP	LFN	LFP	QE	Total errors	Total Score
LAB-01	0	0	2	0	0	2	90
LAB-02	0	0	1	0	0	1	95
LAB-03	0	0	0	0	0	0	100
LAB-04	1	0	1	0	0	2	85
LAB-05	0	0	0	0	0	0	100
LAB-06	0	0	0	0	0	0	100
LAB-07	0	0	1	0	2	3	85
LAB-08**	0	0	0	0	0	0	100
LAB-09*	-	-	-	-	-	-	-
LAB-10	0	0	0	0	0	0	100
LAB-11**	0	0	0	0	0	0	100
LAB-12	0	0	0	0	0	0	100
LAB-13	0	0	0	0	0	0	100
LAB-14	1	0	2	0	0	3	80
LAB-16	0	0	0	0	0	0	100
LAB-17	0	0	1	0	0	1	95
LAB-18	0	0	1	0	0	1	95
LAB-19**	0	0	0	0	0	0	100
LAB-20	0	0	1	0	0	1	95
LAB-21**	1	0	2	0	1	4	75
LAB-22	0	0	1	0	0	1	95
LAB-23	0	0	3	0	0	3	85
LAB-24	0	0	2	0	0	2	90
LAB-25	0	0	0	0	0	0	100
LAB-26	0	0	0	0	0	0	100
LAB-27	0	0	0	0	0	0	100
LAB-28	2	0	0	0	0	2	80
LAB-34*	-	-	-	-	-	-	-

** Report not received*
*** Report received late*

5. Discussion

Panel testing is one the method used for external quality assessment (EQA). The panel testing is mainly used to evaluate the proficiency level of lab technician's microscopic examination of sputum smears. However, panel testing cannot evaluate the overall performance of individual/lab technicians in performing sputum microscopy; it needs to be supplement by other external assessment methodology.

Microscopic examination is a critical step in determining the reliable smear result in sputum microscopy that has direct implication on treatment and its monitoring. The knowledge on AFB bacilli morphology, microscopic skills and techniques of an individual greatly affect the outcome of result and its interpretation. Thus, maintaining an acceptable level of proficiency in smear microscopic examination of all lab technicians performing sputum microscopy is imperative which can only be assessed by periodic panel testing.

Major errors; particularly the reporting of high false positive (HFP) error is a matter of serious concern since patient will be administered with highly toxic ATT drug for 6-8 months for tuberculosis infection. Nevertheless, reporting high false negative (HFN) error will deny patient from medication and ultimately become a source of tuberculosis infections to general populations. Minor errors; low false negative (LFP) and low false negative (LFN) errors have similar consequences of HFP and HFN. QE is mere a technical error which bear no impact in treatment and monitoring.

The main problem associated with high false positive and negative reporting is due to lack of knowledge on AFB bacilli morphology by lab technicians or use of nonfunctional microscope. The later problem is unlikely since all hospitals are supplied with a separate new microscope dedicated for AFB microscopy.

The same above problems could be cited for reporting the low false positive and negative errors. In addition, following irregular screening technique or screening insufficient microscopic fields are other possible problems. The quantification error problem is purely due to lack of knowledge in grading system based on number of fields examined.

6. Conclusions

In 8th and 9th round of panel testing, **21.05%** and **23.07%** of labs performance were found unacceptable respectively. The overall findings from two round of panel testing in 2008 showed not much difference in terms of performance improvement. Very few hospitals are consistent in their proficiency while some hospitals struggle to meet the acceptable standard level.

7. References

1. External Quality Assessment for AFB Smear Microscopy. Supported through the Association of Public Laboratories and Centers for Disease Control and Prevention Cooperative agreement. U60/CCU303019.
2. SAARC TB and HIV/AIDS Center, Report of 4th round external proficiency testing of smear microscopy in National TB reference Laboratories in SAARC region, STC 2007. P.o. Box No. 9517, Kathmandu, Nepal.
3. Laboratory Services in Tuberculosis Control, Organization and Management Part I. WHO/TB/98.258.

Annex-1

Result of 8th round proficiency testing of individual district labs

1. District Laboratory: LAB-01

Table 5: Performance of District laboratory code number 01

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	4	Negative	Negative	No error
2	40	Negative	Negative	No error
3	99	Scanty (3 AFB/100 F)	Scanty (5 AFB/100 F)	No error
4	141	Scanty (4 AFB/100 F)	Scanty (5 AFB/100 F)	No error
5	177	Scanty (4 AFB/100 F)	Scanty (5 AFB/100 F)	No error
6	193	Scanty (5 AFB/100 F)	Scanty (5 AFB/100 F)	No error
7	213	1+	2+	No error
8	241	Scanty (3AFB/100 F)	1+	No error
9	302	1+	1+	No error
10	342	Negative	1+	Major error

Only one High false Negative (HFN) was reported in a panel of 10 slides.

2. District Laboratory: LAB-02

Table 6: Performance of District laboratory code number 02

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	12	Negative	Negative	No error
2	58	Negative	Negative	No error
3	74	Scanty (7 AFB/100 F)	Scanty (5 AFB/100 F)	No error
4	76	Negative	Scanty (5 AFB/100 F)	Minor error
5	187	Negative	Scanty (5 AFB/100 F)	Minor error
6	189	Scanty (3 AFB/100 F)	Scanty (5 AFB/100 F)	No error
7	191	Scanty (2 AFB/100 F)	Scanty (5 AFB/100 F)	No error
8	204	2+	2+	No error
9	290	1+	1+	No error
10	318	1+	1+	No error

Two High False Negative (HFN) were observed in a panel of 10 slides

3. District Laboratory: LAB- 05

Table 7: Performance of District Laboratory code number 05

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	2	Negative	Negative	No error
2	14	Negative	Negative	No error
3	47	Negative	Negative	No error
4	52	Negative	Negative	No error
5	78	Scanty (9 AFB/100 F)	Scanty (5 AFB/100 F)	No error
6	82	1+	Scanty (5 AFB/100 F)	No error
7	125	Scanty (6 AFB/100 F)	Scanty (5 AFB/100 F)	No error
8	151	Scanty (8 AFB/100 F)	Scanty (5 AFB/100 F)	No error
9	181	1+	Scanty (5 AFB/100 F)	No error
10	194	2+	2+	No error

Three Lab Technicians examined the slides and recorded their results. No error of any type was reported in a panel of 10 slides.

4. District Laboratory: LAB- 06

Table 8: Performance of District laboratory code number 06

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	16	Negative	Negative	No error
2	43	Negative	Negative	No error
3	83	Scanty (6 AFB/ 100 F)	Scanty (5 AFB/100 F)	No error
4	90	Scanty (4 AFB/ 100 F)	Scanty (5 AFB/100 F)	No error
5	134	Negative	Scanty (5 AFB/100 F)	Minor error
6	143	1+	Scanty (5 AFB/100 F)	No error
7	208	2+	2+	No error
8	260	1+	1+	No error
9	299	1+	1+	No error
10	330	Scanty (8 AFB/100 F)	Scanty (5 AFB/100 F)	No error

Only one Low False Negative (LFN) was reported in a panel of 10 slides.

5. District Laboratory: LAB- 08

Table 9: *Performance of District Laboratory code number 08*

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	13	Negative	Negative	No error
2	55	Negative	Negative	No error
3	91	Negative	Scanty (5 AFB/100 F)	Minor error
4	112	Negative	Scanty (5 AFB/100 F)	Minor error
5	139	Negative	Scanty (5 AFB/100 F)	Minor error
6	146	Negative	Scanty (5 AFB/100 F)	Minor error
7	216	1+	2+	No error
8	283	2+	1+	No error
9	307	1+	1+	No error
10	308	1+	1+	No error

Two Lab Technicians examined the slides and recorded their results. Four Low False Negative were reported in a panel of 10 slides.

6. District Laboratory: LAB- 09

Table 10: Performance of District Laboratory code number 09

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	6	Negative	Negative	No error
2	69	Scanty (8 AFB/100 F)	Scanty (5 AFB/100 F)	No error
3	148	Negative	Scanty (5 AFB/100 F)	Minor error
4	161	Scanty (7 AFB/100 F)	Scanty (5 AFB/100 F)	No error
5	175	1+	Scanty (5 AFB/100 F)	No error
6	223	3+	2+	No error
7	253	1+	1+	No error
8	258	2+	1+	No error
9	332	1+	1+	No error
10	Missing	Missing	Missing	Missing

Out of 10 slides of a panel, one slide was missed placed. Nine Lab Technicians read the slides and recorded their results. One Low False Negative was reported in a panel of 9 slides.

7. District Laboratory: LAB- 11

Table 11: Performance of District Laboratory code number 11

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	56	Negative	Negative	No error
2	60	Negative	Negative	No error
3	72	Scanty (2 AFB/100 F)	Scanty (5 AFB/100 F)	No error
4	117	Scanty (4 AFB/100 F)	Scanty (5 AFB/100 F)	No error
5	155	Scanty (2 AFB/100 F)	Scanty (5 AFB/100 F)	No error
6	188	Scanty (3 AFB/100 F)	Scanty (5 AFB/100 F)	No error
7	201	3+	2+	No error
8	276	1+	1+	No error
9	314	3+	1+	Minor error
10	320	2+	1+	No error

One Quantification error was reported in a panel of 10 slides.

8. District Laboratory: LAB- 12

Table 12: Performance of District Laboratory, code number 12

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	5	Negative	Negative	No error
2	64	Negative	Negative	No error
3	87	Scanty (1-9 AFB/100 F)	Scanty (5 AFB/100 F)	No error
4	111	Negative	Scanty (5 AFB/100 F)	Minor error
5	131	Scanty (1-9 AFB/100 F)	Scanty (5 AFB/100 F)	No error
6	159	Scanty (1-9 AFB/100 F)	Scanty (5 AFB/100 F)	No error
7	214	2+	2+	No error
8	240	1+	1+	No error
9	275	1+	1+	No error
10	300	1+	1+	No error

One Low False Negative was reported in a panel of 10 slides.

9. District Laboratory: LAB- 13

Table 13: Performance of District Laboratory code number 13

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	18	Negative	Negative	No error
2	48	Negative	Negative	No error
3	73	Scanty (6 AFB/100 F)	Scanty (5 AFB/100 F)	No error
4	156	2+	Scanty (5 AFB/100 F)	Minor error
5	168	1+	Scanty (5 AFB/100 F)	No error
6	179	Scanty (5 AFB/100 F)	Scanty (5 AFB/100 F)	No error
7	221	3+	2+	No error
8	261	2+	1+	No error
9	281	Scanty (4 AFB/100 F)	1+	No error
10	327	2+	1+	No error

One Quantification error was reported in a panel of 10 slides

10. District Laboratory: LAB- 14

Table 14: Performance of District Laboratory code number 14

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	21	Negative	Negative	No error
2	45	Scanty (3 AFB/100 F)	Negative	Minor error
3	94	Scanty (5 AFB/100 F)	Scanty (5 AFB/100 F)	No error
4	105	1+	Scanty (5 AFB/100 F)	No error
5	137	1+	Scanty (5 AFB/100 F)	No error
6	176	Negative	Scanty (5 AFB/100 F)	Minor error
7	205	2+	2+	No error
8	229	2+	1+	No error
9	252	2+	1+	No error
10	324	1+	1+	No error

Two Lab Technicians read the slides and recorded their results. One Low False Negative and one Low False positive were reported in a panel of 10 slides.

11. District Laboratory: LAB- 16

Table 15: Performance of District Laboratory code number 16

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	15	Negative	Negative	No error
2	122	Scanty (3 AFB/100 F)	Scanty (5 AFB/100 F)	No error
3	126	1+	Scanty (5 AFB/100 F)	No error
4	129	Scanty (7 AFB/100 F)	Scanty (5 AFB/100 F)	No error
5	165	1+	Scanty (5 AFB/100 F)	No error
6	224	2+	1+	No error
7	238	1+	1+	No error
8	259	1+	1+	No error
9	265	1+	1+	No error
10	Missing	Missing	Missing	Missing

Out of 10 slides of a panel, one slide was missed placed. No error of any type was reported in a panel of 9 slides.

12. District Laboratory: LAB- 19

Table 16: *Performance of District Laboratory code number 19*

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	8	Negative	Negative	No error
2	58	Negative	Negative	No error
3	96	Negative	Scanty (5AFB/100 F)	Minor error
4	109	Scanty (5AFB/100 F)	Scanty (5AFB/100 F)	No error
5	110	Scanty (8 AFB/100 F)	Scanty (5AFB/100 F)	No error
6	160	Scanty (3 AFB/100 F)	Scanty (5AFB/100 F)	No error
7	225	2+	2+	No error
8	245	1+	1+	No error
9	256	1+	1+	No error
10	321	2+	1+	No error

One Low False Negative was observed in a panel of 10 slides.

13. District Laboratory: LAB- 20

Table 17: Performance of District Laboratory code number 20

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	1	Negative	Negative	No error
2	22	Negative	Negative	No error
3	93	1+	Scanty (5 AFB/100 F)	No error
4	95	1+	Scanty (5 AFB/100 F)	No error
5	106	Scanty (6 AFB/100 F)	Scanty (5 AFB/100 F)	No error
6	135	Scanty (5 AFB/100 F)	Scanty (5 AFB/100 F)	No error
7	222	2+	2+	No error
8	247	1+	1+	No error
9	263	1+	1+	No error
10	303	1+	1+	No error

No error of any type was observed in a panel of 10 slides.

14. District Laboratory: LAB- 22

Table 18: Performance of District Laboratory code number 22

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	37	Negative	Negative	No error
2	42	Negative	Negative	No error
3	70	Scanty (6 AFB/100 F)	Scanty (5 AFB/100 F)	No error
4	124	Scanty (5 AFB/100 F)	Scanty (5 AFB/100 F)	No error
5	140	Negative	Scanty (5 AFB/100 F)	Minor error
6	169	Negative	Scanty (5 AFB/100 F)	Minor error
7	197	2+	1+	No error
8	273	Negative	1+	Major error
9	286	Scanty (5 AFB/100 F)	1+	No error
10	315	Negative	1+	Major error

Two High False Negative and two Low False Negative were reported in a panel of 10 slides

15. District Laboratory: LAB- 23

Table 19: Performance of District Laboratory code number 23

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	39	Negative	Negative	No error
2	54	Negative	Negative	No error
3	84	Scanty	Scanty (5 AFB/100 F)	No error
4	115	Scanty	Scanty (5 AFB/100 F)	No error
5	158	Scanty	Scanty (5 AFB/100 F)	No error
6	173	Scanty	Scanty (5 AFB/100 F)	No error
7	203	2+	1+	No error
8	270	1+	1+	No error
9	306	1+	1+	No error
10	340	2+	1+	No error

No error of any type was observed in a panel of 10 slides.

16. District Laboratory: LAB- 25

Table 20: Performance of District Laboratory code number 25

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	116	Negative	Scanty (5 AFB/100 F)	Minor error
2	152	Negative	Scanty (5 AFB/100 F)	Minor error
3	182	Scanty (9 AFB/100 F)	Scanty (5 AFB/100 F)	No error
4	211	3+	2+	No error
5	236	Scanty (8 AFB/100 F)	1+	No error
6	295	1+	1+	No error
7	298	1+	1+	No error
8	304	2+	1+	No error
9	310	Scanty (8AFB/100 F)	1+	No error
10	336	2+	1+	No error

Two Low False Negative were reported in a panel of 10 slides

17. District Laboratory: LAB- 28

Table 21: Performance of District Laboratory code number 28

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	57	Negative	Negative	No error
2	61	Negative	Negative	No error
3	102	Scanty	Scanty (5 AFB/100 F)	No error
4	123	Scanty	Scanty (5 AFB/100 F)	No error
5	145	Negative	Scanty (5 AFB/100 F)	Minor error
6	157	Scanty	Scanty (5 AFB/100 F)	No error
7	196	2+	2+	No error
8	243	1+	1+	No error
9	291	1+	1+	No error
10	326	Scanty	1+	No error

One Low False Negative was observed in a panel of 10 slides.

Result of 9th round panel testing of individual District Laboratories

1. District Laboratory: LAB- 01

Table 22: Performance of District laboratory code number 01

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	21	2+	2+	No error
2	86	Negative	Scanty (5 AFB/100 F)	Minor error
3	111	2+	1+	No error
4	143	1+	1+	No error
5	145	2+	1+	No error
6	194	Negative	Scanty (5 AFB/100 F)	Minor error
7	239	3+	3+	No error
8	240	3+	3+	No error
9	255	Negative	Negative	No error
10	300	Negative	Negative	No error

Two Low false Negative (LFN) were reported in a panel of 10 slides.

2. District Laboratory: LAB- 02

Table 23: Performance of District laboratory code number 02

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	9	1+	2+	No error
2	90	Scanty (4 AFB/100 F)	Scanty (5 AFB/100 F)	No error
3	109	Negative	Scanty (5 AFB/100 F)	Minor error
4	113	1+	1+	No error
5	142	1+	1+	No error
6	150	1+	1+	No error
7	237	3+	3+	No error
8	249	3+	3+	No error
9	295	Negative	Negative	No error
10	310	Negative	Negative	No error

No error of any types was observed in a panel of 10 slides.

3. District Laboratory: LAB- 03

Table 24: Performance of District Laboratory code number 03

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	11	2+	2+	No error
2	98	Scanty	Scanty (5 AFB/100 F)	No error
3	115	2+	1+	No error
4	141	1+	1+	No error
5	157	1+	1+	No error
6	203	3+	3+	No error
7	210	3+	3+	No error
8	288	Negative	Negative	No error
9	307	Negative	Negative	No error
10	338	Negative	Negative	No error

Two Lab Technicians read the slides and recorded their results. No error of any type was reported in a panel of 10 slides.

4. District Laboratory: LAB- 04

Table 25: Performance of District Laboratory, code number 04

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	64	Negative	2+	Major error
2	107	Negative	Scanty (5 AFB/100 F)	Minor error
3	116	1+	1+	No error
4	166	Scanty (9 AFB/100 F)	1+	No error
5	171	Scanty (8 AFB/100 F)	1+	No error
6	212	3+	3+	No error
7	232	3+	3+	No error
8	286	Negative	Negative	No error
9	306	Negative	Negative	No error
10	331	Negative	Negative	No error

One High False Negative (HFN) and one Low False Negative (LFN) were reported in a panel of 10 slides.

5. District Laboratory: LAB- 05

Table 26: Performance of District Laboratory code number 05

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	17	1+	2+	No error
2	31	2+	2+	No error
3	81	Scanty (4 AFB/100 F)	Scanty (5 AFB/100 F)	No error
4	144	1+	1+	No error
5	176	1+	1+	No error
6	230	3+	3+	No error
7	244	3+	3+	No error
8	254	Negative	Negative	No error
9	298	Negative	Negative	No error
10	318	Negative	Negative	No error

Two Lab Technicians read the slides and recorded their results. No error of any type was observed in a panel of 10 slides.

6. District Laboratory: LAB- 06

Table 27: Performance of District laboratory code number 06

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	16	2+	2+	No error
2	67	Scanty (7 AFB/100 F)	Scanty (5 AFB/100 F)	No error
3	88	Scanty (4 AFB/100 F)	Scanty (5 AFB/100 F)	No error
4	119	1+	1+	No error
5	148	1+	1+	No error
6	193	Scanty (5 AFB/100 F)	Scanty (5 AFB/100 F)	No error
7	204	3+	3+	No error
8	257	Negative	Negative	No error
9	297	Negative	Negative	No error
10	342	Negative	Negative	No error

Two Lab Technicians read the slides and recorded their results. One Low False Negative (LFN) was observed in a panel of 10 slides.

7. District Laboratory: LAB- 07

Table 28 Performance of District Laboratory code number 07

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	42	2+	2+	No error
2	77	Negative	Scanty (5 AFB/100 F)	Minor error
3	118	2+	1+	No error
4	139	2+	1+	No error
5	156	2+	1+	No error
6	173	2+	1+	No error
7	226	4+	3+	Minor error
8	251	4+	3+	Minor error
9	279	Negative	Negative	No error
10	320	Negative	Negative	No error

One Low False Negative (LFN) and two Quantification Error (QE) were reported in a panel of 10 slides.

8. District Laboratory: LAB- 08

Table 29: *Performance of District Laboratory code number 08*

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	2	3+	2+	No error
2	79	Scanty	Scanty (5 AFB/100 F)	No error
3	103	Scanty	Scanty (5 AFB/100 F)	No error
4	125	1+	1+	No error
5	167	1+	1+	No error
6	218	3+	3+	No error
7	248	3+	3+	No error
8	281	Negative	Negative	No error
9	309	Negative	Negative	No error
10	339	Negative	Negative	No error

Two Lab Technicians read the slides and recorded their results. No error of any type was observed in a panel of 10 slides.

9. District Laboratory: LAB- 10

Table 30: Performance of District Laboratory code number 10

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	45	2+	2+	No error
2	71	Scanty (5 AFB/100 F)	Scanty (5 AFB/100 F)	No error
3	117	1+	1+	No error
4	146	1+	1+	No error
5	195	1+	Scanty (5 AFB/100 F)	No error
6	228	3+	3+	No error
7	245	3+	3+	No error
8	260	Negative	Negative	No error
9	291	Negative	Negative	No error
10	328	Negative	Negative	No error

Three Lab Technicians read the slides and recorded their results. No error of any type was reported in a panel of 10 slides.

10. District Laboratory: LAB- 11

Table 31: Performance of District Laboratory code number 11

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	1	3+	2+	No error
2	37	2+	2+	No error
3	73	Scanty (2 AFB/200 F)	Scanty (5 AFB/100 F)	No error
4	120	Scanty (2 AFB/200 F)	1+	No error
5	166	2+	1+	No error
6	216	3+	3+	No error
7	247	3+	3+	No error
8	275	Negative	Negative	No error
9	319	Negative	Negative	No error
10	324	Scanty (3 AFB/200 F)	Scanty (5 AFB/100 F)	No error

No error of any type was reported in a panel of 10 slides.

11. District Laboratory: LAB- 12

Table 32: Performance of District Laboratory code number 12

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	13	2+	2+	No error
2	44	2+	2+	No error
3	101	Scanty	Scanty (5 AFB/100 F)	No error
4	126	1+	1+	No error
5	163	1+	1+	No error
6	222	3+	3+	No error
7	246	3+	3+	No error
8	256	Negative	Negative	No error
9	296	Negative	Negative	No error
10	325	Negative	Negative	No error

No error of any type was reported in a panel of 10 slides.

12. District Laboratory: LAB- 13

Table 33: Performance of District Laboratory code number 13

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	4	3+	2+	No error
2	25	3+	2+	No error
3	66	Scanty (7 AFB/100 F)	Scanty (5 AFB/100 F)	No error
4	92	Scanty (9 AFB/100 F)	Scanty (5 AFB/100 F)	No error
5	121	2+	1+	No error
6	161	2+	1+	No error
7	192	Scanty	Scanty (5 AFB/100 F)	No error
8	225	3+	3+	No error
9	271	Negative	Negative	No error
10	327	Negative	Negative	No error

Four Lab Technicians read the slides and recorded their results. No error of any type was reported in a panel of 10 slides.

13. District Laboratory: LAB- 14

Table 34: Performance of District Laboratory code number 14

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	3	2+	2+	No error
2	26	2+	2+	No error
3	82	Negative	Scanty (5 AFB/100 F)	Minor error
4	108	Negative	Scanty (5 AFB/100 F)	Minor error
5	151	1+	1+	No error
6	170	1+	1+	No error
7	206	Negative	3+	Major error
8	214	3+	3+	No error
9	289	Negative	Negative	No error
10	317	Negative	Negative	No error

Three Lab Technicians read the slides and recorded their results. One High False Negative (HFN) and two Low False negative (LFN) were reported in a panel of 10 slides.

14. District Laboratory: LAB- 16

Table 35: Performance of District Laboratory code number 16

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	97	Scanty (2 AFB/100 F)	Scanty (5 AFB/100 F)	No error
2	122	1+	1+	No error
3	138	1+	1+	No error
4	162	1+	1+	No error
5	231	3+	3+	No error
6	241	2+	3+	No error
7	264	Negative	Negative	No error
8	287	Negative	Negative	No error
9	311	Negative	Negative	No error
10	341	Negative	Negative	No error

No error of any type was observed in a panel of 10 slides.

15. District Laboratory: LAB- 17

Table 36: Performance of District Laboratory code number 17

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	5	1+	2+	No error
2	28	1+	2+	No error
3	41	2+	2+	No error
4	68	Negative	Scanty (5 AFB/100 F)	Minor error
5	158	1+	1+	No error
6	209	3+	3+	No error
7	234	2+	3+	No error
8	266	Negative	Negative	No error
9	323	Negative	Negative	No error
10	329	Negative	Negative	No error

Three Lab Technicians read the slides and recorded their results. One Low False Negative (LFN) were reported in a panel of 10 slides.

16. District Laboratory: LAB- 18

Table 37: Performance of District Laboratory code number 18

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	7	3+	2+	No error
2	80	Negative	Scanty (5 AFB/100 F)	Minor error
3	110	1+	Scanty (5 AFB/100 F)	No error
4	124	2+	1+	No error
5	149	2+	1+	No error
6	168	2+	1+	No error
7	211	2+	3+	No error
8	269	Negative	Negative	No error
9	308	Negative	Negative	No error
10	335	Negative	Negative	No error

One Low False Negative (LFN) was reported in a panel of 10 slides.

17. District Laboratory: LAB- 19

Table 38: *Performance of District Laboratory code number 19*

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	72	Scanty (4 AFB/100 F)	Scanty (5 AFB/100 F)	No error
2	84	Scanty (2 AFB/100 F)	Scanty (5 AFB/100 F)	No error
3	100	Scanty (7 AFB/100 F)	Scanty (5 AFB/100 F)	No error
4	129	1+	1+	No error
5	137	1+	1+	No error
6	165	2+	1+	No error
7	215	3+	3+	No error
8	267	Negative	Negative	No error
9	326	Negative	Negative	No error
10	336	Negative	Negative	No error

No error of any type was reported in a panel of 10 slides.

18. District Laboratory: LAB- 20

Table 39: Performance of District Laboratory code number 20

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	8	2+	2+	No error
2	23	2+	2+	No error
3	76	Negative	Scanty (5 AFB/100 F)	Minor error
4	135	1+	1+	No error
5	147	1+	1+	No error
6	159	2+	1+	No error
7	229	3+	3+	No error
8	265	Negative	Negative	No error
9	285	Negative	Negative	No error
10	316	Negative	Negative	No error

One Low False Negative (LFN) was reported in a panel of 10 slides.

19. District Laboratory: LAB- 21

Table 40: Performance of District Laboratory code number 21

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	74	Negative	Scanty (5 AFB/100 F)	Minor error
2	99	Negative	Scanty (5 AFB/100 F)	Minor error
3	131	Negative	1+	Major error
4	136	Scanty (4 AFB/100 F)	1+	No error
5	187	3+	1+	Minor error
6	189	1+	Scanty (5 AFB/100 F)	No error
7	227	3+	3+	No error
8	276	Negative	Negative	No error
9	305	Negative	Negative	No error
10	314	Negative	Negative	No error

One High False Negative (HFN), two Low False Negative (LFN) and one Quantification error (QE) were reported in a panel of 10 slides.

20. District Laboratory: LAB- 22

Table 41: Performance of District Laboratory code number 22

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	6	2+	2+	No error
2	48	3+	2+	No error
3	94	Negative	Scanty (5 AFB/100 F)	Minor error
4	130	1+	1+	No error
5	154	2+	1+	No error
6	217	3+	3+	No error
7	268	Negative	Negative	No error
8	282	Negative	Negative	No error
9	321	Negative	Negative	No error
10	334	Negative	Negative	No error

Two Lab Technicians read the slides and recorded their results. One Low False Negative (LFN) was reported in a panel of 10 slides.

21. District Laboratory: LAB- 23

Table 42: Performance of District Laboratory code number 23

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	12	2+	2+	No error
2	78	Negative	Scanty (5 AFB/100 F)	Minor error
3	87	Negative	Scanty (5 AFB/100 F)	Minor error
4	186	1+	1+	No error
5	195	Negative	Scanty (5 AFB/100 F)	Minor error
6	219	3+	3+	No error
7	270	Negative	Negative	No error
8	280	Negative	Negative	No error
9	299	Negative	Negative	No error
10	313	Negative	Negative	No error

Three Low False Negative (LFN) was reported in a panel of 10 slides.

22. District Laboratory: LAB- 24

Table 43: Performance of District Laboratory code number 24

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	14	2+	2+	No error
2	70	Negative	Scanty (5 AFB/100 F)	Minor error
3	91	Negative	Scanty (5 AFB/100 F)	Minor error
4	133	1+	1+	No error
5	177	Scanty	1+	No error
6	182	1+	1+	No error
7	188	Scanty	Scanty (5 AFB/100 F)	No error
8	224	3+	3+	No error
9	274	Negative	Negative	No error
10	301	Negative	Negative	No error

Two Lab Technicians read the slides and recorded their results. Two Low False Negative (LFN) was reported in a panel of 10 slides.

23. District Laboratory: LAB- 25

Table 44: Performance of District Laboratory code number 25

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	102	Scanty (4 AFB/100 F)	Scanty (5 AFB/100 F)	No error
2	114	1+	1+	No error
3	153	1+	1+	No error
4	174	1+	1+	No error
5	208	3+	3+	No error
6	235	3+	3+	No error
7	261	Negative	Negative	No error
8	290	Negative	Negative	No error
9	302	Negative	Negative	No error
10	340	Negative	Negative	No error

No error of any types was reported in a panel of 10 slides.

24. District Laboratory: LAB- 26

Table 45: Performance of District Laboratory code number 26

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	29	1+	2+	No error
2	83	Scanty	Scanty (5 AFB/100 F)	No error
3	106	Scanty	1+	No error
4	134	1+	1+	No error
5	185	1+	1+	No error
6	191	Scanty	Scanty (5 AFB/100 F)	No error
7	221	3+	3+	No error
8	272	Negative	Negative	No error
9	278	Negative	Negative	No error
10	309	Negative	Negative	No error

Two Lab Technicians read the slides and recorded their results. No error of any type was reported in a panel of 10 slides.

25. District Laboratory: LAB- 27

Table 46: Performance of District Laboratory code number 27

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	46	2+	2+	No error
2	96	Scanty (1 AFB/100 F)	Scanty (5 AFB/100 F)	No error
3	117	Scanty (7 AFB/100 F)	1+	No error
4	140	Scanty (1 AFB/100 F)	1+	No error
5	164	Scanty (6 AFB/100 F)	1+	No error
6	205	3+	3+	No error
7	238	3+	3+	No error
8	284	Negative	Negative	No error
9	315	Negative	Negative	No error
10	333	Negative	Negative	No error

No error of any type was reported in a panel of 10 slides.

26. District Laboratory: LAB- 28

Table 47: Performance of District Laboratory code number 28

Sl. No.	Slide No.	Result Obtained	Expected Result	Error Type
1	18	Negative	2+	Major error
2	49	2+	2+	No error
3	104	Scanty (2 AFB/100 F)	Scanty (5 AFB/100 F)	No error
4	132	Negative	1+	Major error
5	155	Scanty (1 AFB/100 F)	1+	No error
6	206	3+	3+	No error
7	242	3+	3+	No error
8	259	Negative	Negative	No error
9	292	Negative	Negative	No error
10	322	Negative	Negative	No error

Two High False Negative (HFN) were reported in a panel of 10 slides.

Annex II

National Tuberculosis Reference Laboratory
Public Health Laboratory
Report on Proficiency Testing of District Hospital Laboratories
2008

Preparation of Panel Testing Smear Slides of known content:

The following procedure is a self-explanatory laboratory method for producing multiple test slides from AFB positive and negative samples.

Sodium hydroxide (NaOH) method

1. **Materials Required:**
   - 4% NaOH
   - 40% Formaldehyde
   - Distilled water
   - Centrifuge
   - Centrifuge tubes (50 mL plastic screw cap)
   - Water bath 55-60 ºC
   - New slides
   - Bacteriological incubator

2. **Positive specimen** (fresh specimen, no more than 2 cays old, are preferred)

Amount: 3 mL or more.
AFB load: >2+ AFB by Ziehl-Neelsen direct smear.
Color: White to light green; blood stained specimens should be avoided.
Thickness: Watery (less mucus) specimens are preferred to increase consistency.

Known positive sputum samples were pooled and homogenized in a vortex mixer after the addition of glass beads.

3. **Negative specimen** (fresh specimen, not more than 2 days old, are preferred)

Amount: 5 mL or more
Color: White to green
Thickness: Watery (less mucus) specimens were preferred to increase consistency

Known negative sputum samples (>20 WBC/field) were pooled and homogenized in a vortex mixer after the addition of glass beads. Requisite number of negative slides was prepared from the pool of negative samples.

4. Preparation of Positive stock

Method

Step 1 3 mL of >2+ AFB positive sputum was transferred to a 50 mL screw capped container.
Step 2 1 drop (approx. 50µl) of 40% formaldehyde per mL of sputum was added and vortex well.
Step 3 Incubated for 1 hour at room temperature (25-30ºC).
Step 4 1 mL of 4% NaOH was added. The container was closed tightly with a screw cap having an intact rubber liner and mixed thoroughly by shaking.
Note: When sputum is collected in a suitable wide mouthed container, NaOH is directly added to the container.
Step 5 The specimen was Vortex approximately for 4-5 minutes.
Step 6 Upto 20 mL of distilled water was added and mixed well.
Step 7 Incubated in a water bath for 30 minutes at 55-60ºC, mixed occasionally by inverting the tube during incubation.
Step 8 Upto 40 mL of distilled water was added and mixed by inversion.
Step 9 Carefully transferred the treated specimen to a sterile plastic centrifuge tube.
Step 10 Centrifuged for 20 minutes at 3000 x g. Pipetted off the supernatant fluid and discarded it into a discarding jar having a strong disinfectant such as 5% phenol. 0.5-1 mL of distilled water was added to re-suspend the pellets.
Step 11 Smears were made from these suspensions on separate, clean, grease-free new slides for Z-N staining.

5. Preparation of negative stock

Method

Step 1 3-5 mL aliquots of AFB-negative sputum were transferred to a 50 mL screw caped container.
Step 2 1 drop (approx. 50µl) of 40% formaldehyde per mL of sputum was added and vortex well.
Step 3 Incubated for 1 hour at room temperature (25-30ºC).
Step 4 1 mL of 4% NaOH was added. The container was closed tightly with a screw cap having an intact rubber liner and mixed thoroughly by shaking.
Step 5 Then, vortexed the specimen approximately for 2-3 minutes.
Step 6 Upto 20 mL of distilled water was added and mixed.
Step 7 Incubated in a water bath for 10 minutes at 55-60ºC.
Note: The negative stock was heated for the shorter period to preserve white blood cells.

6. Evaluation of Positive Stock preparations

Step 1 If foam is formed on the top of the stock solution; the contents were pipetted from beneath the foam into a fresh tube.
Step 2 Using a standard microbiological loop 2-3 test smears (approx 1x2 cm in size) were made from the suspension for evaluation of the stock prepared.
Step 3 Used a well leveled surface for drying the smears.

Positive stock: It was optimal to have concentration of 50-60 AFB / field.

7. Dilution procedure

Step 1 Suitable AFB concentration was choosed on a case-to-case basis within suggested range. For better results, 20 AFB/field for 3+, 5 AFB/field for 2+, 50 AFB/100 field for 1+, 5 AFB/100 field and 2 AFB/100 field for exact numbers of AFB.

Step 2 3-4 mL of each suspension was made in order to be able to generate sufficient amount of smears. For preparation of positive or exact number of bacteria, following formula was used for calculation of the dilution factor from stock positive solution:

N = (DC/AC) x A

Where;

N = the amount of drops of positive sputum to be added
DC = desired AFB concentration
AC = actual AFB concentration
A = the amount of drops in a given volume that was estimated during calibration.

Example

AFB concentration in the stock suspension (AC) is 60 AFB/field and we have to prepare 4 mL (A = 80 drops) of 2+ suspension (DC = 5 AFB/field) x 80 drops.
N = 6.6 drops (approx. 7 drops), so 7 drops of positive stock preparation is mixed with 73 (80-7) drops of the negative preparation.

Annex III

National Reference Laboratory of Tuberculosis
Public Health Laboratory
Report on Proficiency Testing of District Hospital Laboratories
2008

Ziehl-Neelsen staining for AFB

Principle
Mycobacterium tuberculosis is known as AFB because it resists decolorisation by acid. This acid fastness is due to the presence of mycolic acid in the cell wall.
In this method, the primary stain (Carbol fuchsin) is heated, which facilitates the stain to penetrate the waxy covering of mycobacteria and resist decolourisation by weak acid. Those bacteria that resist decolourisation by acid are called Acid Fast Bacilli (AFB). This property differentiates AFB from other bacteria, cells and mucus which get decolorized by the action of weak acid. The counter stain (Methylene blue) is used to stain other materials and gives a contrast background for easy visibility of the Acid Fast Bacilli.

Materials Required

- 0.3% Carbol fuchsin stain
- 20% Sulphuric acid
- 1% Methylene blue
- Distilled water
- Filter paper
- Forceps
- Match box
- Spirit lamp
- Staining rack
- Microscope
- Cedar wood oil
- Lens papers
- Clean soft cloth
- Slide boxes

Method

Step 1 Heat fixed sputum smears were placed on the staining rack.
Step 2 Flood the smear with Carbol fuchsin stain. Heat the slides from underside with a spirit lamp until vapour just began to rise. Leave the stain for about 5 minutes.
Step 3 Rinse the stain with clean water and drain off excess water on the slide.
Step 4 Decolorize the smear by covering the whole slide with 20% Sulphuric acid for about 3-4 minutes or until the smear was sufficiently decolorized. Decolorisation is repeated, if necessary. Rinse well with clean water.
Step 5 Cover the smear with methylene blue for 1 minute. Gently rinse the slide with clean water.
Step 6 Wipe the back of the slide clean and place upright on the slide rack to air dry.
Step 7 Examine the smear under oil immersion objective for the presence or AFB.

Result: The AFB was stained bright pink.