

Report on Blinded rechecking of AFB smears of District Hospital Laboratories For 2008



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Acronyms

1.	AFB	Acid Fast Bacilli
2.	NEQAS	National External Quality Assurance Scheme
3.	IUATLD	International Union Against Tuberculosis and Lung Diseases
4.	PHL	Public Health Laboratory
5.	NTRL	National Tuberculosis Reference Laboratory
6.	TB	Tuberculosis
7.	QA	Quality Assurance
8.	QC	Quality Control
9.	QI	Quality improvement-
10.	WHO	World Health Organization
11.	ZN	Ziehl- Neelsen staining

Executive Summary

Blinded rechecking is the most effective method of external quality assessment (EQA) program especially for AFB smear. The blinded rechecking is conducted on AFB smears stored by district labs every day and sent to PHL every quarter. The assessment is primarily focus on smear quality determine by six characteristics of smears to evaluate the overall performance standard of individual/lab technicians in performing routine sputum microscopy.

The blinded-rechecking results for four quarters of 2008 revealed low standard sputum microcopy in the district. This external quality assessment result substantiates the low sputum positivity rate in the country. The evaluation also revealed the reporting of false results by some labs.

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.

Blinded rechecking is the most effective assessment method of NEQAS compare to panel testing and onsite evaluation for sputum microscopy. Blinded rechecking provides the insight of laboratory personnel capability and standard in carrying out day to day sputum microscopy. Blinded rechecking is time consuming and requires more number of competent human resources to conduct it regularly. However, it is the only assessment method that provides evidence about technical problem and individual's performance can be corrected and improved through this particular assessment method.

NEQAS on sputum microscopy network covers twenty eight district hospital laboratories providing sputum microscopy service in the country. Blinded rechecking is conducted every quarterly for an entire year.

2. Objective

To improve the standard of sputum microscopy in the country

3. Materials and Methods

3.1 Determining sample size for blinded rechecking

The sample size for blinded rechecking was determined based on data collected from district labs in 2007. The slide positivity rate (SPR) was **7.5%** from an average number of **12000** negative slides examined in 2007. Considering SPR and total number of negative slides examined, the number of slides to be blinded was statistically determined keeping minimum controllers sensitivity at **75%** and acceptance number as **zero**. Using the statistic table No. 1 reference lab need to recheck 105 blinded slides annually for each district laboratories which accounts to 26-28 slides per quarter in a year.

Table 1: EQA sample size table.

Sensitivity Relative to the Controllers At 75%

		Slide Positivity Rate						
		2.5%	5.0%	7.5%	10.0%	13.0%	15.0%	18.0%
Negatives Examined Annually	Acceptance Number	Total Sample Required						
		100	d=0	78	63	54	47	40
200	d=0	123	91	71	59	48	42	37
300	d=0	154	105	80	64	52	45	38
400	d=0	175	115	85	68	53	47	39
500	d=0	192	121	89	69	54	47	40
700	d=0	215	129	93	72	55	48	40
1000	d=0	236	136	96	73	56	49	41
2000	d=0	267	145	102	77	59	51	41
5000	d=0	289	152	104	78	59	51	43
10000	d=0	297	154	105	78	60	51	43
20000	d=0	302	155	106	79	60	52	43
50000	d=0	305	156	106	79	60	52	43

3.2 Slide collection and sampling methodology

District labs should store all routine slides in sequential order as per the records of TB lab register and send to PHL at the end of every quarter through ambulances.

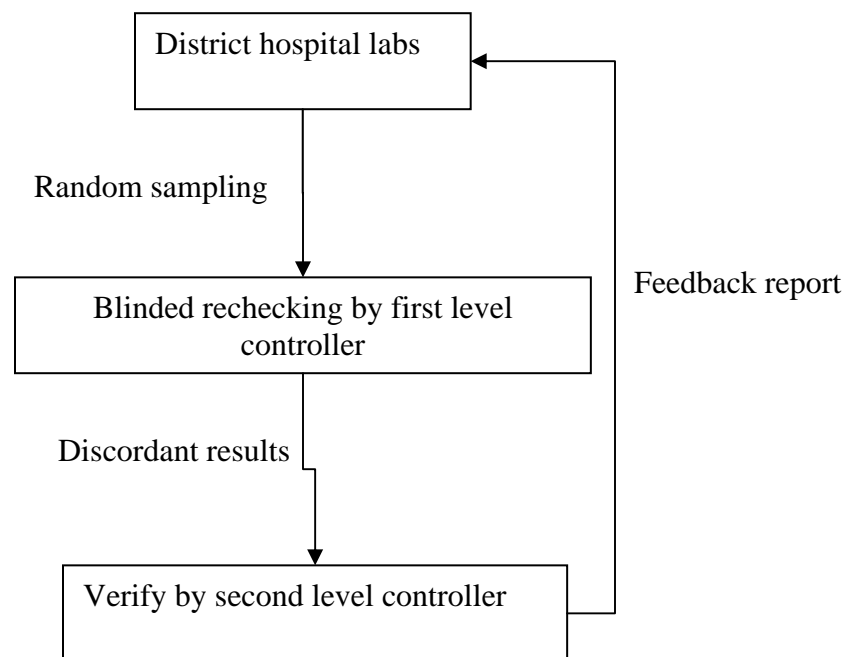
The representative slide numbers required for blinded rechecking for every quarter are randomly selected from total slides received at regular interval covering entire slides irrespective of results

reported. The results of the district laboratories were blinded to the controllers to avoid bias while rechecking. The selection is done using the TB lab record form sent by labs long with slides.

3.3 Rechecking procedures

Rechecking is primarily focused on six characteristics of smear to assess the over all standard of sputum microscopy followed by results reported. The controllers apply the same international standards criteria adopted by national and international guidelines for carrying out routine sputum microscopy. The results of district are decoded only after controllers examine the smear quality and results. If there is any discrepancy between the first level controller and district result, second controller will re-examine to verify the results. The feedbacks are sent back to respective labs for implementing corrective measures (Figure 1)

Figure 1: Organization of Rechecking Process



Six characteristics of smears used for assessment are specimen quality, appropriate size, staining quality, thickness, evenness and cleanliness. The criteria for grading of each characteristic are mention below in Table 2.

Table 2. Assessment criteria of smear slide

Properties	Grading		
	Good	Medium	Poor
1. Sputum Quality	Presences of > 25 leucocytes/field at 100X.	Presence of < 25 Leucocytes/field but > 5 leucocytes/field. Less than 5 epithelial cells/field.	Absence of leucocytes in the smear. Presence of unspecified cells and presence of more than 10 epithelial cells/ field.
2. Size	Smear size of 1x2cm. Oval in shape and smear centrally placed in the slide.	Acceptable size of approximately 1x2cm. Shape of smear round or oval and smear placed at sub-terminal end of slide.	Smear size smaller than 1x2cm and larger than 1x2cm. Irregular in shape. Smear placed at terminal end of slide.
3. Evenness	Sputum spread evenly on the glass slide not too thick and not too thin. Presence of leucocytes in every field screened at 100X for 100 to 200 fields.	Presence of leucocytes in 70% of smear screened at 100X for 100-200 fields.	Leucocytes present in 50% of smear screened at 100X for 100-200 fields. More than 50% empty fields of smear screened at 100X.
4. Thickness	Mono-layer smear on the glass slide Whole depth of the smear layer can be focused sharply in each field.	Few over-lapping cells seen. Whole depth of the smear layer can be focused sharply in 70% of field.	Smears too thick with over-lapping cells seen in entire smear. Too thin smear. Whole depth of the smear layer can be focused sharply in less than 50% of the field.
5. Cleanness	Clean smear. Smear free from stain deposit, crystals produced by overheating of stain and absence of debris.	Some portion of smear seen with stain deposits only but more than 70% of the smear is clean.	Dirty smear, cannot screen the smear on the slide. Smear found with stain deposits, crystals and debris.
6. Staining quality by microscopic observation	AFB and background clearly distinguished. AFB in bright red color. Leucocytes stained blue and absence of stain particles.	AFB and background clearly distinguishable in 80% of the smear. AFB light red in color. Presence of stain particles in some places of the smear.	Not able to distinguish AFB and background. AFB faintly red in color. AFB covered with methylene blue. Remaining of fuchsin color on the background. Remaining of methylene blue color on the un-smeard parts.

Scores for grading:

Good= 10
 Medium= 5
 Poor= 0

% Calculation of scores= $\frac{\text{Sum of Total Score for slides rechecked}}{\text{Total no. of slides rechecked}} \times 100 = _ \%$

3.4 Evaluation table for errors

Table 3: Reports tabulated and analyzed by using the following guideline

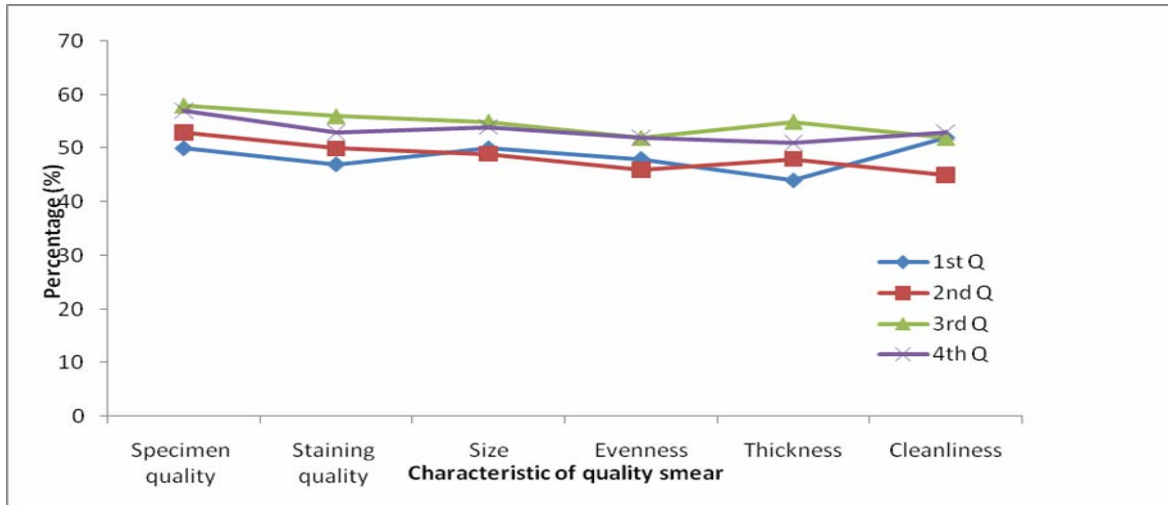
Result of Laboratory	Result of Controlling Laboratory (PHL)				
	Negative	1-9 AFB/100 F	1+	2+	3+
Negative	Correct	LFN	HFN	HFN	HFN
1-9 AFB/100 F	LFP	Correct	Correct	QE	QE
1+	HFP	Correct	Correct	Correct	QE
2+	HFP	QE	Correct	Correct	Correct
3+	HFP	QE	QE	Correct	Correct

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

4. Results

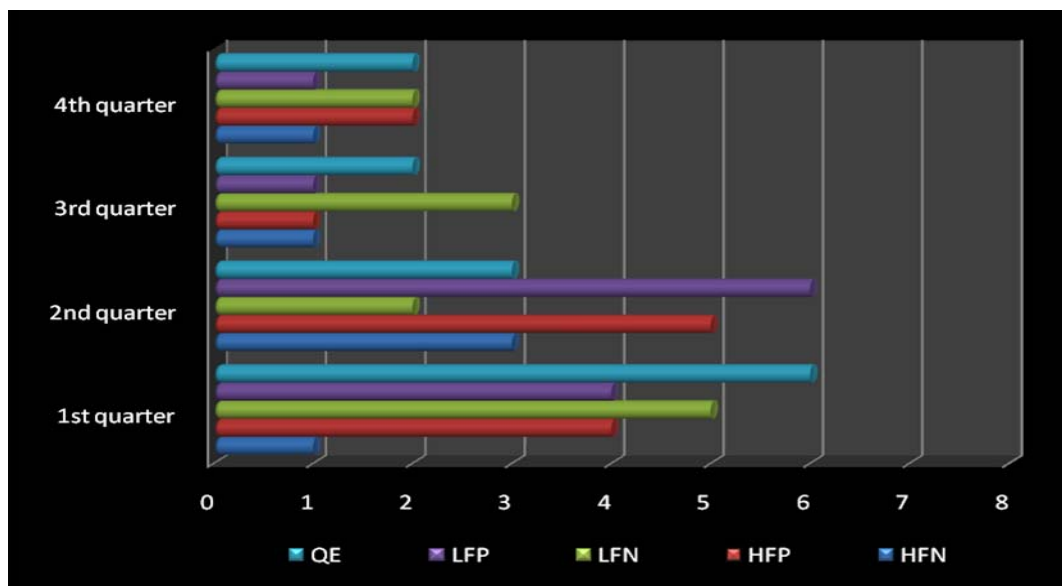
Results of 1st, 2nd, 3rd and 4th quarter 2008 of all district labs participating in NEQAS are shown in tabulation. Almost all the labs failed to meet the minimum acceptable standard of smear quality in 2008 however; some labs have shown steady improvement in their performances (Figure 1). The detail results of each hospital are given in tables.

Figure 1: Smear quality standard of 2008 in the country



In the results interpretation and reporting, few labs were found reporting false positive and some labs still missing out AFB (false negative) (Figure 2). Reporting of low false negative and quantification errors are expected especially if lab technicians are new but not high/low false positive and high false negative.

Figure 2: Errors reported in 2008 by district hospital labs



4.1 District laboratory (LAB-01)

Table 4: Performance of District laboratory code number 01

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	53	73	-	74
2	Slides Rechecked	27	26	-	26
3	High False Negative	0	0	-	0
4	High False Positive	0	0	-	0
5	Low False Negative	0	0	-	0
6	Low False Positive	0	0	-	0
7	Quantification Error	0	0	-	0
8	Overall Agreement Rate (%)	100	100	-	100
9	Total Score	100	100	-	100
i.	Specimen quality (%)	69	54	-	60
ii.	Staining quality (%)	61	38	-	54
iii.	Size (%)	54	46	-	38
iv.	Evenness (%)	46	46	-	48
v.	Thickness (%)	65	46	-	50
vi.	Cleanliness (%)	44	46	-	38

4.2 District laboratory (LAB-02)

Table 6: Performance of District laboratory code number 02

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	60	113	62	72
2	Slides Rechecked	26	27	26	26
3	High False Negative	0	0	0	0
4	High False Positive	0	0	0	0
5	Low False Negative	2	0	0	0
6	Low False Positive	0	1	0	0
7	Quantification Error	0	1	0	0
8	Overall Agreement Rate (%)	92.31	92.59	100	100
9	Total Score	98.46	97.41	100	100
i.	Specimen Quality (%)	25	30	48	42
ii.	Staining (%)	6	26	50	40
iii.	Size (%)	16	26	19	33
iv.	Evenness (%)	6	26	42	39
v.	Thickness (%)	6	26	42	40
vi.	Cleanliness (%)	6	22	67	40

4.3 District laboratory (LAB-03)

Table 9: Performance of District Laboratory code number 03

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	-	54	-	-
2	Slides Rechecked	-	27	-	-
3	High False Negative	-	1	-	-
4	High False Positive	-	0	-	-
5	Low False Negative	-	0	-	-
6	Low False Positive	-	0	-	-
7	Quantification Error	-	0	-	-
8	Overall Agreement Rate (%)	-	96.30	-	-
9	Total Score	-	96.30	-	-
i.	Specimen Quality (%)	-	67	-	-
ii.	Staining (%)	-	65	-	-
iii.	Size (%)	-	74	-	-
iv.	Evenness (%)	-	59	-	-
v.	Thickness (%)	-	56	-	-
vi.	Cleanliness (%)	-	43	-	-

4.4 District laboratory (LAB-04)

Table 10: Performance of District Laboratory, code number 04

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	138	-	-	187
2	Slides Rechecked	26	-	-	26
3	High False Negative	0	-	-	0
4	High False Positive	1	-	-	0
5	Low False Negative	0	-	-	0
6	Low False Positive	0	-	-	0
7	Quantification Error	0	-	-	0
8	Overall Agreement Rate (%)	96.15	-	-	100
9	Total Score	96.15	-	-	100
i.	Specimen Quality (%)	62	-	-	56
ii.	Staining (%)	40	-	-	44
iii.	Size (%)	46	-	-	46
iv.	Evenness (%)	29	-	-	46
v.	Thickness (%)	31	-	-	46
vi.	Cleanliness (%)	25	-	-	44

4.5 District laboratory (LAB-05)

Table 11: Performance of District Laboratory code number 05

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	218	213	253	317
2	Slides Rechecked	27	26	26	26
3	High False Negative	0	0	0	0
4	High False Positive	0	0	0	0
5	Low False Negative	0	0	0	1
6	Low False Positive	0	0	0	0
7	Quantification Error	1	0	1	0
8	Overall Agreement Rate (%)	96.30	100	96.15	96.15
9	Total Score	98.15	100	98.08	99.23
i.	Specimen Quality (%)	61	60	62	71
ii.	Staining (%)	61	62	75	71
iii.	Size (%)	52	60	58	67
iv.	Evenness (%)	55	60	56	65
v.	Thickness (%)	48	52	62	63
vi.	Cleanliness (%)	60	58	65	73

4.6 District laboratory (LAB-06)

Table 5: Performance of District laboratory code number 06

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	34	31	31	20
2	Slides Rechecked	26	27	27	20
3	High False Negative	0	0	0	0
4	High False Positive	0	0	0	0
5	Low False Negative	0	0	0	0
6	Low False Positive	0	0	0	0
7	Quantification Error	0	0	0	0
8	Overall Agreement Rate (%)	100	100	100	100
9	Total Score	100	100	100	100
i.	Specimen Quality (%)	56	34	44	55
ii.	Staining (%)	15	33	43	50
iii.	Size (%)	37	31	44	43
iv.	Evenness (%)	15	31	44	50
v.	Thickness (%)	29	31	43	50
vi.	Cleanliness (%)	15	31	41	50

4.7 District laboratory (LAB-07)

Table 16: Performance of District Laboratory code number 07

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	-	22	-	54
2	Slides Rechecked	-	22	-	25
3	High False Negative	-	0	-	0
4	High False Positive	-	0	-	0
5	Low False Negative	-	0	-	0
6	Low False Positive	-	0	-	0
7	Quantification Error	-	0	-	0
8	Overall Agreement Rate (%)	-	100	-	100
9	Total Score	-	100	-	100
i.	Specimen Quality (%)	-	59	-	52
ii.	Staining (%)	-	48	-	72
iii.	Size (%)	-	48	-	54
iv.	Evenness (%)	-	48	-	52
v.	Thickness (%)	-	48	-	52
vi.	Cleanliness (%)	-	41	-	72

4.8 District laboratory (LAB-08)

Table 15: Performance of District Laboratory code number 08

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	63	80	-	33
2	Slides Rechecked	27	26	-	25
3	High False Negative	0	0	-	0
4	High False Positive	0	0	-	0
5	Low False Negative	0	0	-	0
6	Low False Positive	0	0	-	0
7	Quantification Error	0	0	-	0
8	Overall Agreement Rate (%)	100	100	-	100
9	Total Score	100	100	-	100
i.	Specimen Quality (%)	33	54	-	68
ii.	Staining (%)	17	33	-	70
iii.	Size (%)	30	33	-	68
iv.	Evenness (%)	24	33	-	68
v.	Thickness (%)	19	33	-	66
vi.	Cleanliness (%)	26	33	-	72

4.9 District laboratory (LAB-09)

Table 17: Performance of District Laboratory code number 09

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	198	229	146	191
2	Slides Rechecked	28	26	26	25
3	High False Negative	0	0	0	0
4	High False Positive	0	0	0	0
5	Low False Negative	0	0	0	0
6	Low False Positive	1	0	0	0
7	Quantification Error	1	0	0	0
8	Overall Agreement Rate (%)	92.86	100	100	100
9	Total Score	97.50	100	100	100
i.	Specimen Quality (%)	29	33	54	68
ii.	Staining (%)	23	27	27	54
iii.	Size (%)	36	31	33	62
iv.	Evenness (%)	50	31	33	58
v.	Thickness (%)	38	29	33	58
vi.	Cleanliness (%)	60	29	27	62

4.10 District laboratory (LAB-10)

Table 18: Performance of District Laboratory code number 10

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	248	315	266	199
2	Slides Rechecked	26	27	26	26
3	High False Negative	0	0	0	0
4	High False Positive	0	0	0	0
5	Low False Negative	1	0	0	0
6	Low False Positive	1	0	0	0
7	Quantification Error	0	0	0	0
8	Overall Agreement Rate (%)	92.31	100	100	100
9	Total Score	98.46	100	100	100
i.	Specimen Quality (%)	69	44	62	46
ii.	Staining (%)	81	48	79	38
iii.	Size (%)	77	43	69	46
iv.	Evenness (%)	65	41	56	46
v.	Thickness (%)	62	41	58	46
vi.	Cleanliness (%)	65	37	63	44

4.11 District laboratory (LAB-11)

Table 19: Performance of District Laboratory code number 11

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	37	104	131	96
2	Slides Rechecked	28	27	25	26
3	High False Negative	0	0	0	0
4	High False Positive	0	0	0	0
5	Low False Negative	0	0	0	0
6	Low False Positive	0	0	0	0
7	Quantification Error	0	0	0	0
8	Overall Agreement Rate (%)	100	100	100	100
9	Total Score	100	100	100	100
i.	Specimen Quality (%)	54	54	62	56
ii.	Staining (%)	48	57	60	56
iii.	Size (%)	32	54	60	56
iv.	Evenness (%)	55	50	48	56
v.	Thickness (%)	20	56	50	56
vi.	Cleanliness (%)	50	46	48	56

4.12 District laboratory (LAB-12)

Table 13: Performance of District Laboratory code number 12

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	70	65	92	39
2	Slides Rechecked	28	26	26	25
3	High False Negative	0	0	0	0
4	High False Positive	1	0	0	0
5	Low False Negative	0	0	1	0
6	Low False Positive	0	0	0	0
7	Quantification Error	0	0	0	0
8	Overall Agreement Rate (%)	96.43	100	96.15	100
9	Total Score	96.43	100	99.23	100
i.	Specimen Quality (%)	54	77	44	60
ii.	Staining (%)	52	60	38	62
iii.	Size (%)	50	85	36	66
iv.	Evenness (%)	57	46	38	62
v.	Thickness (%)	57	44	38	58
vi.	Cleanliness (%)	70	58	38	62

4.13 District laboratory (LAB-13)

Table 21: Performance of District Laboratory code number 13

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	296	497	467	421
2	Slides Rechecked	29	26	25	25
3	High False Negative	0	1	0	0
4	High False Positive	0	0	0	0
5	Low False Negative	0	0	2	0
6	Low False Positive	2	0	0	0
7	Quantification Error	1	0	0	0
8	Overall Agreement Rate (%)	89.66	96.15	92.00	100
9	Total Score	96.90	96.15	98.40	100
i.	Specimen Quality (%)	55	96	92	96
ii.	Staining (%)	41	77	90	48
iii.	Size (%)	40	69	86	44
iv.	Evenness (%)	65	69	86	48
v.	Thickness (%)	36	83	82	40
vi.	Cleanliness (%)	65	69	90	52

4.14 District laboratory (LAB-14)

Table 22: Performance of District Laboratory code number 14

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	277	211	-	140
2	Slides Rechecked	28	27	-	25
3	High False Negative	0	0	-	0
4	High False Positive	0	1	-	0
5	Low False Negative	0	0	-	0
6	Low False Positive	0	1	-	0
7	Quantification Error	0	0	-	1
8	Overall Agreement Rate (%)	100	92.59	-	96.00
9	Total Score	100	95.56	-	98.00
i.	Specimen Quality (%)	75	57	-	56
ii.	Staining (%)	41	48	-	56
iii.	Size (%)	43	44	-	58
iv.	Evenness (%)	57	48	-	60
v.	Thickness (%)	45	48	-	60
vi.	Cleanliness (%)	75	48	-	58

4.15 District laboratory (LAB-16)

Table 23: Performance of District Laboratory code number 16

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	37	30	37	52
2	Slides Rechecked	27	27	26	25
3	High False Negative	0	0	0	0
4	High False Positive	0	0	0	0
5	Low False Negative	1	0	0	0
6	Low False Positive	0	1	0	0
7	Quantification Error	0	0	1	0
8	Overall Agreement Rate (%)	96.30	96.30	96.15	100
9	Total Score	99.26	99.26	98.08	100
i.	Specimen Quality (%)	78	44	77	60
ii.	Staining (%)	76	63	90	58
iii.	Size (%)	65	39	87	58
iv.	Evenness (%)	44	41	67	58
v.	Thickness (%)	63	50	75	58
vi.	Cleanliness (%)	69	61	81	46

4.16 District laboratory (LAB-17)

Table 24: Performance of District Laboratory code number 17

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	-	-	-	-
2	Slides Rechecked	-	-	-	-
3	High False Negative	-	-	-	-
4	High False Positive	-	-	-	-
5	Low False Negative	-	-	-	-
6	Low False Positive	-	-	-	-
7	Quantification Error	-	-	-	-
8	Overall Agreement Rate (%)	-	-	-	-
9	Total Score	-	-	-	-
i.	Specimen Quality (%)	-	-	-	-
ii.	Staining (%)	-	-	-	-
iii.	Size (%)	-	-	-	-
iv.	Evenness (%)	-	-	-	-
v.	Thickness (%)	-	-	-	-
vi.	Cleanliness (%)	-	-	-	-

4.17 District laboratory (LAB-18)

Table 25: Performance of District Laboratory code number 18

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	230	219	328	189
2	Slides Rechecked	26	27	26	26
3	High False Negative	0	0	0	0
4	High False Positive	2	3	0	0
5	Low False Negative	0	0	0	0
6	Low False Positive	0	0	0	0
7	Quantification Error	0	0	0	0
8	Overall Agreement Rate (%)	92.31	88.89	100	100
9	Total Score	92.31	88.89	100	100
i.	Specimen Quality (%)	58	48	62	67
ii.	Staining (%)	56	39	62	65
iii.	Size (%)	62	39	56	69
iv.	Evenness (%)	42	39	50	58
v.	Thickness (%)	50	39	50	62
vi.	Cleanliness (%)	39	35	60	56

4.18 District laboratory (LAB-19)

Table 26: Performance of District Laboratory code number 19

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	65	109	121	75
2	Slides Rechecked	27	26	26	26
3	High False Negative	0	0	0	0
4	High False Positive	0	0	0	0
5	Low False Negative	0	0	0	0
6	Low False Positive	0	0	0	0
7	Quantification Error	0	0	0	0
8	Overall Agreement Rate (%)	100	100	100	100
9	Total Score	100	100	100	100
i.	Specimen Quality (%)	61	29	46	48
ii.	Staining (%)	52	27	40	38
iii.	Size (%)	33	25	40	42
iv.	Evenness (%)	40	23	40	38
v.	Thickness (%)	30	23	40	40
vi.	Cleanliness (%)	60	27	40	38

4.19 District laboratory (LAB-20)

Table 27: Performance of District Laboratory code number 20

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	40	65	-	79
2	Slides Rechecked	26	27	-	25
3	High False Negative	0	0	-	0
4	High False Positive	0	0	-	0
5	Low False Negative	0	0	-	0
6	Low False Positive	0	0	-	0
7	Quantification Error	0	0	-	0
8	Overall Agreement Rate (%)	100	100	-	100
9	Total Score	100	100	-	100
i.	Specimen Quality (%)	71	48	-	56
ii.	Staining (%)	94	46	-	48
iii.	Size (%)	67	48	-	50
iv.	Evenness (%)	67	48	-	48
v.	Thickness (%)	60	48	-	48
vi.	Cleanliness (%)	63	48	-	48

4.20 District laboratory (LAB-21)

Table 29: Performance of District Laboratory code number 21

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	-	-	-	-
2	Slides Rechecked	-	-	-	-
3	High False Negative	-	-	-	-
4	High False Positive	-	-	-	-
5	Low False Negative	-	-	-	-
6	Low False Positive	-	-	-	-
7	Quantification Error	-	-	-	-
8	Overall Agreement Rate (%)	-	-	-	-
9	Total Score	-	-	-	-
i.	Specimen Quality (%)	-	-	-	-
ii.	Staining (%)	-	-	-	-
iii.	Size (%)	-	-	-	-
iv.	Evenness (%)	-	-	-	-
v.	Thickness (%)	-	-	-	-
vi.	Cleanliness (%)	-	-	-	-

4.21 District laboratory (LAB-22)

Table 28: Performance of District Laboratory code number 22

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	112	145	85	82
2	Slides Rechecked	28	26	26	25
3	High False Negative	0	0	0	1
4	High False Positive	0	0	0	0
5	Low False Negative	0	0	0	1
6	Low False Positive	0	0	0	0
7	Quantification Error	0	0	0	0
8	Overall Agreement Rate (%)	100	100	100	92.00
9	Total Score	100	100	100	95.20
i.	Specimen Quality (%)	50	37	40	58
ii.	Staining (%)	48	48	85	62
iii.	Size (%)	50	44	46	64
iv.	Evenness (%)	46	33	54	56
v.	Thickness (%)	50	44	54	52
vi.	Cleanliness (%)	43	19	54	56

4. 22 District laboratory (LAB-23)

Table 30: Performance of District Laboratory code number 23

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	58	98	19	19
2	Slides Rechecked	26	27	19	19
3	High False Negative	0	0	0	0
4	High False Positive	0	0	0	2
5	Low False Negative	0	1	0	0
6	Low False Positive	0	0	0	0
7	Quantification Error	0	0	0	1
8	Overall Agreement Rate (%)	100	92.59	100	84.21
9	Total Score	100	97.41	100	86.84
i.	Specimen Quality (%)	60	63	58	58
ii.	Staining (%)	29	72	58	47
iii.	Size (%)	31	61	53	47
iv.	Evenness (%)	45	59	58	50
v.	Thickness (%)	33	69	58	50
vi.	Cleanliness (%)	50	57	53	47

4.23 District laboratory (LAB-24)

Table 31: Performance of District Laboratory code number 24

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	51	90	86	76
2	Slides Rechecked	26	28	26	25
3	High False Negative	0	0	0	0
4	High False Positive	0	0	1	0
5	Low False Negative	0	0	0	0
6	Low False Positive	0	0	1	0
7	Quantification Error	0	0	0	0
8	Overall Agreement Rate (%)	100	100	92.31	100
9	Total Score	100	100	95.38	100
i.	Specimen Quality (%)	69	41	48	58
ii.	Staining (%)	31	29	23	56
iii.	Size (%)	52	27	35	56
iv.	Evenness (%)	46	25	33	58
v.	Thickness (%)	46	25	35	56
vi.	Cleanliness (%)	27	27	35	58

4. 24 District laboratory (LAB-25)

Table 8: Performance of District Laboratory code number 25

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	58	77	58	40
2	Slides Rechecked	27	26	26	26
3	High False Negative	0	0	0	0
4	High False Positive	0	0	0	0
5	Low False Negative	0	0	0	0
6	Low False Positive	0	0	0	0
7	Quantification Error	0	0	0	0
8	Overall Agreement Rate (%)	100	100	100	100
9	Total Score	100	100	100	100
i.	Specimen Quality (%)	32	44	37	44
ii.	Staining (%)	44	48	38	46
iii.	Size (%)	64	40	54	70
iv.	Evenness (%)	65	54	52	50
v.	Thickness (%)	50	50	57	48
vi.	Cleanliness (%)	65	50	44	40

4.25 District laboratory (LAB-26)

Table 32: Performance of District Laboratory code number 26

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	28	17	23	28
2	Slides Rechecked	28	17	23	28
3	High False Negative	0	0	0	0
4	High False Positive	0	0	0	0
5	Low False Negative	0	0	0	0
6	Low False Positive	0	0	0	0
7	Quantification Error	0	0	0	0
8	Overall Agreement Rate (%)	100	100	100	100
9	Total Score	100	100	100	100
i.	Specimen Quality (%)	70	62	52	50
ii.	Staining (%)	79	56	48	50
iii.	Size (%)	64	56	52	50
iv.	Evenness (%)	48	50	52	50
v.	Thickness (%)	55	76	52	50
vi.	Cleanliness (%)	61	53	48	50

4.26 District laboratory (LAB-27)

Table 12: Performance of District Laboratory code number 27

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	101	141	138	107
2	Slides Rechecked	27	26	26	26
3	High False Negative	1	1	1	0
4	High False Positive	0	1	0	0
5	Low False Negative	1	1	0	0
6	Low False Positive	0	3	0	1
7	Quantification Error	3	1	0	0
8	Overall Agreement Rate (%)	81.40	73.08	96.15	96.15
9	Total Score	90.0	87.31	96.15	99.23
i.	Specimen Quality (%)	64	65	65	31
ii.	Staining (%)	43	56	54	27
iii.	Size (%)	45	62	54	40
iv.	Evenness (%)	36	60	54	29
v.	Thickness (%)	36	62	48	29
vi.	Cleanliness (%)	70	58	39	29

4.27 District Laboratory (LAB-28)

Table 7: Performance of District Laboratory code number 28

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	-	40	23	9
2	Slides Rechecked	-	26	23	9
3	High False Negative	-	0	0	0
4	High False Positive	-	0	0	0
5	Low False Negative	-	0	0	0
6	Low False Positive	-	0	0	0
7	Quantification Error	-	0	0	0
8	Overall Agreement Rate (%)	-	100	100	100
9	Total Score	-	100	100	100
i.	Specimen Quality (%)	-	54	67	39
ii.	Staining (%)	-	69	52	56
iii.	Size (%)	-	65	46	50
iv.	Evenness (%)	-	50	48	33
v.	Thickness (%)	-	42	91	33
vi.	Cleanliness (%)	-	69	52	61

4.28 District laboratory (LAB-34)

Table 14: Performance of District Laboratory code number 34

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	-	-	-	-
2	Slides Rechecked	-	-	-	-
3	High False Negative	-	-	-	-
4	High False Positive	-	-	-	-
5	Low False Negative	-	-	-	-
6	Low False Positive	-	-	-	-
7	Quantification Error	-	-	-	-
8	Overall Agreement Rate (%)	-	-	-	-
9	Total Score	-	-	-	-
i.	Specimen Quality (%)	-	-	-	-
ii.	Staining (%)	-	-	-	-
iii.	Size (%)	-	-	-	-
iv.	Evenness (%)	-	-	-	-
v.	Thickness (%)	-	-	-	-
vi.	Cleanliness (%)	-	-	-	-

4.29 District laboratory.

Table 29: Performance of District Laboratory, PHL, Thimphu

Sl. No.	Properties	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
1	Slides Received	960	1162	1140	833
2	Slides Rechecked	27	27	26	25
3	High False Negative	0	1	0	0
4	High False Positive	0	0	0	0
5	Low False Negative	0	0	0	0
6	Low False Positive	0	0	0	0
7	Quantification Error	0	0	0	0
8	Overall Agreement Rate (%)	100	96.30	100	100
9	Total Score	100	96.30	100	100
i.	Specimen Quality (%)	85	65	77	62
ii.	Staining (%)	87	69	54	64
iii.	Size (%)	93	65	71	60
iv.	Evenness (%)	94	69	65	62
v.	Thickness (%)	94	69	73	60
vi.	Cleanliness (%)	85	69	52	64

5. Discussion

Blinded rechecking is the most widely used method of external quality assessment (EQA) program especially for AFB smear in assessing the overall performance of a lab personnel in performing sputum microscopy. The blinded rechecking assessment is primarily focused on smear quality to evaluate the overall performance of individual/lab technicians. Moreover, the results are also evaluated to find out discrepancies in results interpretation. The real asset of blinded rechecking is that it reveals the true picture of sputum microscopy standard at every district hospital labs.

The blinded rechecking is based on six characteristics (specimen quality, size, staining quality, thickness, evenness and cleanliness) which invariably affect the smear quality. The smear quality determines the sensitivity and reliability of results in AFB smears. The current standard of smear quality revealed by NEQAS underscores the needs to understand the technical importance of each smear properties to achieve smear quality as discussed below.

5.1 Specimen quality

Quality of specimen (sputum) greatly affects the smear result since AFB is often detected in mucoid or mucopurulent part of sputum. Thus, the quality of sputum samples accepted by lab technicians and smear prepared from quality sputum determines the result outcome. To get quality specimen, it is very important to demonstrate patients on sample collection procedures.

5.2 Size

As per the international standard, to declare an AFB smears as negative (No AFB seen), the minimum fields required to screen are 100 fields and 200 fields for scanty. To get minimum of 100-200 fields, the smear size recommended is 1x 2 cm. The smear size (1x2cm) usually provides 300 plus fields.

5.3 Staining quality

The ZN staining is a specific stain for AFB. The most important step of ZN staining is the decolourization. Inadequate decolourization often leaves behind lot of stain artifacts that leads to reporting of high false positive. Similarly, over decolourization de-stain primary dye from AFB bacilli resulting to false negative result.

5.4 Thickness

The thicknesses of smear affect the staining quality especially if the smear is too thick. Ideally, the thickness of the smear should be monolayer to take up proper staining since the time kept for staining procedures are based on monolayer of cells and debris.

5.5 Evenness

The unevenness of the smear affects the outcomes of results because the result interpretations of smears are based on number of field screened. The unevenness smears lead to false counting of fields when no smear is found in some part of slides. The common error associated with unevenness is high and low false negative results.

5.6 Cleanliness

The dirty smear usually contributes in reporting high and low false positive error. This is very common error with technicians who lack knowledge on morphology of AFB. In unclean smear; any artifact stained with primary dye of ZN stain mimic the AFB while screening the smears.

6. Conclusion

The blinded rechecking evaluation results for four quarters of 2008 show low standard of sputum microscopy at districts.

7. References

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