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Original Research Article

A study of Revised National Tuberculosis Control Programme quality assurance of sputum microscopy at tuberculosis unit

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ABSTRACT

Background: Since, Revised National Tuberculosis Control Programme (RNTCP) relies on sputum microscopy for diagnosis, categorization of patients and assessment of treatment, the credibility, success and sustainability of the programme depends on the lab networks. The objectives of this study were to Quality assurance of Sputum Microscopy under RNTCP in Tuberculosis Unit.
Methods: A cross-sectional analytic study was conducted at DMC ML Chest hospital, DMC LLRM Hospital and DMC ESI Hospital. On-site evaluation was conducted once a month by Senior Tuberculosis Lab. Supervisor (STLS) of the DMCs (First Controller). This visit included a comprehensive assessment of laboratory safety procedure, conditions of equipment as well as technical components of AFB smear microscopy which includes prepare, staining and reading of smears. This also included examination of five positive and five negative smears in unblinded manner, to observe the quality of smear and staining as well as condition of microscope at each DMCs. A check list prepared for collection and analysis of standard data to point out remedial action.
Results: The overall slide positivity rate (SPR) was 20.17% and 23.99% for ZN-stained slides and fluorescence stained slides respectively. On-site evaluation of all DMCs revealed a good result except of DMC- LLR in maintenance of microscope and well organized and clean working areas. The overall concordance for RBRC slides result of ZN-stained slides was 98.64% and overall discordance was 1.35%. The overall agreement in the re-reading of fluorescent stained slides was 100%. The overall sensitivity, specificity, positive predictive value and negative predictive value of blinded re-checked fluorescence stained slides were 100% each respectively.
Conclusions: The overall performance of all DMCs is acceptable.

Keywords: Assurance, Microscopy, Quality, Sputum, Study

INTRODUCTION

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), infects almost one-third of the world population and kills around two million people worldwide each year. About 80% of the global TB burden occurs in low-income countries, where pulmonary disease and its transmission are most serious public health problems. Among bacterial pathogens of human, Mycobacterium tuberculosis is best known for its slow growth rate and its acid-fast lipid-rich cell wall. Culture of mycobacterium is too slow for practical diagnosis, while their acid-fastness allows rapid detection in clinical specimens. In many countries with a high prevalence of TB, direct sputum smear microscopy remains the most cost effective tool for diagnosing patients with infectious tuberculosis and for monitoring their progress on treatment. Sputum microscopy is an essential component

of the DOTS strategy recommended by the World Health Organization (WHO) and the International Union against Tuberculosis and Lung Disease (IUATLD).[1] Since 1993, RNTCP utilizes the DOTS strategy, is being implemented in India. By March 2006, RNTCP had expended to cover a billion of population. To date, entire country is fully covered under RNTCP. At this period cure and treatment success have constantly been 85% and there has been 7 fold reductions in TB deaths under RNTCP.[2] Since RNTCP relies on sputum microscopy for diagnosis, categorization of patients and assessment of treatment, the credibility, success and sustainability of the programme depends on the lab networks. Well-functioning lab network with easy access to high quality smear microscopy service is the highest priority for RNTCP. Poor quality microscopy services may lead to failure to detect persons with infectious TB who will continue to spread infection, leading to unnecessary treatment of non-cases and error in reading of follow up cases may lead to prolonged treatment of the cases or premature discontinuation of treatment.[3] Hence, present study was carried out to study quality assurance of sputum microscopy under RNTCP in tuberculosis unit.

METHODS

A cross-sectional analytic study was conducted from June 2012 to July 2013 at DMC ML Chest hospital, DMC LLRM Hospital and DMC ESI Hospital of Tuberculosis Unit GSVM Medical College Kanpur. In one of the DMCs (DMC- MLCH) fluorescence staining was used from June 2012 to March 2013 and Z-N staining was used in rest of the 2 DMCs (LLR and ESIH) and for rest of the time in 3rd DMC. Data of DMC ESIH was collected from February 2013 to July 2013. On-site evaluation was conducted once a month by Senior Tuberculosis Lab. Supervisor (STLS) of the DMCs (First Controller). This visit included a comprehensive assessment of laboratory safety procedure, conditions of equipment as well as technical components of AFB smear microscopy which includes prepare, staining and reading of smears. This also included examination of five positive and five negative smears in unblinded manner, to observe the quality of smear and staining as well as condition of microscope at each DMCs. A check list prepared for collection and analysis of standard data to point out remedial action. Random Blinded Rechecking (RBRC) of routine slides from the DMCs done as per system utilizing Lot Quality Assurance Sampling (LQAS) method to calculate the sample size. Using systemic random blinded sampling procedure the sample slides of sufficient number were selected from lab register of each DMCs which represent all slides of the DMC, by STLS which was not aware of original result of peripheral laboratory technician (LT), for Random Blinded Rechecking (RBRC)of routine slides from the DMCs.

The LT entered the slide numbers that were selected by STLS in a separate form (Annexure) along with results. This form was enclosed in sealed envelope by LT. This envelope and slides were picked up by STLS and handed over to DTO. Authors observed the RBRC conducted by DTO. The results of the above three DMC's were collected from District Tuberculosis Centre (DTC), Kanpur city every month. A total of 370 ZN-stained and 110 fluorescence stained slides were randomly collected and examined by STLS at DTC. For each DMC a separate chart of RBRC results was prepared and comparative RBRC performance of each DMC monitored. The discrepant results were resolved by a second controller (umpire).

Statistical analysis

Data processing and statistical analysis were performed using the Graph Pad Instat and MedCalc softwares (Windows version 3.10 and 12.75 respectively). The percentages of different types of errors were calculated for each DMC. The sensitivity, specificity, Positive predictive value (PPV), Negative predictive value (NPV) of smear reading by was calculated. Chi square test was used to see any association with different variable. The agreement in reading between the SM and STLS was done using kappa statistics and p value less than 0.05 was considered to be statistically significant.

RESULTS

Table 1 shows on-site evaluation slide volume and slide positivity rate. Out of 17732 examined slides 3937 (22.35%) were smear positive and 13768 (77.65%) were smear negative for AFB. Slide positivity rate (SPR) is highest in DMC-MLCH with ZN-staining (26.06%) and least in DMC-ESIH (9.02%). SPR was above accepted limit (>10%) in DMC-LLR and MLCH while it is below accepting limit in DMC-ESIH (9.02%).

Table 1: On-site evaluation slide volume and slide positivity rate.

DMC	Total no. of sample examined	No. of smear positive	No. of smear negative	SPR (%)
LLR (ZN)	3958	632	3326	15.96
ESI (ZN)	532	48	484	9.02
MLCH (ZN)	3829	998	2831	26.06
Total	8319	1678	6641	20.17
MLCH (Fm)	9413	2259	7127	23.99
Total	17732	3937	13768	22.35

(Fm): Fluorescent microscopy; (ZN): ZN-staining; SPR: Slide positivity rate.

Table 2: Calculation of overall sensitivity and specificity of on-site evaluation (OSE) slide results (ZN-stained slides).

DMC: LLR (ZN)+ ESI (ZN)+ MLCH (ZN)	STLS results		Total
SM result	Positive	Negative	
Positive	166	4	170
Negative	0	170	170
True Total	166	174	340
Kappa=0.976 (CI=95%)			
Sensitivity=100%			
Specificity=97.70%			
Positive Predictive Value=97.65%			
Negative Predictive Value=100%			

ZN: ZN-Staining.

The sensitivity, specificity, positive predictive value and negative predictive value for DMC- LLRH were 100%, 97.22%, 97.14% and 100% respectively for DMC-MLCH (Fm and ZN) were 100%, 100%, 100% and 100% respectively for DMC-ESIH were 100%, 93.75%, 93.33% and 100% respectively. There was a good agreement between results of STLS and SM at each DMCs as kappa values at DMC- LLRH, MLCH and ESIH were 0.971, 1.000 and 0.933 (CI=95%) respectively (Table 2).

All DMCs have slides with good smear size (>70%) with highest percentage (92%) in MLCH with Fluorescence microscope and least in ESIH (78.3%). Also, there were no significant differences between DMCs in smear size (p value >0.05). All DMCs have slides with good thickness (>70%) with highest percentage (91%) in MLCH with Fluorescence microscope and least in ESIH (75%). Also, there were no significant differences between DMCs in smear thickness (p value >0.05). All DMCs have slides with good evenness (>70%) with highest percentage (90%) in MLCH and least in ESIH (75%). Also, there were no significant differences between DMCs in smear evenness (p value >0.05) (Table 3).

Table 3: Quality of on-site evaluation (OSE) slides.

Parameters		LLR (ZN)	ESI (ZN)	MLCH (ZN)	MLCH (Fm)	Total
Staining quality	Good	122 (87.14%)	46 (76.66%)	32 (80%)	90 (90%)	290 (85.29%)
	Poor	18 (12.85%)	14 (23.34%)	8 (20%)	10 (10%)	50 (14.71%)
Test of significance for staining: LLR (ZN), ESI (ZN) and MLCH (ZN): X2 = 3.703, p value = 0.1570; MLCH (ZN) and MLCH (Fm): X^2 = 1.736, p value = 0.1877						
Slide smear size	Good	114 (81.5%)	47 (78.3%)	33 (82.5%)	92 (92%)	286 (84.11%)
	Poor	26 (18.57%)	13 (21.67%)	7 (17.5%)	8 (10%)	54 (15.88%)
Test of significance for smear size: LLR (ZN), ESI (ZN) and MLCH (ZN): X2 = 3.458, p value = 0.8412; MLCH (ZN) and MLCH (Fm): X^2 = 1.794, p value = 0.1805						
Smear thickness	Good	112 (80%)	45 (75%)	32 (80%)	91 (91%)	280 (82.35%)
	Poor	28 (20%)	15 (25%)	8 (20%)	9 (9%)	60 (17.64%)
Test of significance smear thickness: LLR (ZN), ESI (ZN) and MLCH (ZN): X2 = 0.6723, p value=0.7145; MLCH (ZN) and MLCH (Fm): X^2 = 2.291, p value = 0.1301						
Smear evenness	Good	122 (87.14%)	48 (80%)	36 (90%)	90 (90%)	296 (87.05%)
	Poor	18 (12.85%)	12 (20%)	4 (10%)	10 (10%)	44 (12.94%)
Test of significance for evenness: LLR (ZN), ESI (ZN) and MLCH (ZN): X2=2.448, p value=0.2941; MLCH (ZN) and MLCH (Fm): X2=0.000, p value=1.000						

Fm: Fluorescence microscopy; ZN: ZN-Staining.

The overall concordance and discordance for ZN-stained smear were 98.64% and 1.35% respectively. Also, there were no significant differences between designated microscopy centers (DMCs). Out of total 370 randomly selected ZN-stained slides from all DMCs 47 slides were read positive by STLS and 52 slides were read positive by SM.

There was 100% concordance for smear positive slides. The overall concordance in the results of STLS and SM was 98.64% and overall discordance was 1.35%. Out of total 110 randomly selected fluorescence stained slides 26 slides were read positive by STLS and SM too. There was 100% concordance in the results of STLS and SM. Out of 182 randomly selected slides of DMC- LLR, checked by STLS at DTC only 4 slides were misread by SM as positive (HFP). The Concordance and discordance in results of STLS and SM were 97.80% and 2.19% respectively. While out of 44 randomly selected ZN-stained slides of DMC-MLCH checked by STLS at DTC only 1 slide was misread by SM as positive (HFP). The Concordance and discordance in results of STLS and SM were 97.72% and 2.27% respectively. There were no discrepancies in the results of STLS and SM of the

randomly selected ZN-stained slides of DMC ESIH and fluorescence stained slides of DMC-MLCH at DTC. And overall concordance in the results of STLS and SM was 100% (Table 4).

Table 4: Overall concordance and discordance in the STLS and sputum microscopy (SM) results: RBRC slides (all ZN-stained and fluorescence stained slides separately).

DMC	Concordant slides		Discordant slides	
	Number	%	Number	%
LLRH (ZN)	178	97.80	4	2.19
ESIS (ZN)	144	100	0	0
MLCH (ZN)	43	97.72	1	2.27
MLCH (Fm)	110	100	0	0

Fm: Fluorescence microscopy; ZN: ZN-Staining; Test of significance for concordance of STLS and SM results between designated microscopic centers (DMCs): LLRH (ZN), ESIH (ZN) and MLCH (ZN): X2= 3.231; p value = 0.1988; LLR (ZN) and MLCH (Fm): X2= 10.094, p value = 0.2955.

The sensitivity, specificity, positive predictive value and negative predictive value for DMC-LLR were 100%, 97.40%, 87.50% and 100% respectively; for DMC-MLCH (Fm and ZN) were (100% and 100%), (100% and 96.88%), (100% and 92.31%) and (100% and 100%) respectively; for DMC-ESIH were 100%, 100%, 100% and 100% respectively. The overall sensitivity, specificity, positive predictive value and negative predictive value for the ZN-stained slides of all DMCs examined by STLS and SM were 100%, 98.42%, 90.38% and 98.83% respectively (Table 5). All DMCs have good stained smear (>70%) with highest in MLCH with fluorescent microscopy (91.81%) and least in MLCH with ZN-staining (84.09%). Also, there was no significant difference between DMCs (p value >0.05).

Table 5: Calculation of overall sensitivity and specificity of random blinded rechecking (RBRC) slide results (ZN-stained slides).

DMC: LLR(ZN)+ ESI (ZN)+ MLCH(ZN)	STLS results		Total
SM result	Positive	Negative	
Positive	47	5	52
Negative	0	312	312
True Total	47	317	364
Kappa=0.942 (CI = 95%)			
Sensitivity=100%			
Specificity=98.42%			
Positive Predictive Value=90.38%			
Negative Predictive Value = 98.83%			

ZN: ZN-Staining.

Table 6: Quality of random blinded rechecking (RBRC) slides staining quality.

Parameters		LLR (ZN)	ESI (ZN)	MLCH (ZN)	MLCH (Fm)	Total
Staining quality	Good	162 (89.1%)	126 (87.5%)	37 (84.09%)	101 (91.81%)	426 (88.75%)
	Poor	20 (10.98%)	18 (12.5%)	7 (15.91%)	9 (8.2%)	54 (11.25%)
Test of significance for staining: LLR (ZN), ESI (ZN) and MLCH (ZN): X2 = 0.828, p value = 0.1570; MLCH (ZN) and MLCH (Fm): X2=1.271, p value=0.2596						
Slide smear size	Good	159 (87.36%)	124 (86.11%)	33 (82.5%)	98 (89.09)	417 (89.09)
	Poor	23 (12.64%)	20 (13.88%)	8 (18.18%)	12 (10.90%)	63(13.13%)
Test of significance for smear size: LLR (ZN), ESI (ZN) and MLCH (ZN): X2 = 0.9188, p value 0.6317; MLCH (ZN) and MLCH (Fm): X2 = 0.8979, p value = 0.3434						
Smear thickness	Good	164 (90.10%)	123 (85.41%)	37 (84.09%)	100 (90.9%)	424 (88.33%)
	Poor	18 (9.9%)	21 (14.58%)	7 (15.90%)	10 (9.1%)	56 (11.66%)
Test of significance smear thickness: LLR (ZN), ESI (ZN) and MLCH (ZN): X2=1.265, p value=0.2607; MLCH (ZN) and MLCH (Fm): X2=0.8745, p value=0.3497						
Smear evenness	Good	165(90.65%)	129 (89.58%)	38 (86.36%)	102 (92.72%)	434 (90.41%)
	Poor	17 (9.35%)	15 (10.42%)	6 (13.64%)	8 (7.27%)	46 (9.58%)
Test of significance for evenness: LLR (ZN), ESI (ZN) and MLCH (ZN): X; = 0.7150, p value = 0.6994; MLCH (ZN) and MLCH (Fm): X2 = 0.8663, p value = 0.3520						

Fm: Fluorescence microscopy; ZN: ZN-Staining.

All DMCs have good smear size (>70%) with highest in MLCH with fluorescent microscopy (89.09%) and least in MLCH with ZN-staining (81.81%). Also, there was no significant difference between DMCs (p value >0.05). All DMCs have good smear thickness (>70%) with highest in MLCH with fluorescent microscopy (90.09%) and least in MLCH with ZN-staining (84.09%). Also, there was no significant difference between DMCs (p value >0.05). All DMCs have good smear evenness (>70%) with highest in MLCH with fluorescent microscopy (92.72%) and least

in MLCH with ZN-staining (86.36%). Also, there was no significant difference between DMCs (p value >0.05).

DISCUSSION

Authors study out of 17732 examined slides 3937 (22.35%) were smear positive and 13768 (77.65%) were smear negative for AFB. In a similar study by Mulat M et al, found that the overall slide positivity rate (SPR) was 9%. Slide positivity rate among public and private centers was 8.9 and 11% respectively and he concluded that this difference was due to difference in terms of TB patient visit at these centers.[4]

Evaluation of DMCs through on-site evaluation showed that overall achievement of DMCs was good. All DMCs are good in infrastructure, Standard Operating Procedure (SOP), reagents and equipment maintenance of microscope, biosafety and waste disposal, Training status of staffs and Data management except Maintenance of microscope (64.28%) and well organized and cleaned working areas (64.28%) in DMC- LLR. In a similar study by Mulat M et al, showed that overall achievement of laboratories was 69.2% and lowest score was recorded on maintenance of microscope (53.5%) and highest score was recorded on data management (89.0%).[4]

In another study conducted by Kumar A et al, showed that 44.15% errors in laboratory checklist-items were identified in ten IRLs.[5] Majority of errors occurred in EQA (92.6%), internal quality control (90%), staining reagents/equipment (56.6%), infrastructure (42%), and bio-safety practices (40%).[5] Parissa F et al, found that 68.4% of TB laboratories were using commercially prepared staining kits of inferior quality and 72% of TB technicians examined each slide for less than 7 minutes whereas the recommended standard time is 15-20 minutes.[6]

Authors study all DMCs had good smear slide qualities (>70%) each in terms of smear staining, smear size, smear thickness and smear evenness. In a similar study conducted BY Addo K et al, showed that staining quality, smear cleanness, thickness, size and evenness was found to be 79%, 69%, 46%, 67% and 60% with improvements to 90%, 86%, 79%, 80% and 74% after the establishment of the QA system, respectively.[1]

Out of 182 randomly selected slides of DMC- LLR, checked by STLS at DTC 4 slides were misread by SM as positive (HFP). The Concordance and discordance in results of STLS and SM were 97.80% and 2.19% respectively. While out of 44 randomly selected ZN-stained slides of DMC-MLCH checked by STLS at DTC 1 slide was misread by SM as positive (HFP). The Concordance and discordance in results of STLS and SM were 97.72% and 2.27% respectively. There were no discrepancies in the results of STLS and SM of the randomly selected ZN-stained slides of DMC ESI and fluorescence stained slides of DMC-MLCH at DTC. And overall concordance in the results of STLS and SM was 100%. Also, there was a good agreement between results of STLS and SM for each DMCs as kappa values of results for DMC- LLR, MLCH (Fm and Sm) and ESIH were 0.920, (1.000 and 0.940) and 1.000 respectively. In a similar study on RNTCP: Quality control of sputum microscopy at sub-district level Sarin R et al, found that there was 100% concordance with slides reported as smear positive by the Microscopist and the discordance in smear negative slides was 2% in one TB Unit (TU) and 6.4% in the other.[7] The discordant slides were then given to STLS of other TU for umpire reading. Hundred per cent concordance was found between the two STLS in respect of umpired slides.[7]

In a similar study Paramasivan CN et al, concluded consistency of positives ranged from 38% to 100%, indicating under-reading at some sites.[8]

The negative consistency was better, however, with only five of the totals of 95 readers in all rounds yielding a consistency of less than 100%. Considering overall agreement, seven of the eight centers showed an agreement of over 90%. Four of the eight centers gave no false-positive result. For the remaining centers the false positivity rate varied from 2% to 7%.[8]

Shargie BE showed a good agreement was recorded in the readings of AFB among the peripheral and reference laboratories. This study supported our study.[9]

In our study, 100 per cent concordance has been observed between microscopists and the STLS in respect of sputum smear positive slides for all the DMCs, even when using a blind design. In contrast Nguyen TNL et al, concluded false-negative error was more common than false-positive error.[10]

Basra D et al, showed that the overall agreement on reading was 89.2%, the overall sensitivity was 88.5% and specificity was 100%.[3] In this study the sensitivity, specificity, positive predictive value and negative predictive value for DMC-LLR were 100%, 97.40%, 87.50% and 100% respectively for DMC-MLCH (Fm and ZN) were (100% and 100%), (100% and 96.88%), (100% and 92.31%) and (100% and 100%) respectively; for DMC-ESI pandunagar were 100%, 100%, 100% and 100% respectively.

The overall sensitivity, specificity, positive predictive value and negative predictive value for the ZN-stained slides of all DMCs examined by STLS and SM were 100%, 98.42%, 90.38% and 98.83% respectively.[3]

CONCLUSION

The overall performance of all DMCs is acceptable. The overall agreement in the re-reading of fluorescent stained slides was 100%. The overall sensitivity, specificity, positive predictive value and negative predictive value of

blinded re-checked ZN-stained slides were 100%, 98.42%, 90.38% and 98.83% respectively. The overall sensitivity, specificity, positive predictive value and negative predictive value of blinded re-checked fluorescence stained slides were 100% each respectively.

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