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Research Paper

Data of Manufactured Panel Test Smears In The Who-Rntcp, Gujarat, India

Clinical Microbiology

KEYWORDS : Consistency, EQA, Proficiency, Validation

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ABSTRACT

Aim: To present an overview of manufacturing and validation data about proficiency panel test smears in the RNTCP-EQA, India guideline.

Material and Methods: The Intermediate Reference Laboratory, Ahmedabad; Gujarat state, India is a well-functioning tuberculosis reference laboratory and has clinical laboratory resources and instruments required for manufacturing and validating proficiency panel test slides. Manufacturing and validating proficiency panel test smears is an important integral part of EQA guideline, and the Intermediate Reference Laboratory; Ahmedabad is implementing it since 2005, an overview of which is presented in this article. All tuberculosis laboratory procedures described here had been performed in biological safety cabinets along with universal safety precautions as per the WHO-RNTCP guidelines.

Results: For each panel batch, it took two to three weeks for abiding manufacturing and validation procedure. The smear characteristics were pleasingly different from those of routine Z-N smear. Four to six intermediate reference laboratory personnel validated consistency of each batch of slides by selecting a sample of six slides from each AFB grade, staining and reading it. True consistency level was found for each AFB grade in every validated batch.

Conclusion: Manufacturing panel slides requires considerable resources like well-equipped laboratory, dedicated laboratory staff and meticulous documentation of validation process. Inclusion of N-acetyl-L-cysteine is a key part of sputum digestion process. The technical quality of smears manufactured at the Intermediate Reference Laboratory, Ahmedabad was satisfactory. With this labour intensive and time consuming process, EQA has come closer to gold standard Z-N smear, at least in proficiency panel testing if not in re-checking mechanism. Validated panel smears can be successfully used for assessing proficiency of the laboratory staff in sputum smear microscopy.

Introduction

Tuberculosis case detection using sputum microscopy among persons seeking care for prolonged cough is one of the five elements of the global Directly Observed Treatment Short-Course Chemotherapy strategy. World Health Organization (WHO) has recommended establishment of a microscopy services network endowed with responsibility for quality control so that a sputum microscopy network with built-in quality control be accessible to symptomatic persons.[1] The core activities of a national tuberculosis control programme like, the Revised National Tuberculosis Control Programme (the RNTCP, India) in a low income country include case finding by sputum smear microscopy, and the delivery of effective chemotherapy to patients. The national tuberculosis reference laboratory (NRL) must concentrate its efforts to support these activities within the national laboratory network. Usually, it is not in the position to carry out all tasks related to training, supervision, and proficiency testing of sputum smear microscopy in the entire country, nor would this be an efficient use of its resources. Therefore, decentralisation of certain activities is highly desirable, and the national tuberculosis reference laboratory should take the leading role in encouraging regional or provincial laboratories to take part in carrying out the essential tasks of the national laboratory network.[2]

To optimise quality assurance, decentralisation of the supervision and monitoring of the laboratory network is essential, and capacity building of the states to undertake these activities becomes a priority in the external quality assessment (EQA) framework.3 The national reference laboratory provides training to all intermediate reference laboratory personnel who are responsible for EQA activities. Laboratory personnel are made knowledgeable in all operational and technical elements of acid fast bacilli (AFB) smear microscopy and develop sufficient expertise to observe technicians performing routine tasks. The national reference laboratory namely, National Institute for Research in Tuberculosis (erstwhile Tuberculosis Research Centre); Chennai, is entrusted with the task of EQA monitoring of the Intermediate Reference Laboratory (IRL); Ahmedabad; Gujarat state, India.

Historically, WHO has used the term proficiency testing for a process of sending smears from the reference laboratory to the peripheral sites. The reference laboratory may use known positive and negative patient specimens to produce a large collection of positive slides with a consistent, pre-determined quantity of AFB per slide as well as negative slides with authentic leucocyte background material. By using such manufactured slides, all laboratory technicians involved in the panel testing exercise will receive an identical set of slides, which should minimise variation in expected results due to variation in the consistency of smears. Well-manufactured slides with good consistency should result in demonstration of good performance by the technicians being evaluated. However, the process for preparing slides requires a high degree of technical proficiency and a reference laboratory with appropriate equipment including a bio-safety cabinet. If manufactured slides are used for panel testing, every effort must be made to validate the consistency of slides prior to sending out test panels. This will ensure the reliability of panel testing results and document that reading errors do not represent a problem in the manufacturing process. Producing individual batches of slides with an identical number of AFB, especially low positives, requires practice to achieve slide-to-slide consistency.4 Each batch of slides must be validated by selecting a sample of six slides, staining, and reading by different technicians to document consistency of every batch.[3] Reference laboratories should develop the capacity to produce and validate batches of fifty to one hundred slides that can be stored for future use in preparing proficiency panel test sets.[3,4]

Material and Methods

The Intermediate Reference Laboratory, Ahmedabad is a well-functioning tuberculosis reference laboratory and has clinical laboratory resources and instruments required for manufacturing and validating proficiency panel test slides. Consistent with the above mentioned decentralisation background, it looks after

quality assurance network of more than 700 designated microscopy centres in the state of Gujarat and Union Territories of Daman, Diu and Dadara Nagar Haveli. Manufacturing and validating proficiency panel test smears is an important integral part of EQA guideline, and the Intermediate Reference Laboratory; Ahmedabad is implementing it since 2005, an overview of which is presented in this article. The programme requires stringent record keeping and monitoring by programme officers. Since this activity is a part of the programme officers' duty, ethical clearance is not necessary. We analysed prospectively filed slide manufacturing data for their critical evaluation.

All tuberculosis laboratory procedures described here had been performed in biological safety cabinets along with universal safety precautions as per the WHO-RNTCP guidelines.3,4

By using the WHO-RNTCP guidelines, fifty to one hundred slides of each AFB grade, per batch were prepared.

The consistency of each batch of slides was validated by selecting a sample of six slides from each AFB grade, staining, and reading it by four to six independent technicians.

The heat fixed slides of different AFB grades were kept in separate, designated wooden boxes and stored in shelves for up to six months, for usage in proficiency panel testing. The Intermediate Reference Laboratory, Ahmedabad consistently used only manufactured and validated panel slides in proficiency panel testing during on-site evaluation visits. A different panel set was used for each district laboratory technician.

Results

The Intermediate Reference Laboratory, Ahmedabad prepared panel smears from time to time depending on their requirement during on-site evaluation visits. We manufactured two to three such batches each year. For each panel batch, it took two to three weeks for abiding manufacturing and validation procedure as described above. Four to six intermediate reference laboratory personnel checked consistency of each batch of slides by selecting a sample of six slides from each AFB grade, staining and reading it. The smear characteristics were pleasingly different from those of routine Z-N smear. Sputum was easy to spread and dry, had nice authentic blue background studded with plenty of leucocytes and minimal background noise. The intermediate reference laboratory readers' AFB smear results were plotted on validation log chart. The mean (M) and standard deviation (SD) values were computed for each AFB grade from corresponding six slide test results. The manufactured slides were considered valid if two standard deviation results met WHO/CDC consistency criteria. We found true consistency level for each AFB grade in every validated batch. The technical quality of manufactured smears was satisfactory and acceptable for panel test exercise. Results of one such validated panel smear batch are given in the table. However, it is safe to assume that validation data of other batches are almost identical to results cited in the table.

These slides were used for assessing proficiency of district laboratory readers during EQA visits, Gujarat. Each reader was given a panel slide set of five heat fixed un-stained sputum slides. The panel set essentially constituted at least one AFB negative, one AFB positive, and three slides of any grade. Consistency level in AFB grade agreement for negative slides, scanty slides, and other grade positive slides was more than 99%, 95%, and 98% respectively. High false positive error was not observed during field experience (data already published).7

DISCUSSION

Important administrative and technical concepts in relation to manufacturing and validation of proficiency panel test smears are discussed below.

Since the introduction of new quality control guideline in 2005, the Intermediate Reference Laboratory; Ahmedabad, Gujarat state, India is taking regular part in the state level implementation of EQA activities. The tuberculosis laboratory network of designated microscopy centres is supported and supervised by the co-sponsoring organisation, the Intermediate Reference Laboratory; Ahmedabad, which in-turn is overseen by the Supra-National Reference Laboratory namely National Institute for Research in Tuberculosis, Chennai, India.

Maintaining high proficiency in routine tuberculosis smear microscopy carried out in peripheral health facilities is one of the major tasks of the national tuberculosis reference laboratory. The IUATLD suggests proficiency panel testing by manufactured and validated panel test smears.2 Panel testing using sets of slides developed in the central laboratory and administered to the peripheral laboratory is a mechanism that can be implemented with minimal resources.4

The Japan study- aimed at evaluating two methods for preparation of panel test slides using NaOH or N-acetyl-L-cysteine-found the procedure easy to manufacture many slides, but requiring some expensive reagents. It concluded by suggesting addition of avirulent mycobacteria in artificial sputum to culminate the problem of harvesting a large amount of AFB negative sputa for dilution of heavily positive stock.5 We perceived panel preparation quite demanding in terms of infrastructure, man-power, and time period it consumes. However, N-acetyl-L-cysteine does play a key role in sputum digestion process as suspension acquires almost watery consistency. We found digested sputa quite useful in dispensing them during dilution procedure, and smear preparation.

The national level laboratories provide training and technical support to the Intermediate Reference Laboratory; Ahmedabad, so that quality assurance activities are planned and implemented in a well-organised fashion, with the aim to improve quality and performance of the designated microscopy centre network of general health service under the RNTCP.

The RNTCP encourages usage of manufactured slides, because it can assess several aspects of laboratory technician's technical performance, including preparation of staining reagents, staining procedure, reading and reporting of results. By using validated slides with different grades of positivity, all laboratory technicians involved in panel testing will receive a standardised set of slides, which will minimise variation in expected results due to differences in smear consistency.3 Manufactured slides of different grades must be stored in separate, AFB grade designated boxes. This precaution can act as a safeguard against cross-contamination problem due to storage of slide. For example, AFB negative slides if stored in a box previously utilised for storing AFB positive slides, may result in false positive result during panel testing exercise.

Kubica GP et al have commented that use of N-acetyl-L-cysteine as the mucolytic agent improves the release of bacilli by better mucolysis.6 Sodium citrate exerts a stabilising effect on the N-acetyl-L-cysteine by chelating heavy metal ions present in the specimen. Association of Public Health Laboratories had cautiously advocated its use for improving quality of the smears.4 Dave PV et al had used manufactured and validated slides for assessing the proficiency of Gujarat technicians in sputum smear microscopy, and documented their high level of precision and overall consistency level of more than 98% in Z-N grade agreement. Having found consistency level significantly different between scanty slides and other grade slides, their study sug-

gested that panel testing exercise can be made more effective by raising the proportion of lower grade positive slides in panel set of each reader.7 Martinez-Guarneros A et al evaluated proficiency of Mexican technicians by panel testing slides having known numbers of AFB. The analysis of the results showed that the number of low-positive slides in the set was the critical factor most closely associated with proficiency results.8

The feasibility and methodology of proficiency programmes in developing countries have been described as controversial.9 Baker M et al distributed validated panels to 20 microscopy centres and found EQA panel testing feasible for an unbiased representative evaluation of the overall quality of smear microscopy in Haiti. However, a slide group was considered valid and was included in to the panel if the results from five independent readers did not exceed one standard deviation from the mean. They manufactured 'scanty' smears by a validated protocol to avoid clumping effect in them,10 which is different from the WHO-RNTCP protocol.

The programme officers may consider infusing additional resources when too many unacceptable smear results are obtained from the field during the proficiency panel testing exercise. Additional resources can be in the form of on-site visit, correction of problem identified in individual laboratories or technician, replacement of microscope, re-training, and follow-up panel testing.

Apart from being labour intensive and time consuming, another limitation of panel testing is that it does not monitor routine performance, and hence it should be considered to be less effective than random blinded re-checking of routine slides. For this reason, panel testing is not performed as a routine in designated microscopy centres, as they will have regular on-site evaluation and blinded re-checking.3 This approach, however, requires a more sophisticated smear preparation by the reference laboratory using more homogenous sputum specimens with the desired numbers of bacilli per slide, which are difficult to prepare. In principle, this method generates panel slides by a method originally described by Smithwick.11 This protocol manufactures slides having carefully balanced bacillary load and unequivocally known results. This way, EQA has come closer to gold standard Z-N smear, at least in proficiency panel testing if not in re-checking mechanism.

Conclusion

Manufacturing panel slides requires considerable resources like well-equipped laboratory, dedicated laboratory staff and meticulous documentation of validation process. Inclusion of N-acetyl-L-cysteine as the mucolytic agent along with sodium citrate has turned out as an important integral part of sputum digestion process. This is a labour intensive and time consuming process. This protocol manufactures slides having carefully balanced bacillary load and unequivocally known results. This way, EQA has come closer to gold standard Z-N smear, at least in proficiency panel testing if not in re-checking mechanism. The technical quality of smears manufactured at the Intermediate Reference Laboratory, Ahmedabad was satisfactory. Validated panel smears can be successfully used for assessing proficiency of the laboratory staff in sputum smear microscopy.

Table: Validation data of manufactured slides, year 2011

			AFB result by IRL reader						consistency	
	Grade	Slide #	Reader I	Reader II	Reader III	Reader IV	M- 2 SD	M+ 2 SD	WHO/CDC standarad	IRL consistency result
Manufactured slides	Negative	1	0	0	0	0	----	0	0 AFB/ 100 fields	TRUE
		2	0	0	0	0				
		3	0	0	0	0				
		4	0	0	0	0				
		5	0	0	0	0				
		6	0	0	0	0				
	Scanty	1	5	7	6	5	6	7	<9 AFB/ 100 fields	TRUE
		2	6	5	7	6				
		3	5	7	6	7				
		4	6	6	5	7				
		5	7	6	7	6				
		6	5	7	6	6				
	1+	1	43	38	42	45	38	42	<99 AFB/ 100 fields	TRUE
		2	36	43	41	39				
		3	38	39	40	42				
		4	42	41	39	38				
		5	39	38	41	40				
		6	41	40	40	38				
	2+	1	7	6	5	6	5	7	<10 AFB/ field	TRUE
		2	7	7	6	7				
		3	5	5	6	7				
		4	7	6	6	5				
		5	6	5	6	7				
		6	7	6	7	7				
	3+	1	14	16	17	12	14	----	>11 AFB/ field	TRUE
		2	13	15	19	11				
		3	15	17	14	12				
		4	18	16	18	13				
		5	14	15	18	12				
		6	15	16	15	13				

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