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QUALITY CONTROL IN ISOLATION AND IDENTIFICATION OF MYCOBACTERIA FROM CLINICAL SPECIMENS

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The importance of laboratory test results in the practice of medicine and the increasing complexity of many modern laboratory procedures makes it essential that quality control (QC) measures be instituted to monitor the rapidly expanding, often automated, laboratory technology. QC is the responsibility of all laboratory personnel. QC procedures should be performed on a regular basis in the Mycobacteriology laboratory to assure reproducibility and reliability of laboratory results. For a QC to be helpful, it must be practicable and workable. Most of the clinical laboratories in the developing countries lack a well organised QC network. On the contrary, the majority of the clinical laboratories in the USA are under the jurisdiction of one or more accreditation agencies.

Some of the positive aspects of QC programmes are as follows : 1) Potential problem in the isolation and identification of micro-organisms can be greatly reduced by monitoring media and reagents before using them in clinical specimens ; 2) serious and costly breakdown of equipments can be. minimised by routine monitoring and maintenance ; 3) laboratory aspects can be more expeditious and accurate because the use of poor and inadequate media, equipment and technique is minimised and 4) the QC programme can also be a learning technique enabling. recognition or identification of problem areas that might otherwise have been overlooked.

The keys to the development of a workable quality control programme are a) adequately trained, interested and committed personnel and b) common sense in approaching a practical problem. The US Public Health Service, CDC, Atlanta Publication on "Public Health Mycobacteriology - A Guide for. the level I I I laboratory " (1985) observed the following as their general recommendations (1).

1) All quality control records should be retained in files or note books for atleast 2 years.

2) Procedural manual(s) should be available for every routine procedure performed in the laboratory. Any change should be indicated clearly.

3) All containers of media, constituents of media, stains and reagents should show the date received and the date first opened. Purchases should be maintained to a 6 months supply. Unsatisfactory materials should be removed.

4) Standard procedures must be followed. If any procedure is to be changed it must be after experimental control studies and

5) Laboratories should maintain the number and variety of cultures needed to check the quality of tests performed.

For maintaining quality, in addition to the in-house monitoring of laboratory personnel, space and equipment, it is important that other quality checks be made on specimens to be processed ; transportation of samples; microscopy ; culture media ; digestion-decontamination ; culture examination ; media, reagents and biochemical tests and the final report.

Specimen :

It is important to note the quality of the sputum; ie., is it thick, mucoid, purulent, or is it more like saliva. The latter is important, primarily when a negative sputum smear report is sent. Also important for the negative smear or culture report is the quantity of the sputum specimen submitted. If the quantity is less than 5 ml and the report is negative, an alternate sputum is to be obtained in adequate quantity and examined.

Transportation :

Best results are obtained with specimens that are transported and examined without much delay. Any delay may adversely affect the viability of tubercle bacilli. A study reported from Tuberculosis Research Centre, Madras has highlighted this fact (2). In the first part of this study, 41 specimens stored for upto 28 days at room temperature showed that smear results were not affected by storage, the positivity being 83% before storage and 80-83% even

after 28 days. The culture positivity was 88% before storage. and 83%, 68%, 22%, 13%, and 0% after 3, 7, 14, 21, and 28 days of storage respectively. The reduction in positivity on storage attains significance at 7 days (P = 0.05).

Another study involved 163 specimens of sputum for 3, 5 or 7 days, Each specimen was examined before storage and after 2 periods of storage, at random. The smear results were again not affected. There was, however, significant loss of viability, the proportion culture positive being reduced from 92% before storage to 83% at 3 days (P = 0.05), 71% at 5 days (P < 0.01) and 63% at 7 days (P < 0.001).

It is concluded that the sputum should not be stored at room temperature for longer than 3 days for culture but it can be stored for 4 weeks without any loss of smear positivity.

Microscopy :

A number of checks should be done in this area. Each new batch of stains should be checked both on known positive and known negative control smears ; the use of positive control ensures the staining capability of new stain solutions, whereas the negative control smears will confirm that acid-fast contaminants are not present in stain solution(s). It is good practice to confirm positive smears of clinical specimens by a second reader and to retain positive smears for several weeks or months against the likelihood of a request to 're-examine' the smear.

Culture Media :

Records should be kept on all “home made” media ; source and batch number of reagents or powdered base media ; coagulation time and temperature for inspissated egg media ; visual appearance (colour, bubbles, consistency) and firmness of media.

Digestion-Decontamination :

To determine decontaminating capabilities of each new batch of reagent, digest 4-6 sputa, concentrate by centrifugation and inoculate to plates of general bacteriology agar media as well as T.B. media, Acceptable range of contamination is about 3 to 5%. Less than 3% of contamination suggests overly harsh decontamination, whereas percentages much higher than 5% suggests either

too weak a decontaminant or a) incomplete digestion. Additionally, a careful recording of the numbers (percentages) of isolates of *M. gordonae* can serve as a built-in indicator of digestant toxicity. This species commonly seen in most laboratories (10% of routine isolation) is more susceptibility to toxic digestants than *M. tuberculosis,* so recovery of fewer than *5% M. gordonae* (from among all AFB cultured from clinical specimens) suggests an overly harsh decontamination procedure.

As Tuberculosis Research Centre (TRC) every day processes about 500 sputum and extrapulmonary specimens from tuberculosis patients admitted to various controlled clinical studies, several planned quality control procedures are being undertaken on a routine basis. As TRC has also been involved in monitoring the implementation of sh ort course chemotherapy (SCC) under programme conditions in 18 districts of 6 different states in India and since case finding in the field depends on sputum microscopy, several periodic checks are being undertaken to monitor sputum smear results. Also, TRC has established culture laboratories in the' districts to augment case finding and the performance of these laboratories is being monitored by testing alternate specimens obtained from the same patients. The following are some of the results obtained by TRC in various studies.

Culture Results in Quality Control Specimens :

To check the possibility whether there was any carry over of tubercle bacilli from positive specimens to negative ones in the laboratory, autoclaved specimens of sputum were introduced along with the routine test specimens while processing. Along with these sterile autoclaved samples of sputum specimens, sputum samples containing marker strains were introduced. A batch of 10 samples with different set pattern of marker and non-marker strains were introduced with the test samples. The technicians were unaware of the identity of these samples. The results obtained over a period of 10 calender years is presented in the following table :

Culture Results in QC Specimens :

Year	Total No. of QC batches set up	Marker Q.C. specimens Culture negative		Total	Non-marker Q.C. specimens Culture positive	
		No.	%	No.	No.	%
1977	97	5	5.1	512	5	0.98
1978	114	6	5.2	684	2	0.29
1979	160	8	5.0	960	4	0.42
1980	23	14	6.6	1386	6	0.43
1981	112	0	0.0	372	1	0.15
1982	85	2	2.4	510	0	0.00
1983	143	1	0.7	858	1	0.12
1984	97	0	0.0	582	2	0.34
1985	98	0	0.0	588	1	0.17
1986	37	0	0.0	222	0	0.00
All	1174	36	3.1	6974	22	0.31

Of the 6974 autoclaved specimens processed during a decade, 22 specimens yielded positive cultures, (0.31%,) which by itself reflects maintenance of a high standard in the laboratory. Of the 1174 specimens with QC marker strains where live known cultures were introduced, 36 samples (3.1%) yielded negative growth. The negative result may be attributed to the quality of the sputum sample or to excessive pretreatment decontamination procedure employed or to technical error. If every major clinical microbiology laboratory adopts serious in-house QC measures, results obtained in these laboratories can be relied upon with certainty.

Comparison of Culture Results of TRC and Field Laboratories :

Apart from doing bacteriological investigations on patients admitted to Short Course Chemotherapy (SCC) under programme conditions, TRC has established culture facilities, the first one at Pennathur Sanatorium, North Arcot

District and the second one at Govt. TB Sanatorium Gorimedu, Pondicherry, during the year 1985-1986. The laboratory technicians working in these places were trained for 4-6 weeks at TRC before the establishment of these laboratories.

Analysis of the culture results obtained in both these laboratories are presented in the tables below :

Comparison of Bacteriological findings of North Arcot District Field Laboratory Vs. TRC.

		TRC Results					
		Negative	Positive	Cont.	NTM	Total	
Fibld Lnb. Results	Neg.	136	400	18	8	562	(29%)
	Pos.	71	1104	30	3	1208	(63%)
	Cont.	25	107	3	2	137	(7.1%)
	NTM	5	10	0	0	15	
	Total	237 (12%)	1621 (84%)	51 (3%)	13	1922	

The proportion of negative cultures is 29% at NA and 12% at TRC, the difference attaining statistical significance. The proportion of positive cultures is 63% at NA compared to 84% at TRC.

However, the culture positivity at NA had shown a steady increase from 43% during 85-86 to 78% during 88-89 as shown in the table below and this is due to several corrective measures undertaken by TRC staff.

Comparison of Culture Positive Results Year Wise

Year	Culture Positive (%)	
	North Arcot	TRC
85-86	42	78
86-87	59	82
87-88	72	82
88-89	78	83

Analysis of the culture results of 3300 specimens processed during the period 85-89 at Pondicherry and at TRC is shown in the following table :

Comparison of Culture Results of Pondicherry Vs TRC

		TRC Results				
		Negative	Positive	Cont.	NTM	Total
Pondicherry	Neg.	1549	437	59	30	2075
	Pos.	37	1052	22	5	1116 (34%)
	Cont.	43	43	5	4	95
	NTM	2	9	1	2	14
	Total	1631	1541 (47%)	87	41	3300

Thirty-four percent of specimens were culture positive at Pondicherry compared to 47% at TRC. The lower percentage of culture positivity in both the places could have been due to inclusion of sputum specimens collected= from patients during treatment. The difference in the rate of positivity between. two laboratories could also have been due to two different methods used for processing; Petroff's concentration method at the Centre and cetrimide swab (CS) method at Pondicherry. The CS method is less sensitive for smear negative specimens. The contamination rates were similar. This exercise clearly shows that it is possible to establish culture facilities at peripheral laboratories with proper check on the quality of the results obtained.

Comparison of Sputum Smears : In Controlled Clinical Trials :

TRC has been all along conducting controlled clinical trails to find out suitable and easily adaptable regimens for treating tuberculosis patients. Recently, TRC has established a treatment facility at Rajaji Hospital, Madurai and now tuberculosis patients are being admitted to the controlled clinical study both at Madurai and at Madras. However, smear examination alone is done at Madurai and patients are allocated to treatment based on smear results. The sputum specimens are then transported to TRC main laboratory for smear, culture, sensitivity and identification tests. Comparison based on 9422 smears (same specimens but different smears) of assessment cases during a 4 year period (1-4-85 - 31-3-89) is presented in the following table :

Comparison of Smear Results on Assessment Specimens between Madras and Madurai Laboratories of TRC (Irrespective of grades)

		Madras		
		Negative	Positive	Total
Madurai	Neg.	5462 (58%)	384 (4%)	5846
	Pos.	200 (2%)	3376 (36%)	3576
	Total	5662	3760	9422

There was an agreement of 94% observed on 2 different smears taken from same samples when examined at 2 different places irrespective of grades.

Agreement between Madras and Madurai Laboratories of TRC according to Gradation of Smear Results.

		Madras				
		Neg.	1+	2+	3+	Total
Madurai	Neg.	5462	375	9	0	5846
	1+	186	1243	462	9	1900
	2+	11	250	949	136	1346
	3+	3	14	148	163	328
	Total	5662	1882	1568	308	9422

There was an agreement of 83% on grades of positivity also. The minor differences observed between these two places could be attributed to the microscopes used; quality of specimen used for preparing smear; work load, smear size and checking facility which is available only at Madras and not at Madurai.

Quality Control Procedures

Medium or Reagent	Sterility	Organisms	Expected results
Growth on isolation media (LJ)	Check pH colour consistency	M. tuberculosis H_{37} Rv H_{37} Ra	Should be able to support growth of small inoculum in 4-6 weeks.
Media for drug susc. testing	X	M. tuberculosis known sucs. strain TMC 201 or TMC 202	Positive on control inhibited by all drugs
		Drug free medium inoculated with test culture	Verifies ability of the medium
Photochromo -genicity	X	M. Kansasii TMC 1201	Test provides own negative control Positive (colour change after 2 hour light exposure)
Catalase pH 7.0 68°C		M. gastri TMC 1456 M. tuberculosis TMC 201	Negative (No bubbles)
		M. fortuitum TMC 1529 M. gordonae TMC 1324	Positive (bubbles)
Niacin		M. fortuitum TMC 1529	Negative (No change)
		M. tuberculosis TMC 201	Positive (pink or yellow)
Growth in LJ containing para Nitro Benzoic Acid 500 mg./l		M. tuberculosis TMC 201	Negative (No growth)
		M. fortuitum TMC 1529	Positive (Growth)

Comparison of sputum smear results during surveys :

During surveys it is often difficult to obtain even minimum facilities to establish field laboratories in the base camps. In a tuberculosis prevalence survey conducted by TRC during 1989 in a district in the state of Karnataka, 4 field laboratories were established for sputum smear examination. This has been done to avoid undue delay in the start of treatment to the smear positive patients and also to enhance case holding. The same smears along with the sputum samples were transported to TRC, Madras with minimum delay. The comparison of sputum smear results is presented below :

Tuberculosis prevalence survey : Sputum smear results. Base camps Vs TRC.

		TRC				
		0	1-9	1+	2+	Total
Base Camp	0	1532	19	6	0	1557
	1-9	17	15	18	0	50
	1+	8	9	151	57	225
	2+	2	1	11	50	64
	Total	1559	44	186	107	1896

TRC Pos Base Camp Neg : 25; TRC Neg/Base Camp Pos : 27

Such an excellent agreement as observed above ensures the good quality of the survey undertaken in this district.

As a part of our Centre's activities in the District Tuberculosis Programme (DTP) and as mentioned earlier, TRC personnel visit these districts periodically. As case finding and assessing progress of patients during treatment and follow up in DTP is based on sputum smear examination by Ziehl-Neelson method, it was planned to check at the centre samples of positive and negative sputum slides collected from the districts. In all, 1020 sputum slides were collected during 1939 from 13 districts and it was heartening to observe that discrepancy was found only in 5% of slides. Corrective measures were undertaken after the identification of the concerned PHC responsible for these discrepant results. Studies of this nature ensures QC at the peripheral level.

Comparison of Sputum Smear Results : TRC Vs Field Lab.

TRC & Field Pos.	TRC & Field Neg.	TRC Pos Field Neg.	TRC Neg. Field Pos.
555	414	45	6

Quality Control for Media, Reagents and Biochemical Tests :

QC procedures should also provide checks for sterility, growth and biochemical tests. Sterility test should be performed at 25°C and 37°C on a sample of each batch of medium that is autoclaved, inspissated or filter sterilised during preparation. These sterility tests with reagents and media will ensure against 'environmental' contaminants. Growth studies verify that the medium will support the growth of the desired organism(s), whereas positive and negative control organisms are used to document that the medium will produce the expected biochemical test responses.

At TRC, a minimum number of tests are being routinely done to differentiate *M. tuberculosis* from Non-Tuberculous Mycobacteria (NTM). Cultures from patients who repeatedly excrete NJM strains are subjected to a battery of tests employed under Numerical Taxonomy with suitable controls. Similarly, for drug sensitivity testing, adequate controls are always included.

Use of Bacteriophage Typing in Checking Laboratory Cross Contamination :

Wilbur D. Jones (1988) published an interesting article on the use of bacteriophage typing on *M. tuberculosis* cultures to check laboratory cross contamination(s). He has reported on testing 235 *M. tuberculosis* cultures submitted from 31 laboratories. In each instance, either the attending physician questioned the misdiagnosis of tuberculosis or the laboratory supervisior suspected that Laboratory cross contamination occurred.

The phage typing data confirmed that cross contamination incident occurred in all 31 laboratories. In the case of physician suspected cross contamination, it was heartening to find that their patients did not have tuberculosis. This signalled a need for laboratory staff to tighten up on aseptic techniques and to institute more right quality control on technical procedures. Some of the laboratory detected cross contamination were traced to human error, but several were shown to be related to poor between-specimen sterilisation of

sampling needles used in rapid radiometric equipment for Mycobacteriology. QC for radio metric technology calls for periodic chocks on cleanliness of needles to prevent cross contamination of cultures, And thus, Mycobacteriophage typing is a useful adjunct in the investigation of suspected cross-contamination of laboratory cultures of *M. tuberculosis.*

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