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Processing Sputum Specimens in a Refrigerated Centrifuge Does Not Increase the Rate of Isolation of *Mycobacterium tuberculosis*

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A total of 1,047 sputum samples from pulmonary tuberculosis patients was collected in cetyl pyridinium chloride-sodium chloride solution. Each sample was divided into two parts and randomly allocated for the isolation of *Mycobacterium tuberculosis*, with one part to be processed by the standard method and the other by a modified method. In the standard method, the samples were processed by using nonrefrigerated centrifuges, while in the modified method, they were processed by using a refrigerated centrifuge. Fifty-seven samples that yielded contaminants were excluded, and the remaining 990 samples were taken up for analysis. The rates of isolation of *M. tuberculosis* with the standard and modified methods were 48.6 and 48.1%, respectively, and the difference was not statistically significant (McNemar's test; $P > 0.5$). However, 51% of the positive cultures were isolated within 2 weeks with the modified method compared to 37% with the standard method (chi-square test; $P < 0.001$). The results of the study reveal that processing of sputum samples in a refrigerated centrifuge does not improve the rate of isolation but will result in rapid isolation of *M. tuberculosis*.

Isolation of *Mycobacterium tuberculosis* from biological samples is essential in drug resistance surveys and in initiating treatment for cases involving drug resistance (2). In most laboratories in developing and developed countries, sputum samples are processed by using nonrefrigerated centrifuges to isolate *M. tuberculosis.* Processing the sputum samples by using the fixed-angle rotors at 3,000 × *g* for 15 min was found to alleviate the detrimental effect of heat on tubercle bacilli (4). However, no documented evidence is available on the rate of isolation of *M. tuberculosis* from sputum samples processed by using refrigerated and nonrefrigerated centrifuges. Therefore, an attempt was made to ascertain the influence of cold centrifugation on the rate of isolation of *M. tuberculosis* by using sputum samples equally split into two parts.

Cetyl pyridinium chloride-sodium chloride (CPC-NaCl) solution. One percent CPC (Sisco Research Laboratories, Mumbai, India) in 2% NaCl (Qualigens Fine Chemicals, Mumbai, India) was prepared by dissolving 10 g of CPC and 20 g of NaCl in 1,000 ml of distilled water (8). The solution was sterilized by autoclaving for 15 min at 121°C, and 5-ml aliquots were poured into sterile McCartney bottles. The bottles were distributed in cardboard containers to health units, where they were stored at room temperature for 3 to 7 days until they were used to collect sputum samples.

Collection of sputum samples. A total of 1,047 sputum samples were collected from pulmonary tuberculosis patients attending health units in the Valliyur Tuberculosis Unit in Thiruvallur district, Tamil Nadu, India. These were from patients with symptoms suggestive of pulmonary tuberculosis and from those who were monitored while being treated with the regimens prescribed in the Revised National Tuberculosis Control Programme (3). The samples were stored at room temperature in cardboard containers at the health units or laboratories until they were transported (5 to 10 days subsequently) to the central laboratory at Tuberculosis Research Centre, where they were processed for isolation of *M. tuberculosis.*

Processing of sputum samples. Each sputum sample was split into two equal parts that were randomly allocated; one part was allocated to the standard method in which a nonrefrigerated centrifuge (Haereaus megafuge 1.0) was used, and the other was allocated to the modified method in which a refrigerated centrifuge (Beckman J-6B) was used. The temperature of the refrigerated centrifuge was maintained between 4 and 7°C. The nonrefrigerated centrifuges were kept in an air-conditioned laboratory where the temperature was maintained between 21 and 25°C. Horizontal rotors with a relative centrifugal force of 3,000 × *g* and a centrifugation time of 15 min were used for both types of centrifuges. The samples were processed independently for the isolation of *M. tuberculosis* by the procedure described earlier (7). In brief, the samples were centrifuged in the respective centrifuges, and the supernatant was discarded. To the deposit, 20 ml of sterile distilled water was added and centrifuged again. After the supernatant was discarded, a loopful of the deposit was inoculated into each of two Lowenstein-Jensen (LJ) media mixtures and into one LJ medium containing 0.5% sodium pyruvate (LJP). The medium bottles were incubated at 37°C in an incubator and examined for the growth of *M. tuberculosis* every week. The growth of *M. tuberculosis* was graded as follows: 3+ for confluent growth; 2+ for growth of innumerable colonies; 1+ for growth of 20 or more colonies but less than 100 colonies; Cols for growth of 1 to 19 colonies; Neg for no growth of *M. tuberculosis*; and Cont for contamination. All positive cultures were subjected to a niacin test, a 68°C catalase test, and growth on *para*-nitroben-

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TABLE 1. Comparison of culture results obtained by using the standard and modified methods[a]

Result by the standard method	No. of samples with indicated result by the modified method							
	3+	2+	1+	Cols	Pos	Neg	Cont	All
3+	34	22	1	0	57	0	1	58
2+	32	85	64	2	183	4	5	192
1+	1	6	63	35	105	1	3	109
Cols	0	3	20	85	108	23	3	134
Pos	67	116	148	122	453	28	12	493
Neg	0	0	4	19	23	486	17	526
Cont	0	0	0	1	1	8	19	28
All	67	116	152	142	477	522	48	1,047

[a] Results were graded as follows: 3+ for confluent growth; 2+ for innumerable colonies; 1+ for more than 20 colonies but less than 100 colonies; Cols for 1 to 19 colonies; Neg for No growth of *M. tuberculosis*; and Cont for contamination. Pos, total number of positive cultures.

TABLE 2. Growth rates of *M. tuberculosis* with the standard and modified methods

Wk	Cumulative % positive cultures in different media with the indicated method					
	LJ1		LJ2		LJP	
	Standard	Modified	Standard	Modified	Standard	Modified
1	0.9	1.4	0.8	1.5	0.9	1.4
2	33.4	44.7	33.3	45.3	36.8	50.9
3	78.6	84.5	81.5	85.1	79.8	88.3
4	92.9	92.0	92.9	93.9	93.0	94.1
5	96.2	96.2	96.2	96.5	97.6	96.7
6	98.2	98.5	98.7	98.5	99.1	99.3
7	99.3	99.2	99.7	99.2	100.0	100.0
8	100.0	99.9	100.0	100.0	100.0	100.0

zoic acid to confirm the presence of *M. tuberculosis.* The culture results were decoded and matched for comparison.

Biological materials should be processed immediately or within 3 days after collection of the sample for the isolation of *M. tuberculosis*. Any delay beyond 3 days will result in reduced recovery of *M. tuberculosis* from cultures due to contaminants in the specimen overgrowing in the culture medium (5). In the present study, since the samples cannot be transported from the microscopy centers to the central laboratory at Tuberculosis Research Centre, Chennai, India, within 3 days, they were collected in CPC-NaCl solution and stored in the health units until they were transported for culture work. Earlier studies had shown that storage of sputum samples in the CPC-NaCl solution reduced the contamination rate, thereby improving the isolation rate of *M. tuberculosis* (6–9). Moreover, the rate of isolation of *M. tuberculosis* in CPC-NaCl-preserved samples and in samples processed immediately after collection is not significantly different (6).

The culture results obtained with the standard and modified methods are presented in Table 1. Of the 1,047 samples, 57 were contaminated either by one method or by both methods. The remaining 990 samples were considered for the analysis. The rates of isolation of *M. tuberculosis* were 48.6 and 48.1% by the standard and modified methods, respectively. The difference observed between the methods was not statistically significant (McNemar's test; $P > 0.5$). Of the 453 specimens that yielded *M. tuberculosis* by each of the methods, the proportions of cultures yielding grades of 3+, 2+, 1+ and Cols were 14.8, 25.6, 32.7, and 27% by the modified method and 12.6, 40.4, 23.2, and 23.8% by the standard method, respectively. The proportion of cultures with growth at or above levels corresponding to grades of 1+ and 2+ was significantly higher in the standard method than in the modified method ($P < 0.001$). Although a greater proportion of samples showed higher grades by culture by the standard method, there was no difference qualitatively. Forty-eight of 1,047 samples were contaminated by the modified method compared to 28 by the standard method. The observed difference in the rates of contamination between the methods attained statistical significance ($P < 0.002$). This could be attributed to the lower recovery rate by the modified method. Since the study was carried out in CPC-preserved sputum samples, the results of the findings cannot be extrapolated to other methods of processing sputum samples.

Table 2 shows the percentage of positive *M. tuberculosis* cultures isolated at different weeks with each of the methods. It is evident that irrespective of the type of medium, larger proportions of positive cultures were recovered within 2 weeks with the modified method than with the standard method; the recovery rates in LJ1, LJ2, and LJP were 44.7, 45.3, and 51% with the modified method and 33.4, 33.3, and 36.8% with the standard method (chi-square test; $P < 0.001$). The delay in growth of *M. tuberculosis* with the standard method may be attributed to the exposure of the bacilli to the heat generated in the nonrefrigerated centrifuge. On the other hand, the rapid growth observed with the modified method could be due to the soft treatment provided to the bacterium by cold centrifugation. It may be pointed out here that changes in the optimal conditions in processing food were reported to affect the growth and survival of the organism (1).

In conclusion, the study reveals that processing sputum samples in refrigerated centrifuges does not improve the rate of isolation but will result in rapid recovery of *M. tuberculosis*.

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