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Inhibitory Effect of Sodium Dodecyl Sulfate in Detection of *Mycobacterium tuberculosis* by Amplification of rRNA

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The concentrations of sodium dodecyl (lauryl) sulfate (SDS) found in the sediments of the respiratory samples pretreated with SDS-NaOH varied between 3.36 and 12.42 mg/ml. These concentrations of SDS were higher than the level considered critical (≥0.16 mg/ml) to obtain negative results with the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. Extensive washing, after neutralization of sediment, is sufficient to remove all traces of detergent which interfere with the enzymes used by the Mycobacterium Tuberculosis Direct Test assay.

Technological advances in amplifying and detecting specific regions of bacterial DNA or RNA have provided the necessary methodology to improve the laboratory diagnosis of tuberculosis (1–3, 5, 11).

Gen-Probe (San Diego, Calif.) has developed an isothermal transcription-mediated amplification system which detects *Mycobacterium tuberculosis* complex directly in respiratory specimens (1, 6, 8, 9, 12). After amplification of a specific rRNA target via DNA intermediates, the amplicon is detected by the same hybridization protection assay employed in the Gen-Probe test used for culture confirmation. The manufacturers suggest that the Mycobacterium Tuberculosis Direct Test (MTD) be carried out with sediments of sputa and bronchial aspirates which have been decontaminated according to the *N*-acetyl-L-cysteine (NALC)–NaOH or NaOH procedures recommended by the Centers for Disease Control and Prevention (Atlanta, Ga.) (7). Many clinical laboratories, particularly in European countries, pretreat their respiratory specimens with sodium dodecyl (lauryl) sulfate (SDS)-NaOH according to the method described by Tacquet and Tisson (15). The inhibitory effects of SDS in PCR assays have been previously described (4). The aim of the present study was to determine the critical concentrations of SDS that inhibit amplification and detection of *M. tuberculosis* complex in the Gen-Probe MTD assay.

Five sputum samples and five bronchial aspirates from patients without tuberculosis were decontaminated by the standard method of Tacquet and Tisson (15). Briefly, 2 or 3 ml of the specimens was transferred to a 50-ml plastic centrifuge tube, and an equal volume of SDS-NaOH solution (2% NaOH, 3% SDS [Fluka Chemical Company, Buchs, Switzerland]) was added; after being vortexed, the samples were vigorously shaken for 30 min. Later, H_3PO_4 (containing 0.006% bromocresol purple as a pH indicator) was added to neutralize the specimens. After a centrifugation step (3,300 × *g*; 20 min), the pellet obtained was used for determination of residual SDS. SDS concentrations were determined by the modified Eaton (13) two-phase titration method for quantification of anionic active detergents.

Test compound (SDS, Fluka Chemical Company) was prepared as a concentrated stock solution and diluted in distilled water. Two serial twofold dilutions of SDS stock solution were prepared before use in 1.0 ml of distilled water. Concentrations of SDS ranging from 0.025 to 25.6 mg/ml were studied. The inocula were prepared from cultures of *M. tuberculosis* H37Rv in BACTEC 12B broth medium (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.). A vial of 7H12 12B medium (4.0 ml) was inoculated with 0.1 ml of a broth suspension of the same optical density as a no. 1 McFarland standard diluted 1:2. An inoculum of 0.1 ml from 12B broth culture with a GI between 300 and 400 provided an initial concentration of 10^3 to 10^4 CFU/ml, which was used for a series of test tubes. Another series of test tubes was inoculated with 0.1 ml from 12B broth culture with a GI between 700 and 900 (10^5 to 10^6 CFU/ml).

The MTD protocol was performed the same day with the two series of test tubes and consisted of the following steps. For lysis, 50 μl of the sample was added to the mixture, which was sonicated for 15 min in a water bath sonicator at room temperature. For amplification, 25 μl of reconstituted amplification reagent was placed in a reaction tube and covered with 200 μl of oil. Fifty microliters of lysate (or MTD amplification-positive control and MTD amplification-negative control) was transferred to the amplification tube, incubated at 95°C for 15 min, and then cooled at 42°C for 5 min. An enzyme reagent (25 μl) was added, and the mixture was incubated at 42°C for 2 h. Then, 20 μl of the termination reagent was added to each tube, and the mixtures were kept at 42°C for another 10 min. For detection, the reconstituted acridinium-labeled probe (100 μl) was added to the tubes, which were incubated at 60°C for 15 min; then the selection reagent (300 μl) was added, and the mixtures were reincubated at 60°C for 15 min. Appropriate hybridization protection assay-positive and hybridization protection assay-negative control experiments were run according to the recommendations of the manufacturers. Prior to being read in a luminometer (Leader 50; Gen-Probe), the tubes were cooled at room temperature for 5 to 10 min. The cutoff value was set at 30,000 relative light units. The concentrations of SDS found in the sediments of the respiratory samples studied were variable, ranging between 3.36 and 12.42 mg/ml in sputa, with an average value of 9 mg/ml, and between 8.06 and 10.64 mg/ml in bronchial aspirates, with an average value of 8.71 mg/ml (Table 1).

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TABLE 1. Concentrations of SDS in clinical specimens tested

Type of clinical specimen and specimen no.	Reference	SDS concn (mg/ml)
Sputum		
1	E1/391K	3.36
2	E2/550K	9.86
3	E3/237K	11.66
4	E4/477K	10.42
5	E5/921K	12.42
Bronchial aspirate		
6	B1/282K	8.06
7	B2/731K	8.96
8	B3/970K	7.84
9	B4/071K	10.64
10	B5/290K	8.06

As shown in Table 2, in the artificial samples studied which contained inocula of 10^3 to 10^4 CFU/ml, the MTD assay did not detect *M. tuberculosis* when the concentration of SDS in the original sample was ≥6.4 mg/ml. When the concentration of SDS was ≤3.2 mg/ml, the amplification reaction was not inhibited and all the samples were MTD positive with values of >1,500,000 relative light units. In samples with a higher inoculum (10^5 to 10^6 CFU/ml), the results were the same; positive results were obtained from the MTD when SDS concentrations in the sample were ≤3.2 mg/ml (Table 2). Considering the final concentrations of SDS in the sample and the reagents used in the MTD test, it has been possible to calculate that concentrations of ≥0.16 mg of SDS per ml in the test tube inhibit the amplification of 16S rRNA sequences and the detection of *M. tuberculosis* with the Gen-Probe MTD. When the classic method of Tacquet and Tisson (15) was used, the concentrations of SDS found in the centrifuged pellet of all decontaminated samples (Table 1) were higher than the level considered critical to avoid obtaining false negative results with the MTD.

TABLE 2. Inhibitory effect of different SDS concentrations on the detection of *Mycobacterium tuberculosis* by MTD

Test inoculum (CFU/ml) and SDS concn (mg/ml)	RLU[a] value	MTD result
10^3–10^4		
25.6	2,323	−
12.8	2,328	−
6.4	2,338	−
3.2	1,609,000	+
1.6	1,620,000	+
0.8	1,665,000	+
0.4	1,613,000	+
0.2	1,632,000	+
0.1	1,615,000	+
0.05	1,539,000	+
0.025	1,583,000	+
10^5–10^6		
25.6	2,381	−
12.8	2,385	−
6.4	1,995	−
3.2	1,650,000	+
1.6	1,656,000	+
0.8	1,682,000	+
0.4	1,690,000	+
0.2	1,687,000	+
0.1	1,694,000	+
0.05	1,667,000	+
0.025	1,652,000	+

[a] RLU, relative light units.

According to the manufacturer's suggestion, the new MTD should be carried out only with sediments of sputa and bronchial aspirates which have been decontaminated by NALC-NaOH or NaOH procedures (11, 12). The performance of MTD with other decontamination procedures commonly used in mycobacteriological laboratories has not been sufficiently evaluated. Given that the decontamination method of SDS-NaOH is often used in clinical laboratories, especially in Europe, and that it has previously been reported that low concentrations of SDS can inhibit PCR assays (4), we propose to study the possible ways that SDS might interfere with the amplification process of rRNA, which is used in the MTD.

SDS concentrations in the sediments of sputa and bronchial aspirates decontaminated by the method of Tacquet and Tisson (15) were determined by a modified version of the Eaton two-phase titration method (13). This method is subject to relatively low interference by the various other ingredients usually found in detergent formulations.

In this study, we have been able to rule out the possibility that the concentrations of SDS found in the sediments of both sputa and bronchial aspirates decontaminated by the Tacquet-Tisson method (3.36 to 12.42 mg/ml) are capable of inhibiting the amplification of rRNA in the MTD (Table 1). SDS concentrations up to 3.2 mg/ml in the centrifuged pellet (which, as determined by doing the appropriate calculations, is equal to concentrations of ≥0.16 mg of SDS per ml in the tube where the amplification reaction takes place) totally inhibited the process. Recently, Pfyffer et al. (12) have demonstrated that in respiratory clinical samples decontaminated with SDS-NaOH, similar or better results than those obtained with NALC-NaOH may be obtained with the MTD. The sensitivity of the MTD was 93.9%, the specificity was 97.6%, the positive predictive value was 80.7%, and the negative predictive value was 99.3% for the NALC-NaOH series; the corresponding values for the SDS-NaOH series were 97.4, 96.9, 76.0, and 99.7%, respectively. The data from Pfyffer et al. (11, 12) demonstrate that respiratory specimens do not necessarily need to be processed by the NALC procedure; extensive washing after SDS decontamination seems to be very important (11, 14). Obviously, this washing step after neutralization of sediment is sufficient to remove all remaining traces of detergent (12) that might interfere with the enzymes of the MTD assay.

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