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Removal of PCR Inhibitors by Silica Membranes: Evaluating the Amplicor *Mycobacterium tuberculosis* Kit

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The effectiveness of PCR inhibitor removal by silica membranes in combination with the Amplicor *Mycobacterium tuberculosis* kit was analyzed for 655 respiratory and nonrespiratory specimens. The overall inhibition rate was reduced from 12.5%, when applying the Amplicor kit alone, to 1.1% with the addition of silica membrane DNA purification.

Clinical specimens sometimes contain inhibiting substances (inhibitors) that interfere with the performance of the PCR (7, 34). Therefore, a routine procedure suitable for removal of all inhibitors simultaneously is highly desirable (28, 29). Owing to the extremely variable nature of inhibitors, however, no single ideal procedure exists yet (2, 3, 15).

The binding of specimen DNA to silica membranes represents a strategy for eliminating a variety of inhibitors simultaneously (18, 22). The Amplicor *Mycobacterium tuberculosis* kit is especially susceptible to inhibition (30), inasmuch as it does not provide for cleaning of the extracted DNA. As such, this kit is suited for studying the effectiveness of inhibitor removal. The present study had a twofold aim: (i) to analyze the efficiency of PCR inhibitor removal via silica membranes on various types of clinical materials and (ii) to investigate the validity of the PCR results obtained from the Amplicor *M. tuberculosis* kit in combination with silica membrane columns.

Six hundred fifty-five clinical samples were analyzed by PCR in a prospective study over 14 months (P. Charache, Editorial, Clin. Infect. Dis. **23:**1107–1108, 1996). All samples except primarily sterile materials were processed for decontamination by the *N*-acetyl-L-cysteine NaOH method (23). Aliquots (0.2 ml) of this suspension were used (i) to prepare auramine-rhodamine fluorochrome-stained smears (12), (ii) for cultures including radiometric broth (12), and (iii) for PCR-enzyme-linked immunosorbent assay as described by the Amplicor protocol (Amplicor *Mycobacterium tuberculosis* [MTB] Test; Hoffmann-LaRoche, Grenzach-Wyhlen, Germany) with detection of both the *M. tuberculosis* and the internal control (IC) oligonucleotide probes. The resulting preamplification sample contained a 200-μl solution of extracted DNA, 50 μl of which was used for continuing with the Amplicor protocol. A PCR was considered to be inhibited when the IC generated an optical density (OD) of <0.35. These inhibited samples were subjected to the "silica membrane protocol." To remove the inhibitors, 100 μl of the remaining preamplification solution was transferred to a silica membrane (QIAamp DNA Mini Kit; Qiagen, Heidelberg, Germany) and eluted in 50 μl of elution buffer. Twenty-five microliters of this purified sample was reamplified by adding 50 μl of Amplicor master mix, 10 μl of 10× PCR buffer, 8 μl of 50 mM $MgCl_2$, and 17 μl of H_2O. When this PCR again was inhibited, we classified the final result as inhibited. Because the IC samples ($n = 20$) gave ODs similar to those observed with the Amplicor kit (mean, 2.7; standard deviation, 0.15) and the silica membrane protocol (mean, 2.5; standard deviation, 0.25), we used the same cutoff (i.e., OD $\geq$ 0.35) for a positive PCR result.

The sensitivity of both protocols was analyzed by applying a 10-fold serial dilution of *Mycobacterium bovis* BCG in triplicate experiments. The detection limit was as low as 4 CFU/sample in both cases.

Results of the Amplicor protocol with clinical samples appear in Table 1. Nonrespiratory specimens had a higher positive rate than respiratory specimens (7.1 versus 3.3%), which was accompanied by a much higher inhibition rate (18.6 versus 4.0%). The silica membrane protocol (Table 2) reduced the inhibition rates from 12.5% (82 of 655 samples) to 1.1% (7 of 655 samples) overall, from 4.0% (11 of 273 samples) to 0.4% (1 of 273 samples) for respiratory specimens, and from 18.6% (71 of 382 samples) to 1.6% (6 of 382 samples) for nonrespiratory specimens.

TABLE 1. PCR results of 655 specimens analyzed with the Amplicor protocol

Specimen group	No. of specimens analyzed	No. (%) with PCR result		
		Positive	Negative	Inhibited[c]
Respiratory tract[a]	273	9 (3.3)	253 (92.7)	11 (4.0)
Nonrespiratory	382	27 (7.1)	284 (74.3)	71 (18.6)
Lymph nodes	43	5 (11.6)	16 (37.2)	22 (51.2)
Gastric fluid	60	5 (8.3)	50 (83.3)	5 (8.3)
CSF	104	5 (4.8)	93 (89.4)	6 (5.8)
Urine	12		4 (33.3)	8 (66.6)
Other[b]	163	12 (7.4)	121 (74.2)	30 (18.4)
All	655	36 (5.5)	537 (82)	82 (12.5)

[a] Includes bronchial washings ($n = 194$), sputa ($n = 70$), and tracheal aspirates ($n = 9$).
[b] Includes body fluid aspirates ($n = 68$), biopsy specimens ($n = 35$), swabs ($n = 18$), blood ($n = 11$), peritoneal fluid ($n = 10$), bone ($n = 7$), liver ($n = 5$), brain ($n = 4$), pericard aspirate ($n = 3$), and stool ($n = 2$).
[c] OD of the internal control, <0.35.

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TABLE 2. PCR results of 82 initially inhibited specimens analyzed with the silica membrane protocol

Specimen group	No. of specimens analyzed	No. (%) with PCR result		
		Positive	Negative	Inhibited[c]
Respiratory tract[a]	11	1 (9.1)	9 (81.2)	1 (0.4)
Nonrespiratory	71	6 (8.4)	59 (83.1)	6 (1.6)
Lymph nodes	22	5 (22.7)	16 (72.7)	1 (2.3)
Gastric fluid	5		4 (80)	1 (1.66)
CSF	6	1 (16.6)	5 (83.4)	0 (0.0)
Urine	8		7 (87.5)	1 (8.3)
Other[b]	30		27 (90)	3 (1.8)
All	82	7 (8.5)	68 (83)	7 (1.1)

[a] Includes bronchial washings ($n = 2$), sputum ($n = 6$), and tracheal aspirates ($n = 2$).
[b] Includes body fluid aspirates ($n = 9$), biopsy specimens ($n = 11$), swabs ($n = 2$), blood ($n = 3$), peritoneal fluid ($n = 1$), bone ($n = 1$), pericard aspirate ($n = 1$), and stool ($n = 2$).
[c] Percentage calculated based upon all samples (Table 1).

To evaluate the quality of the PCR results, we compared them with culture results (Table 3). By applying defined criteria, all of the discrepant PCR-positive–culture-negative results could be resolved (Table 4), resulting in an overall sensitivity of 0.75 for the Amplicor protocol and 0.78 for the silica membrane protocol. Specificities (1.0 and 1.0) and positive (1.0 and 1.0) and negative (0.98 and 0.97) predictive values gave excellent results for both protocols, respectively.

Lymph nodes proved to be one of the most potent inhibitors of the Amplicor protocol. They were inhibited in 51% of samples (22 of 43 samples); however, they also displayed the highest positive rate (10 of 43 samples). Inasmuch as five of the positive specimens initially were inhibited, half of these PCR diagnoses would have been missed without the silica membrane protocol. Other important materials known to be troublesome for culture (16) and successfully cleaned from inhibitors included cerebrospinal fluid and gastric fluid. Overall, the removal of inhibitors by application of the silica membrane protocol was successful in 91.5% of the samples (75 of 82 samples), thereby proving efficiency in a variety of clinical materials. The overall sensitivity of the Amplicor protocol ranges from 66.6 to 87% (4, 5, 9, 13, 17, 27, 29, 31). This compares well with our findings of overall sensitivities of 75 and 78% (Table 4). In summary, the data demonstrate that the additional performance of the silica membrane protocol does not detract in any way from the quality of the PCR results in clinical samples.

Various attempts have been made to reduce PCR inhibition in diagnostic tests, mostly with regard to a specific material (8, 9, 21). Inexpensive methods, such as boiling, have been effective with urine samples (32) and partially effective with cerebrospinal fluid, depending on the protein level (24, 25, 26). Notably, boiling can also cause inhibition (1). Boiling was found to be as effective as sample dilution with cervical specimens (33). Sample dilution worked particularly well with urine specimens (6, 10) but was inadequate for respiratory tract specimens. Instead, in the case of the latter, the addition of bovine serum albumin neutralized inhibitors in 21 of 22 specimens (14). The addition of bovine serum albumin protects PCR from the effects of blood (2), but this procedure has been analyzed with only a few clinical samples. Phenol-chloroform extraction has been shown to be highly effective (11, 19, 20) but uses toxic substances and is particularly laborious. The silica membranes used in this study add approximately $1 per sample to costs but were effective in a variety of materials. Therefore, it remains to be seen whether a given method or some combination of methods will be best suited to the task of overcoming PCR inhibition.

Finally, the binding of DNA to silica membranes is rapidly performed and easy to handle. Hence, a wider range of different laboratory specimens is now available to confirm the clinical diagnosis of tuberculosis. Given the good sensitivity of our protocol, we think the additional use of silica membranes in a variety of PCR assays represents a significant step in the direction of improved PCR diagnostics.

TABLE 3. Comparison of PCR results with culture results

Protocol	Result	No. with culture result		Sensitivity	Specificity	Positive predictive value	Negative predictive value
		Positive	Negative				
PCR	Positive	30	6	0.71			
Amplicor ($n = 573$)	Negative	12	525		0.99	0.83	0.93
PCR	Positive	4	3	0.67			
Silica membrane ($n = 75$)	Negative	2	66		0.96	0.57	0.97

TABLE 4. Comparison of PCR results with culture results corrected according to clinical findings[a]

Protocol	Result	No. with corrected result		Sensitivity	Specificity	Positive predictive value	Negative predictive value
		Positive	Negative				
PCR	Positive	36	0	0.75			
Amplicor ($n = 573$)	Negative	12	525		1.0	1.0	0.98
PCR	Positive	7	0	0.78			
Silica membrane ($n = 75$)	Negative	2	66		1.0	1.0	0.97

[a] Clinical specimens with the discrepant result PCR positive, culture negative (Table 3) were corrected to PCR positive, clinical result positive if they (i) came from a patient under tuberculostatic therapy ($n = 6$), (ii) showed a positive smear ($n = 2$), or (iii) originated from a patient with culture-positive results in other materials obtained within the same period ($n = 1$).

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