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Use of AmpliWax To Optimize Amplicon Sterilization by Isopsoralen

MARIA DE LA VIUDA,[1] MANFRED FILLE,[1] JUAN RUIZ,[2] AND JABER ASLANZADEH[1]*

Department of Laboratory Medicine[1] and Department of Medicine,[2] University of Connecticut School of Medicine, Farmington, Connecticut

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The photochemical inactivation of amplicons by isopsoralen (IP-10) has been suggested as a possible means to prevent PCR carryover contamination. To evaluate the technique, serial dilutions of amplicons (10^{11} to 10^3) from the *Borrelia burgdorferi* OSP A gene were amplified in the presence of 0, 25, 50, and 100 μg of IP-10 per ml for 45 cycles. The PCR products were exposed to UV light for 15 min to activate IP-10 and sterilize the amplicons. One microliter of each sterilized sample was reamplified for an additional 45 cycles. The PCR products were then resolved in an agarose gel, blotted onto a nylon membrane, and probed with an alkaline phosphatase-conjugated chemiluminescent probe. Although IP-10 at concentrations of 50 and 100 μg/ml effectively sterilized up to 10^{11} amplicons, the compound was inhibitory to PCR. IP-10 at a concentration of 25 μg/ml had slight inhibitory effect on PCR and did not completely sterilize all of the amplicons. Therefore, in subsequent experiments AmpliWax was substituted for mineral oil, and PCR was performed on 10^9 to 10^3 amplicons as described above. Following the amplification, the PCR tubes were cooled to solidify the AmpliWax and inoculated with various concentrations of IP-10. With this technique, PCR products produced from as many as 10^9 target amplicons were effectively sterilized with 200 μg of IP-10 per ml. Similarly, the addition of IP-10 (50 μg/ml) before and after PCR was evaluated for the detection of *B. burgdorferi* in 62 ticks from a region of southern Connecticut where the organism is highly endemic. PCR performed in the presence of 50 μg of IP-10 per ml detected *B. burgdorferi*-specific DNA in 17 of 62 ticks (27%) following gel electrophoresis and in 34 of 62 ticks (55%) following Southern blot hybridization of the PCR products. In contrast, post-PCR addition of IP-10 detected borrelia-specific DNA in 31 of 62 ticks (50%) following gel electrophoresis and in 46 of 62 ticks (64%) following Southern blot hybridization. We conclude that the replacement of mineral oil with AmpliWax can be useful in eliminating the inhibitory effects of IP-10 and other sterilizing agents for post-PCR sterilization of amplicons.

Aerosolized amplicons remain the main source of PCR carryover contamination (3, 19). Although some general precautions and good laboratory practice (11, 23) can reduce the possibility of such contamination, other simple and more effective preventive measures have been proposed. These techniques include exposing the reaction mixture to UV light (8, 20); autoclaving (6); treating the reaction mixture with multiple restriction enzymes (29), DNase (29), bleach (16), or hydroxylamine (1); substituting dUTP for dTTP in every PCR and treating the subsequent PCR mix with uracil DNA glycosylase (2, 12); using anticontamination primers (22) and ribose-modified primers and primer hydrolysis (25); and photochemically modifying the contaminating DNA with psoralen or isopsoralen (IP-10) (4, 9).

The photochemical procedures use psoralen compounds that are known to intercalate between nucleic acid base pairs (4, 9). These compounds, primarily IP-10, are added to the reaction vessel, and then post-PCR activation through exposure to long-wave UV light is carried out. The result is a cross-link between the complementary strands of the PCR product or the formation of monoadducts (depending on the psoralen compound). The presence of these modifications prevents amplicons from further reamplifying (15). However, IP-10 has been demonstrated to have a concentration-dependent inhibitory effect on PCR (9, 18). The inhibitory effects may be partially reduced by the addition of 10% glycerol, bovine serum albumin (BSA), or other organic solvents (9). The objective of this study was to investigate the post-PCR addition of IP-10 to PCR tubes presealed with a commercially available wax bead (AmpliWax; Perkin-Elmer). Solid AmpliWax beads were added to the PCR tubes during setup. Elevated temperatures during the PCR cause the beads to melt, thus forming a vapor barrier usually supplied by mineral oil. Following thermal cycling, the tubes were cooled, causing the beads to form a solid wax barrier. The subsequent addition of IP-10 to the top of the solidified wax, followed by heating and remelting of wax and exposure to UV light, allowed the IP-10 to mix with and inactivate amplicons after completion of the PCR. Our results demonstrate that post-PCR addition of IP-10 to PCR tubes presealed with AmpliWax resulted in complete sterilization of the amplicons and elimination of the inhibitory effects of IP-10 without further modification of the procedure.

MATERIALS AND METHODS

Borrelia burgdorferi 2591 was inoculated into modified Kelly's medium and incubated at 30°C until a concentration equivalent to a 0.5 McFarland standard was attained. The spirochetes were then centrifuged at 35,000 × *g* for 45 min at 4°C and washed twice with phosphate-buffered saline (PBS). After the last wash, the spirochetes were resuspended in PBS and enumerated microscopically by using a Petroff-Hausser bacterial counting chamber (VWR Scientific, Rochester, N.Y.). The spirochete concentration was adjusted to 10^8 organisms per ml and heat inactivated by boiling for 5 min. Four microliters of this suspension was subjected to 45 cycles of PCR amplification with a set of primers that amplified a 156-bp sequence unique to the *B. burgdorferi* OSP A gene (14). The PCR mix contained 5 μl of 10× PCR buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl, 15 mM $MgCl_2$, 0.001% [wt/vol] gelatin), 200 μM each deoxynucleoside triphosphate, 25 pmol of each oligonucleotide primer, 2.5 U of *Taq* polymerase and water to yield a 50-μl reaction volume per tube. The tubes were overlaid with 70 μl of mineral oil and subjected to 45 cycles of PCR at 94°C for 1 min, 50°C for 1 min, and 72°C for 1.5 min. PCR products were visualized in an ethidium

* Corresponding author. Mailing address: Department of Laboratory Medicine, University of Connecticut School of Medicine, 263 Farmington Ave., Farmington, CT 06030. Phone: (860) 679-3132. Fax: (860) 679-1098.

bromide-stained 3% NuSieve–1% SeaKem agarose gel (FMC Bioproducts, Rockland, Maine). The PCR products were recovered from the gel by a method described by Zhen and Swank (28). The final DNA pellet was dissolved in TE buffer (1 M Tris base, 0.5 M EDTA, pH 8), and the amount of amplicons was calculated and adjusted to 10^{11} amplicons per μl (5) with a U2000 UV spectrophotometer (Hitachi Corp., Danbury, Conn.).

Amplicon sterilization. A 10-fold dilution series (10^{11} to 10^3 amplicons per μl) was prepared from the purified PCR products. One microliter of each amplicon dilution was added to individual Microfuge tubes containing PCR mix plus 10% glycerol and 0, 25, 50, or 100 μg of IP-10 (HRI Associates Inc., Concord, Calif.) per ml. Following the PCR, IP-10 was activated by placing the tubes directly on a UV transilluminator (Fotodyne Inc., New Berlin, Wis.) for 15 min. PCR products were electrophoresed in an agarose gel, blotted onto a nylon membrane, and probed with an alkaline phosphotase-conjugated chemiluminescent probe (Lightsmith II; Promega Corp., Madison, Wis.). One microliter of each PCR product treated with the concentrations of IP-10 indicated above was reamplified with a fresh PCR mix as described above to check the efficiency of sterilization.

In order to improve the efficacy of the amplicon sterilization by IP-10, a modified procedure was developed. One microliter of each amplicon dilution (10^9 to 10^3 amplicons per μl) was added to five series of PCR tubes containing 49 μl of PCR reaction mix and an AmpliWax bead (Perkin-Elmer). PCR was performed as described above, and the reaction tubes were cooled to room temperature to allow AmpliWax to form an aerosol-free seal over the amplicon solutions. Each PCR tube series was then inoculated with 0, 25, 50, 100, or 200 μg of IP-10 per ml. The tubes were heated and cooled to melt the wax and mix the IP-10 with the amplicons and were exposed to UV light to activate IP-10 and sterilize the amplicons. One microliter of each IP-10-treated PCR product was placed in a fresh PCR tube and reamplified as described above to check for efficiency of amplicon sterilization.

Effect of IP-10 on PCR detection of *B. burgdorferi*-specific DNA in infected *Ixodes scapularis*. To determine the effects of pre- or post-PCR addition of IP-10 on detection of *B. burgdorferi* from clinical samples, extracts from 62 ticks from a region of southern Connecticut where *B. burgdorferi* is highly endemic were subjected to PCR amplification. Adult ticks collected and individually stored in 70% ethanol were placed on filter paper to air dry. The ticks were then placed in individual Microfuge tubes containing three BSA-coated glass beads and 20 μl of nuclease-free distilled and deionized water. The ticks were crushed with a wooden applicator, heated at 95°C for 5 min, and immediately chilled on ice. Five-microliter aliquots of each tick extract were subjected to PCR amplification in the AmpliWax-sealed reaction tubes inoculated with 50 μg of IP-10 per ml either before or after PCR amplification. The PCR tubes were then exposed to UV light to activate the IP-10 and sterilize the amplicons. PCR products were then detected as described above.

RESULTS

In order to determine the detection limit of the agarose gel electrophoresis and Southern blot hybridization, serially diluted stock amplicons were electrophoresed in an agarose gel, blotted onto a nylon membrane, and probed with an alkaline phosphatase-conjugated chemiluminescent probe. No DNA band was detected in an ethidium bromide-stained agarose gel for any of the amplicon dilutions. However, Southern blotting was positive for a 156-bp band at amplicon amounts of 10^{10} or larger. In addition, all of the amplicon dilutions subjected to PCR amplifications were positive for a 156-bp band detected by the ethidium bromide-stained agarose gel (data not shown).

Photochemical inactivation. To determine the effect of IP-10 on PCR, amplicons (10^{11} to 10^3) were subjected to an amplification reaction in the presence of 0, 25, 50, and 100 μg of IP-10 per ml. For the amplicons treated with 25 μg of IP-10 per ml, all but the last reaction tube, containing 10^3 amplicons, yielded a 156-bp PCR product that was detected by agarose gel electrophoresis, indicating that 25 μg of IP-10 per ml had a slight inhibitory effect on PCR (Fig. 1, First PCR). The subsequent activation of IP-10 in these tubes by UV light sterilized the majority of the amplicons. In fact, when aliquots from these tubes were subjected to a second PCR, no DNA band was detected by agarose gel electrophoresis (Fig. 1, Second PCR). However, when the samples were analyzed by Southern blot hybridization, a 156-bp DNA band was detected in tubes containing 10^4 or more amplicons.

The presence of 50 or 100 μg of IP-10 per ml in the reaction tubes had a more pronounced inhibitory effect on PCR. Following the amplification, no PCR product was detected by agarose gel electrophoresis in any of the tubes inoculated with 10^{11} to 10^3 amplicons. Southern blot analysis detected amplicons only in tubes inoculated with 10^7 or more amplicons (Fig. 2). All of the generated amplicons were effectively sterilized with these concentrations of IP-10 (data not shown).

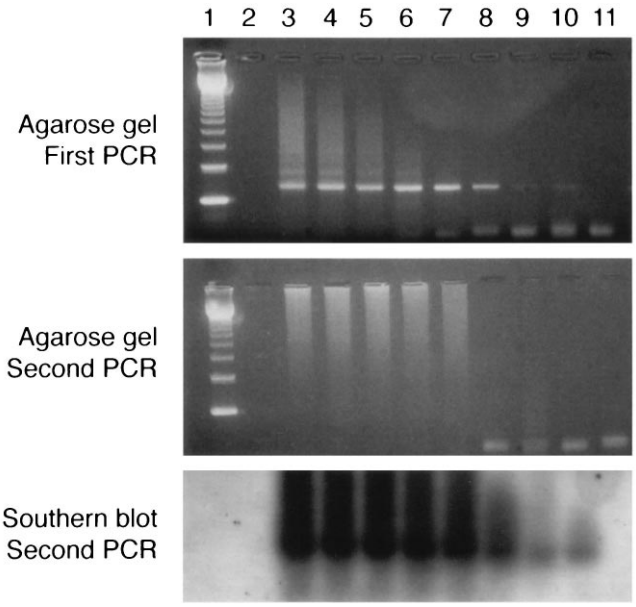

FIG. 1. Agarose gel electrophoresis of PCR products presterilized with 25 μg of IP-10 per ml followed by agarose gel electrophoresis and Southern blot analysis of subsequent PCR of the sterilized amplicons. Lanes: 1, molecular size standard (123-bp ladder); 2, blank well; 3 to 11, serial 10-fold dilution of amplicons (10^{11} to 10^3, respectively).

Modified photochemical inactivation. Five series of PCR tubes inoculated with 10^9 to 10^3 amplicons and an AmpliWax bead were subjected to PCR as described above. Following the PCR, the tubes were cooled to room temperature and each tube series was inoculated with 0, 25, 50, 100, or 200 μg of IP-10 per ml. The tubes were then heated and cooled to mix IP-10 with the amplicons and exposed to UV light. A 1-μl aliquot from each tube was inoculated into a PCR tube containing fresh PCR mix and reamplified as described above. The results from these experiments indicated that IP-10 at concentrations of 25 μg/ml (Fig. 3B) and 50 μg/ml (Fig. 3C) did not completely sterilize the PCR products, and a subsequent PCR was positive for all target dilutions. However, IP-10 at a concentration of 100 μg/ml sterilized all the amplicons generated by PCR with an initial target of 10^5 or less and significantly reduced the concentration of amplicons generated with a higher target inoculum (Fig. 3D). IP-10 at a concentration of 200 μg/ml effectively sterilized all of the amplicons generated from 10^9 to 10^3 amplicons, and there was no PCR product detected by subsequent PCR of the sterilized amplicons (Fig. 3E).

To further evaluate the effect of IP-10 on PCR amplification of the *B. burgdorferi* OSP A gene, PCR was performed with 62 ticks collected from a region of southern Connecticut where *B. burgdorferi* is highly endemic. PCR tubes were presealed with AmpliWax and inoculated (pre- or postamplification) with 50 μg of IP-10 per ml. This concentration of IP-10 was determined to reduce the amplification efficiency without complete inhibition of the PCR (Fig. 2). Table 1 shows the amplification results for each PCR group. PCR performed in the presence of

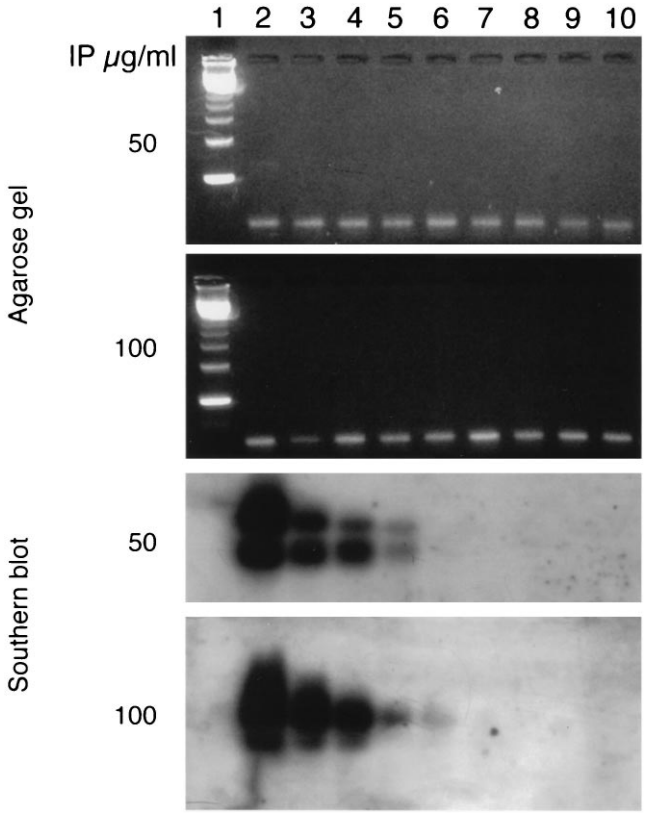

FIG. 2. Agarose gel electrophoresis and Southern blot analysis of PCR products performed in the presence of 50 or 100 μg of IP-10 per ml. Lanes: 1, molecular size standard (123-bp ladder); 2 to 10, serial 10-fold dilution of amplicons (10^{11} to 10^3, respectively).

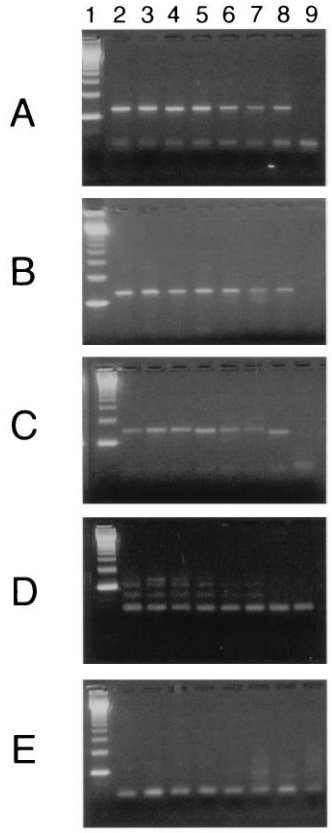

FIG. 3. Agarose gel electrophoresis of amplicons sterilized after PCR by using AmpliWax with 0 (A), 25 (B), 50 (C), 100 (D), or 200 (E) μg of IP-10 per ml. Lanes: 1, molecular size standard (123-bp ladder); 2 to 8, serial 10-fold dilution of amplicons (10^9 to 10^3, respectively); 9, negative control.

IP-10 detected borrelia-specific DNA in 17 of 62 ticks following gel electrophoresis and in 34 of 62 ticks following Southern blot hybridization. In contrast, PCR performed in the absence of IP-10 (with IP-10 added following the amplification in AmpliWax-sealed tubes) detected borrelia-specific DNA in 31 of 62 ticks (all determined to be positive by either gel electrophoresis or blotting in PCRs with IP-10) following gel electrophoresis and in 46 of 62 ticks (a 26% increase in the total number of positive ticks) following Southern blot hybridization of the PCR products. Sixteen ticks were negative for borreliae by both methods. Similarly, 16 ticks were positive for borreliae by gel electrophoresis and Southern blot hybridization by both methods.

DISCUSSION

The introduction of molecular techniques, especially PCR, into clinical microbiology laboratories has provided a powerful set of new tools that has facilitated the detection and diagnosis of infectious diseases (21). In fact, the reported sensitivities of PCR for the detection of some organisms are higher than those of previously known "gold standards" (21). It is precisely this extreme sensitivity that has made the routine use of PCR in clinical laboratories challenging. Contamination of reagents or specimens with previously amplified sequences constitutes its major drawback. Besides physical measures and good laboratory practice, different chemical and enzymatic systems have been reported to be useful measures to prevent carryover contaminations (13). The decision to implement one or more of these procedures depends on each laboratory, with respect to time and technical and economic resources, and on the unique characteristics (percent GC and length) of each amplicon (7, 13).

Post-PCR sterilization by photochemical inactivation of amplicons by IP-10 is a commonly used sterilization technique. However, the agent may have an inhibitory effect on PCR (9, 10, 13). The results from our experiments show the inhibitory effects that increasing concentrations of IP-10 have on the amplification of *B. burgdorferi* DNA. As little as 50 μg of IP-10 per ml completely inhibited the amplification of up to 10^5 target amplicons (Fig. 2) and significantly reduced the ampli-

TABLE 1. Effect of pre- or post-PCR addition of 50 μg of IP-10 per ml on detection of *B. burgdorferi* OSP A DNA in infected *I. scapularis*

No. of ticks	Detection of DNA			
	IP-10 present		IP-10 absent	
	Gel	Blot	Gel	Blot
16	−	−	−	−
7	−	−	−	+
5	−	−	+	+
7	−	+	−	+
10	−	+	+	+
1	+	+	−	+
16	+	+	+	+

fication efficiency of larger amplicon amounts. In addition, the inhibitory effects of IP-10 on PCR may contribute to or act together with other inhibitors that are already present in a clinical sample (heparin, hemoglobin, etc.) (17, 24, 26, 27). Similarly, since the amount of target DNA in a clinical sample is unknown (often probably below the 10^5 DNA copies), the additive effect of even a minimal inhibition by IP-10 may cause false-negative results.

Our results are in contradiction with reports from other investigators that have shown a lesser inhibition of PCR with IP-10. Isaacs et al. (9) found no inhibition of PCR with 400 μg of IP-10 per ml. Rys and Persing (18) observed some inhibitory effect with IP-10 at concentrations of 200 μg/ml and regularly used 100 μg/ml in their experiments. There are several possible explanations for these differences. Isaacs et al. (9) used three different IP-10 compounds (4′-AMDMIP, AMIP, and 6-AMDMIP) and a different PCR target sequence (115 bp of the human immunodeficiency virus genome). They concluded that the sterilization and inhibitory properties of different psoralen compounds vary according to their chemical characteristics. They also argued that the length and base composition of the amplicons could influence these properties, and it is necessary to optimize the conditions for each amplification prior to their routine use. In our study we used a different psoralen compound, IP-10, and targeted a 156-bp sequence from the *B. burgdorferi* OSP A gene. These two factors may explain some of the discrepancy between the two studies. However, Rys et al. (18) used the same *B. burgdorferi* DNA amplicon in their studies and compared three different anticontamination systems. They mixed IP-10 with a known concentration of amplicon, and following the activation of IP-10, they made serial 10-fold dilutions of the amplicons, thus diluting the effective concentration of IP-10 present in subsequent PCR vials. In addition, Rys et al. (18) used a plasmid as a target for their first PCR cycles. The inhibitory effect of IP-10 is related to the stabilization of the double-stranded amplicon and/or the kinetic inhibition of the polymerization (9). The presence of other DNA besides the target sequence may reduce the inhibitory effect by acting as nonspecific binding sites for IP-10, thus decreasing the effective concentration of IP-10 in the reaction tubes. However, the amounts of nonspecific DNA, especially for extraction from sterile sources, are negligible, and the use of IP-10, especially when there are very few target sequences, may cause false-negative results.

In order to overcome the inhibitory effect of IP-10, we have designed a system based on the replacement of mineral oil with AmpliWax beads. Following the PCR, the AmpliWax solidifies and acts as a physical barrier which prevents the release of aerosolized amplicons into the environment. This system allows post-PCR addition of IP-10 into the reaction tubes with no inhibitory effect on the overall yield of the PCR. Using the AmpliWax, we have tested increasing concentrations of IP-10 with serially diluted amplicons to obtain sterilization limits. In addition, results from a study on the use of PCR for detecting *B. burgdorferi* in 62 ticks from an area in southern Connecticut where *B. burgdorferi* is highly endemic showed that when PCR was performed in the presence of IP-10, 34 of 62 ticks were positive for borreliae (17 by gel electrophoresis and 17 by Southern hybridization). In contrast, PCR performed in the absence of IP-10 detected borrelia-specific DNA in 46 ticks, of which only 15 required Southern hybridization. These data clearly show that post-PCR addition of IP-10 is a more reliable way to sterilize PCR products. Moreover, an added advantage of AmpliWax is that much higher concentrations of sterilizing agents may be used regardless of their effect on the amplification reaction.

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REFERENCES

1. **Aslanzadeh, J.** 1993. Application of hydroxylamine hydrocholoride for post-PCR sterilization. Mol. Cell. Probes **7:**145–150.
2. **Carmody, M. W., and C. P. H. Vary.** 1993. Inhibition of DNA hybridization following partial dUTP substitution. BioTechniques **15:**692–695.
3. **Cimino, G. D., K. Metchette, S. T. Issacs, and Y. S. Zhu.** 1990. More false positive problems. Nature (London) **345:**91–92.
4. **Cimino, G. D., K. C. Metchette, J. W. Tessman, J. E. Hearst, and S. T. Isaacs.** 1991. Post-PCR sterilization: a method to control carryover contamination for the polymerase chain reaction. Nucleic Acids Res. **19:**99–107.
5. **Davis, L. G., W. M. Kuehl, and J. F. Battey.** 1994. Basic methods in molecular biology, p. 22–24. Appleton and Lange, Norwalk, Conn.
6. **Dwyer, D. E., and N. Saksena.** 1992. Failure of ultra violet irradiation and autoclaving to eliminate PCR contamination. Mol. Cell. Probes **6:**87–88.
7. **Espy, M. J., T. F. Smith, and D. H. Persing.** 1993. Dependence of polymerase chain reaction product inactivation protocols on amplicon length and sequence composition. J. Clin. Microbiol. **31:**2361–2365.
8. **Fox, J. C., A. K. Mouir, A. Webster, and V. C. Emery.** 1991. Eliminating PCR contamination: is U.V irradiation the answer? J. Virol. Methods **33:**375–382.
9. **Isaacs, S. T., J. W. Tessman, K. C. Metchette, J. E. Hersat, and G. D. Cimino.** 1991. Post-PCR sterilization: development and application to an HIV-1 diagnostic assay. Nucleic Acids Res. **10:**109–116.
10. **Kox, L. F. F., D. Rhienthong, A. Mendo-Miranda, N. Udomsantisuk, K. Ellis, J. van Leeuwen, S. van Heusden, S. Kuger, and H. S. Kolk.** 1994. A more reliable PCR detection of *Mycobacterium tuberculosis* in clinical samples. J. Clin. Microbiol. **32:**672–678.
11. **Kwok, S., and R. Higuchi.** 1989. Avoiding false positives with PCR. Nature (London) **339:**237–238.
12. **Longo, M. C., M. S. Berninger, and J. L. Hartley.** 1990. Use of uracil DNA glycosylase of control carry-over contamination in polymerase chain reaction. Gene **93:**125–128.
13. **Persing, D. H., and G. Cimino.** 1993. Amplification product inactivation methods, p. 105–121. *In* D. H. Persing, T. F. Smith, T. White, and F. C. Tenover (ed.), Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, D.C.
14. **Persing, D. H., S. R. Telford III, P. N. Rys, D. E. Dodge, T. J. White, S. E. Malawista, and ●. Spielman.** 1990. Detection of *Borrelia burgdorferi* DNA in museum specimens of *Ixodes dammini* ticks. Science **249:**1420–1423.
15. **Podzorski, R. P., and D. H. Persing.** 1995. Molecular detection and identification of microorganisms, p. 130–157. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
16. **Prince, A. M., and L. Andrus.** 1992. PCR: how to kill unwanted DNA. BioTechniques **12:**358–360.
17. **Rosssen, L., P. Norskow, K. Holmskom, and O. F. Rasmussen.** 1992. Inhibition of PCR by components of food samples, microbial assays and DNA-extraction solutions. Int. J. Food Microbiol. **17:**37–45.
18. **Rys, P. N., and D. H. Persing.** 1993. Preventing false positives: quantitative evaluation of three protocols for inactivation of polymerase chain reaction amplification products. J. Clin. Microbiol. **31:**2356–2360.
19. **Saksena, N. K., D. Dwyer, and F. Barre-Sinausi.** 1991. A "sentinel" technique for monitoring viral aerosols contamination. J. Infect. Dis. **164:**1021–1022.
20. **Sarkar, G., and S. S. Sommer.** 1990. Shedding light on PCR contamination. Nature (London) **343:**27.
21. **Tompkins, L. S.** 1992. The use of molecular methods in infectious disease. N. Engl. J. Med. **327:**1290–1297.
22. **Van den Brule, A. J. C., E. C. J. Class, M. du Maine, W. J. G. Melchers, T. Helmerhorst, W. G. V. Quint, J. Lindeman, C. J. L. M. Meijer, and J. M. M. Walboomers.** 1990. Use of anticontamination primers in the polymerase chain reaction for the detection of human papilloma virus genotypes in cervical scrapes and biopsies. J. Med. Virol. **29:**20–27.
23. **Victor, T., A. Jordan, R. du Toit, and P. D. Van Helden.** 1993. Laboratory experience and guidelines for avoiding false positive chain reaction results. Eur. J. Clin. Chem. Clin. Biochem. **31:**531–535.
24. **Wadowsky, R. M., S. Laus, S. J. States, and G. A. Enrlich.** 1994. Inhibition of PCR-based assay for *Bordetella pertusis* by using calcium alginate fiber and aluminum shaft components of a nasopharyngenal swab. J. Clin. Microbiol. **32:**1054–1057.
25. **Walder, R. Y., J. R. Hayes, and J. A. Walder.** 1993. Use of PCR primers containing a 3′-terminal ribose residue to prevent cross-contamination of amplified sequences. Nucleic Acids Res. **21:**4339–4343.

26. **Wang, J. T., T. H. Wang, J. C. Sheu, S. M. Lin, J.-T. Lin, and D.-S. Chen.** 1992. Effects of anticoagulants and storage of blood samples on efficacy of the polymerase chain reaction assay for hepatitis C virus. J. Clin. Microbiol. **30:**750–753.
27. **Yoshii, T., K. Tamura, T. Tariguchi, K. Akiyama, and I. Ishiyama.** 1993. Water-soluble eumelamin as PCR-inhibitor and a simple method for its removal. Jpn. J. Legal Med. **47:**323–329.
28. **Zhen, L., and R. T. Swank.** 1993. A simple and high yield method for recovering DNA from agarose gels. BioTechniques **19:**894–898.
29. **Zhu, Y. S., S. T. Isaacs, G. D. Cimino, and J. E. Hearst.** 1991. The use of exonuclease III for polymerase chain reaction sterilization. Nucleic Acids Res. **19:**2511.