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Slow Inactivation of Dry PCR Templates by UV Light

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False-positive polymerase chain reactions (PCR) due to amplification of contaminating DNA are a constant problem despite rigorous safeguards.[1] Ultraviolet (UV) irradiation has been shown to decontaminate solutions effectively.[2,3] Recent work has shown that dry contaminating DNA may be brought into the setup area on hair, skin,[4] or gloves that have touched a surface inadvertently contaminated with dry DNA.[5] Sarkar and Sommer[3] have noted that dry DNA is more resistant to UV inactivation. We did not find literature investigating the efficacy of UV light in inactivating the short sequences of dry DNA that might contaminate a PCR setup area.

To examine the effectiveness of the UV light technique, purified DNA from cells infected with human herpes virus 6 (HHV-6) was dried onto plastic tissue culture petri dishes (Costar; 1 μg DNA/cm^2) and irradiated with two germicidal UV lamps for the times indicated in Figure 1 (ballast UF-36-2, lamps G36T6L, American Ultraviolet Company, Santa Anna, CA). The lamps were mounted on the ceiling of a fume hood at a distance of 1 meter from the working surface. Under these conditions, the incident near-UV radiation as measured at the working surface was 400 μW/cm^2 (Black-Ray UV Meter J-225, San Gabriel, CA). (The University of Washington standard for biosafety hoods requires at least 160 μW/cm^2 at the surface.) The DNA in each specimen was redissolved and serially diluted, and each dilution was amplified with three different HHV-6–specific primer pairs. The sequences selected by these primer sets were the targets whose UV sensitivity was explored. The number of amplifiable templates remaining at each time point was estimated as described in the figure legend.

Approximately 5 x 10^8 copies of each template were present in the unirradiated aliquots used for PCR. This amount of DNA could theoretically contaminate 10^8 reactions (at our detection limit of five copies) and could be found in 1 nl of a fully saturated 100 μl PCR mixture containing 5 x 10^{13} primer pairs. After 30 min of UV irradiation, which is reported to inactivate many DNA species in solution by approximately five orders of magnitude,[2,6] the ability of our dry DNA to serve as a PCR template was not decreased reproducibly (not shown). After 1 hr, amplifiable DNA was reduced to 5 x 10^7 copies (one order of magnitude). After 8 hr of irradia-

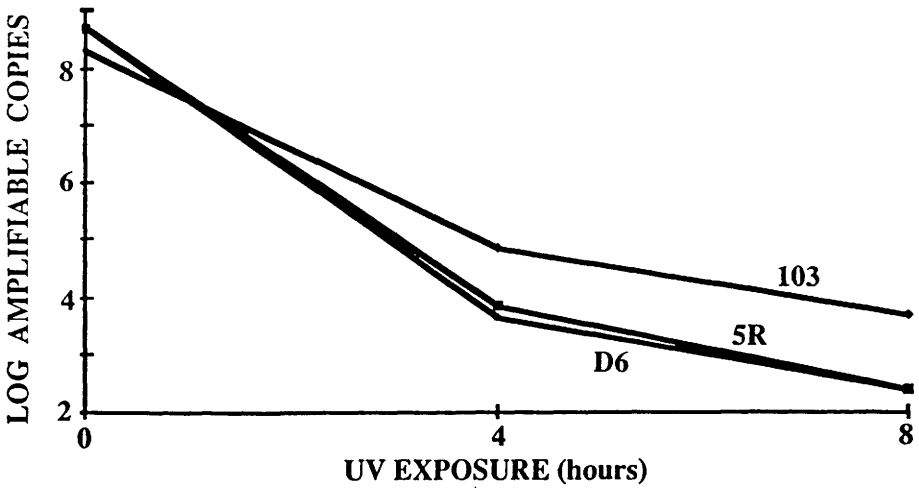

FIGURE 1 UV inactivation of HHV-6 DNA as measured with three primer sets. After irradiation as described in the text, dried HHV-6–containing DNA was exposed to UV light for the times indicated in the figure, dissolved, serially diluted, and subjected to PCR (30 cycles) with each of three primer sets specific for different regions of the HHV-6 genome: 5R (223 bp, 42 dimerizable thymines, D6 (172 bp, 33 dimerizable thymines; sequence kindly provided by R. Honess), and 103 (105 bp, 4 dimerizable thymines; plasmid kindly provided by P. Pellet). The product was detected by hybridization in solution with specific ^{32}P-labeled probes, followed by acrylamide gel electrophoresis and autoradiography. By comparison with the results from simultaneously amplified solutions containing known numbers of HHV-6 genomes, the number of amplifiable target sequences remaining at each time point was estimated.

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tion, 250 amplifiable copies of the longer, thymine-rich D6 and 5R templates remained, as compared to 5000 copies of the shorter, relatively thymine-poor 103 template. DNA in solution has been shown to vary more than 10^4-fold in UV sensitivity depending on sequence length, and on thymine and cytosine content.[2,3,6]

UV irradiation inactivated our dry PCR templates in a time- and sequence-dependent fashion, albeit slowly. Based on these results, contaminating DNA could be significantly reduced after 8 hr or more of irradiation with a germicidal UV bulb. Since UV lights can often be left on for 10 hr or more per day, prolonged UV irradiation may be a practical means of partially inactivating dried PCR templates. However, the 10-fold reduction observed after 1 hr of irradiation may not be adequate for preventing PCR contamination.

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REFERENCES

1. Kwok, S. and R. Higuchi. 1989. Avoiding false positives with PCR. *Nature* **339:** 237–238.
2. Sarkar, G. and S.S. Sommer. 1990. Shedding light on PCR contamination. *Nature* **343:** 27.
3. Sarkar, G. and S. Sommer. 1990. More light on PCR contamination (letter). *Nature* **347:** 340–341.
4. Kitchin, P.A., Z. Szotyori, C. Fromholc, and N. Almond. 1990. Avoidance of false positives (letter). *Nature* **344:** 201.
5. Cone, R., A. Hobson, M.-L. Huang, and M. Fairfax. 1990. Polymerase chain reaction decontamination: The wipe test. *Lancet* **316:** 686–687.
6. Cimino, G., K. Metchette, S. Isaacs, and Y. Zhu. 1990. More false-positive problems (letter). *Nature* **345:** 773–774.
7. Gopal, M., B. Thomson, J. Fox, R. Tedder, and R. Honess. 1990. Detection by PCR of HHV-6 and EBV DNA in blood and oropharynx of healthy adults and HIV-seropositives (letter). *Lancet* **335:** 1598–1599.

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