TECHNICAL NOTE

Manfred N. Hochmeister,[1] *M.D.; Bruce Budowle,*[2] *Ph.D.; Urs V. Borer;*[1] *and Richard Dirnhofer,*[1] *M.D.*

Effects of Nonoxinol-9 on the Ability to Obtain DNA Profiles from Postcoital Vaginal Swabs

REFERENCE: Hochmeister, M. N., Budowle, B., Borer, U. V., and Dirnhofer, R., **"Effects of Nonoxinol-9 on the Ability to Obtain DNA Profiles from Postcoital Vaginal Swabs,"** *Journal of Forensic Sciences*, JFSCA, Vol. 38, No. 2, March 1993, pp. 442–447.

ABSTRACT: Nonoxinol-9, the active ingredient of many spermicide foams and creams, has been shown to inactivate effectively high titres of HIV in vitro. Therefore the early administration of nonoxinol-9, perhaps by a rape victim herself, has been suggested as a potential prophylactic therapy for prevention of a possible HIV infection. For forensic DNA identity testing, it becomes pertinent to determine whether nonoxinol-9 could have an adverse effect on the recovery of high molecular weight DNA from postcoital vaginal swabs and thereby have an impact on restriction fragment length polymorphism (RFLP) analysis. If high molecular weight DNA can not be recovered, it may still be possible to proceed with analyses using PCR-based tests. In order to investigate the potential effects of nonoxinol-9, inserts, gels, or sponges containing nonoxinol-9 were applied either 15 min pre- or 15 to 60 min post coitus. Postcoital vaginal swabs were taken one and six h after sexual intercourse, the DNA was isolated and DNA identity typing was performed.

The results demonstrate that nonoxinol-9 has no negative effect on the ability to obtain DNA profiles, either RFLP or PCR-based, from postcoital vaginal swabs. The quantity of extractable high molecular weight DNA obtained (as determined by slot-blot analysis) was comparable with that from uncontaminated postcoital vaginal swabs. RFLP patterns and PCR-based typing results at the HLA-DQ alpha and D1S80 loci from the nonoxinol-9 treated swabs were consistent with the uncontaminated control swabs and the corresponding whole blood samples of the donors. Therefore an early prophylactic administration of the topical anti-HIV agent nonoxinol-9 is not an impedient for obtaining DNA profiles from evidentiary material.

KEYWORDS: toxicology, pathology and biology, AIDS, HIV, nonoxinol-9, deoxyribonucleic acid (DNA), vaginal swabs, identification of semen, RFLP, PCR

Approximately 2.5 million women in the United States and many more throughout the world use spermicides in the form of vaginal creams, jellies, foams, and sponges to

The names of commercial manufacturers are provided for identification only and inclusion does not imply endorsement by the Federal Bureau of Investigation.

Received for publication 30 March 1992, revised manuscript received 29 June 1992; accepted for publication 3 July 1992.

[1]Forensic scientist, research assistant, and director respectively, Department of Forensic Medicine, University of Bern, Switzerland.

[2]Research chemist, Forensic Science Research and Training Center, Laboratory Division, FBI Academy, Quantico, VA.

prevent pregnancy [*1*]. The active ingredient of most spermicides is nonoxinol-9, a highly polar molecule that immobilizes sperm by disrupting the outer lipoprotein surface of the cell membrane. Several in vitro studies have demonstrated that nonoxinol-9 inhibits a variety of sexually transmissible infectious agents, including those responsible for gonorrhea, syphilis, chlamydial infection, genital herpes, candidiasis, and trichomoniasis [*2*]. Moreover, several studies [*3–5*] demonstrated that nonoxinol-9 inactivates HIV in vitro at a concentration of less than 0.05% within 30 s of exposure. Hicks et al. [*3*] concluded that in vitro results could be extrapolated to the clinical setting, and that preparations containing nonoxinol-9 could rapidly inactivate HIV in vivo.

There are more than 90 000 rape cases brought to the attention of official investigators per year in the United States. In most of these cases, the transmission of HIV is of particular concern to the rape victim, due to its life-threatening consequences. Foster and Bartlett [*6*] suggest that a treatment for victims of sexual assaults should include early administration of nonoxinol-9 as a potential prophylactic therapy. While the utmost concern in a rape case is the health and well-being of the victim, there also is a desire to identify the assailant. Generally, identity testing is accomplished by typing polymorphic genetic markers in recovered semen and a blood sample from a suspect. The results of comparisons of genetic marker profiles can either inculpate or exculpate a suspect as a potential source of the evidentiary material. The effects of an antiHIV or antispermicidal agent on the ability to type genetic markers should be known, so the proper choice can be made to select the most effective method for the analysis of evidentiary materials, especially when the quantity of the material is limited. Foster and Bartlett state that nonoxinol-9 has no effect on typing acid phosphatase, the antigen p30, peptidase A phosphoglucomutase, or ABO blood groups. An in vitro study carried out by Sheridan et al. [*7*] demonstrated that DNA can be isolated from sperm cells after five days exposure to nonoxinol-9. The purpose of the present study was to determine whether nonoxinol-9 would have an adverse effect on the recovery of the high molecular weight DNA from semen recovered from postcoital vaginal swabs and thereby have an impact on restriction fragment length polymorphism (RFLP) analysis, or on PCR-based typing results.

Material and Methods

The following spermicides were used in this study: Conceptrol™ Contraceptive Inserts, each containing 150 mg nonoxinol-9, or 8.3% by weight (Advanced Care Products Ortho Pharmaceutical Corp. Raritan, NJ); Conceptrol™ Single Use Contraceptives Unscented Gel Prefilled Applicators (each containing 75 mg nonoxinol-9, or 9.4% by weight); and Todays Sponge™ Vaginal Contraceptive Sponges, 1000 mg nonoxinol-9 (Whitehall Laboratories Inc., New York, N.Y.).

Whole blood samples, drawn by venipuncture, were collected from five male and five female donors and stored in EDTA tubes. Control (nonoxinol-9 free) postcoital vaginal swabs were collected on cotton-tip swabs one hour and six h after sexual intercourse from female donors. Conceptrol™ Inserts, Conceptrol™ Gels or Todays Sponge™ were applied vaginally either 15 min before, or 15 or 60 min after sexual intercourse; vaginal swabs were collected one hour or six h after intercourse (because nonoxinol-9 containing spermicides are foam forming agents, it was crucial to properly swab the vaginal vault and to be aware not to swab mostly foam). The samples were dried with a swab dryer (Kinderprint Company, Martinez, CA) and stored at −20°C until DNA analysis was carried out. A total of 192 samples were processed.

The quantity of human DNA in all samples was determined using the slot-blot method described by Waye et al. [*8*] and by ethidium bromide agarose gel electrophoresis. RFLP analysis was performed at the loci D2S44, D10S28, D17S26, and D4S139 according to

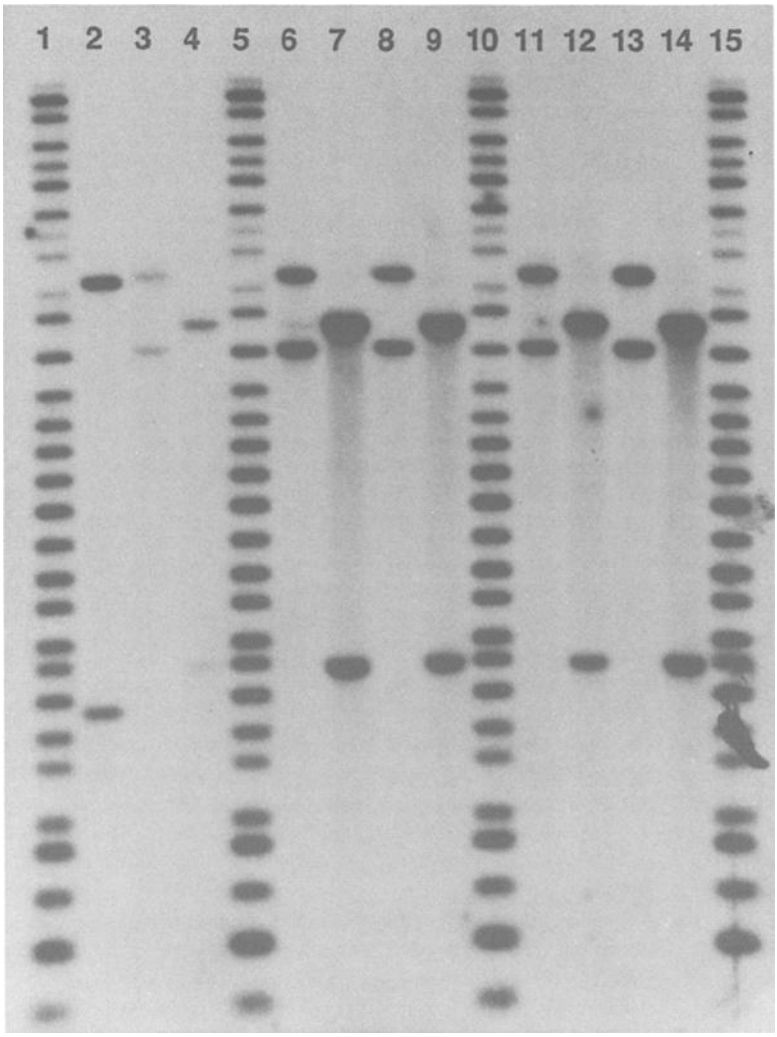

FIG. 1—*Example of RFLP profiles of the D17S26 locus demonstrating the effects of nonoxinol-9 containing spermicides. In all these samples the spermicides were administered 15 min after sexual intercourse and the swabs were taken six h after intercourse. Lane 1, 5, 10, 15 = 0.5 – 22 kb size standard (BRL, Bethesda Research Laboratories, Gaithersburg, MD). Lane 2 = Allelic control DNA K562/Hae III (BRL). Lane 3 = Female control blood sample (blood stain); Lane 4 = Male control blood sample (blood stain); Lane 6 = control (nonoxinol-9 free) postcoital vaginal swab, female fraction; Lane 7 = control (nonoxinol-9 free) postcoital vaginal swab, male fraction. Lane 8 = female fraction of vaginal swab after exposure to Conceptrol™ Contraceptive Inserts; Lane 9 = male fraction of vaginal swab after exposure to Conceptrol™ Contraceptive Inserts; Lane 11 = female fraction of vaginal swab after exposure to Conceptrol™ Single Use Contraceptives Unscented Gel Prefilled Applicators; Lane 12 = male fraction of vaginal swab after exposure to Conceptrol™ Single Use Contraceptives Unscented Gel Prefilled Applicators; Lane 13 = female fraction of vaginal swab after exposure to Todays Sponge™ Vaginal Contraceptive Sponges; Lane 14 = male fraction of vaginal swab after exposure to Todays Sponge™ Vaginal Contraceptive Sponges. All specimen lanes contained about 500 ng of human genomic DNA. The autoradiography exposure time was 48 h.*

TABLE 1—*Effect of nonoxinol-9 on RFLP results.*

Measurements	No. of Measurements	Range of Measurements[a]
Male blood compared with male fraction of control (nonoxinol-9 free) postcoital swabs	20	±0.41%
Female blood compared with female fraction of control (nonoxinol-9 free) postcoital swabs	20	±0.36%
Male fraction of nonoxinol-9 free postcoital swabs compared with male fraction of nonoxinol-9 containing postcoital swabs	80	±0.51%
Female fraction of nonoxinol-9 free postcoital swabs compared with female fraction of nonoxinol-9 containing postcoital swabs	80	±0.58%

[a]This values represent all measurements for RFLP loci D2S44, D10S28, D17S26, and D4S139.

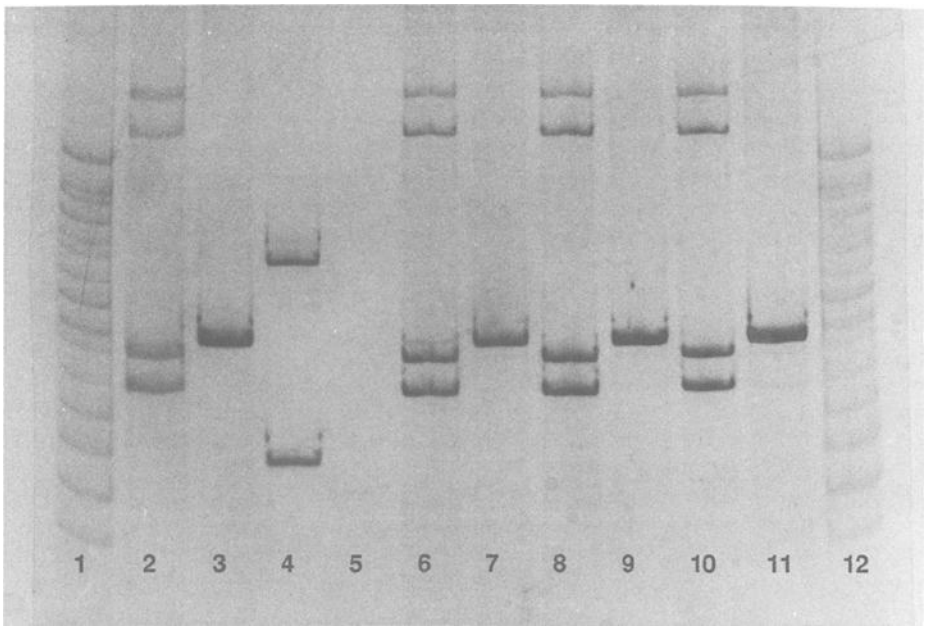

FIG. 2—*Example of silver-stained amplified fragment length polymorphism (AMP-FLP) profiles of the D1S80 locus demonstrating the effects of nonoxinol-9 containing spermicides. In all these samples, the spermicides were administered 15 min after sexual intercourse and the swabs were taken 6 h after intercourse. Lane 1 and 12 = a D1S80 allelic ladder (Cetus Corporation, Emeryville, CA); Lane 2 = female control blood sample (blood stain); Lane 3 = male control blood sample (blood stain); Lane 4 = a D1S80 control DNA Tpe 18,31 (Cetus Corp.); Lane 5 = negative control; Lane 6 = female fraction of vaginal swab after exposure to Conceptrol™ Contraceptive Inserts; Lane 7 = male fraction of vaginal swab after exposure to Conceptrol™ Contraceptive Inserts; Lane 8 = female fraction of vaginal swab after exposure to Conceptrol™ Single Use Contraceptives Unscented Gel Prefilled Applicators; Lane 9 = male fraction of vaginal swab after exposure to Conceptrol™ Single Use Contraceptives Unscented Gel Prefilled Applicators; Lane 10 = female fraction of vaginal swab after exposure to Todays Sponge™ Vaginal Contraceptive Sponges; Lane 11 = male fraction of vaginal swab after exposure to Todays Sponge™ Vaginal Contraceptive Sponges. Additional extra bands can be seen at times (for instance, Lanes 2,6,8,10). These bands are probably due to mispriming events or heteroduplex formation. The extra bands are not due to the presence of nonoxinol-9, because they can be observed in control samples, also. Since they are outside the area where allele bands reside on the gel, they do not affect profile interpretation.*

the method of Budowle and Baechtel [9]. Additionally 20 ng of human DNA were amplified and typed for the D1S80 and HLA-DQ alpha loci as described previously [10,11].

Results and Discussion

High molecular weight DNA could be recovered from all postcoital vaginal swabs exposed to nonoxinol-9 as well as from the corresponding nonoxinol-9 free control swabs and whole blood samples. The quantity of human DNA recovered from the postcoital vaginal swabs exposed to nonoxinol-9 (average 1.1 μg male and 1.5 μg female DNA per swab) as determined by slot-blot analysis did not show any differences compared with the nonoxinol-9 free control swabs (average 1.0 μg male and 1.5 μg female DNA per swab), neither were there any noticeable differences in the quality of recovered DNA between the postcoital vaginal swabs exposed to nonoxinol-9 and the control vaginal swabs, based on ethidium bromide agarose gel electrophoresis.

The RFLP profiles from control and nonoxinol-9 exposed samples were similar in all cases for all loci analyzed (an example is shown in Fig. 1). Based on the observed measurement error, nonoxinol-9 had no effect on RFLP results (Table 1).

All samples were also subjected to PCR and typed for HLA-DQ alpha and D1S80. Each sample was amplified and typed in duplicate to demonstrate reproducibility and minimize potential contamination effects on interpretation of DNA patterns. The types of the HLA-DQ alpha locus as well as the D1S80 locus derived from the nonoxinol-9 treated samples were consistent with those of the corresponding control vaginal swab and whole blood samples from the donors (Fig. 2). No false positive or negative results were observed.

A potential prophylactic treatment against transmission of HIV and other sexually transmitted diseases in sexual-assault cases is the administration of nonoxinol-9 containing spermicides as soon as possible after sexual intercourse [6]. If this suggested approach becomes an accepted practice, it is pertinent for the forensic-science community to be aware of the effects that nonoxinol-9 has on DNA typing results obtained from postcoital vaginal swabs. The data here demonstrate that sperm and nonsperm cells exposed to nonoxinol-9 in vivo are still amenable to RFLP analysis for identity typing purposes.

Prior to this study it was unknown whether recovered DNA exposed to nonoxinol-9 in vivo might have been substantially degraded or significantly diluted. Because PCR-based genetic markers are beginning to be applied to forensic situations, HLA-DQ alpha and D1S80 typing were an essential part of this study. The DNA was not affected adversely and RFLP analysis was demonstrated to be a viable approach. PCR-based testing demonstrated at the HLA-DQ alpha and D1S80 loci also appears to be feasible.

In conclusion, these results compliment previous reports from these laboratories [10–15]. When DNA was exposed to a chemical insult (in this case nonoxinol-9) no false positive or false negative resulted. Therefore, this study supports the reliability of RFLP and PCR-based typing procedures for forensic applications.

References

[1] Pratt, W. F., Mosher, W. D., Bachrach, C. A., et al., "Understanding U.S. Fertility: Findings from the National Survey of Family Growth Cycle III," Washington, D.C., Population References Bureau, 1984.

[2] Rosenberg, M. J., Rojanapithayakorn, W., Feldblum, P. J., and Higgins, J. E., "Effect of the Contraceptive Sponge on Chlamydial Infection, Gonorrhea, and Candidiasis," *Journal of the American Medical Association*, Vol. 257, No. 17, 1987, pp. 2308–2312.

[3] Hicks, D. R., Martin, L. S., Francis, D. P., et al., "Inactivation of HTLV-III/LAV Infected Cultures of Normal Human Lymphocytes by Nonoxinol-9 In Vitro," *The Lancet I*, 1985, pp. 1422–1423.

[4] Malkovsky, M., Newell, A., and Dalgleish, A. G., "Inactivation of HIV by Nonoxinol-9," *The Lancet I*, 1988, p. 645.

[5] Polsky, B., Baron, P. A., Gold, J. W. M., et al., "In Vitro Inactivation of HIV-1 by Contraceptive Sponge Containing Nonoxinol-9, *The Lancet*, 1988, p. 1456.

[6] Foster, I. M. and Bartlett, J., "Anti-HIV Substances for Rape Victims," *Journal of the American Medical Association*, Vol. 261, No. 23, 1989, p. 3407.

[7] Sheridan, K. T., Foster, I. M., and Cotton, R. W., "Effect of Nonoxinol-9 on Recovery of DNA from Sperm," Abstract, International Symposium on the Forensic Aspects of DNA Analysis, FBI Academy Quantico, Virginia, 1989.

[8] Waye, J. S., Presley, L. A., Budowle, B., Shuttler, G. G., and Fourney, R. M., "A Simple and Sensitive Method for Quantifying Human Genomic DNA in Forensic Specimen Extracts," *BioTechniques*, Vol. 7, No. 8, 1989, pp. 852–855.

[9] Budowle, B. and Baechtel, F. S., "Modifications to Improve the Effectiveness of Restriction Fragment Length Polymorphism Typing," *Applied and Theoretical Electrophoresis*, Vol. 1, 1990, pp. 181–187.

[10] Comey, C. T. and Budowle, B., "Validation Studies on the Analysis of the HLA-DQ Alpha Locus Using the Polymerase Chain Reaction," *Journal of Forensic Sciences*, Vol. 36, No. 6, November 1991, pp. 1633–1648.

[11] Hochmeister, M. N., Budowle, B., Borer, U. V., et al., "Typing of Deoxyribonucleic Acid (DNA) Extracted from Compact Bone from Human Remains," *Journal of Forensic Sciences*, Vol. 36, No. 6, November 1991, pp. 1649–1661.

[12] Hochmeister, M. N., Budowle, B., and Baechtel, F. S., "Effects of Presumptive Test Reagents on the Ability to Obtain Restriction Fragment Length Polymorphism (RFLP) Patterns from Human Blood and Semen Stains," *Journal of Forensic Sciences*, Vol. 36, No. 3, November 1991, pp. 656–661.

[13] Hochmeister, M. N., Budowle, B., Jung, J., et al., "PCR-Based Typing of DNA Extracted from Cigarette Butts," *International Journal of Legal Medicine*, Vol. 104, 1991, pp. 229–233.

[14] Sajantila, A., Stroem, M., Budowle, B., et al. "The Polymerase Chain Reaction and Post-Mortem Forensic Identity Testing: Application of Amplified D1S80 and HLA-DQalpha Loci to Identification of Fire Victims," *Forensic Science International*, Vol. 51, 1991, pp. 23–34.

[15] Adams, D. E., Presley, L. A., Baumstark, A. L., et al., "Desoxyribonucleic Acid (DNA) Analysis by Restriction Fragment Length Polymorphisms of Bloodstain and Other Body Fluid Stains Subjected to Contamination and Environmental Insults," *Journal of Forensic Sciences*, Vol. 36, No. 5, September 1991, pp. 1284–1298.

Address requests for reprints or additional information to
M. N. Hochmeister, M.D.
Department of Forensic Medicine
Institut für Rechtsmedizin
University of Bern
Bühlstrasse 20
CH-3012 Bern Switzerland