# TECHNICAL NOTE

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# AmpliType® PM and HLA DQα Typing from Pap Smear, Semen Smear, and Postcoital Slides

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**ABSTRACT:** Deoxyribonucleic acid (DNA) samples extracted from stained pap smear, semen smear and postcoital slides were amplified by the polymerase chain reaction (PCR) and typed for the AmpliType® PM and HLA DQα alleles. HLA DQ α and PM types consistent with blood controls from the donors were obtained. Stained cells fixed to slides can provide a valuable source of material for determining a genetic profile of the sample donor, particularly in sexual assault cases and possibly in missing person cases involving women.

**KEYWORDS:** criminalistics, DNA, AmpliType® PM, HLA DQα, PCR, sexual assault evidence

Restriction fragment length polymorphism (RFLP) analysis is currently the method of choice for individualizing blood and body fluid stains from various samples submitted to forensic science laboratories because of its high discrimination power [*1,2*]. The RFLP approach is most suitable for samples containing at least 50 ng of high molecular weight DNA. However, evidence samples frequently are degraded or limited in quantity and are not always able to yield RFLP results. The development of DNA typing systems based on the polymerase chain reaction (PCR) [*3*] allows genetic information of the sample donor to be obtained from extracted DNA that is either high quality or compromised [*4–6*].

The commercially available AmpliType® PM and HLA DQ α PCR Amplification and Typing kits contain all the necessary reagents for forensic DNA amplification and subsequent detection of alleles. The PM kit allows simultaneous amplification of the following six genetic loci: HLA DQA1, Low Density Lipoprotein Receptor (LDLR), Glycophorin A (GYPA), Hemoglobin G Gammaglobin (HBGG), D7S8 and Group Specific Component (GC). The DNA probe strip in the PM kit is used to type the five non-DQA1 loci, each of which contains two or three alleles. The HLA DQα kit contains reagents to amplify and type a polymorphic region of the human leukocyte antigen (DQA1) locus [*7*]. The HLA DQα DNA probe strips allow six DQA1 alleles to be distinguished resulting in 21 possible genotypes [*8*].

This study was undertaken to determine if DNA extracted from stained and unstained semen and pap smear slides and stained postcoital slides can be amplified and typed using the AmpliType® PM and HLA DQα kits. The ability to obtain genetic information from these types of samples is important because stained vaginal smear slides, containing sperm, occasionally are submitted as evidence for sexual assault cases instead of a vaginal swab [*9*]. Also, it is possible that a pap smear slide could be used as a source of reference material for the analysis of remains or bloodstains thought to have arisen from a known missing woman.

## Materials and Methods

Blood and semen samples were collected from volunteers. For each semen sample slides were prepared with 5 μL of the semen smeared on the glass and left at room temperature overnight to dry. One slide was then stained with 'Christmas tree' stain [*10*] and the other slide was left unstained. Pap smears were collected in duplicate. One of the slides was stained with Christmas tree stain and the other left unstained. Several slides stained with the Gynecological stain [*11*] were obtained from the hospital. Postcoital swabs obtained from volunteers were smeared on slides and stained with Christmas tree and Gynecological stain. All stained and unstained slides were mounted with a Pro-Texx* Mounting Medium, and the cover slips were removed with xylol. The rape kit vaginal smear slides had been stained previously with Christmas tree stain. Since no unstained slides were available from the rape kits, slides were chosen from the kits where the semen donors' identity were not in question (consentual cases). The ages of the slides used in this research varied from one day to seventeen months. However, the ages of the slides provided by the hospital were unknown.

The smeared region of each neat semen and pap smear slide was swabbed with cotton-tipped sterile applicators wet with autoclaved distilled water. The swabs were then processed for DNA extraction. These swabs and the corresponding reference blood samples from the semen and pap smear donors were extracted with Chelex [*12*]. The postcoital and rape kit slides also were swabbed with cotton-tipped sterile applicators wet with autoclaved distilled water. How-

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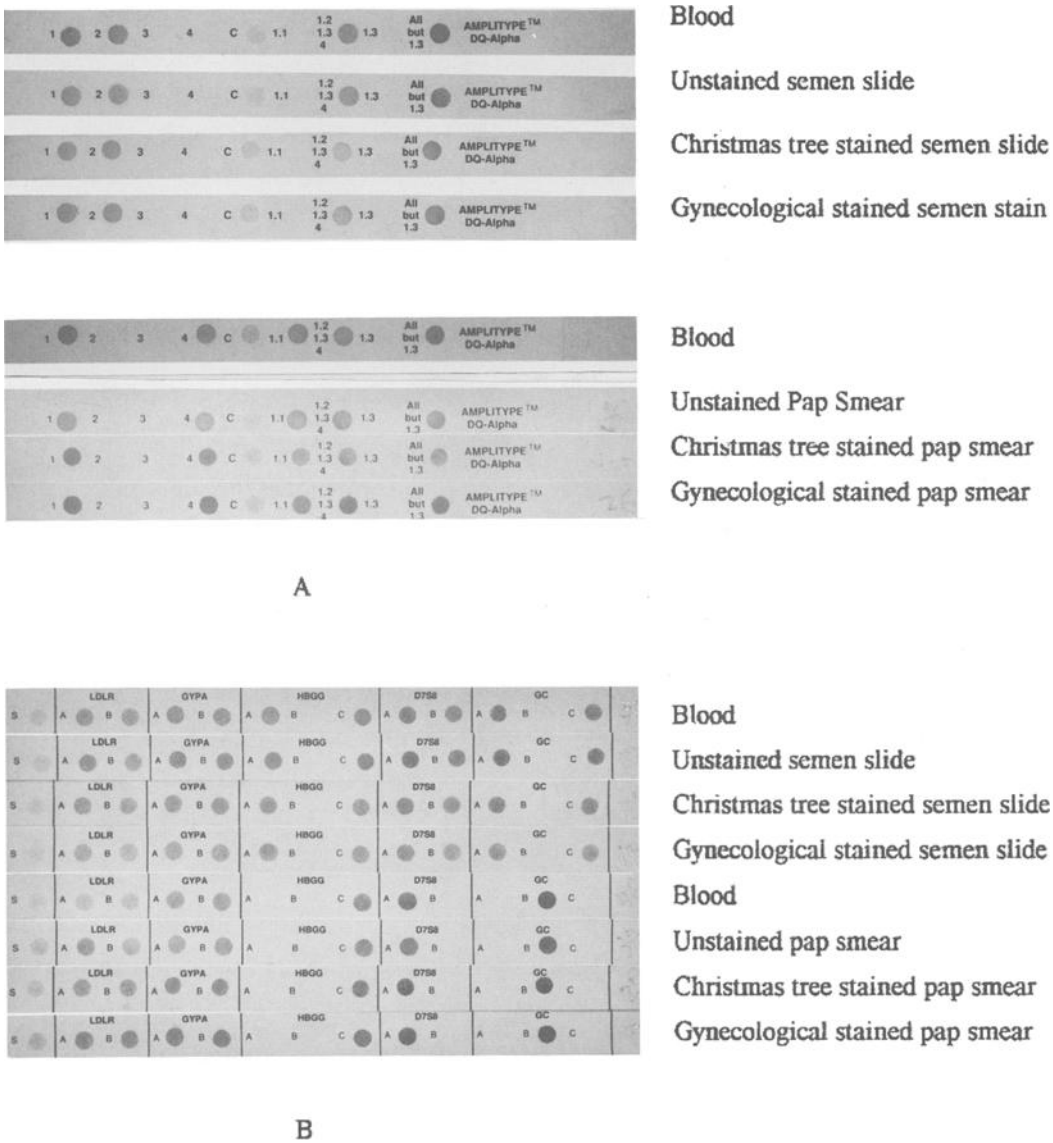



FIG. 1—*AmpliType HLA DQα* (A) *and AmpliType PM* (B) *DNA probe strip typing results from blood, semen and pap smears from two individuals. The source of the extracted DNA is indicated at the right of the strips.*

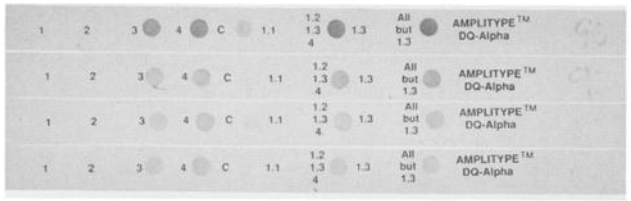
ever, because these samples contained both sperm and epithelial cells, they underwent differential lysis [*13*] prior to Chelex extraction. Only the sperm fractions from these samples were extracted and evaluated.

Twenty microliters of extracted DNA from each sample were subjected to PCR amplification as recommended. The detailed procedures for the amplification are included in the AmpliType® PM and HLA DQα kits. The PCR amplified products were then evaluated by gel electrophoresis following the protocol in the AmpliType® User Guide [*13*].

AmpliType PM and HLA DQα typing was performed according to the recommended protocols using the DNA probe strips included in the kits with one minor deviation. Twenty microliters of some HLA DQα amplified samples were used for hybridization with the probes instead of the recommended 35 μL.

## Results and Discussion

Sexual assault kits submitted to the Criminalistics Laboratory in Nebraska are required to contain vaginal swabs as well as vaginal smear slides. In some of the smaller counties or rural hospital facilities, emergency room personnel may prepare two vaginal smear slides, which they stain themselves for microscopic visualization of sperm. They do not include any vaginal swabs in the kit and instead enclose the already stained slides for genetic marker analysis of the semen donor. Consequently, it was important to evaluate the ability to obtain reliable DNA typing results from stained slides. Neat semen smeared on slides, pap smear slides containing no spermatozoa and postcoital smear slides were used to evaluate the ability to extract and PCR amplify DNA from cells that have been fixed to slides and stained. Several slides from

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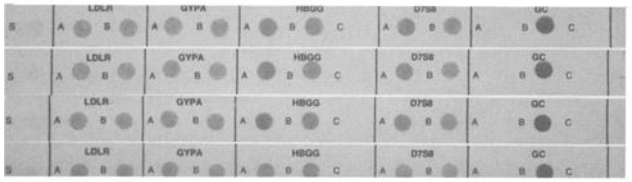


B

FIG. 2—*AmpliType HLA DQα* (A) *and AmpliType PM* (B) *DNA probe strip typing results from blood and the sperm fraction of a postcoital smear slide. The source of the extracted DNA is indicated at the right of the strips.*

TABLE 1—*Results of HLA DQα and PM typing from various stained slides.*

| Slides | Number of Slides | | | PM Results | DQα Results | Age |
|---|---|---|---|---|---|---|
| | Christmas Tree Stained | Gynecological Stained | Unstained | | | |
| A. Pap Smear | 2 | 2 | 2 | + | + | 2 weeks |
| | 4 | 4 | 4 | + | + | 5 months |
| | 3 | 3 | 3 | + | + | 12 months |
| | 5 | 5 | 5 | + | + | 15 months |
| | 1 | 1 | 1 | + | + | 17 months |
| | 1 | | | − | − | 3 months |
| | | 1 | | − | − | 3 months |
| | | | 1 | + | + | 3 months |
| B. Semen Smear | 1 | 1 | 1 | + | + | 2 weeks |
| | 2 | 2 | 2 | + | + | 10 months |
| | 2 | 2 | 2 | + | + | 15 months |
| | 1 | 1 | 1 | + | + | 17 months |
| C. Hospital Provided Slides | | | | | | |
| Semen | 2 | 2 | 2 | + | + | Unknown |
| Pap Smear | 3 | 3 | 3 | + | + | Unknown |
| D. Postcoital Smears | 1 | 1 | 1 | + | + | 1 day |
| | 1 | 1 | 1 | + | + | 15 months |
| | 1 | 1 | 1 | + | + | 17 months |
| E. Rape Kits | 2 | NA | NA | + | + | 1 day |
| | 3 | NA | NA | + | + | 5 months |
| | 3 | NA | NA | + | + | 8 months |
| | 1 | NA | NA | − | − | 1 day |
| | 1 | NA | NA | − | − | 5 months |
| | 1 | NA | NA | − | − | 6 months |
| | 1 | NA | NA | − | − | 8 months |

+ indicates correct typing results. − indicates no results were obtained. NA indicates not available.

sexual assault cases in which the semen donor's identity was not in question were also processed. The numbers and kinds of slides that were amplified and typed are listed in Table 1.

DNA extracted from stained and unstained semen and pap smear slides typed correctly for both the AmpliType® PM and HLA DQα Systems (Fig. 1). The cellular material removed from the nine stained and unstained postcoital smear slides and the 12 stained rape kit slides was processed by differential lysis and extraction prior to amplification. Only DNA extracted from the sperm fraction was amplified and typed in both AmpliType systems; the epithelial cell fractions were not extracted. The types obtained from all of the sperm fractions were the same as the corresponding reference blood types (Fig. 2). The amount of DNA was not quantitated for this study and no attempt was made to count the number of spermatozoa or cells in each slide. However, 24 slides out of 24 (100%) smeared with 5 μL semen as well as 54 routinely collected pap smear slides out of 54 (100%) yielded adequate DNA for amplification and typing, and 8 of the 12 stained rape kit slides (67%) yielded enough "amplifiable" DNA for both PM and HLA DQα typing (Table 1). It is possible that the other four stained rape kit slides did not contain sufficient DNA for amplification.

The Christmas tree staining protocol used to stain the semen, pap and postcoital smear slides is the method of choice in the Nebraska State Patrol. Criminalistics Laboratory for visualizing sperm. However, many of the hospitals in Nebraska use the gynecological stain protocol for staining the slides contained in rape kits. DNA extracted from pap, semen and post-coital smear slides stained with Christmas tree stain and the gynecological stain amplified and typed correctly (Figs. 1 and 2). The reagents used for Christmas tree and Gynecological staining do not interfere with the ability to extract and amplify DNA from smeared slides using the procedures described in the Material and Methods section. DNA extracted from stained and unstained slides from the same individual was successfully amplified and typed.

In conclusion, AmpliType® PM and HLA DQα typing can be performed after PCR amplification of DNA extracted from stained and unstained pap, semen and postcoital smear slides. No mistyping results were obtained. Clearly, stained postcoital smear slides containing sperm can be used to obtain genetic information about the assailant in sexual assault cases. In addition, given the successful results obtained from the stained pap smear slides and the fact that these slides are retained by many hospitals in Nebraska for several years, they could serve as a reference sample, for example, in cases involving the analysis of remains or biological fluids believed to have originated from a known missing woman.

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