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# Evaluation of the AmpliType® PM DNA Test System on Forensic Case Samples

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**ABSTRACT:** Evidence material from sexual assault cases which had been submitted to the laboratory for routine processing were examined to determine the usefulness of the AmpliType® PM PCR Amplification and Typing Kit developed by Roche Molecular Systems for forensic evidence. In all cases in which a conclusive answer was reached for the AmpliType PM system, the results agreed with or surpassed results previously obtained with RFLP testing. The AmpliType PM DNA test system has promise as a quick and easy method for elimination or inclusion of suspects.

**KEYWORDS:** pathology and biology, PCR, DNA typing, RFLP, sex assault samples, AmpliType® PM

Over the last several years RFLP DNA typing [*1–3*] using a series of variable number tandem repeat (VNTR) loci has become widespread in the forensic community. Although this procedure produces very high levels of discrimination it has the disadvantage of requiring a relatively long time to complete and requiring high molecular weight DNA. By using the polymerase chain reaction (PCR) [*4,5*] with the HLA DQα locus [*6*] these two disadvantages were overcome, but with a greatly reduced power of discrimination. Analyzing additional markers via individual amplifications is relatively easy in PCR based testing because the amount of DNA required per test is only about 10 to 20% of that required for RFLP testing. An alternative route to add additional markers is through amplification of several loci using several sets of primers in one tube under the same set of conditions (multiplexing).

This alternative approach has been utilized in a new kit developed by Roche Molecular Systems known as the AmpliType® PM PCR amplification and typing kit. The AmpliType PM system uses the same reverse dot blot format as the DQα test [*7*]. The markers which are amplified are HLA DQα, LDLR (low density lipoprotein receptor) [*8*], GYPA (glycophorin A) [*9*], HBGG (hemoglobin G gamma globin) [*10*], D7S8 [*11*], and GC (group specific component) [*12*]. The last five loci listed are typed using a single reverse dot blot strip containing immobilized sequence specific probes and DQα is typed using a separate strip. The chromosomal locations, PCR product size, and number of alleles for each of these five loci are given in Table 1. The HLA DQα locus has been thoroughly investigated elsewhere [*6,13–15*] and will not be discussed further here.

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TABLE 1—*Summary of loci used on the AmpliType® PM typing strip.*

| | LDLR | GYPA | HBGG | D7S8 | GC |
|---|---|---|---|---|---|
| Chromosome | 19 | 4 | 11 | 7 | 4 |
| PCR product (bp) | 214 | 190 | 172 | 151 | 138 |
| Alleles | 2 | 2[a] | 3 | 2 | 3 |

[a]The African American population exhibits a third allele detected but not discriminated by the strip.

This paper investigates the usefulness of the five loci LDLR, GYPA, HBGG, D7S8, and GC included in the AmpliType PM system to determine their potential in the forensic setting. Results obtained with these five loci were compared with results previously obtained from the same evidence samples using RFLP testing.

## Methods and Materials

### *Samples*

Cuttings from 16 cases submitted to the Georgia Bureau of Investigation Division of Forensic Sciences were taken. Each of these items had been previously analyzed using RFLP typing. The unknown samples were stored either frozen or refrigerated after receipt in the laboratory. Evidence associated with these cases included 15 vaginal swabs (collected on cotton swabs), 1 semen stain from panties and 38 known blood samples. Cases were selected so that RFLP matches, exclusions, and inconclusive results would be tested using the AmpliType PM system.

### *DNA Extraction*

DNA was extracted from the unknown stain materials using the Chelex extraction method [*16,17*]. DNA from the known blood samples had been previously extracted using the phenol:chloroform method and stored at −70°C. Approximately one-third of one swab from each case was used for the extraction. The efficiency of the initial epithelial cell lysis was checked by microscopy prior to sperm lysis with DTT and proteinase K. Appropriate negative controls were also included.

### *DNA Quantification*

Approximately one half of the DNA samples obtained from the vaginal swabs were quantitated using the D17Z1 locus (Oncor) as described by Waye et al. [*18*]. Known amounts of K562 DNA (Gibco Life Technologies) were used as the standard.

### *DNA Amplification*

The AmpliType® PM amplification is a fixed volume reaction requiring 40 μL reaction mix (provided by Roche Molecular Systems), 40 μL 6 mM $MgCl_2$ and 20 μL DNA. The samples were amplified in a Perkin Elmer DNA Thermal Cycler 480 using the following parameters: 94°C 1 min, 60°C 30 s, 72°C 30 s for 32 cycles with a 7 min incubation at 72°C at the conclusion of the cycles. Verification of amplification at all six loci was determined by electrophoresis of the products through 2.0% or 2.5% ultrapure agarose (Gibco BRL) in 1X TBE [*19*] buffer containing ethidium bromide at 75V for 10 to 20 min.

*DNA Typing*

The types for each of the five loci for these samples were determined as follows. The product was heated to 95°C for 3 to 10 min and 20 μL added to 3 mL of hybridization solution (5X SSPE, 0.5% w/v SDS) in a tray containing AmpliType PM typing strips. Hybridization was for 15 min at 55°C. The solution was aspirated from each strip, followed by a brief wash with 5 mL wash solution (2.5X SSPE, 0.1% w/v SDS). This wash was aspirated from the strips and 3 mL of hybridization solution/enzyme conjugate was added to each strip. Binding of the horse radish peroxidase/streptavidin complex to the biotinylated primers used to amplify the products was for 5 min at 55°C. After the solution was removed by aspiration the strips were again briefly washed with the wash solution, followed by the stringent wash for 12 min at 55°C. The wash solution was removed by aspiration, the strips rinsed with wash solution and then 5 mL citrate buffer (0.1M sodium citrate, pH 5.0) was added. This was shaken gently for 5 min at room temperature, aspirated off, and replaced with 5 mL color development solution (5 mL citrate buffer, 0.25 mL tetramethylbenzidene (TMB), 5 μL 3% hydrogen peroxide per strip). Development was allowed to proceed 10–25 minutes at room temperature.

## Results and Discussion

The amplification sensitivity of this system is very close to that obtained with the DQα system. In each of the samples for which a human DNA quantity was measured prior to amplification, the quantity estimated was a very good indicator of potential amplification success. All samples which contained at least 3 to 5 ng DNA per 20 μL amplified successfully. The positive control DNA included with the kit reagents contains 2 ng DNA per 20 μL.

Of the 16 semen stains extracted, 13 produced a type in the sperm fraction and 8 produced a type in the non-sperm fraction. The sperm fraction DNA type from three of the swabs was derived from victim DNA (Case's #1, #11, #12), rather than sperm DNA. These swabs were observed to have only a few or no sperm heads present in the microscopic examination. Each of the 38 known blood samples produced unequivocal results which were easily interpreted.

DNA results other than the victim's DNA type were obtained from 9 of the 16 evidence samples tested. These results (presumably from sperm DNA) were compared to the DNA types from the individuals submitted as suspects in the cases. A summary of the results is shown in Table 2. RFLP results from cases in which the suspect's patterns matched with sperm DNA patterns from the swabs were derived from at least four loci (Cases #2, #13). While Case #13 had resulted in a match using RFLP testing, Case #3 produced inconclusive RFLP results because of an insufficient amount of sperm DNA. In these two cases (Cases #3, #13) AmpliType® PM dots were observed which indicated the presence of an additional person, but because of incomplete separation of victim non-sperm DNA from sperm DNA, no conclusive determination of types from these loci was possible. However, the typing strip results from both cases indicated a suspect could be included as the sperm donor because the probe strips were designed to have balanced dot intensities within a locus for a single heterozygous DNA source. Dots of unequal intensity were observed at several loci in both cases.

Comparison of the dot intensity within loci allowed deduction of types at some loci even when separation of DNA from sperm and non-sperm cells was not complete. This feature may prove to be very useful in routine casework. This point is shown clearly in Fig. 1 where the differential extraction was not complete (Case #6). In this case the non-sperm fraction HBGG locus produced 3 dots with the A allele being approximately twice as intense as the B and C alleles (indicating that at least 2 A alleles were present). By comparison with the known sample from the victim in the case (HBGG AA), it is possible

TABLE 2—*Summary of results from the 16 stains tested with the 5 loci on the AmpliType® PM typing strip.*

| Case # | Non-sperm | Sperm | PM result | RFLP result |
|---|---|---|---|---|
| 1 | + | +[a] | NI | NI |
| 2 | + | + | I | I |
| 3 | + | +[d] | NI, I[c] | NI |
| 4 | + | + | E | E |
| 5 | + | NR | NI | NI |
| 6 | Inc | + | E | E |
| 7 | NR | + | E | E |
| 8 | NR | + | E | E |
| 9 | NR | + | E | E |
| 10 | NR | + | E | E |
| 11 | NR | +[a] | NI | E |
| 12 | NR | +[a] | NI | NI |
| 13 | NR | +[c] | I[c] | I |
| 14 | + | + | NE[b] | NI |
| 15 | + | + | I | NI |
| 16 | + | NR | NI | NI |

[a]Only the victim's DNA types were seen in the sperm fraction.

[b]Both suspects submitted had the same profile over all 5 loci. Neither suspect could be excluded from having donated the sperm DNA isolated from this swab.

[c]This sample appeared to be a mixture of non-sperm DNA from the victim and sperm DNA. Incomplete separation of the sperm/non-sperm DNA and common alleles between the two potential donors prevented a conclusive result, but intensity variations within the dots indicated that the suspect would have been included.

[d]This sample was a mixture of non-sperm DNA from the victim and sperm DNA. Two potential sperm donors were submitted, no alleles corresponding to only suspect #1 were observed. Alleles consistent with suspect #2 were observed, indicating a possible inclusion. All of the cases in which RFLP testing gave no information concerning the sperm donor were the result of an insufficient amount of sperm DNA. NR, NI, I, E, NE—no result obtained from that fraction, no information concerning sperm donor, include suspect, exclude suspect, and suspects not excluded, respectively.

to assign the A alleles to the victim and know that the B and C alleles originated with another individual(s).

The faint dots visible on the sperm fraction strip in Fig. 1 (which would not be interpreted in casework) initially appear to possibly include the suspect. However, closer examination of the potential dots contributed by the submitted known samples shows this not to be the case. This conclusion can be reached because no victim DNA appears in this fraction (no A allele found at GC locus on strip 2) and the suspect does not have the faint A allele observed at the HBGG locus. Therefore, neither the primary semen donor observed on strip 2 nor the secondary donor responsible for the faint dots were submitted as knowns in this case.

In another case (Case #14), there was adequate separation of the sperm and non-sperm DNA, but the two submitted suspects possessed the same type at all five of the loci on the AmpliType PM strip. These two suspects do have different RFLP profiles. Inconclusive RFLP testing from this case was the result of an insufficient amount of sperm DNA obtained from the swab. Further testing with additional markers such as DQα or D1S80 may have resolved this case. Because both suspects could have donated the sperm DNA found in this case, but no differences were observed using these five loci, a conclusion that neither suspect could be excluded was reached. This is strictly a semantic point, as it would be just as correct to say that both suspects could be included. The terminology of 'included' was used in those cases (Cases #2, #3, #15) where only one individual in the case produced the same types at the loci of interest even though it is possible for other individuals to produce that same series of genotypes.

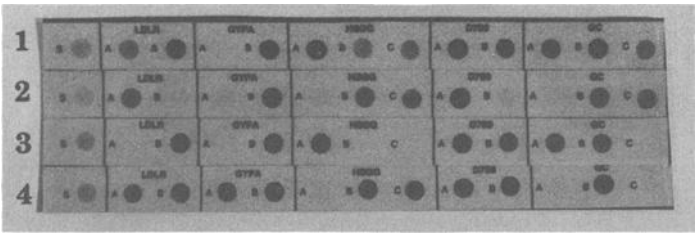


FIG. 1—*This set of strips are the results from Case #6 (Table 2). The intensity differences between the dots in the HBGG locus area clearly indicate the value of the intensity balanced probe dots. Strips 1–4—non-sperm fraction, sperm fraction, victim known, suspect known respectively. The faint dots that are less intense than the control S dot appear to be the result of sperm present from a second sexual partner. These dots would not be interpreted in casework because of their low intensity.*

As expected there were no instances in which RFLP testing produced a match between a suspect and the evidence, while AmpliType PM testing produced an exclusion. There was only one instance in which RFLP testing proved inconclusive because of insufficient DNA and the AmpliType® PM testing produced a conclusive result with regard to the submitted suspect. The AmpliType PM test results would have included the suspect in that particular case (Case #15).

## Conclusions

The AmpliType PM test has the potential to greatly facilitate DNA testing in forensic laboratories. This test has the advantages of providing results in a much shorter time frame than RFLP, while at the same time providing relatively good discriminatory power (Table 3). This gives laboratories the potential of quickly screening and excluding suspects, so that investigators can focus their attention on apprehending the correct individual. Although the number of samples studied here was small, in all but one case in which a sperm DNA type was obtained, there was a clear exclusion or inclusion of the submitted suspect. In addition, these results agreed 100% with exclusionary results that had been previously obtained from RFLP testing.

TABLE 3—*Discrimination levels provided by the five loci on the PM typing strip.*

| Case # | Black | Caucasian | Hispanic | Georgia Black |
|---|---|---|---|---|
| 2 | $4.7 \times 10^2$ | $3.5 \times 10^3$ | $1.8 \times 10^3$ | $3.2 \times 10^2$ |
| 13 | $1.3 \times 10^2$ | $6.2 \times 10^3$ | $1.1 \times 10^3$ | $9.0 \times 10^1$ |
| 14 | $1.4 \times 10^2$ | $2.0 \times 10^6$ | $4.5 \times 10^4$ | $1.0 \times 10^2$ |
| 15 | $5.5 \times 10^1$ | $3.8 \times 10^4$ | $3.5 \times 10^3$ | $3.5 \times 10^1$ |

NOTE: The four cases in which the suspects were included (not excluded) are shown here. Each of the numbers is the reciprocal of the calculated frequency. The first three columns are calculated using data from 100 individuals provided in the kit insert provided by RMS. The last column is calculated using data obtained from 65 individuals submitted to the GBI-DOFS laboratory. The addition of DQα would increase each these numbers by one to two orders of magnitude. All calculations using the PM loci assumed Hardy-Weinberg and linkage equilibrium. In comparison to RFLP pattern frequencies in Cases #2, #13, the calculated frequency of occurrence for the suspect's pattern in the Black population was approximately 1 in $8 \times 10^9$ (5 loci) and 1 in $3 \times 10^6$ (4 loci) respectively.

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## References

[1] Giusti, A., Baird, M., Pasquale, S., Balazs, I., and Glassberg, J., "Application of Deoxyribonucleic Acid (DNA) Polymorphisms to the Analysis of DNA Recovered from Sperm," *Journal of Forensic Sciences,* Vol. 31, No. 2, April 1986, pp. 409–417.

[2] 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.

[3] Budowle, B., Waye, J. S., Schutler, G. G., and Baechtel, F. S., "HaeIII-A Suitable Restriction Endonuclease for Restriction Fragment Length Polymorphism Analysis of Biological Evidence Samples," *Journal of Forensic Sciences,* Vol. 35, No. 3, May 1990, pp. 530–536.

[4] Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A., and Arnheim, N., "Enzymatic Amplification of β-globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia," *Science,* Vol. 230, 1985, pp. 1350–1354.

[5] Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A., "Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase," *Science,* Vol. 239, 1988, pp. 487–491.

[6] Helmuth, R., Fildes, N., Blake, E., Luce, M. C., Chimera, J., Madej, R., Gorodezky, C., Stoneking, M., Schmill, N., Klitz, W., Higuchi, R., and Erlich, H. A., "HLA-DQα Allele and Genotype Frequencies in Various Human Populations, Determined by Using Enzymatic Amplification and Oligonucleotide Probes," *American Journal of Human Genetics,* Vol. 47, 1990, pp. 515–523.

[7] Saiki, R. K., Walsh, P. S., Levenson, C. H., and Erlich, H. A., "Genetic Analysis of Amplified DNA with Immobilized Sequence-Specific Oligonucleotide Probes," *Proceedings of the National Academy of Sciences, USA,* Vol. 86, 1989, pp. 6230–6234.

[8] Yamamoto, T., Davis, C. G., Brown, M. S., Schneider, W. J., Casey, M. L., Goldstein, J. L., and Russell, D. W., "The Human LDL Receptor: A Cystein-Rich Protein with Multiple Alu Sequences in Its mRNA," *Cell,* Vol. 39, 1984, pp. 27–38.

[9] Siebert, P. D. and Fukuda, M., "Molecular Cloning of a Human Glycophorin B cDNA: Nucleotide Sequence and Genomic Relationship to Glycophorin A," *Proceedings of the National Academy of Sciences, USA,* Vol. 84, 1987, pp. 6735–6739.

[10] Slightom, J. L., Blechl, A. E., and Smithies, O., "Human Fetal $^{G}\gamma$- and $^{A}\gamma$-Globin Genes: Complete Nucleotide Sequences Suggest That DNA Can Be Exchanged between These Duplicated Genes," *Cell,* Vol. 21, 1980, pp. 627–638.

[11] Horn, G. T., Richards, B., Merrill, J. J., and Klinger, K. W., "Characterization and Rapid Diagnostic Analysis of DNA Polymorphisms Closely Linked to the Cystic Fibrosis Locus," *Clinical Chemistry,* Vol. 36, 1990, pp. 1614–1619.

[12] Yang, F., Brune, J. L., Naylor, S. L., Cupples, R. L., Naberhaus, K. H., and Bowman, B. H., "Human Group-Specific Component (Gc) Is a Member of the Albumin Family," *Proceedings of the National Academy of Sciences, USA,* Vol. 82, 1985, pp. 7994–7998.

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

[14] Comey, C. T., Budowle, B., Adams, D. E., Baumstark, A. L., Lindsey, J. A., and Presley, L. A., "PCR Amplification and Typing of the HLA-DQα Gene in Forensic Samples," *Journal of Forensic Sciences,* Vol. 38, No. 2, March 1993, pp. 239–249.

[15] Blake, E., Mihalovich, J., Higuchi, R., Walsh, P. S., and Erlich, H., "Polymerase Chain Reaction (PCR) Amplification and Human Leukocyte Antigen (HLA)-DQα Oligonucleotide Typing on Biological Evidence Samples: Casework Experience," *Journal of Forensic Sciences,* Vol. 37, No. 3, May 1992, pp. 700–726.

[16] Walsh, P. S., Metzger, D. A., and Higuchi, R., "Chelex 100 As a Medium for Simple Extraction of DNA for PCR-Based Typing from Forensic Material," *BioTechniques,* Vol. 10, 1991, pp. 506–513.

[17] AmpliType® User Guide, Version 2, Perkin-Elmer, Norwalk, CT.

[18] Waye, J. S., Michaud, D., Bowen, J. H., and Fourney, R. M., "Sensitive and Specific Quantification of Human Genomic Deoxyribonucleic Acid (DNA) in Forensic Science Specimens: Casework Examples," *Journal of Forensic Sciences,* Vol. 36, No. 4, July 1991, pp. 1198–1203.

[19] Maniatis, T., Fritsch, E. F., and Sambrook, J., *Molecular Cloning: A Laboratory Manual*, 2nd ed., 1989, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

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