Letters to the Editor

Reliability of the HLA-DQα PCR-Based Oligonucleotide Typing System

Dear Sir:

In testimony concerning the admissibility of human lymphocyte antigen (HLA)-DQα PCR-based oligonucleotide typing results in a rape/murder case (*California v. Mello*, 1989), it was asserted [*1*] that this dot blot typing system was unreliable because it occasionally failed to identify the DQA1.2 allele. We wish to point out that this allegation was based on a misconception.

Six sequence-defined alleles, DQA1.1, 1.2, 1.3, 2, 3, and 4, are detected by the 8 oligonucleotide probes used in the HLA-DQα typing system [*2–6*]. (The DQA4 allele can be further subtyped; however, the 4.1 and the less common 4.2 and 4.3 subtypes are not distinguished with these probes and type simply as DQA4.) The allegation of unreliability was based on the PCR/oligonucleotide analysis of 5 individuals who were assumed to contain a DQA1.2 allele but for whom the PCR/oligonucleotide probe typing results did not indicate the presence of the DQA1.2 allele. Sample 2, which types reproducibly by the PCR/oligonucleotide method as DQA1.2/DQA4, was cited in the testimony because the witness thought it was a DQA1.2/DQA1.2 homozygote (see Table 1). The assumption that these 5 individuals did, in fact, contain the DQA1.2 allele was based on restriction fragment length polymorphism (RFLP) patterns using the *Taq*I enzyme and the DQα cDNA probe, pDCH-1. These 5 samples, as well as an additional 35 samples, were recently typed for HLA-DQα polymorphism by the PCR/oligonucleotide reverse dot blot method [*4*]. The results from these 40 samples, coded so that the typing was performed as a blind trial, were interpreted independently by representatives of two different laboratories. The DQα typing results were identical to the initial PCR/oligonucleotide dot blot typing results, including those for the 5 samples originally cited as evidence of unreliability. Thus, DQα oligonucleotide typing results are reproducible.

This finding raises the possibility that an incorrect assignment of the sequence-defined DQA1.2 allele based on the presence of a specific *Taq*I fragment may have led to this alleged discordant typing. With this DQα probe, *Taq*I digestion produces 5 fragments to which the type designations 1a, 1b, 1c, 2, and 3 have been assigned. Although preliminary work suggested a correlation between some of these and the sequence-defined alleles, the results of the Tenth International HLA Workshop indicate that this is not the case. In particular, the *Taq*I "1b" fragment, presumed to be associated with the DQA1.2 allele, is also found with other DQα alleles. As shown below, it is also associated with the DR8, DQA4.2/4.3 haplotype found in these 5 samples (Table 1).

As the inventor of an issued patent (U.S. Patent 4,582,788) on HLA RFLP typing and an inventor on two issued PCR patents (U.S. Patents 4,683,195 and 4,800,159), one of us (HAE) is in a unique position to appreciate the strengths and limitations of these two methods. In general, it should not be surprising that RFLP patterns do not correspond in a one-to-one fashion with the coding sequence polymorphism of the DQα second exon. In particular, for DQα typing, the official RFLP report of the Tenth International HLA Workshop (Simons et al. in Ref 7) states that some of the DQα *Taq*I fragments "are of doubtful validity since they bear no apparent relation to DR or DQ specificities" and refers to "the lack of genetically meaningful association" (Ref 7, p. 978).

Table 1 shows the results of repeated DQα PCR/oligonucleotide typing, RFLP analysis, and DR PCR typing for 6 samples, including the 5 cited in the testimony. These results suggest that the DR8 samples containing the "1b" *Taq*I fragment were incorrectly assumed to have the DQA1.2 allele. Sample 2 does, in fact, contain a DQA1.2 allele

TABLE 1—*Comparison of PCR/oligonucleotide and RFLP typing on selected samples.*[a]

Sample	PCR/Oligonucleotide, DQα Type	4.2/4.3 Oligonucleotide	RFLP, kilobases	PCR/Oligonucleotide, DRβ Type
C	(1.2), [4]	–	(1b), [2]	(2), [3]
1[b]	(4), [4]	+	[1b], (2)	(3), [8]
2[b,c]	(1.2), [4]	+	[1b], (1b)	(2), [8]
3[b,d]	(1.1), △3, [4]	+	(1a), [1b]	(1), △4, [8]
4[b]	(3), [4]	+	[1b], (3)	[8], (4)
5[b]	(4), [4]	+	[1b], (2)	[8], (3)

[a]The inferred haplotypes, based on known patterns of linkage disequilibrium, are denoted by boxes, circles, and triangles. Subsequent oligonucleotide typing with the oligonucleotide probe HE46 [7] indicated that the 5 samples *all* contained the DQA4.2/4.3 subtype.

[b]The 5 samples cited in the California *v.* Mello testimony.

[c]The *Taq*I RFLP pattern obtained with a DRβ cDNA probe was not typical of any known DR type (KL, data not shown).

[d]Sample 3 is a mixture and contains 3 DRβ and 3 DQα alleles. The DR4, DQA3 haplotype was a minor contaminant (~10%) and was not detected by RFLP analysis. The DRβ typing was performed by PCR amplification and hybridization with HRP-oligonucleotide probes (*9–11*). (In Ref *7*, the 1b *Taq*I fragment is identified as 6.19 kb.)

(attributed to the DR2 haplotype) as well as the DQA4 allele (attributed to the DR8 haplotype). Subsequent oligonucleotide typing with the probe HE46 [*8*], which distinguishes DQA4.2 and 4.3 from the DQA4.1 subtype, confirmed that these DR8 cells all contain the DQA4.2 or 4.3 allele (Table 1). Thus, the simplest explanation of the oligonucleotide typing results and the RFLP patterns is that these 5 samples cited in the *California v. Mello* admissibility hearings do not, in fact, contain the DQA1.2 allele, except Sample 2, as noted above. Therefore, the allegation made during the *California v. Mello Frye* hearing that PCR/oligonucleotide typing is unreliable was based on an erroneous interpretation of RFLP results.

Henry A. Erlich, Ph.D.
Senior Scientist and Director
and
Russell Higuchi, Ph.D.
Scientist
Human Genetics Department
Cetus Corporation
1400 53rd St.
Emeryville, CA 94608

Kay Lichtenwalter, Ph.D.
Rebecca Reynolds, Ph.D.
and
George Sensabaugh, D.Crim.
School of Public Health
University of California at Berkeley
Berkeley, CA 94720

References

[1] Testimony of Dr. M. C. King in California v. Mello, 1989.
[2] Saiki, R. K., Bugawan, T. L., Horn, G. T., Mullis, K. B., and Erlich, H. A., "Analysis of Enzymatically Amplified β-globin and HLA-DQα DNA with Allele-Specific Oligonucleotide Probes," *Nature*, Vol. 324, 1986, pp. 163–166.
[3] Higuchi, R., von Beroldingen, C. H., Sensabaugh, G. F., and Erlich, H. A., "DNA Typing from Single Hairs," *Nature*, Vol. 332, 1988, pp. 543–546.
[4] Saiki, R. 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 of the U.S.A.*, Vol. 86, 1989, pp. 6230–6234.
[5] Horn, G. T., Bugawan, T. L., Long, C., and Erlich, H. A., "Allelic Sequence Variation of the HLA-DQ Loci: Relationship to Serology and Insulin-Dependent Diabetes Susceptibility," *Proceedings of the National Academy of Sciences of the U.S.A.*, Vol. 85, 1988, pp. 6012–6016.
[6] Gyllensten, U. B. and Erlich, H. A., "Generation of Single-Stranded DNA by the Polymerase Chain Reaction and Its Application to Direct Sequencing of the HLA-DQα Locus," *Proceedings of the National Academy of Sciences of the U.S.A.*, Vol. 85, 1988, pp. 7652–7656.
[7] *Immunobiology of HLA*, Tenth International HLA Workshop, B. Dupont, ed., 1989, p. 984.
[8] Erlich, H. A. and Bugawan, T., "HLA Class II Gene Polymorphism: DNA Typing, Evolution, and Relationship to Disease Susceptibility," *PCR Technology: Principles and Applications for DNA Amplification*, H. Erlich, ed., Stockton Press, New York, 1989, p. 193.
[9] Scharf, S. J., Freidmann, A. Brautbar, C., Szafer, F., Steinman, L., Horn, G., Gyllensten, U., and Erlich, H. A., "HLA Class II Allelic Variation and Susceptibility to Pemphigus Vulgaris," *Proceedings of the National Academy of Sciences of the U.S.A.*, Vol. 85, 1988, pp. 3504–3508.
[10] Scharf, S. J., Freidmann, A., Steinman, L., Brautbar, C., and Erlich, H. A., Specific HLA-DQB and HLA-DRBI Alleles Confer Susceptibility to Pemphigus Vulgaris," *Proceedings of the National Academy of Sciences of the U.S.A.*, Vol. 86, 1989, pp. 6215–6219.
[11] Erlich, H. A., Scharf, S., Long, C. M., and Horn, G. T., (1989) "Analysis of Isotypic and Allotypic Sequence Variation in the HLA-DRβ Region Using the In Vitro Enzymatic Amplification of Specific DNA Segments," *Immunobiology of HLA*, B. Dupont, ed., 1989, p. 181.

New Tubular Hollow Point Ammunition

Dear Sir:

I recently came across commercially available ammunition whose unique projectile shape, high velocity, and overall construction will be of interest to firearms examiners and medical personnel. These rounds are the .38 Special + P and .44 Special Ultramag tubular bullets sold under the PMC™ (Precision Made Cartridges) label by the Eldorado™ Cartridge Corp. (Boulder City, Nevada). Figure 1 shows the unique projectile shape (intact and cross-sectioned), a complete round, and the major components (minus the powder charge) of the .38 Special + P ammunition.

The .38 caliber 66-grain Tubular Hollow Point (THP) and .44 caliber 110-grain THP are projectiles whose hollow cavity extends completely through the bullet. The bullet composition is homogeneous and not copper-plated lead. The relatively sharp leading edge of the bullet, coupled with the minimal frontal area and structural integrity inherent in an all-copper projectile, may produce remarkable penetration, atypical terminal ballistic performance, and unique ricochet characteristics.

The low weight of these two projectiles means that the velocity at which they are delivered can be markedly increased. The manufacturer's literature covering their 1990 sporting ammunition lists the muzzle velocity of the .38 Special + P 66-grain THP at

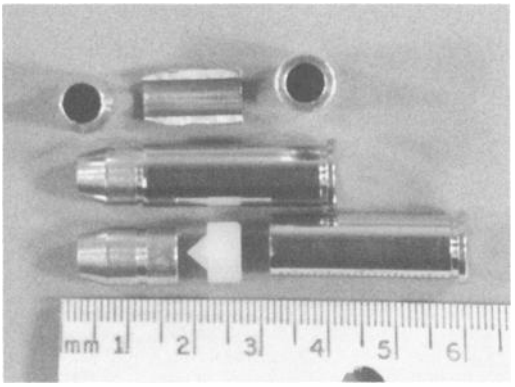

FIG. 1—*The .38 Special + P tubular hollow point (THP):* (top row) *complete projectile viewed from the ogive, the horizontally cross-sectioned round, the complete projectile viewed from the base:* (middle) *the complete .38 Special + P round;* (bottom) *the complete projectile viewed horizontally, the nonmetallic follower, and the cartridge case.*

1542 ft/s (470 m/s) and that of the .44 Special 110-grain THP at 1200 ft/s (366 m/s).[1] These velocities are in stark contrast to those of the typical solid projectile, commercially loaded, .38 Special + P ammunition, which has a muzzle velocity in the ±1000-ft/s (305-m/s) range, and the .44 Special ammunition, whose velocities are in the ±825-ft/s (251-m/s) range.[2]

The overall construction of the .38 Special + P ammunition is typical, with the exception of a nonmetallic follower behind the THP which acts as a gas seal. This follower, which does not appear to be attached by any means to the THP, has a tapered nose that may facilitate alignment with the THP during firing. Like a shotgun shell wad or sabot from other types of ammunition, the follower can inflict wounds and may provide a method of determining the range of fire based on separation or nonseparation of the follower from the THP before impact with the target. However, unlike a sabot, which encases a subcaliber rifle bullet, preventing the bullet from engaging the rifling, this follower allows the THP to engage the barrel rifling directly. The result is that both the THP and the follower can be compared with each other, and both the projectile and the follower can be compared with a suspect firearm to determine whether a particular weapon fired that round.

Given that this ammunition is not yet available in many retail stores, I would be interested in hearing of criminal cases that involved the use of the THP.

Paul A. Halvorsen, B.S., M.P.A.
M.F.S. Candidate
The George Washington University
P.O. Box 8590
Temple Hills, MD 20757

[1]Data extracted from the "PMC™ Sporting Ammunition for 1990" consumer handout. Copyright 1990 by Eldorado™ Cartridge Corp. (Box 308, Boulder City, NV 89005).

[2]Data contained in the 1990 "Ammunition Guide" consumer handout published by Winchester/Olin Corp. (East Alton, IL 62024) list .38 Special + P ammunition using projectiles that weigh 95, 110, 125, and 158 grains, having muzzle velocities of 1100, 995, 945, and 890 ft/s (335, 303, 288, and 271 m/s), respectively. Also listed are .44 Special 200- and 246-grain rounds having muzzle velocities of 900 and 755 ft/s (274 and 230 m/s), respectively. These rounds are available from commercial sources. Data for handloaded ammunition were not reviewed, as the component parts for the THP (bullet and follower) are not listed as commercially available in the "PMC™ Sporting Ammunition for 1990" consumer handout.

Role of Forensic Science in a Democracy

Dear Sir:

We are witness to electrifying changes in the world. Historical processes that most expected would take generations are happening overnight. Who would have imagined a year ago that we would be seeing the beginnings of democracy in Central America, Eastern Europe, and the Soviet Union? We look on in awe as the human condition strives towards freedom—freedom of individual expression, free elections, legitimate dissent, and a free press, to name but a few.

Modern Western society is built on the pillars of democratic institutions and the rule of law. The individual, not the state, is paramount. Personal rights usually take precedence over what may be in the best interests of society. In the United States, for example, there is a tension between the law and the state which provides numerous checks and balances to safeguard individual liberties.

Forensic science provides one of many checks and balances critical to the administration of justice. Judges and juries require experts to explain technical matters that are beyond the knowledge or understanding of lay people. The courts have also recognized imperfections in our legal system. The facts presented are not always what they seem. Experience has shown that abuse of authority and improper and sometimes unlawful police procedures have occurred in the course of criminal investigations. Eyewitness testimony is not as certain as some believe. Juries, too, recognize that impartial scientific evaluation of physical evidence clarifies issues and frequently illuminates other evidence presented to the trier of fact.

For forensic science practitioners to perform their function properly within the legal system, they must exercise independence and integrity. Stated simply, forensic scientists cannot be biased for or against an investigation in which they are involved. Their job is to champion their expert opinions, based on accepted, properly performed scientific inquiry. Forensic scientists who understand their role in a democratic criminal justice arena help to protect individual rights and freedoms while ensuring that justice is delivered.

We wish our Soviet, Eastern European, and Central American colleagues every success in their efforts to bring about democracy in their respective countries. We should also endorse mutual cooperation in efforts to advance the forensic sciences.

Barry A. J. Fisher, M.S.
Director, Scientific Services Bureau
Los Angeles County Sheriff's Department
2020 W. Beverly Blvd.
Los Angeles, CA 90057-2494