TECHNICAL NOTE

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Report of the Blind Trial of the Cetus AmpliType HLA DQα Forensic Deoxyribonucleic Acid (DNA) Amplification and Typing Kit

REFERENCE: Walsh, P. S., Fildes, N., Louie, A. S., and Higuchi, R., **"Report of the Blind Trial of the Cetus AmpliType HLA DQα Forensic Deoxyribonucleic Acid (DNA) Amplification and Typing Kit,"** *Journal of Forensic Sciences*, JFSCA, Vol. 36, No. 5, Sept. 1991, pp. 1551–1556.

ABSTRACT: The AmpliType™ HLA DQα forensic DNA amplification and typing kit is designed for the qualitative analysis of the human leukocyte antigen (HLA) DQα alleles present in deoxyribonucleic acid (DNA) extracted from forensic samples. The AmpliType kit is the first forensic DNA typing product based on the GeneAmp™ polymerase chain reaction (PCR) process [*1–3*]. The kit was evaluated by five forensic science laboratories (test sites) to assess their ability to perform DNA typing using PCR on sample types typically encountered by forensic laboratories. None of the DNA-containing samples was mistyped. Of the 180 DNA-containing samples analyzed, results were reported for 178 (98.9%). Of the 178 samples with results, all were correctly typed. Two sites did not report a result for one sample each. Four of the five laboratories experienced no significant levels of contamination in the DNA-containing samples. At the one site with the highest number of DNA-containing samples with contamination, the typing results were not compromised. This site was able to correct the contamination problem through simple procedural changes and stricter attention to sterile technique. Blank controls were important to monitor contamination. In conclusion, the trial demonstrated that forensic science laboratories are capable of setting up a PCR-based DNA typing laboratory and successfully using the AmpliType HLA DQα forensic DNA amplification and typing kit to analyze forensic samples.

KEYWORDS: forensic science, deoxyribonucleic acid (DNA), genetic typing

The AmpliType™ HLA DQα forensic DNA amplification and typing kit[2] is comprised of deoxyribonucleic acid (DNA) amplification reagents [including polymerase chain reaction (PCR) mixes], DNA probe strips, and typing reagents to analyze qualitatively the human leukocyte antigen (HLA) DQα alleles present in forensic science samples [*4–7*].

Received for publication 8 Nov. 1990; revised manuscript received 9 Jan. 1991; accepted for publication 14 Jan. 1991.

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DNA is first extracted from a sample and then amplified using the PCR reaction mix. The resulting amplified DNA is then hybridized to a DNA probe strip and subsequently washed to remove unbound material. Enzyme conjugate (horseradish peroxidase) and chromogen (3,3′,5,5′-tetramethylbenzidine) are used to develop the hybridized DNA probe strip, with the bound DNA visualized colorimetrically by the formation of blue dots on the probe strip. As shown on Fig. 1 (photograph of a developed strip of DQα 1.1,4), the strip contains probes to detect the six common alleles (DQα 1.1, 1.2, 1.3, 2, 3, and 4). A result can be determined only when the control (C) dot is visible. Dots darker than C are considered positive. (Details for interpretation of strips are described in the AmpliType User Guide.) There are 21 different genotypes detected using the AmpliType kit.

This trial was designed to determine whether forensic scientists who have not previously used DNA amplification techniques can set up a PCR-based DNA typing laboratory, properly prepare samples for analysis by PCR, and use the AmpliType kit to amplify and type samples commonly encountered in a forensic science laboratory. Five forensic science laboratories participated in the trials: Connecticut State Police Forensic Science Laboratory, Meriden, Connecticut; Illinois State Police, Bureau of Forensic Sciences, Carbondale, Illinois; Metro-Dade Police Crime Laboratory, Miami, Florida; North Carolina State Bureau of Investigation, Raleigh, North Carolina and Orange County Sheriff's Department Crime Laboratory, Santa Ana, California. For this report, each site was assigned a color-coded designation to maintain anonymity (the color codes were blue, green, purple, red, and yellow).

Participants from each laboratory had three opportunities to apply the kit to samples typical of forensic casework. First, each trial participant completed a three-day training session at Cetus Corp., where they analyzed four samples. Upon completion of the training, the participants set up their laboratories for PCR-based DNA analysis. Next, a panel of 23 "known" samples was provided to each laboratory. Each group typed these samples correctly. Finally, each site received 35 "blind" samples (including blank controls). The results of the blind trial are discussed in this report.

Identification and Preparation of Blind Samples

The blind trial included 6 different sample types: purified DNA, bloodstains, plucked hairs, a semen stain, buccal swabs, and postcoital samples. The 4 postcoital samples (and 2 swabs used as postcoital blank controls) were separated by the laboratories into male and female fractions [8], which were each considered to be a separate sample. The shaft of 1 hair sample was used as a hair blank control. In total, each participating laboratory analyzed 36 DNA-containing samples, including 8 postcoital fractions and 6 blank controls (1 reagent control, 4 fractions from the swab controls, and 1 hair shaft blank). Each of the 42 samples was analyzed in duplicate. Except for the purified DNA and hair, the samples were cut into thirds, and separate extractions were performed on two of the

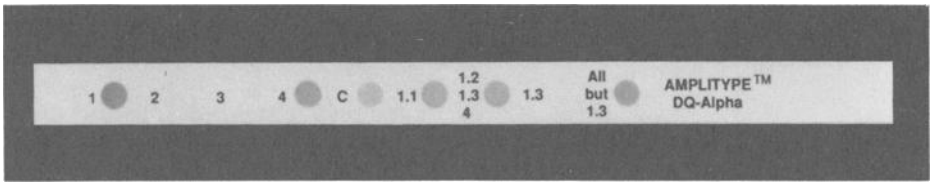

FIG. 1—*Photograph of a developed AmpliType HLA DQα probe strip (type DQα 1.1, 4).*

three pieces. Each of these extracts was then amplified and typed. For the purified DNA and hair samples, a single extract was amplified and typed twice. In total, each site generated 84 typing results (developed DNA probe strips) from the 42 samples. Each site analyzed identical samples and blank controls.

Bloodstain samples were made from blood specimens of known DQα type. Hair samples were collected by plucking and were selected for the presence of sheath material. The semen stain was ten years old. Buccal samples were collected by vigorous swabbing. Postcoital samples were collected by vaginal swabbing or by drainage onto a clean panty liner. After air drying, all samples were stored at −20°C until use. A list of samples and blank controls is provided in Table 1.

All six DQα alleles that are distinguished by this typing system were represented among the samples. Of 21 possible genotypes, 15 were represented. The genotypes not represented were 1.1,1.3; 1.2,2; 1.3,2; 1.3,3; 2,2; and 2,4.

Blind Trial Results

The laboratories (test sites) were asked to prepare, amplify, and type the blind trial samples and to record the results on a form provided by Cetus. The sites were also asked to photograph the developed strips and to submit the photographs along with the recorded results to Cetus upon completion of the study. After test site Purple found contamination in amplifications from some DNA-containing samples and blank controls (as indicated by extraneous dots on the developed probe strips), this laboratory proposed and completed additional testing of those samples. The remaining third piece of sample for 10 DNA-containing samples and 4 blank controls was reexamined. No incorrect result was reported during either the initial or repeat testing by test site Purple. Test site Red also performed additional studies on bloodstain samples after finding apparent PCR inhibition by a component present in bloodstain extracts.

The results reported by the test sites for DNA-containing samples were grouped into three categories: "DNA result reported," "incorrect result reported," and "no result reported." The "DNA result reported" category included DNA-containing samples for which the site reported a DNA type. "Incorrect result reported" samples were DNA-containing samples for which the type reported by the site differed from the reference type. "No result reported" samples were DNA-containing samples for which the site did not report a type.

TABLE 1—*Blind trial list of samples.*[a]

Number of Samples	Sample Type	Number of Strips	Comments
3	Purified DNA samples	6	
1	Reagent control	2	
13	Bloodstains	26	including 1 ten-year-old sample
5	Plucked hair samples	12	the shaft of one hair was used as a negative control
6	Buccal swabs	12	
1	Semen stain	2	ten-year-old stain
4	Postcoital samples	16	separated into male and female fractions
1	Cotton liner control	4	separated into male and female fractions
1	Dacron swab control	4	separated into male and female fractions

[a]Total = 35 samples; total developed strips = 84.

As shown in Table 2, 178 out of 180 (98.9%) of the samples were successfully typed ("DNA results reported") and no sample produced an "incorrect result." Two samples (1.1%) were reported as "no result." For a few samples, no result was obtained for one of the two duplicates. However, the correct DQα type was always reported for the duplicate giving a result. No discrepancies were reported between duplicates for samples where results were reported for both duplicates.

Photographs of the strips were used to evaluate contamination. This analysis was performed by a Cetus reviewer. Contamination for a DNA-containing sample was defined by the reviewer as the presence of any dots other than those expected from the DQα type of the sample. Contamination for a blank control was defined by the reviewer as the presence of any dots, particularly noting whether the C dot was detectable. The number of blank control strips in which detectable dots were present but a C dot was absent is footnoted in Table 3. In those cases noted, the detectable contaminants were very faint.

Four of the five test sites experienced little or no contamination of the DNA-containing samples. Several sites, however, did experience contamination of the blank control samples. Results are shown in Table 3. Test site Purple, which experienced the highest number of samples having contamination, was able to adjust its sample preparation procedures to resolve the problem. This test site repeated 14 samples (10 DNA-containing samples and 4 blank controls) and experienced no contamination.

Discussion

The test sites had little or no prior experience with either PCR or the AmpliType HLA DQα kit prior to the study and were given only limited training prior to the evaluation. Nevertheless, of the 180 DNA-containing samples analyzed, results were reported for 178 (98.9%). Of the 178 samples with results, all were correctly typed. One site did not report a result because of the presence of mixed alleles from incomplete separation of the male fraction from the female fraction of a postcoital sample. Also, one of the sites reported "no result" for one sample which did not produce a clear type for either duplicate strip. In no case did a site report an incorrect result for any DNA-containing sample, even in the presence of background contamination.

Due to the sensitivity of PCR, contamination has been a concern [9]. Little or no contamination occurred during the testing of the panel of known samples. Table 3 summarizes the contamination found during the blind trial. Two of the five laboratories (Red and Yellow) experienced essentially no contamination in either samples or blank controls. Two sites (Blue and Green) experienced a moderate number of blank controls with contamination and low numbers of samples with contamination. At the site where the most contamination was experienced (Purple), the typing results were not compromised, even prior to repeat testing. The site was able to correct the problem through implementation of simple procedural sample handling precautions.

TABLE 2—*Blind trial summary results from DNA-containing samples.*

Site	DNA Result Reported	Incorrect Result Reported	No Result Reported
Blue	35/36	0/36	1/36
Green	36/36	0/36	0/36
Purple	36/36	0/36	0/36
Red	35/36	0/36	1/36
Yellow	36/36	0/36	0/36
Total	178/180 (98.9%)	0/180 (0%)	2/180 (1.1%)

TABLE 3—*Blind trial summary comparison of contamination by test site.*

Site	Contaminated Strips	
	DNA Samples, total No.	Blanks, total No.[a]
SITES EXPERIENCING LOW LEVELS OF SAMPLE CONTAMINATION[b]		
Yellow	0	0
Red	1	1
Green	1	6
Blue	1	5
SITE EXPERIENCING HIGH LEVELS OF SAMPLE CONTAMINATION		
Purple[c]	8	9
Purple (repeat testing)	0	0

[a]Four of the contaminated blanks had no detectable C dot. The contaminating dots present were barely visible.

[b]Original testing at all sites was composed of 72 DNA-containing samples and 12 blank controls. Repeat testing for test site Purple was composed of 10 DNA-containing samples and 4 blank controls.

[c]For one strip from a DNA-containing sample, the intensity of one dot, because of contamination, was greater than the C dot. The site interpreted the dot as probable contamination, based both on the absence of this extra dot on the duplicate strip and on the presence of other contaminating dots (less intense than the C dot) on this strip. Based on this assumption, site Purple was able to determine correctly a type for the sample.

Blank controls were found to be an important monitor of contamination. At those sites experiencing low instances of contamination, only blank controls were affected and no C dots were visible on the strip. At those sites with increased numbers of blank controls with contamination, a few DNA-containing samples were affected. The site experiencing the greatest number of contaminated blank control strips, test site Purple, also had a considerable number of sample strips with contaminant dots. Yet, even this site reported the correct types for all DNA-containing samples. Although very high levels of contamination in a particular sample might be expected to interfere with typing, especially with samples containing low amounts of DNA, the levels seen in this trial did not affect the results. In casework, the possibility that light dots may not be contaminants but might result from the unequal mixture of two genotypes should be considered. The circumstances of the evidence (for example, a sample known to be mixed body fluids) and the results of retesting should be taken into account.

Contamination found during the trials appeared to originate primarily from cross-contamination during sample handling. This conclusion is based on several observations. First, the contaminants were a range of types, suggesting multiple sources. Second, no DNA-containing sample showed contamination at more than one site, suggesting that the samples themselves were not the source of the contamination. Third, many of the contaminant types corresponded to the types of the samples handled previously within the same set. Finally, and most significantly, test site Purple was able to eliminate contamination by a few simple changes designed to reduce sample cross-contamination. These changes included taking more time and handling fewer samples (no more than approximately 16 samples per set), using a microfuge tube decapper that does not touch the

inside of the cap (such as one from Robbins Scientific), and avoiding physical transfer between samples and gloves, dissecting surfaces, and cutting implements.

Inhibition of amplification by extracts from bloodstains was observed in this trial. Inhibition of PCR by components of blood (for example, porphyrin compounds) has been previously reported [7]. The Red site, in particular, initially found inhibition from most of their bloodstain samples. This inhibition was overcome by simple dilution of the bloodstain sample DNA extract to be amplified. The inhibition does not appear to be purely sample dependent since each bloodstain sample was successfully amplified by at least four of the five test sites.

In summary, these trials demonstrated that a forensic science laboratory inexperienced with PCR can set up a PCR-based DNA typing program and successfully use the AmpliType HLA DQα forensic DNA amplification and typing kit to analyze forensic samples. Blank controls were shown to be a useful monitor of contamination. Two of the laboratories were able to type a blind sample set with no contamination; even those laboratories which experienced contamination still successfully typed samples; and the laboratory with the most severe contamination could eliminate it with simple procedural changes. The few problems encountered by the sites were corrected with minor procedural modifications and stricter attention to sterile technique. These modifications have been incorporated into either the AmpliType kit package insert or the AmpliType User Guide where appropriate. In particular, additional precautions regarding sample handling have been included to minimize contamination. Procedures designed to address inhibition by bloodstains also have been included.

Acknowledgments

We would like to thank Mike DiGuglielmo, John Hartman, Dr. Roger Kahn, Rob Kiester, Dr. Henry Lee, David Metzger, and Elaine Pagliaro for their participation in the trial. We are also grateful to Jan Bashinsky, Ed Blake, Rock Harmon, and Mark Nelson for review of the manuscript.

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