TECHNICAL NOTE

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A Systematic Analysis of PCR Contamination*

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ABSTRACT: In light of the strict legal scrutiny surrounding DNA typing at this time, it has become necessary to systematically address the issue of PCR contamination. To precisely define the parameters affecting PCR contamination under casework analysis conditions, PCR amplification reactions were intentionally compromised by employing sub-standard laboratory technique and by introducing secondary sources of DNA.

The PCR parameters considered for potential sources of contamination include amplification set-up, amplification product handling, aerosol DNA and storage. In addition, analyst technique was evaluated by modifying or eliminating standard safeguards.

Under the circumstances normally encountered during casework analysis, PCR contamination was never noted. Significantly, using the dot blot detection method, contamination was never observed when nanogram quantities of genomic DNA were mishandled or aerosolized. Contamination occurred only when amplification product was carelessly manipulated or purposefully sprayed near or directly into open tubes containing water or genomic DNA. Although standard precautions should be employed during PCR-based DNA typing, our data indicates that contamination during amplification procedures is not prevalent when detected by dot blot analysis.

KEYWORDS: forensic science, DNA typing, polymerase chain reaction, contamination, HLA-DQA1, LDLR, GYPA, HBGG, D7S8, GC, validation

Forensic organizations such as the Technical Working Group on DNA Analysis Methods (TWGDAM) and the American Society of Crime Laboratory Directors (ASCLD) have endorsed guidelines designed to minimize the possibility of contamination during PCR analysis (1,2). Some individuals in the forensic and legal communities favor even more rigorous safeguards. Many forensic laboratories currently employ PCR methods, and new PCR systems are being introduced with the advent of STR analysis. With increasing numbers of DNA tests available and the legal scrutiny surrounding PCR typing, it is necessary to systematically address the issue of PCR contamination.

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We have considered four general aspects of PCR typing during which contamination might occur: amplification set-up, handling of amplification product, aerosolization, and DNA storage. To define precisely the parameters affecting PCR contamination, PCR reactions were intentionally compromised by employing sub-standard laboratory techniques and by introducing secondary sources of DNA. Our standard casework protocols for the PM + DQA1 test were used, and detection was by reverse dot blot. The results demonstrate that contamination was possible, primarily when gross deviations from standard laboratory procedures were practiced. Our study provides evidence that contamination is difficult to achieve when using the dot blot detection method in PCR analysis.

Methods

General

Unless otherwise noted, gloves and plugged pipette tips were used throughout all procedures, which were the standard casework analysis protocols for the Connecticut State Police Forensic Science Laboratory. For all experiments, water (20 μL), genomic DNA (10 ng) or amplification product (1 μL) were amplified using the Amplitype PM and/or DQA1 systems (Perkin Elmer Biosystems, Foster City, CA). We routinely amplify 10 ng of genomic DNA for our casework samples. All amplification tubes were spun down before further use. For the aerosol experiments, DNA was extracted from 200 μL of liquid blood using the QIAamp Blood Kit (QIAGEN Inc., Valencia, CA), and 10 ng of the recovered genomic DNA were amplified. The success of amplification was assessed by electrophoresis of ten percent of the reaction volumes in 1% agarose gels, visualized by ethidium bromide staining according to standard protocols. "No amp" was noted when there was no visible amplification product; when any visible product was present, genotypes were determined according to manufacturer's protocols (4). All experiments were performed using genotypes which are easily resolved in mixtures. The genotypes and experimental conditions are summarized in Table 1.

Amplification Set-up

To address the issue of poor technique, amplifications were set up on a bench top in either the genomic DNA preparation room or the amplification product analysis room. All tubes were open throughout the process, and the standard use of gloves and plugged tips was omitted. To detect whether genomic DNA could be carried

TABLE 1—*Summary of genotypes and experimental conditions.*

Genotypes	#1 "contaminating" genotype (4.1)	
	#2 starting genotype (1.2, 1.3)	
	#3 analyst genotype (1.2, 2)	
Conditions	Amplification set-up	poor technique
		residual genomic DNA carryover
		contamination by analyst
	Handling	centrifugation
		ejecting tips
		product gel
		opening/closing tubes
		detection (strips)
	Aerosol	amp product or genomic DNA
		upward or downward
	Storage	

over from one tube to another, amplification reactions were set up without changing the pipette tip between samples, thereby adding the residual genomic DNA (less than one drop) to the next tube (blank) containing water (20 μL). Possible contamination by the analyst was evaluated by adding hair (~5 mm with and without a root), dead skin, blood (1 μL), saliva (1 μL), and sneezing over tubes containing either water or genomic DNA.

Handling

To evaluate the potential for aerosol contamination during microcentrifugation, capped and uncapped tubes of amplification product were spun for one minute at full speed next to capped or uncapped tubes of water (20 μL), genomic DNA (10 ng), or amplification product (20 μL) of another genotype. We normally perform a brief full speed pulse spin. Capped or uncapped tubes of water, genomic DNA, or amplification product were placed on the bench next to the microfuge. The effect of aerosol generated during pipetting was evaluated by ejecting tips containing residual genomic DNA or amplification product in front of open tubes of water (20 μL) or genomic DNA (10 ng).

To address the issue of possible contamination during routine manipulation of amplification product, tubes containing amplification product (20 μL) were opened, 10 μL of product were removed to a new tube and a product gel was loaded. A rack with open tubes of water (20 μL) was on the bench during this process. In addition, tubes of amplification product were opened and closed 1 time, 5 times, and 20 times in front of open tubes of water (20 μL) or genomic DNA (10 ng). Tubes of two different amplification products were alternately opened and closed 20 times (i.e., open #1, open #2, close #1, close #2) without changing gloves. The experiment was repeated using one tube of amplification product and one tube of genomic DNA. Finally, the effect of cross-contamination during strip processing was examined by adding amplification product (1–10 μL) to a tray containing hybridization solution and a different amplification product (20 μL). The strips were processed according to the manufacturer's instructions, and the genotypes recorded.

Aerosol

Amplification product (20 μL) or genomic DNA (10 ng) was sprayed (using a Rainin pipettor) upward at a 45° angle 6 in., 3 ft., and 6 ft. from open tubes containing water (20 μL), genomic DNA (10 ng), liquid blood (200 μL) or amplification product (20 μL). The same protocol was performed spraying amplification product directly downward in front of open tubes of water only.

Storage

Open tubes of water (20 μL) or genomic DNA (10 ng) were stored next to open tubes of amplification product (20 μL) of a different genotype in the freezer. The tubes were arranged such that each experimental tube was adjacent to a tube containing amplification product. Each day (for five days), one tube each of water and genomic DNA was removed and amplified.

Results and Discussion

Genotypes which are easily resolvable in mixtures were used in this study. Genomic DNA #1 was designated as the contaminating genotype (4.1), genomic DNA #2 as the starting genotype (1.2,1.3), and genomic DNA #3 as the genotype of the analyst (1.2,2). Experiments were designed to address four general aspects of PCR analysis: amplification set-up, handling, aerosol and storage (Table 1).

Amplification Set-Up

Attempts were made to compromise amplification set-up in several manners (Table 2). First, separate reactions containing genomic DNA #1 and #2 were prepared either in the genomic DNA extraction room or the amplification product analysis room. Routine contamination preventative measures were omitted; no gloves or plugged tips were used, and the tubes were open at all times. In each case, only the original genotype was detected after amplification. Secondly, five amplification reactions were set up with genomic DNA #1. The pipette tip was not ejected after the addition of the genomic DNA to the reaction tubes, and the residual liquid was added to a blank tube of water. No product was detected upon amplification of these blank tubes. Finally, the possibility of contamination by the analyst was evaluated by adding hair (with or without the root), dead skin, blood, saliva, or by sneezing into a blank tube of water or genomic DNA #1. None of the water tubes yielded amplification product, and only genotype #1 was detected in the tubes with genomic DNA. No amplification product was detected in the tube designated genomic DNA #1 plus blood, presumably due to heme inhibition (5).

TABLE 2—*Amplification set-up.*

Condition	Input	Result
Poor technique	genomic #1	#1
	genomic #2	#2
Genomic carry-over	water + residual genomic #1	no amp
Analyst	water + hair (root)	no amp
	hair (no root)	no amp
	skin	no amp
	blood	no amp
	saliva	no amp
	sneeze	no amp
	genomic #1 + hair (root)	#1
	hair (no root)	#1
	skin	#1
	blood	no amp
	saliva	#1
	sneeze	#1

Handling

Handling of amplification product was broken down into distinct situations normally encountered during PCR analysis: centrifugation, tip ejection, product gel analysis, opening and closing tubes, and typing of amplification product on strips.

Centrifugation

In separate trials, capped or uncapped tubes of amplification product #1 were centrifuged next to and across from capped or uncapped tubes of water, genomic DNA #2 and amplification product #2. Capped and uncapped tubes of water and genomic DNA #2 were positioned on the bench near the microcentrifuge during this procedure. The results of these experiments are summarized in Table 3. Contamination was noted only when uncapped amplification product was spun next to uncapped water tubes. Genomic DNA #2 and amplification product #2 yielded only genotype #2 under all conditions tested, with no evidence of contamination by amplification product #1. No contamination was seen in any of the tubes on the bench adjacent to the microcentrifuge (data not shown).

Tip Ejection and Product Gel Analysis

Pipette tips containing residual genomic DNA #1 or amplification product #1 were ejected in front of a rack with open tubes of water or genomic DNA #2. After amplification, no product was recovered from the water tubes, and only genotype #2 was detected in the genomic DNA #2 tubes. There was no evidence of genotype #1 in any of the reactions (data not shown). Next, tubes containing amplification product #1 were opened, 10 μL aliquots were removed to new tubes and loaded onto a gel in front of a rack with tubes of water. No amplification product was obtained from any of the water tubes after amplification (data not shown).

Opening/Closing Tubes

Tubes containing amplification product #1 were opened and closed 1, 5, and 20 times in front of open tubes of water or genomic DNA #2. One third of the water tubes and genomic DNA tubes were closed and removed at each interval. These tubes were amplified, and the results are shown in Table 4. Amplification product was never detected in the water tubes, even after the amplification product #1 tubes had been opened and closed 20 times. Furthermore, only genotype #2 was detected in the tubes of genomic DNA #2, with no evidence of contamination by amplification product #1. In addition, tubes of amplification product #1 and genomic DNA #2 were alternately opened and closed 20 times, and then amplified. In this case, both genotypes #1 and #2 were detected in the amplification reaction from genomic DNA #2 (see Table 4). This experiment was repeated using amplification product #1 and amplification product #2, and no cross-contamination was seen (data not shown).

TABLE 3—*Handling—centrifugation.*

Input		Result
Amp product #1 capped +	water capped	no amp
	water uncapped	no amp
	genomic #2 capped	#2
	genomic #2 uncapped	#2
	amp product #2 capped	#2
	amp product #2 uncapped	#2
Amp product #1 uncapped +	water capped	no amp
	water uncapped	#1
	genomic #2 capped	#2
	genomic #2 uncapped	#2
	amp product #2 capped	#2
	amp product #2 uncapped	#2

TABLE 4—*Handling—opening/closing tubes.*

Input	Result
Water 1×*	no amp
5×	no amp
20×	no amp
Genomic DNA #2 1×*	#2
5×	#2
20×	#2
Amp Product #1†	#1
Genomic DNA #2†	#1, #2

*Amp product #1 tubes were opened and closed 1×, 5×, and 20× in front of open tubes of water or genomic DNA #2.

† Amp product #1 and genomic DNA #2 tubes were alternately opened then closed 20×.

TABLE 5—*Handling—typing on strips.*

Input (μL of amp product #2)*	Genotype
0	1.2, 1.3
0.5	1.2, 1.3
1.0	1.2, 1.3
2.5	1.2, 1.3, 4.1 < C
5–10	1.2, 1.3, 4.1†

*20 μL of genotype #1 amp product + indicated amount of genotype #2 amp product.

† At 5 μL, 4 > C but 4.1 < C, 6–10 μL, 4.1 > C.

Detection

The effect of adding a small amount of a second amplification product during strip hybridization was investigated. Twenty μL of amplification product #2 (genotype 1.2, 1.3) were added to DQA1 strips along with one to 10 μl of secondary amplification product #1 (genotype 4.1; Table 5). The presence of the secondary genotype (4.1) was detected definitively (according to laboratory and manufacturer's guidelines) at 5 μL of amplification product. The 4.1 allele was first observed with 2.5 μL of secondary amplification product, but the intensity of this dot was less than that of the control.

Aerosol

Amplification product #1 was sprayed upward at various distances from open tubes of water, genomic DNA, liquid blood or amplification product (all genotype #2). The results are presented in Table 6. The genotype of the aerosolized DNA (genotype #1) was detected in all of the water tubes. A mixture of the original genotype (#2) and that of the aerosolized DNA (#1) was seen in the genomic DNA and liquid blood tubes at the greatest distance from the source of aerosol. The expected genotypes were obtained from all other target tubes. No contamination was seen in any of the target tubes when genomic DNA was used as the source of aerosol

TABLE 6—*Aerosol.*

Input	Result (at Distance Noted)		
	6 in.	3 ft	6 ft
water*	#1	#1	#1
genomic DNA #2*	#2	#2	#1, #2
liquid blood #2*	#2	#2	#1, #2
amp product #2*	#2	#2	#2
water†	no amp	no amp	no amp

*20 μL amp product #1 aerosolized upward.
†20 μL amp product #1 aerosolized downward.

(data not shown). The experiment was repeated by spraying amplification product downward at the same distances from tubes containing water, since the water tubes all showed contamination in the previous experiment. In this case, no contamination was seen in tubes at any distance (Table 6.)

Storage

Open tubes of water or genomic DNA #2 were stored in the freezer for one to five days next to open tubes of amplification product #1. Each day, a duplicate set of both water and genomic DNA tubes was removed and amplified. No amplification was seen in the water tubes, and the genomic DNA tubes yielded genotype #2 (data not shown).

We have found that it is possible to compromise the results of PCR analysis, but only under extreme circumstances which should not occur during normal laboratory practice. Contamination was detected by the dot blot method only when amplification product was carelessly manipulated or purposefully sprayed near or directly into open tubes containing water or genomic DNA. Deliberate spraying of amplification product adversely affected the amplification results, but this type of "contamination" was due to overt operator error and required substantial effort.

We saw no evidence of contamination during various stages of PCR analysis, even when introducing deliberate procedural errors which might occasionally occur under standard laboratory conditions. Results were never compromised during amplification set-up, even when reactions were assembled in the amplification product analysis area without the use of conventional precautions such as gloves and plugged tips. Contamination of PCR reactions by body fluids, shed cells or hairs of the analyst was never observed. Although a laminar hood is good protection against contamination, our data indicate that it is not necessary for PCR analysis using a dot blot detection system.

Amplification product was handled per standard laboratory practice (i.e., microcentrifugation, opening and closing tubes, ejecting tips, and loading gels), even very carelessly, without evidence of contamination. The exceptions were when open tubes of amplification product were centrifuged next to open tubes of water, or when genomic DNA and amplification product tubes were opened and closed together 20 times. However, these circumstances were artificially generated and are not typically encountered during casework analysis. Typing on strips was only affected when a secondary amplification product which constituted at least 25% of the volume of the primary product was added at the hybridization step.

We never saw contamination when nanogram quantities of genomic DNA were mishandled or aerosolized during PCR analysis. The "accidental" addition of residual genomic DNA to a new blank tube during set-up did not affect typing results. These findings are significant because they represent errors which are more realistic when performing routine PCR tests.

Conclusion

In conclusion, we have found that PCR contamination as detected by dot blot analysis is much less evident than generally supposed. Significant contamination occurred only with gross deviations from basic preventative protocols, and could not be generated by simple acts of carelessness. The physical separation of amplification product from genomic DNA recommended by TWGDAM is sufficient to prevent all instances of PCR contamination we observed. Furthermore, this physical separation is only necessary during PCR set-up, and we observed no contamination of samples through storage of genomic DNA and amplification product together, even when the tubes were open in the freezer.

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