TECHNICAL NOTE

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Substitution of H_2O for Formamide in the Sample Preparation Protocol for STR Analysis Using the Capillary Electrophoresis System: The Effects on Precision, Resolution, and Capillary Life*

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ABSTRACT: Water has been evaluated as a suitable solvent to replace formamide in the preparation of samples for capillary electrophoresis analysis. Critical aspects relevant to forensic analysis were studied to test the validity of the substitution. Correct assignment of allele labels was reproducibly performed on known samples, and size determination was found to be precise. Three hundred injections, regardless the method of preparation, may be run on a single capillary without loss of precision or resolution.

KEYWORDS: forensic science, short tandem repeats, DNA typing, capillary electrophoresis, resolution D3S1358, vWA, FGA, Amelogenin, THO1, TPOX, CSF1PO, D5S818, D13S317, and D7S820

Formamide has been used to effectively denature DNA for many applications. However, it poses a variety of health hazards and has been definitively linked to both liver and kidney toxicity in humans (1). Epidemiological studies have also shown an association with an increased incidence of testicular cancer (2–4). Additionally, animal studies have indicated a potential for embryo toxicity (5) and a possible role as a teratogen (6). In addition to implication as a health hazard, formamide has a potentially negative effect on capillary electrophoresis analysis. Formamide, which was not completely deionized or became ionized during storage or while on the autosampler, will be preferentially injected into a capillary (7). The effect of preferential formate ion injection will be particularly detrimental in forensic samples of either low DNA concentration or unequal mixtures. Replacing formamide with water will also eliminate the cost of the chemical, as well as its disposal. Thus, the present study was undertaken to determine if it was necessary to use this chemical for DNA identification applications with the capillary electrophoresis system. Analyses were performed at the following loci: D3S1358, vWA, FGA, Amelogenin, THO1, TPOX, CSF1PO, D5S818, D13S317, and D7S820 (8–16).

Our results indicate that samples may be prepared for forensic STR analysis in water instead of formamide with no change in results. Precision in size calling and reproducibility of allele assignment were comparable for both methods of sample preparation. Furthermore, no appreciable difference in resolution or fluorescene intensity was observed. Results reveal that without loss of resolution or precision, 300 samples can be injected on a single capillary by both methods, three times the number currently guaranteed by the manufacturer. This can represent a significant cost savings for high throughput, analytical laboratories such as state DNA databanks.

Materials and Methods

Allelic ladders supplied with the AmpF*l*STR Profiler I Kit (Perkin Elmer Applied Biosystems [PE ABI], Foster City, CA) contain pooled PCR products of all common alleles expected from amplification. The allelic ladders were prepared in a 0.2 mL thin walled tube by adding 24 uL deionized formamide (Amresco, Solon, OH), 1 uL Genescan 350 ROX (PE ABI) and 0.5 μL each of the blue, green and yellow ladders. Deionized formamide was frozen until use. Alternatively, 24 μL polished water was substituted for deionized formamide. The WinLab (Winokur, Bethel, CT) water polisher was used to produce American Society for Testing and Materials (ASTM) Type I (maximum specific conductance 0.06 micromho/cm, minimum specific resistance 18.0 megohms-cm) water, which was dispensed from the polisher on each day of use. Prior to loading, all samples were heat denatured for 3 min, then cooled on ice for 3 min. Samples were then transferred to a 48 tube autosampler and analyzed with the ABI Prism™ 310 Genetic Analyzer (PE ABI). Fragments were separated using a linear polymer, Performance Optimized Polymer 4 (POP4) (PE ABI), injected from a 1 mL syringe (Hamilton, Reno, NV) into a 47 cm by 50 μm i.d. capillary (PE ABI). The running buffer used was Genetic Analyzer Buffer with EDTA (PE ABI) diluted to 1 × concentration. The run module used was GS STR POP4 (1 mL) F (PE ABI) unmodified from the manufacturer. Injection conditions were 5 s at 15 kV. Run conditions were 60°C and 15 kV. Size determination (base pairs) was

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performed using the GeneScan™ software version 2.1 (PE ABI). Heavy smooth options were used for analysis, as recommended by the manufacturer. Allele assignment was then performed using Genotyper™. Data were collected during runs in which conditions such as polymer age, capillary age, and room temperature were monitored.

Results and Discussion

All 10,472 alleles (88 alleles per Profiler ladder, injected 119 times), regardless of whether they were prepared in formamide or Type I H_2O, were correctly assigned using the Genotyper software. Here "injection" is used to indicate the cycle for processing one sample, one time (one line on an injection sheet). "Run" is used to indicate all of the injections from a single injection list (the entire injection sheet). Precision was also comparable for both solvents as measured by standard deviation in base pairs. For example, the average standard deviation for allelic ladder prepared in formamide and injected over three separate runs was 0.0580 bp, while the average standard deviation for samples prepared in Type I H_2O and injected over three separate runs was 0.0582 bp. These values were tightly clustered within the required $+/-$ 0.5 bp window for samples prepared in formamide or H_2O. When the average standard deviation at each allele was compared for 30 injections of allelic ladder prepared in Type I H_2O versus formamide, injected over three separate runs, differences were generally evenly distributed across the size range of the multiplex, 111–317 bp, and neither method of preparation exhibited any advantage over this range.

Peak width and distance between two peaks determines degree of resolution. Therefore, resolution was evaluated using the formula, $R = [2 \ln 2]^{1/2}(T_2 - T_1)/(HW_1 + HW_2)$ where T represents the scan number (describes the location of the data point), and HW represents the width in data points at half height (17). A resolution value for comparison was obtained by dividing observed base pair difference between adjacent peaks by the value calculated from the formula above. Average resolution between alleles X and Y at the Amelogenin locus for samples prepared in water was 1.24 bp, compared to 1.27 bp for those prepared in formamide. Between alleles 14 and 15 for the CSF1PO locus, average resolution for samples prepared in water was 1.58 bp, versus 1.81 bp for those prepared in formamide. Forensic STR analysis requires separation between single bases, as for alleles 9.3 and 10 at the THO1 locus. Separation need not be to baseline for the adjacent peaks to be recognized as distinct and labeled as such by the Genotyper software. In this study, regardless of solvent, these two peaks were resolved by the Genotyper software. Furthermore, resolution was maintained over time (runs lasting approximately 24 h) for both methods of sample preparation.

In addition to software options, temperature changes of only a few degrees can dramatically affect the run. Room temperature was monitored throughout this study, during which it was noticed that even placement of the instrument within a room (near a window as opposed to an internal wall for example) can dramatically reduce precision of size determination. During a typical run lasting 10 h, the temperature varied only 1.07°C from 19.69°C to 20.76°C. In the range, 15°C to 30°C, the important factor was the variation in temperature, not the actual temperature of the room. When the variation was more than 3°, precision was poor, as evidenced by large standard deviations in base pair assignment.

Currently the manufacturer guarantees no more than 100 injections per capillary. For a high throughput operation such as DNA Databank in which a plate of 96 samples may be run, this means changing the capillary with each plate. We found that, for both solvents, water and formamide, at least 300 samples may be injected without compromising reproducible allele assignment. Size determination remained precise. Ladder prepared in H_2O had an average standard deviation of 0.072 bp at 100 injections and 0.066 bp at 300 injections on the capillary. Samples prepared in formamide had an average standard deviation of 0.073 bp at 100 injections and 0.077 bp at 300 injections. No significant change in the distribution of peaks over data points (an indication of compression of data or a change in migration rate) was noted. For example, in samples prepared in formamide or water, the 75 bp peak consistently fell around data point 3550, while the 350 bp peak consistently fell around the 5990 datapoint. The same degree of resolution was retained between the 9.3 and 10 alleles at the THO1 locus regardless of solvent, while resolution was also maintained at the largest alleles where loss of resolution is likely to be observed. The study was repeated with capillaries from a different lot with the same results: accurate size calling and allele labeling, no loss of resolution, or compression of data and no change in migration rate. Since using each capillary for 300 injections reduces turn around time between runs and decreases costs, it holds advantages for high throughput laboratories such as state DNA databanks.

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