BRIEF COMMUNICATION

Jaiprakash G. Shewale,[1] Ph.D.; Stephen L. Richey,[1] B.S.; and Sudhir K. Sinha,[1] Ph.D.

Detection and Correction of a Migration Anomaly on a 310 Genetic Analyzer

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ABSTRACT: During STR analysis on the 310 Genetic Analyzer, retarded migration of GS500ROX size standards and alleles in some samples was observed. The contribution of reagents, capillary and performance optimized polymer POP 4 to the observed anomaly was experimentally eliminated. Variation in electrophoresis temperature between 55°C and 65°C did not alter the rate of migration of GX500ROX size standard and sample alleles. An eroded connector for the cathode mounted on the heat plate assembly caused the abnormal migration. Hence, it is important to verify the mobility of all fragments in the size standard for each sample to avoid any erroneous allele calls by the automated data analysis software.

KEYWORDS: forensic science, short tandem repeats, analysis, capillary electrophoresis, DNA typing, polymerase chain reaction

In recent years, short tandem repeat (STR) sequences have become useful markers for human identification (1,2). The high degree of polymorphism associated with a large number of alleles at each locus provides a high level of discrimination between individuals. STR analysis using Perkin Elmer- Applied Biosystems 310 Genetic Analyzer (310 GA) involves amplification of STR loci by using polymerase chain reaction (PCR) followed by automated capillary electrophoresis and fluorescent signal detection and processing. The data is automatically analyzed by GeneScan® software and alleles are typed by using the Genotyper® software. An internal size standard is co-injected with each sample for accurate sizing of the PCR products. This approach has been well accepted in forensic analysis because of various advantages over conventional methods of DNA analysis such as restriction fragment length polymorphism (2–5).

During STR analysis using the Profiler Plus™ amplification kit and GeneScan® GS 500 ROX size standard on the 310 GA, a unique phenomenon of shift in the electrophoresis pattern was observed. This migration anomaly was detected on only one of the three instruments. The possible factors contributing to this occurrence were investigated. Since 310 GAs are widely used for DNA analysis for convicted Felon Database and forensic casework, it is important that the forensic scientists are aware of this phenomenon and take appropriate QA/QC measures.

Materials and Methods

The samples were blood stains on S & S paper (Schleicher & Schuell GmbH, Post Fach, Dassel, Germany) submitted for DNA profiling. The methods used for isolation of DNA, amplification by using Profiler Plus™ amplification kit and analysis on the 310 GA were performed as described in the user manuals of Perkin-Elmer. The Profiler Plus™ amplification kit, performance optimized polymer POP 4, size standard GS 500 ROX, 10X electrophoresis buffer for 310 GA, formamide and other supplies were obtained from Perkin Elmer, Foster City, CA. The electrophoresis was performed at 60°C and 15 K V; the resulting current ranged between 7 to 8 μA.

Results and Discussion

Normal and altered electopherograms of the samples are presented in Figs. 1–4. A careful observation of the profiles reveals that the phenomenon is a result of delayed migration of all size fragments. Furthermore, the extent of the shift in migration was independent of size fragment length (Figs. 1 and 3). The slower migration was not restricted to GS500ROX size standard, but was also observed for comigrating STR alleles (Figs. 2 and 4). This slower migration resulted in incorrect calls of both the size standards and sample alleles. The 139.00 size fragment was called as 257.34, 210.60, and 266.63 in samples 1, 2 and 3, respectively (Figs. 1 and 3). Similarly, alleles 16 and 18 at D3S1358 for sample 1 were called as 24 and 27 at the FGA locus, and the two off-ladder (OL) alleles called at this locus were actually alleles 15 and 17 from the vWA locus (Figs. 2 and 4). This type of shift in the migration during the electrophoresis was randomly observed for certain samples in a 96 well plate. Moreover, the extent of the mobility shift was not uniform. The allele calls for positive amplification control and for alleles in allelic ladder were correct. The amplified product was not detected in buffer control for extraction and negative amplification control.

The migration of size standards and alleles during the capillary electrophoresis is controlled by various factors. Some prominent factors are current, pH and conductivity of the buffer, temperature, partial replacement of the polymer after each electrophoresis run, and charge of the migrating species. The reagents such as electrode

[1] ReliaGene Technologies, Inc., 5525 Mounes St. Suite 101, New Orleans, LA 70123.

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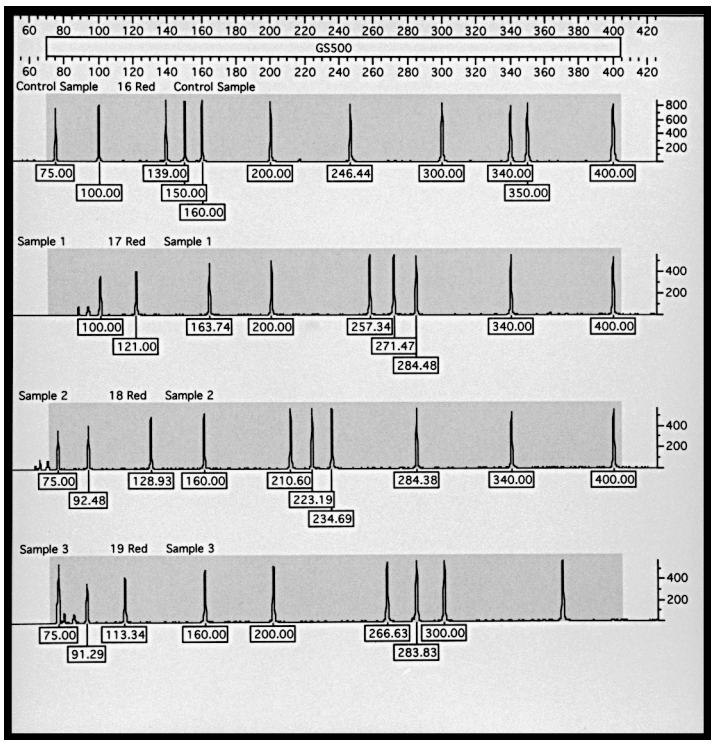

FIG. 1—*Genotyper profiles of the electopherogram of internal size standard GS500ROX of control and samples exhibiting delayed migration.*

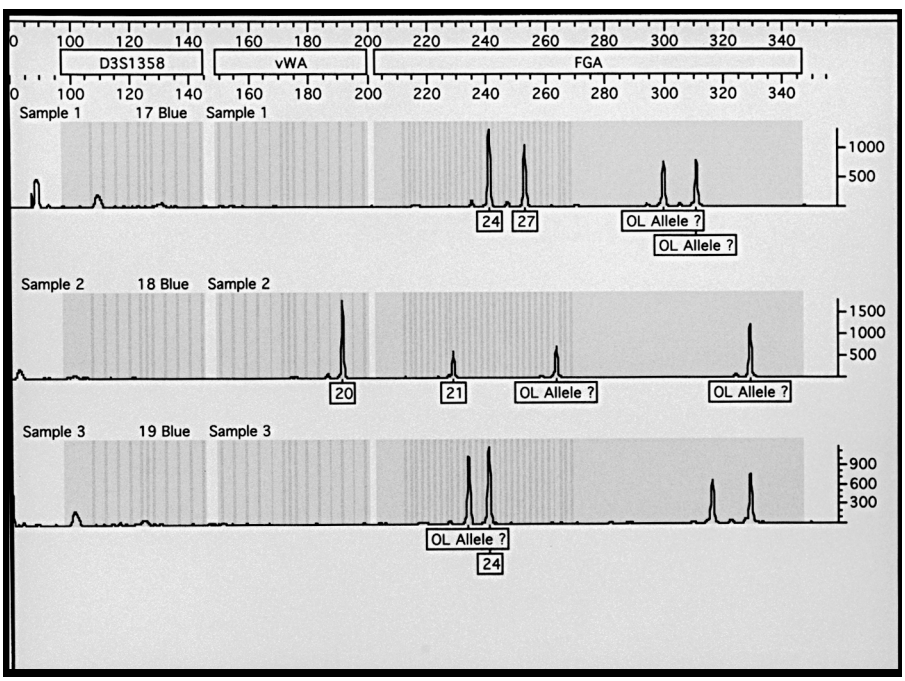

FIG. 2—*Genotyper profiles of the electopherogram of alleles at D3S1358, vWA, and FGA loci (blue window) of samples exhibiting delayed migration.*

buffer, performance optimized polymer POP4 (a gel medium for electrophoresis) and formamide (which reacts with DNA molecule thereby denaturing irreversibly) were replaced. The capillary was also changed. The syringe pump force that controls the movement of syringe plunger was increased in order to ensure total replacement of polymer POP4 from the capillary after each electrophoresis run. A new syringe was installed to eliminate a possibility of polymer oozing from the syringe plunger instead of filling into the capillary. None of these changes corrected the delayed migration of size standards, thereby excluding the role of reagents, capillary and

other mechanical factors contributing to this phenomenon. The same samples, when injected on different 310 GA or reinjected on the same 310 GA, did not exhibit the shift in migration. Thus, the phenomenon was selectively observed on one particular instrument and was due to a malfunctioning during that specific injection.

An examination of the data collection log did not show any changes in the gel temperature, current, voltage and laser power. The electrophoresis was performed by varying the temperature from 55°C to 65°C; the migration of alleles was normal (data not shown). Thus, the temperature within this range did not alter the

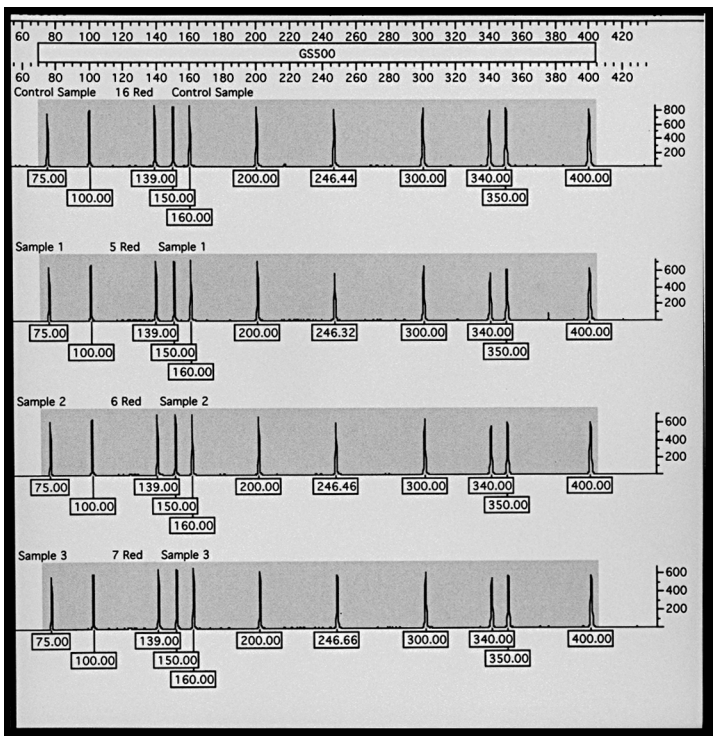

FIG. 3—*Genotyper profiles of the electopherogram of internal size standard GS500ROX of control and samples after replacing the heat plate assembly.*

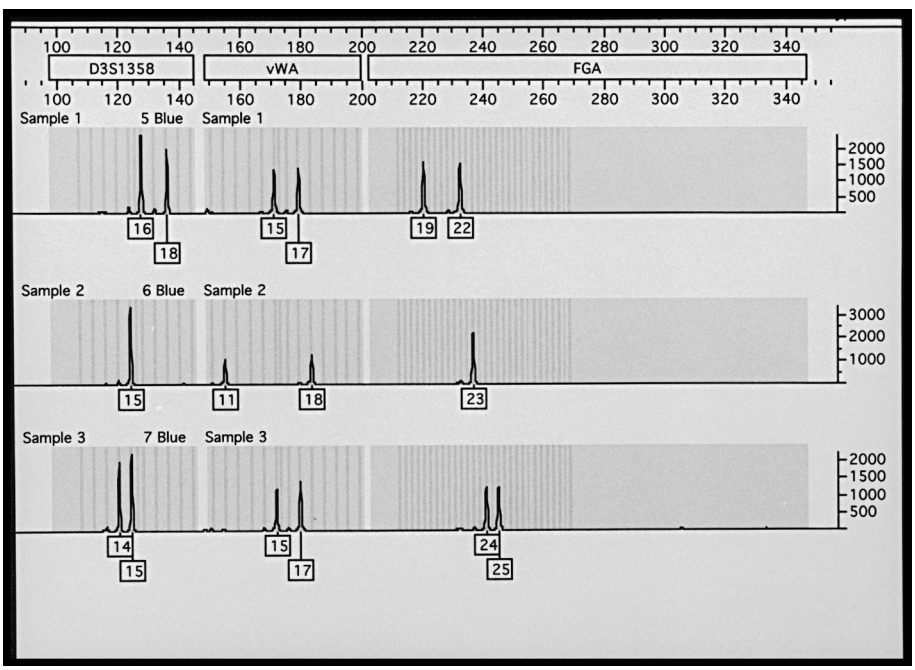

FIG. 4—*Genotyper profiles of the electopherogram of alleles at D3S1358, vWA, and FGA loci (blue window) of samples after replacing the heat plate assembly.*

rate of migration of denatured DNA fragments of sizes between 75 and 400 base pairs. As a final measure, a critical inspection of the heat plate assembly was performed which revealed that the connector for the cathode was eroded. The heat plate assembly was replaced. The results of electrophoresis of the samples on same 310 GA unit after replacing the heat plate assembly are presented in Figs. 3 and 4. The migration of size standards was normal resulting in correct size calls. The retarded migration phenomenon has not been observed in four months (35–45 injections per day), after replacing the heat plate. The results indicate that the delayed migration of size standards and sample alleles could be due to variation or fluctuation in current resulting from the eroded connector but was not recorded.

The erroneous allele calls by Genotyper® software due to the retarded migration can be detected only by careful observation of the data for each sample. Our study warrants additional quality measures during STR analysis on the 310 GA, particularly for analysis of forensic evidence samples where the quantity of sample can be limited. In addition to checking the GeneScan® data and Genotyper® allele calls, the analyst must ensure that all size fragments present in the size standard have migrated within an acceptable data point range and the size calls are correct.

In conclusion, we recommend that ascertaining the size call of all fragments present in the internal size standard be an integral part of standard operation procedure for analysis of STR alleles using the 310 GA.

Acknowledgments

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Additional information and reprint requests:
Sudhir K. Sinha
President and Laboratory Director
5525 Mounes St. Suite 101
New Orleans, LA 70123