Bruce Budowle,[1] *Ph.D. and Robert C. Allen,*[2] *Ph.D.*

Electrophoresis Reliability: I. The Contaminant Issue

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ABSTRACT: The effects of the common contaminants—soil, oil, gasoline, salt, acid, base, bleach, and detergent—on various forensically used genetic marker systems were studied. The predicted effects of the various contaminants on the proteins and the electrophoretic separations agreed with the observed results. A contaminant that affected protein conformation also adversely affected the integrity of the electrophoretic system, thus signalling an anomaly. It also was pointed out that the ideal control study for the effects of contaminants on genetic markers in evidentiary material is often provided to forensic scientists—that is victim's blood on victim's clothing and other substrata. The data presented in this paper support the validity and reliability of electrophoretic analyses of evidentiary material with respect to the contaminant issue.

KEYWORDS: forensic science, electrophoresis, reliability, validity, isoelectric focusing, contaminant, evidentiary material, blood

The Supreme Court of the State of Michigan recently decided in the case of *People v. Young* (Docket number 67373) [*1*] that the "general scientific acceptance of the reliability of serological electrophoresis of evidentiary bloodstains among impartial and disinterested experts in the scientific community has not been established" (majority's opinion, 3-2 decision) [*1*]. The Court's decision was based upon their perception that there was a lack of independent validation studies on the multisystem analysis in question, that there was a lack of comprehensive control tests evaluating the effects of different environmental contaminants on serological evidence, and that the results needed to be subjected to the scrutiny of the scientific community.

It was unfortunate that the evidence supporting the reliability of electrophoretic analysis of evidentiary material was not conveyed adequately and/or the Court did not fully appreciate the multitude of available data. Electrophoresis is a well-defined and accepted technique for the separation of proteins and nucleic acids in many scientific disciplines, including forensic serology. It is not possible to address the issue of impartial and disinterested parties; that is an interpretation of the Court. However, in the Court's own words, "Ideally the community would be scientists with *direct* empirical experience with the procedure in question"

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[1]Research chemist, Forensic Science Research and Training Section, Laboratory Division, FBI Academy, Quantico, VA.

[2]Full professor, Department of Pathology, Medical University of South Carolina, Charleston, SC.

[*1*] and "A certain degree of interest must be tolerated if scientists familiar with the theory and practice of a new technique are to testify at all" [*1*]. Further, the Court stated, "there is a large number of nonforensic scientists using electrophoresis who are capable of evaluating the reliability of electrophoresis of evidentiary bloodstains if presented with the information they need to fill the gaps in their own knowledge and experience." Therefore, whether a court decides to accept testimony from experts who have the theoretical understanding and practical experience of electrophoresis as it applies to forensic serology or chooses nonforensic scientists, it is evident that the Supreme Court of the State of Michigan was requesting information on the reliability and validity of electrophoresis of evidentiary material to "fill the gaps" [*1*].

Electrophoresis, simply stated, is the movement of charged particles in an electric field. The routinely used electrophoretic methods can be divided into two groups: continuous zone electrophoresis (CZE) and multizonal electrophoresis. Isoelectric focusing (IEF) is a special case of the latter. CZE relies on a constant pH environment and IEF uses a pH gradient to separate charged particles, particularly proteins. Maintaining the pH integrity of the systems and producing no artifacts are of the utmost importance. Extensive work has been devoted to defining proper parameters of these electrophoretic systems so they may be used reliably and reproducibly. The present authors [*2–41*] as well as others [*42–93*] have published many articles regarding the development and uses of electrophoretic techniques (there have been approximately 3000 journal articles per year over the last 20 years regarding electrophoresis; obviously space limits listing even a part of these reports). The scientific community believes in and relies on these methods, and the literature supports the validity and reliability of electrophoresis as an analytical tool. However for the Court (majority's opinion) the issue remains whether or not evidentiary material which may be exposed to a myriad of environmental insults can yield false positive results.

Such a scenario sounds quite plausible to the layman, but to the scientist it demonstrates a lack of understanding of the basics of protein chemistry and electrophoresis. Basic principles of biochemistry address the effects of altering the conformation of proteins, particularly enzymes. Temperature and pH extremes will denature protein molecules so they are no longer functional and thus cannot express activity. Although there are many human genetic markers that can be resolved by electrophoresis [*94,95*], only a dozen or so have proved to be sufficiently practical for use in forensic serology [*95*]. These genetic markers have been shown through long experience to persist in recognizable forms in body fluid stains. Further, the behavior of the markers (aging studies, band pattern interpretation) is well defined and well understood so that experienced individuals can reliably interpret the electrophoretic profiles derived from evidentiary material. For example, it is well known that certain genetic markers, such as erythrocyte acid phosphatase [*94*], glyoxalase I [*94*], and esterase D [*94*], contain sulfhydryl groups that can be oxidized when exposed to air. These enzymes must be extracted in the presence of a reducing agent (for example, dithiothreitol) to reverse any environmentally encountered oxidation effects. Otherwise, recognizable patterns would not be obtained after electrophoretic analysis. Again, the Court appears to accept what has been defined in the laboratory environment, but it decided that data needed to be gathered on the effects of contaminants on the electrophoretic pattern and its subsequent interpretation, such as the effects of two very common contaminants, soil and gasoline (as indicated by the majority opinion) [*1*]. This study not only includes these two contaminants, but oil, bleach, salt, detergent, acid, and base, as well.

Materials and Methods

A series of controlled experiments were carried out to demonstrate the effects of contaminants on the electrophoretic process for analysis of evidentiary materials. Contaminants that could affect protein conformation, those that alter pH and ionic strengths, and those that

impart charge changes will, in themselves, be evident. Any contaminant that has these properties will distort the electrophoretic gel pattern in such a manner that its influence can be recognized readily by the operator.

The array of contaminants used in this study was 10% sodium dodecyl sulfate (SDS) (an anionic detergent), chlorine bleach, 0.1*M* phosphate buffer (salt), leaded gasoline, 10W-40 motor oil, 1.0*M* sodium hydroxide (high pH), 1.0*M* glacial acetic acid (low pH), and common soil from outside the laboratory building. The series of materials chosen for this study are representative of commonly encountered contaminants.

Whole blood samples were obtained by finger prick from donors of known phenotypes at the FBI Academy. The common variants of the following genetic marker systems were represented: phosphoglucomutase-1 (PGM), erythrocyte acid phosphatase (EAP), hemoglobin (Hb), glyoxalase I (GLO), adenylate kinase (AK), adenosine deaminase (ADA), group specific component (Gc), and transferrin (Tf). Liquid bloods were mixed with each of the contaminants in ratios of 20:1, 10:1, 5:1, and 1:1 and analyzed in liquid form. Further bloodstains were prepared in two different manners, each with the same blood-to-contaminant ratios as the liquid samples. In the first series, the contaminants were placed on washed cotton cloth and allowed to dry for at least 1 h. Next, liquid blood was applied to the contaminated surface and air-dried. In the second series of experiments, the stains were prepared in the reverse manner—blood was deposited first followed by addition of the contaminant.

All samples were subjected to CZE or IEF and assayed for the following genetic markers: PGM, EAP, Hb, GLO, AK, ADA, Gc, and Tf using previously described methods [*25,26,35,39,96–99*]. More than 50 gels were analyzed (comprising 750 samples).

Results and Discussion

It was important to demonstrate that basic physicochemical properties of a given protein could enable a scientist to predict the effects of such contaminants on the genetic markers in the samples. Further, any contaminant that would affect protein conformation of the genetic marker under analysis would also adversely affect the integrity of the electrophoretic system, thus signalling an anomaly to the analyst. The ability of such contaminants to distort the sample separation track on an electrophoretic gel is an added benchmark for the detection of environmentally encountered contaminants.

Tables 1 through 6 indicate predicted events versus observed results with the various types of contaminants on the genetic markers and on the electrophoretic separation. Based on physicochemical principles and practical experience, the observed effects of the contaminants on the genetic markers were predicted correctly. The disturbances in the system that were observed during the electrophoretic run also were as expected. The results were the same for the three types of samples investigated. As expected, soil, gasoline, and oil had no effect on the genetic markers or the electrophoretic run (Figs. 1, 2, and 3). Soil is insoluble, and predictably, it settled to the bottom of the extract in the tube (either by gravity or following centrifugation) and did not associate with the soluble proteins. The physical laws governing nonpolar and polar interactions do not allow the mixing of nonpolar organics and aqueous biological liquids. Thus, there is no oil/gasoline influence on the proteins or the subsequent electrophoretic separation pattern.

The types of contaminants that influence protein integrity are those that can impart an extreme change in the pH environment of the sample (for example, acid, base, bleach), impart a charge change to the protein (such as the anionic detergent SDS), increase the ionic strength of the sample (salts), oxidatively affect protein structure (bleach), or dissociate proteins having subunit structure (SDS). Acid, base, and bleach (at all ratios) altered the pH integrity of the electrophoretic gels so much that the sample tracks were distorted dramatically (Figs. 2, 3, and 4). Bleach and SDS affected the migration of the Hb present in all samples such that it was noticeably different from the untreated controls (Tables 1 through

TABLE 1—*Predictions and observations of contaminant effects of electrophoretic analyses of phosphoglucomutase-1.*

Contaminant	Prediction	Observation	Basis of Prediction
Soil	no effect	no effect	insolubility
Oil	no effect	no effect	nonpolar
Gasoline	no effect	no effect	nonpolar
Phosphate buffer	retard migration of Hb, high salt cause arcing of protein patterns, no other effects on protein	same as prediction	high salt concentration
Acid	distorted sample track, no enzymatic activity	same as prediction	denaturation and pH shift
Base	distorted sample track, no enzymatic activity	same as prediction	denaturation and pH shift
Bleach	distorted sample track, Hb migrate anodally, no enzymatic activity	same as prediction	denaturation, charge shift, pH shift
SDS	Hb migrate anodally, no enzymatic activity	same as prediction	denaturation, charge shift, pH shift

TABLE 2—*Predictions and observations of contaminant effects of electrophoretic analyses of erythrocyte acid phosphatase.*

Contaminant	Prediction	Observation	Basis of Prediction
Soil	no effect	no effect	insolubility
Oil	no effect	no effect	nonpolar
Gasoline	no effect	no effect	nonpolar
Phosphate buffer	retard Hb migration, no other effect on protein	same as prediction[a] except conversion of C CB and CB B	high salt concentration
Acid	distorted sample track, no enzymatic activity	same as prediction	denaturation and pH shift
Base	distorted sample track, no enzymatic activity	same as prediction	denaturation and pH shift
Bleach	distorted sample track, Hb migrate anodally, no enzymatic activity	same as prediction	denaturation, charge shift, pH shift
SDS	Hb migrate anodally, no enzymatic activity	same as prediction	denaturation, charge shift, pH shift

[a]Phenomenon occurs only when EAP is analyzed in polyacrylamide gels cast by the capillary technique, but no phenotype conversion is observed in polyacrylamide gels cast by the flap technique.

6). Such profound distortions from the normal performance of a system should be apparent even to a novice in the field of electrophoresis. Note that in subsequent assay for the specific enzymatic markers, no activity was demonstrated (Fig. 5) or the sample phenotypes clearly were inconclusive (Figs. 2 and 3). The effect of these contaminants also was apparent when the gels were assayed with a general protein stain; the protein profiles obtained were noticeably distorted from their usual appearance (Fig. 3).

TABLE 3—*Predictions and observations of contaminant effects of electrophoretic analyses of glyoxalase I.*

Contaminant	Prediction	Observation	Basis of Prediction
Soil	no effect	no effect	insolubility
Oil	no effect	no effect	nonpolar
Gasoline	no effect	no effect	nonpolar
Phosphate buffer	no effect on protein, slow migration of sample	no effect	high salt concentration
Acid	distortion at origin, no enzymatic activity	same as prediction, Hb precipitated at origin	denaturation and pH shift
Base	distortion at origin, no enzymatic activity	same as prediction,	denaturation and pH shift
Bleach	gel distortion, no enzymatic activity	same as prediction, Hb migrates anodally	denaturation, charge shift, pH shift
SDS	gel distortion, no enzymatic activity	same as prediction, Hb migrates anodally	denaturation, charge shift, pH shift

TABLE 4—*Predictions and observations of contaminant effects of electrophoretic analyses of group specific component and transferrin.*

Contaminant	Prediction	Observation	Basis of Prediction
Soil	no effect	no effect	insolubility
Oil	no effect	no effect	nonpolar
Gasoline	no effect	no effect	nonpolar
Phosphate buffer	distort pH gradient, distort protein profile	same as prediction	high salt concentration
Acid	distort pH gradient, distort protein profile	same as prediction	denaturation and pH shift
Base	distort pH gradient, distort protein profile	same as prediction	denaturation and pH shift
Bleach	distort pH gradient, distort protein profile, Hb migrate anodally	same as prediction	denaturation, charge shift, pH shift
SDS	Hb migrate anodally, unrecognizable protein profile	same as prediction	denaturation, charge shift, pH shift

A concentration of 0.05*M* phosphate buffer was required before any separation pattern distortion could be observed. Arc-shaped band patterns result from high salt concentration and have been described previously [*8,100,101*]. The only unexpected observation was the effect of high phosphate buffer concentrations (0.02 and 0.05*M*) on EAP. The C phenotype shifted to a CB and the CB phenotype converted to a B. Again, the electrophoretic system itself signalled the presence of a contaminant to the analyst. The high salt concentration also retarded the migration of hemoglobin (Hb). Thus, a noticeable difference in the migration rate of Hb in the unknown sample compared with the control sample immediately suggests a possible sample contamination to the scientist. Subsequent to this study it was discovered

TABLE 5—*Predictions and observations of contaminant effects of electrophoretic analyses of hemoglobin.*

Contaminant	Prediction	Observation	Basis of Prediction
Soil	no effect	no effect	insolubility
Oil	no effect	no effect	nonpolar
Gasoline	no effect	no effect	nonpolar
Phosphate buffer	distorted, wavy profile no other effect on proteins	same as prediction	high salt concentration
Acid	distort pH gradient, distort protein profile	same as prediction	denaturation and pH shift
Base	distort pH gradient, distort protein profile	same as prediction	denaturation and pH shift
Bleach	distort Hb migration, distort pH gradient	same as prediction	denaturation, charge shift, pH shift
SDS	Hb migrates anodally	same as prediction	denaturation, charge shift, pH shift

TABLE 6—*Predictions and observations of contaminant effects of electrophoretic analyses of adenylate kinase and adenosine deaminase.*

Contaminant	Prediction	Observation	Basis of Prediction
Soil	no effect	no effect	insolubility
Oil	no effect	no effect	nonpolar
Gasoline	no effect	no effect	nonpolar
Phosphate buffer	slow migration of proteins, no other effects	same as prediction, Hb migrated slower than controls	high salt concentration
Acid	distort gel origin, no enzymatic activity	same as prediction	denaturation and pH shift
Base	distort gel origin, no enzymatic activity	same as prediction	denaturation and pH shift
Bleach	distort gel origin, no enzymatic activity, Hb migrate anodally	same as prediction	denaturation, charge shift, pH shift
SDS	no enzymatic activity, Hb migrate anodally	same as prediction	denaturation, charge shift, pH shift

that this phenotype conversion of EAP was due to the gel and not the high salt concentration.[3] When EAP was analyzed in polyacrylamide gels cast by the capillary technique, the phenotype conversion was observed. In fact, this phenomenon at times occurred without the presence of high salt. However, when EAP analysis was undertaken in a polyacrylamide gel cast with the flap technique no phenotype conversion was observed (even in the presence of 0.05*M* phosphate buffer).

In all instances the predicted effects agreed with the observed results. When no effect on

[3]B. Budowle, unpublished data.

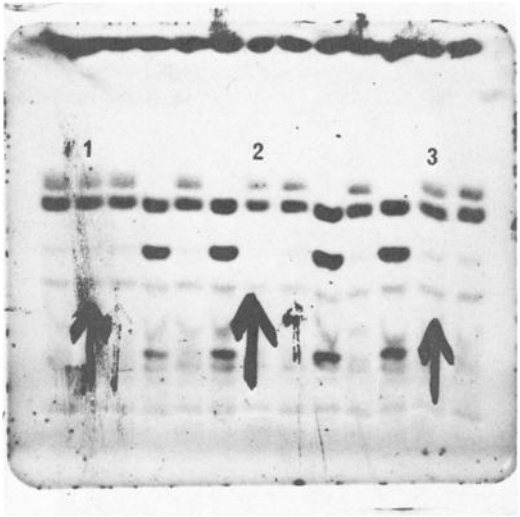

FIG. 1—*A PGM gel displaying effects of oil. The phenotypes from left to right are: 1+1−, 1+1−, 1+1−, 1+2+, 1+1−, 1+2+, 1+1−, 1+1−, 1+2+, 1+1−, 1+2+, 1+1−, and 1+1−. Samples numbered 1, 2, and 3 were liquid bloods mixed with oil in a 1:5 ratio. The cathode is at the top.*

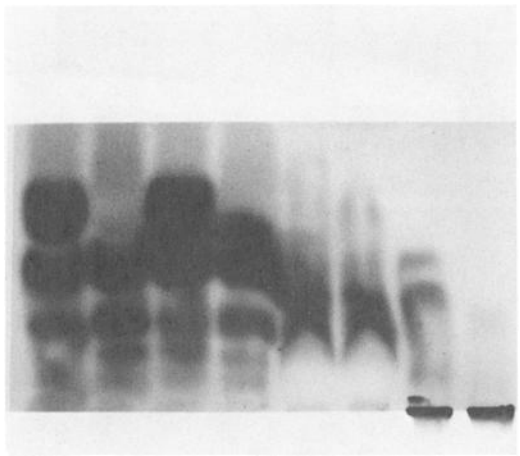

FIG. 2—*An AK gel displaying effects of contaminants. The phenotypes from left to right are 2−1, 1, 2−1, 1, inconclusive, inconclusive, inconclusive, and inconclusive. Samples 1 and 2 were controls. Samples 3 and 4 were bloodstains diluted 1:5 with gasoline. Samples 5 and 6 were bloodstains diluted 1:10 with acid. Samples 7 and 8 were diluted 1:10 with SDS. The cathode is at the top.*

the electrophoretic separation or the genetic markers was expected, none was observed. Contaminants that are known to affect proteins signalled their presence by distorting the sample tracks of the separation or altering Hb migration or both. Thus, they were readily detected as an abnormality by the operator. In those instances where a chemical contaminant denatured a protein, no enzyme activity was observed. Even for EAP (analyzed in capillary cast gels), a retarded Hb migration suggested high salt contamination. The data support the experience

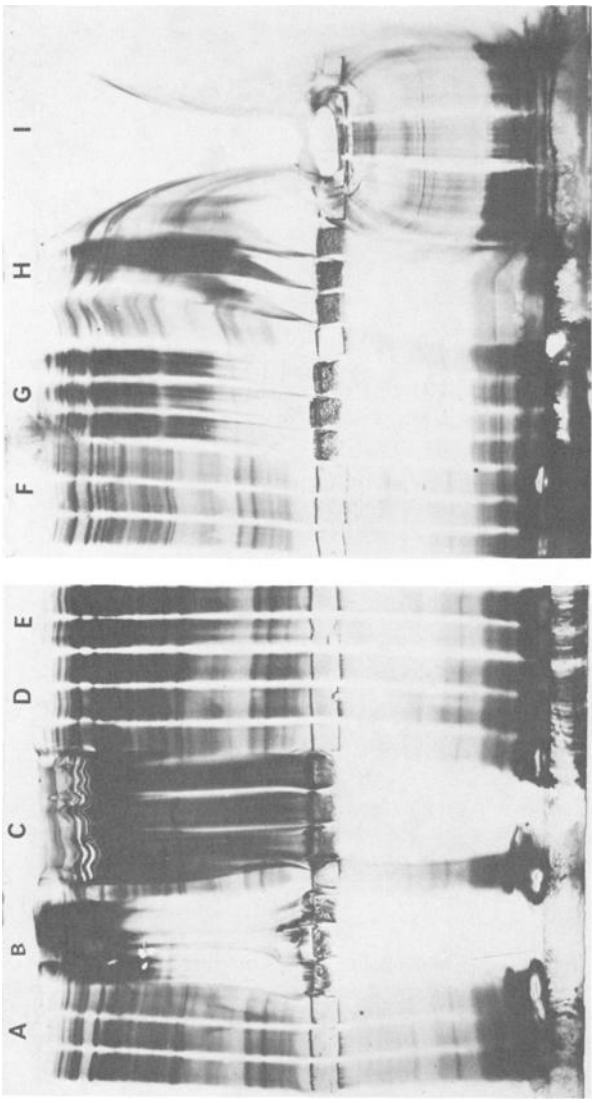

FIG. 3—*General protein stained (with silver) gels displaying effects of contaminants. These are IEF gels with a pH gradient of 5 to 7. The cathode is at the bottom. Samples A and F were normal controls. Samples B were contaminated with bleach (1:5). Samples C were contaminated with SDS (1:10). Samples D were contaminated with gasoline (1:5). Samples E were contaminated with soil (1:5). Samples G were contaminated with bleach (1:1). Samples H were contaminated with SDS (1:1). Samples I were contaminated with acid (1:20).*

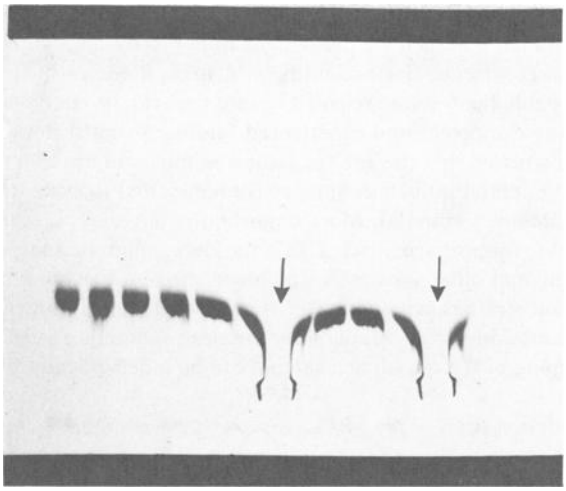

FIG. 4—*An IEF gel designed for PGM subtyping. Displayed are the migration of Hb from the sample tabs. The arrows indicate samples contaminated with base (1:10). The cathode is at the top.*

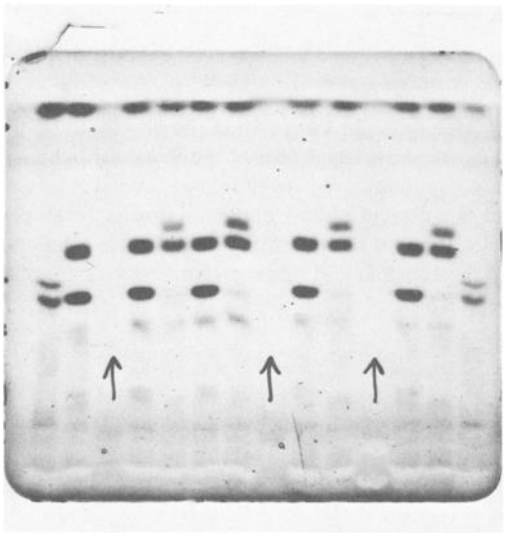

FIG. 5—*A PGM gel displaying effects of bleach. The phenotypes from left to right are: 2+2−, 1+2+, negative, 1+2+, 1+1−, 1+2+, 1+1−, negative, 1+2+, 1+1−, negative, 1+2+, 1+1−, and 2+2−. The arrows indicate bloodstains that were mixed with bleach in a 1:5 ratio. The phenotypes from left to right of these samples are: 1+2+, 1+1−, and 2+2−. The cathode is at the top.*

of these authors that strong chemical or reactive contaminants affect the sample in such a way that the final result may be interpreted readily as negative or at least inconclusive. The capability of detecting the presence of contaminants, which distort or affect the pattern, provides a built-in control in electrophoretic analysis of body fluid stains. This allows the experienced forensic scientist to have greater confidence in the conclusive results obtained from electrophoretic analysis of evidentiary materials.

This set of experiments is, perhaps, self-evident. The data demonstrate that the effects of the types of contaminants studied are predictable from a knowledge of protein behavior and the individual marker systems. Basic scientific principles, the scientific literature, and practical experience enable the forensic scientist to interpret results confidently.

Furthermore, the competent and experienced forensic scientist does not work in a vacuum. It should be stressed that the analyst gathers as much information as possible regarding the case (and potential influences such as contaminants) to evaluate properly the data obtained from evidentiary material. More importantly, in reality, an ideal control study is naturally provided to forensic scientists. This is the electrophoretic analysis of victim's blood on victim's clothing and other substrata. The blood shed by a victim onto his/her clothing and surrounding substrata is exposed to the same myriad of environmental insults as other questioned stains submitted to the laboratory for electrophoretic analysis. The accuracy of electrophoretic typing of the questioned sample can be independently verified with the victim's whole blood.

During the validation trials of the FBI Laboratory's electrophoretic techniques for identification of genetic markers derived from evidentiary material, attention is paid to data regarding the victim's known blood and the victim's shed blood. If discrepancies in analysis had occurred, serious doubt would be cast regarding the validity of that particular electrophoretic method as a valid indicator of genetic marker data. The electrophoretic methods that are implemented in the Serology Unit of the FBI Laboratory satisfy this as well as other basic requirements.

Conclusion

In conclusion, this paper provides supporting data for the reliability of electrophoretic analyses of evidentiary material. The information should "fill the gaps" concerning the contaminant issue raised by the Supreme Court of the State of Michigan, the legal profession, and the forensic science community. There should be no doubt that even though body fluid stains may be exposed to a myriad of environmental insults, when placed in its proper perspective, electrophoretic analyses of evidentiary material are valid and reliable. In a following paper the issues of multisystem and single system analyses and scientific scrutiny will be addressed.

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Address requests for reprints or additional information to
Bruce Budowle
FSRTC
FBI Academy
Quantico, VA 22135