

## Correspondence

**Commentary on Tomczak PD, Buikstra JE.** Analysis of blunt trauma injuries: Vertical deceleration versus horizontal deceleration injuries. *J Forensic Sci* 1999; 44(2):253–62.

Sir:

The recent article by Tomczak and Buikstra attributes the injuries in a decomposed body to impact by a truck or other vertical-front vehicle on a pedestrian. We are concerned about the interpretation of these results and the implications of this methodology, should it be adopted more widely.

Although it is difficult to determine the exact nature of the injuries from the article, they appear to consist of fractures at the vertebral ends of left ribs 1, 3–12 as well as sternal fractures of left ribs 5–9. Right ribs are also injured, including rib 1 and 9. Spinal injuries are limited to T4, and T7 to L1 and consist of spinous process, transverse process and articular facet fractures. Side of injury is not specified in the spine. Additional injuries included the right scapula and left clavicle. No injuries are noted on the cranium, pelvic ring, upper limb or lower limb.

This isolation of trauma to the upper torso appears incompatible with the usual pattern of vehicle-pedestrian accidents as reported in the article, even when these involve vans or trucks. The more typical pattern of the car to “run-under” the victim is altered with vertical-faced vehicles. Instead the victim is thrown forward where they will also suffer additional injuries upon impact with the road or other objects in their pathway. Head injuries are the most common cause of death in vehicle-pedestrian accidents. Neck injuries may result from the initial impact. Pelvic fractures are common in vehicle-pedestrian accidents. It is highly unusual for these circumstances not to result in lower limb fractures, especially as the bumper strikes the lower leg. “Boot-top” fractures of the tibia and fibula are common due to the bumper of the vehicle striking the legs. These may occur at remarkably slow speeds.

In the case under discussion, it seems difficult to reconstruct an impact which would strike the upper to mid back without inflicting damage to the head, pelvis or lower limbs. We are concerned that other possible mechanisms of injury were not sufficiently considered. Instead, it appeared that the only assessment was which of two mechanisms (vehicle-pedestrian impact or fall from a tree) was most probable.

In the article, a possible cause of death or incapacitation is presented. While such speculations are common within the archaeological literature, it is inappropriate for forensic anthropologists to include such interpretations in a forensic case. The cause and manner of death, including possible soft tissue injuries and the capability of the victim to move following injury should be left to the forensic pathologist.

Finally, avulsion fractures are small fragments of bone that are detached from the bony prominences by the tension produced by the attached ligaments or tendons (1). Tight bonds between the Sharpey’s fibers and the adherent soft tissue prevent failure at the

insertion point and failure is displaced to the surrounding bone. The illustrations of the scapula and of the two vertebrae are both described as being “avulsion” fractures. However, the massive scapula injuries seem much more likely to be caused by direct trauma. Similarly the vertebral fractures illustrated are not consistent with the definition of avulsion fractures and are more likely due to either impact, rotational or shearing injuries. While such fragments could be displaced by the attached muscles following fracture, this does qualify as an avulsion fracture.

While we applaud the efforts of Tomczak and Buikstra to explore more deeply into the trauma, we are cautious about the ability to make such detailed interpretations.

### Reference

1. Rogers LF. Radiology of skeletal trauma. 2nd ed. New York: Churchill Livingstone, 1992.

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### Authors’ Response

Sir:

In reply, we thank Dr. Galloway and Dr. Mason for their comments. As mentioned in the article, we agree that it is most common to find skull injuries and fractures of the pelvis and lower extremity associated with vehicular-pedestrian accidents. However, the presence of extensive and severe trauma to the thoracic region and the absence of such “characteristic” vehicular-pedestrian injuries suggest that the individual under study was not struck by a car or van, but most likely a larger vehicle, such as a truck. If the point of impact was higher than that associated with a car (i.e., thoracic area) one would not expect “boot-top” fractures of the tibia and fibula. For instance, the individual who was struck by a truck in the study (Case #2) did not display skull nor lower extremity fractures. Instead, injury was concentrated to the thoracic region. In contrast, all of the individuals who were struck by cars in this study suffered lower extremity injuries and 80% (4/5) suffered cranial fractures. While the number of cases studied in this investigation are limited, there appears to be a distinction in injuries suffered by individuals struck by cars versus a larger vehicle (i.e., truck).

Additionally, a comprehensive literature review suggested that extensive blunt trauma to the thoracic region was most likely due to vertical deceleration or horizontal deceleration trauma, thereby limiting the focus of our investigation. While Galloway and Mason suggest that we do not explore a sufficient number of possible mechanisms of injury, our research on extensive blunt trauma, as well as the context in which the body was found best support the

scenarios put forth. We would certainly welcome any additional probable scenarios to explain the trauma observed.

The focus of this article was to examine the extensive injuries the individual sustained in order to ascertain the most probable manner of death. As we are aware that cause of death is a medical determination, there is no attempt in this article to ascertain cause of death. We are simply stating that the severe injuries sustained by this individual most likely seriously incapacitated him.

Finally, several of the injuries to the scapula and vertebrae have been attributed to contraction of particular muscles. However, our understanding of avulsion fractures as a result of forcible tearing or pulling suggested that these injuries could also be classified as avulsion fractures. For instance, fractures of the inferior and superior scapular angle, where there is muscle attachment, are often classified as avulsion fractures.

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**Commentary on** Introna F, Di Vella G, Campobasso CP. Determination of postmortem interval from old skeletal remains by image analysis of luminol test results. *J Forensic Sci* 1999; 44(3):535–8.

Sir:

I have a few questions for the authors followed by some comments on luminol. What was the history of the bones examined in the study? Were the bones from burials or were they from non-buried, relatively pristine bodies? Did the bones undergo any cleaning procedures prior to luminol treatment?

A forensic scientist must always be very careful when interpreting luminol results. In this study, the authors took appropriate steps to eliminate false positives that could result from plant peroxidases; however, other sources of contamination can cause false luminol positive reactions. Copper, copper salts, ferricyanide, iron ions, cobalt ions, and sodium hypochlorite (bleach) can cause luminol to fluoresce (1–3). Any of these substances could come in contact with bones, particularly bones that have been buried in mineral rich soil and bones that have been cleaned with tap water and/or bleach. I have seen luminol react with copper salts that have leached into the fabric surrounding the copper rivets of blue jeans. I have also seen luminol react with black fingerprint powder. When using the suggested method for aging bones, the scientist must be aware of other substances that can cause variation in the fluorescent intensity of luminol. Standards, such as known bone samples of varying PMI, and controls, such as a soil sample collected from the area surrounding the bone, clothing associated with the remains, and bone cleaning materials, should be used in conjunction with this type of analysis.

## References

1. Albrecht HO. Über die Chemiluminescenz des Aminophthalsäurehydrazids. *Z Phys Chem (Leipzig)* 1928;136:321–30.
2. Grodsky M, Wright K, Kirk PL. Simplified preliminary blood testing. An improved technique and comparative study of methods. *J Crim Law Criminol Police Sci* 1951;42:95–104.
3. Steigmann A. Luminol light reactions. *Chem Industry*: 889–890 through *Chem. Abstr.* 1942;36:1848.

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## Authors' Response

Sir:

Thank you very much for the comments regarding our article: "Determination of postmortem interval from old skeletal remains by image analysis of luminol test results." We do really appreciate them and certainly agree that forensic scientists must always be very careful when interpreting luminol results.

The goal of our study is testing a simple and easy distinction method between two broad groups of skeletal remains frequently examined during forensic investigations: "modern" (less than 50 years) and "ancient" (more than 50 years) bones. The paper is a preliminary effort to the evaluation of correlating the time since death with blood remnants in bone tissue. Luminol is very sensitive, reacting rapidly to the most minute traces of blood, but it is a presumptive test, capable of delivering both false positives and false negatives. For example it does not differentiate between human and animal blood (1).

Major sources of false positives are chemical oxidants, catalysts, and salts of heavy metals such as copper and nickel. To avoid the possible influence of the most common substances (such as iodine, rust, household bleach, formalin and plant peroxidases such as are found in horseradish, citrus fruits, bananas, watermelon and numerous vegetables), we washed in distilled water all the bone samples and heated them to 100°C for a period of 5 min prior to testing with luminol solution. This temperature does not appreciably affect the heme portion of the hemoglobin responsible for the luminescence reaction and destroys the plant peroxidases.

However, as you stated in your comments, metal surfaces such as copper, copper salts, ferricyanide, iron ions, cobalt ions and sodium hypochlorite (bleach) are particularly likely to yield false positives. To avoid the possible influence of these substances we followed procedures as reported in a previous paper on this topic (2) collecting bone powder from the inner compact tissue of the mid-shaft of each femur. Compact bone is, in fact, far less susceptible to physical and/or surface contamination than trabecular bone with its large surface area to volume ratio and multiple cavities that easily become filled with contaminating soil and clay particles. After removing the periosteal (outer) and endosteal (inner) surfaces and pulverizing the compact tissue samples into a fine bone powder using a grinder no other particular cleaning procedures were used except a second washing in distilled water.

Regarding the history of the bone samples examined, the femora belonging to the "ancient" group examined (fourth and fifth group with PMI ranging between 50 and over 80 years) were from human remains found in different ossuaries (crypts) of old Roman Catholic churches. For these latter bones the original burial conditions are still not well defined and for some skeletons completely unknown. However, based on the negative results of image analysis of luminol tests for this latter "ancient" group we can exclude manifest false positives since only one femur (PMI ranging between 50 and 60 years) recorded from the powdered bone than the other groups). The most of femora (33 out of 60) belonging to the "modern" group (first, second and third group with PMI ranging between 1 month and 35 years) were from skeletal remains found outdoors, in open fields, during forensic investigations. The rest of femora belonging to "modern" group (27 out of 60) came from cemetery exhumations. These bodies were buried in wooden coffins embedded both beneath the soil and in cement niches for urns; actually, we do not know exactly which coffins were lined with metal (zinc) plate or which kind of clothing was associated with the remains. Consequently, it was not possible to standardize the variations caused by burial environments, since the examined material came from different sites such as

bodies nonburied (found outdoors) and from not well defined burial conditions (church burials and cemetery exhumations).

Exchanges of elements, anyway, between bone and the surrounding soil after burial have been reported (3–5) but never examined systematically. To the best of our knowledge the most recent paper on this topic has been published in 1998 by Shinomiya et al. (6). In 1980 the inorganic substance content of skeletal remains was used by Foldes et al. (7) as a means of determining the duration of burial in the ground. In this latter paper the authors demonstrated that trabecular bone is highly susceptible to post-mortem absorption of inorganic contaminant substances than compact bone. They also observed that the metals content in bones gradually increases with age measuring higher concentrations in archaeological skeletal remains than in recent bone samples; differences in metal content were observed between bones and soil samples collected from the surroundings of the burial site. Variation of trace metals in ancient and contemporary bones were also discussed by several other authors in relation to the mineralization of bones and the surrounding environment (8–10).

Actually, we are going deeper in our research analyzing soil samples from the cemetery where the buried bones come from, and even if the study is still going on, we can anticipate that no clear false positives seem to have occurred to the femura of the “modern” group. This is supported from the mineral content of bone and soil samples measured by atomic-absorption-spectrophotometry. Between the “modern” bone samples and several contemporary controls no significant differences in the Fe, Zn, Pb, Cu, Cb, Mg and Mn content have been observed—unlike the high concentration of metals measured from cemetery soil samples according to the results illustrated by Foldes et al. (1980).

However, regarding the postmortem absorption of inorganic substances such as salts of heavy metals by the skeleton, we think that much more has to be investigated. Since several substances can cause variation in the fluorescent intensity of luminol, we agree with you, of course, that controls, such as soil samples collected from the area surrounding the skeletal remains, clothing associated with them and bone cleaning materials should be tested when available in conjunction with the luminol test. This procedure of testing could exclude occasional false positives or negatives and validate the results obtained from the analysis. Since our JFS paper is a preliminary attempt to classify the correlation between the intensity and distribution of chemiluminescence from bone powder (compact tissue) and postmortem interval (PMI), our results provide only a glimpse of the potential of a luminol test as a chemical and physical method for dating human skeletal remains. We hope in the future to share our experience with other investigators and that our efforts continue to stimulate research and open discussions in this field. Further comments or suggestions are welcome and they are helpful to us.

## References

1. Grundermann KD. Chemiluminescence in organic compounds. *Angew Chem Int Ed* 1965;4(7):566–73.
2. Colonna M, Introna F Jr, Di Nunno C, Dattoli V. Valutazioni tanaocronologiche su resti scheletrici ed applicazione sperimentale del Luminol test. *Zacchia* 1987;5(4):65–80.
3. Mosenbach R. The chemical and mineral composition of inorganic substances of bones and dentin of existing and fossil vertebrates structure and size of the middle grains dependent on the geologic age. *Bull Group Eur Rech Sci Stomatol Odontol* 1974;17:59–85.
4. Lambert JB, Szpunar CB, Buikstra JE. Chemical analysis of excavated human bone from middle and late Woodland sites. *Archaeometry* 1979;21:115–29.
5. Jaworowski Z, Barbalat F, Blain C, Peyre E. Heavy metals in human and animal bones from ancient and contemporary France. *Sci Total Environ* 1985;43:103–26.
6. Shinomiya T, Shinomiya K, Orimoto C, Minami T, Tohno Y, Yamada

M. In- and out-flows of elements in bones embedded in reference soils. *Forensic Sci Int* 1998;98:109–18.

7. Foldes V, Kosa F, Viragos-Kis E, Rengei B, Ferke A. Atomabsorptions-spektrophotometrische Untersuchung des Gehaltes an anorganischen Substanzen von Skelettbefunden zur Ermittlung der Dauer des Be-grabenseins in der Erde. *Arch Kriminol* 1980;166(3–4):105–11.
8. Waldron HA. Postmortem absorption of lead by the skeleton. *Am J Phys Anthropol* 1981;55(3):395–8.
9. Jaworowski Z, Barbalat F, Blain C, Peyre E. Chronologic course of the content of lead, cadmium and zinc in human bones in France. *CR Acad Sci III* 1984;299(10):409–12.
10. Hisanaga A, Hirata M, Tanaka A, Ishinishi N, Eguchi Y. Variation of trace metals in ancient and contemporary Japanese bones. *Biol Trace Elem Res* 1989;22(3):221–31.

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**Commentary on** Hochmeister MN, Budowle B, Sparkes R, Rudin O, Gehrig C, Thali M, Schmidt L, Cordier A. Validation studies of an immunochromatographic 1-step test for the forensic identification of human blood. *J Forensic Sci* 1999;44:597–602.

Sir:

In their validation of a device for identification of human hemoglobin in bloodstains, Hochmeister et al. (1) tested bloodstains from a variety of animal species. The domestic ferret (*Mustela puterius fero*) was not among the animals tested. Examination of a database of amino acid sequences of proteins (2) reveals that the hemoglobins from humans, several primates, and ferrets share a common amino acid sequence from residues 67 to 73 of the alpha chain, namely TNAVAHV.<sup>1</sup> This sequence differs from that of the corresponding segment of hemoglobin from mouse (ASAAGHL) and rabbit and goat (both TKAAGHL) and is therefore potentially immunogenic for the production of monoclonal and polyclonal antibodies. Such antibodies are the critical components of immunochromatographic devices. Among the amino acid differences between mouse and human hemoglobin, the TNAVAHV sequence shows maximal discrimination between human and other commonly encountered animal hemoglobins and is the likely candidate for contribution to the epitope recognized by a monoclonal antibody selected for this purpose.

Ferrets are occasionally encountered as companion animals in the United States and are potential sources of bloodstains. Because of this hemoglobin sequence homology, it is necessary to indicate the reactivity toward ferret blood in validation studies of immunoassays for identification of human hemoglobin in bloodstains.

## References

1. Hochmeister MN, Budowle B, Sparkes R, Rudin O, Gehrig C, Thali M, et al. Validation studies of an immunochromatographic 1-step test for the forensic identification of human blood. *J Forensic Sci* 1999;44:597–602.
2. PIR-International Protein Sequence Database. National Biomedical Research Foundation, Georgetown University Medical Center (DC), 1999. The “PROWL” search program (<http://prowl1.rockefeller.edu>) was used.

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<sup>1</sup> Single-letter abbreviations for the amino acid residues are: A, Alanine; G, Glycine; H, Histidine; K, Lysine; L, Leucine; N, Asparagine; S, Serine; T, Threonine; and V, Valine.

## Authors' Response

Sir:

We thank Dr. Rowley for her valuable comments regarding the article "Validation studies of an immunochromatographic 1-step test for the forensic identification of human blood". As demonstrated in the paper, whole blood samples from human donors and several primates tested positive for human hemoglobin to a dilution of 1:100 000 when sterile water was used to dilute the samples.

In response to Dr. Rowley's letter, we obtained whole blood from a domestic ferret (*Mustela puterius fero*) by venipuncture and serially diluted the blood to 1:100 000 with sterile water. Indeed, the blood sample tested positive for human hemoglobin using the Hexagon OBTI Test to a dilution of 1:100 000.

Therefore, our statement: "In the species specificity experiments only human and primate blood tested positive with the assay. These data suggest that the assay is primate specific" can now be modified to "in the species specificity experiments only blood from human, primate, and domestic ferret (*Mustela puterius fero*), which shares a common amino acid sequence from residues 67 to 73 of the alpha chain with human, and primate hemoglobin, tested positive with the assay. These data suggest that although the assay tends to be primate specific, positive results also may be obtained from whole blood from the domestic ferret (*Mustela puterius fero*)."

However, in forensic casework, the practical implications of this cross reactivity with ferret blood is minimal, since one can assume that the number of cases where ferret blood may be found at the scene is low and crime scene investigation can determine if a pet ferret was possibly at the scene. Most important, if the blood sample yields a typical human DNA profile (1), we can reasonably deduce that the blood is of human origin. Therefore, this simple test is still an excellent tool for the forensic laboratory, even if its limitations (positive reaction with human blood, as well as primate blood and ferret blood) are considered.

## Reference

1. Sparkes R, Kimpton C, Watson S, Oldroyd N, Clayton T, Barnett L, et al. The validation of a 7-locus multiplex STR test for use in forensic casework. *Int J Leg Med* 1996;109:186-94.

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**Commentary on** Koons, RD, Buscaglia J. The forensic significance of glass composition and refractive index measurements. *J Forensic Sci* 1999;44(3):496-503.

Sir:

We wish to congratulate the authors on their work. However, we feel that the very data that they have presented appears to be amenable to the opposite conclusion to the one given by the authors and feel that forensic application of their conclusion may be seriously misleading.

The aims of this paper appear to be to demonstrate that elemental analysis and refractive index together have such good discriminatory power that to attach further statistical analysis to any evidentiary item is pointless. We start by making a general point. The discriminatory power of a technique is interesting per se. However, it cannot be discerned from this paper. Not only is the methodology

for developing this number suspect but the discrimination of refractive index and elemental composition is inextricably linked. Of much greater interest would have been the discrimination of elemental analysis conditional on refractive index.

The authors set to prove their point by showing that the range of probabilities of two random pieces of glass sharing "indistinguishable" attributes is in the "very unlikely" range. They present a concept that they call the "information content". We reject this concept as a valid measure of discrimination for the very reasons that the authors give in their own work, and are concerned that the concept is given any credence at all.

In presenting the probability that two pieces of glass from different sources would "match by chance" the authors have answered the pre-data question, which is "What is the probability I would make a mistake if I carried out this matching procedure?" rather than the post-data question, which is "How much does this evidence increase the likelihood that it was the accused who broke it?" It is, of course, the latter in which the court is interested (1,2). Such a question can only be answered by a Bayesian analysis of the evidence and despite the authors' claims to the contrary, database collections of glass samples are the most reliable way we have of assessing the value of such evidence. Furthermore, if we analyze a simple case in the Bayesian framework, it becomes evident that statistics are actually more necessary than in the DNA situation. For example, take a case where a single group of glass has been recovered from a suspect. A small sample of glass has been taken from the crime scene and the evidence has been measured using some analytical method (RI or elemental composition). The likelihood ratio (LR) under consideration is, as in any case,

$$LR = \frac{\Pr(\text{Evidence} | \text{Contact})}{\Pr(\text{Evidence} | \overline{\text{Contact}})}$$

When the LR is coupled with the jurors' prior odds on *Contact* it yields the posterior odds on *Contact* having seen the evidence. When the LR in this particular case is calculated using the notation of Evett and Buckleton (3) it becomes

$$LR = T_0 + \frac{T_L P_0}{S_1 P_L} \cdot lr_{cont} \approx \frac{T_L P_0}{S_1 P_L} \cdot lr_{cont}$$

where  $\frac{T_L P_0}{S_1 P_L}$  represents expert knowledge about the number of fragments that might be transferred, persisted and were recovered, the number of fragments from a single source, and the number of sources. The quantity  $lr_{cont}$ , introduced by Walsh et al. (4) for RI and Curran et al. (5) for elemental information, represents the ratio of "match" strength to the relative rarity of the glass in the population. In a simple two stage approach, where the LR is calculated only if the samples pass some sort of matching criterion, then

$$lr_{cont} \approx \frac{1}{\hat{P}}$$

where  $\hat{P}$  is the relative rarity of the glass. This quantity can only be calculated from a database of glass samples. It is clear that in this case the form of  $lr_{cont}$  is very similar to the LR for a single contributor stain in a DNA case. With STR loci in DNA analysis there is effectively no measurement error in determining the match, and therefore the numerator of  $lr_{cont}$  is 1 in simple cases. However, if

one accepts (and the authors clearly do) that there is measurement error in the analysis of glass, either due to variability within the glass itself or due to the operating precision of the instruments, then there is always some chance that, due to the aforementioned errors, the samples will match. This must be reflected in any analysis of the evidence, and proves once again that the Bayesian approach is necessary (6,7). Failure to do so can seriously disadvantage an innocent defendant.

The authors set to prove the “discriminatory power” of elemental analysis and refractive index by, *inter alia*, testing for significant pairwise correlation between the variables used to describe the samples. We are concerned at the reemergence of “fixed bin” type approaches and had hoped that the faint praise given to fixed binning in the National Research Council report on the evaluation of forensic DNA (8) may have dissuaded future authors from following this inferior approach. Given that a binning approach has been discussed it is unclear to us whether the correlations were calculated from binned data or preferably from the continuous data. A test for correlation after binning is inferior, as much information in the data has been destroyed. The binning strategy for refractive index is curious. We are unaware of any published justification for the use of bins of width 0.0002. However, there are other published approaches (4,9) for the estimation of the RI density (which is the ultimate goal). The origin of the  $12\sigma$  value used to construct the fixed bins is interesting. It appears to be the result of considerations based on a normal distribution, which is difficult to justify. If this assumption of normality is the reason, then perhaps it also could be the basis for their comments about the conservative nature of this binning strategy. However the comparison of small samples such as these (three replicates from each of two samples), where the standard deviation is unknown, is more usually performed by a *t*-test on four degrees of freedom. This is especially important as there appears to be no evidence that standard deviation is constant across samples. In addition the authors appear to make the error of basing their analysis of within source variation on “perfect” samples whereas in casework typically one sample is seriously constrained, the one recovered from the clothing, and may be small, dirty, and over-representing surface fragments. Such an error is potentially serious. It is very unclear to us how the data in Fig. 1 has been processed and different approaches are feasible. The very fact that the authors refer to “weighing” samples leads one to believe that these are substantial fragments, atypical of recovered glass.

Correlation tests can be misused to imply that if correlation between a pair of elements is low, then one may multiply the frequencies of the individual elements, to get the joint frequency of a set of element concentrations. Such an analysis is sometimes sensible, but has been rendered suspect by the authors’ serious editing of the sample data 204 from 1545 samples) which will have the effect of emphasizing difference. The data set in itself appears to be an odd set collected from casework rather than the more useful set of glass on persons unassociated with crime. Binning (if done before the correlation coefficients were calculated) further invalidates this analysis as it destroys information content. Figures 1 and 2 suggest that the data is highly skewed, and thus relationships between any pair of elements, if they exist, are unlikely to be linear. Therefore, even if there is correlation, a linear correlation coefficient is unlikely to detect it. The conclusion that the correlation coefficients observed prove that “all variables are independent” is not substantiated by the presented data and in fact is a hypothesis that is both unprovable and almost certainly wrong. The authors have unwittingly fallen into the problem of “the curse of dimensionality,” a phrase coined by mathematician Richard Bellman (10) who observed that the effort required to solve the problem increases exponentially with increase in

dimension. Scott (11) estimates that for “well-behaved” data in 8 dimensions, approximately  $10^8$  observations would be required to estimate the multivariate density accurately. The current data set is in 11 dimensions and almost certainly not “well-behaved”. Every statistical text the authors of this letter consulted suggested dimension reduction as the only feasible way to approach such problems, an approach used by Curran et al. (5,12) in a Bayesian context. We note that the presented correlation coefficients do not detect the probable association between refractive index and composition. This is most probably because of the serious data editing. The use of an unprovable (and probably false) assumption of independence may result in a serious underestimate of the joint probability of observing a particular set of elemental measurements.

In summary, while we agree that elemental composition and refractive index combined do have good discriminatory power (13–18), the body of literature for the use of statistics and the Bayesian approach in particular is overwhelming. We believe the authors are doing the legal and forensic community a disservice to suggest otherwise.

## References

1. Robertson, BW, Vignaux GA. DNA evidence: wrong answers or wrong questions. *Genetica* 1995;96:145–52.
2. Robertson B, Vignaux GA. *Interpreting evidence: evaluating forensic science in the courtroom*. John Wiley & Sons Ltd., 1995.
3. Evett IW, Buckleton JS. The interpretation of glass evidence: a practical approach. *J Forensic Sci Soc* 1990;30:215–23.
4. Walsh KAJ, Buckleton JS, Triggs CM. A practical example of glass interpretation. *Sci Just* 1996;36:213–8.
5. Curran JM, Triggs CM, Almirall JR, Buckleton JS, Walsh KAJ. The interpretation of elemental composition measurements from forensic glass evidence II. *Sci Just* 1997;37:245–9.
6. Lindley DV. A statistical paradox. *Biometrika* 1957;44:187–92.
7. Lindley DV. A problem in forensic science. *Biometrika* 1977;64:207–13.
8. NRC. *NRC II: The evaluation of forensic DNA evidence*. Washington, D.C.: National Academy Press, 1996.
9. Evett IW. The interpretation of refractive index measurements II. *Forensic Sci Int* 1978;12:37–47.
10. Bellman RE. *Adaptive control processes: a guided tour*. Princeton, N. J.: Princeton University Press, 1961.
11. Scott DW. *Multivariate density estimation*. New York: John Wiley & Sons, Inc., 1992.
12. Curran JM, Triggs CM, Almirall JR, Buckleton JS, Walsh KAJ. The interpretation of elemental composition measurements from forensic glass evidence. *Sci Just* 1997;37:241–4.
13. Coleman R, Goode G. Comparison of glass fragments by neutron activation analysis. *J Radioanal Chem* 1973;15:367–88.
14. Hickman DA. Elemental analysis and the discrimination of sheet glass samples. *Forensic Sci Int* 1983;23:213–23.
15. Hickman DA. Glass types identified by chemical analysis. *Forensic Sci Int* 1987;33:23–46.
16. Koons R, Fiedler C, Rawalt R. Classification and discrimination of sheet and container glasses by inductively coupled plasma-atomic emission spectrometry and pattern recognition. *J Forensic Sci* 1988;33:49–67.
17. Koons R, Peters C, Rebbert P. Comparison of refractive index, energy dispersive x-ray fluorescence and inductively coupled plasma atomic emission spectrometry for forensic characterization of sheet glass fragments. *J Anal Atomic Spectrom* 1991;6:451–6.
18. Almirall JR. Characterization of glass evidence by the statistical analysis of their inductively coupled plasma/atomic emission spectroscopy and refractive index data. *American Academy of Forensic Sciences Meeting*. 1996, Nashville, TN.

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## Authors' Response

Sir:

One of our intended purposes in writing the referenced article, in addition to presenting our research results, was to stimulate discussion among forensic scientists concerning the important topic of the use of statistics in evaluating items of trace evidence. We wish to thank Curran et al. for initiating this discussion and giving us the opportunity to clarify and expand upon a few points that we made in our original paper. The letter writers indicate that our aim was to show that statistics are "pointless". Nothing could be further from the truth. In fact, we are proponents of the appropriate and correct use of statistics in the evaluation of evidence. However, we do not advocate the calculation of purportedly exact statistical measures that may be interpreted without consideration of the underlying uncertainties. We wish the readers to recognize the difficulty, if not impossibility, of calculating frequency of occurrence statistics when using highly discriminating analytical techniques to evaluate evidence whose characteristics vary over both location and time. Although our paper is concerned with the elemental analysis of glass, similar considerations apply when evaluating many items of trace evidence using well-accepted methodologies. The following comments address specific points raised by Curran et al.

The discriminatory power of a technique is not only interesting, but it is also quite useful to scientists making decisions whether or not to use the technique. It should also be of interest to triers of fact when considering what significance to place on analytical results in legal proceedings. In our paper, we do not use or explicitly calculate "discriminatory power" (a term used by the letter writers). However, the data we present clearly indicates the high degree of discrimination among glass sources obtained using a combination of refractive index (RI) and elemental analysis. The link between RI and elemental composition is not significant in this work. The RI of a glass fragment is a direct result of both its total chemical composition and thermal history, and it is independent of any single element concentration. There is no need to consider elemental analysis conditional upon RI as suggested by the letter writers. In fact, our study, which consists of all evidentiary glass for which the FBI Laboratory obtained triplicate analyses from 1990 to 1996, includes no two sources with the same elemental composition, regardless of RI.

The concept of information content is a valid measure of discrimination, within the context that it is used in our paper. The information content, as we defined it, is a measure of the maximum number of distinguishable sources that could possibly exist within the compositional range exhibited in a set of samples. It is a useful measure of the relative discrimination capability of a given technique and serves as a benchmark for comparison of alternate techniques for a given analysis. For example, several forensic laboratories are currently considering the use of ICP-MS instead of ICP-AES for compositional analysis of glass. The question of whether one obtains better discrimination capability by determining 30 elements with relative standard deviations (RSDs) in the 10–50% range by ICP-MS or the 10 elements with 1–5% RSDs by ICP-AES can be answered by comparing the information content of the two methods. As we pointed out in our paper, the information content provides no information about the distribution of glass specimens within the elemental and RI combinations. Despite Curran et al.'s concern that information content be given any credence at all, this measure (although calculated differently than we defined it in our paper) has been widely used and has stood as a landmark

concept in information theory as applied to analytical spectroscopy for over 20 years (1).

We agree that we have answered Curran et al.'s pre-data question and not their post-data question. The purpose of our article was to demonstrate that the analytical method used provides information that can be used for excellent source discrimination—a pre-data question. The post-data question as posed by the authors is applicable to evaluation of evidence in a case framework, a situation not addressed in our article. We agree that their post-data question is best answered by a Bayesian approach, precisely because the question is framed within that approach. We think it important to note, however, that the discrimination capability of the analytical method is an intrinsic part of the calculation of the likelihood ratio and any assessment of the significance of the evidence. The Bayesian approach is one method of assessing the significance of a finding of indistinguishability between glass fragments recovered from a suspect and those from a broken glass object. In simple cases, where probability distributions for all measured parameters in the appropriate crime scene and alternate hypothesis environments and transfer and persistence parameters are known, the Bayesian approach may be viable. Additionally, we never stated that glass databases are not the most reliable way of assessing the value of evidence. We agree that *appropriate* databases are the best way of calculating frequency of occurrence statistics. However, we state again that, when using highly discriminating analytical methods and considering items of evidence whose distributions vary over both location and time, it may not be possible to obtain the databases needed for the Bayesian approach. Application of any statistical approach to probability calculations when population distributions are unknown is dangerous and may produce misleading results.

A major portion of the letter consists of a primer on the calculation of likelihood ratios, which is a summary of the authors' work in this area. We suggest that interested readers read the original articles (2,3) for a full derivation of the equations used in the letter. What is not mentioned in the letter and the authors' other articles is the uncertainty associated with each term in their equations. In the denominator of their first equation, the probability of the evidence given no contact depends upon having a database of glass from wherever the defendant's alibi may be. The values, which must be used for the transfer and persistence terms, are highly subjective and subject to order of magnitude errors in realistic case situations (see Reference 2 for examples). The quantity  $lr_{\text{cont}}$  is an interesting approach, particularly when coupled with the use of Hotelling's  $T^2$  for multivariate data. However, as pointed out by Curran et al.,  $lr_{\text{cont}}$  is roughly proportional to  $1/P$ . An important point that we have made in our paper is that the value of  $P$  is extremely small. One can dispute the details of the calculation of the rarity of a particular glass, but it is indisputable that as more discriminating methods of analysis are used, the probability of two different sources of glass being indistinguishable decreases and the likelihood ratio increases. Our comment concerning likelihood ratio calculations, to which Curran et al. seem to have such a strong objection, is that the number cannot be calculated with any degree of precision. However, this is unimportant if an analytical method is used that assures that the number is so large as to be highly significant for indistinguishable specimens.

We agree that there is some error associated with each measurement in glass. In fact, there is measurement error associated with any analytical measurement in any field of endeavor. There is a vast field of chemistry literature detailing non-Bayesian methods

of dealing with analytical error. The discrimination potential of a method is determined by the magnitude of the measurement error plus sample heterogeneity relative to the range across similar samples. The fact that measurement error exists does not “prove once again that the Bayesian approach is necessary”. The equations of likelihood ratio are an interesting academic exercise and provide a framework for qualitative consideration of the factors involved in assessing the significance of matching analytical data. We appreciate the discussion of this method and leave it to the readers of this journal to determine whether the calculation of likelihood ratios is a reasonable and legally acceptable approach for presentation of evidence to a court of law.

We do not feel that we used a “fixed bin” approach inappropriately here. Our bins are not of fixed width, a point which we discussed in detail in our paper. The selection of bin widths based on measurement precisions is an appropriate method for comparison of specimens of similar compositions. The bin means are fixed in our calculations of our measures of information content and most common composition. However, as we state in our article, if we were to use our data to calculate the frequency of occurrence for an evidentiary specimen, we would use a floating bin for each variable with a position and width based on the analytical mean and standard deviation calculated from replicate samples of the evidentiary specimen. The justification of 0.0002 as a bin width for RI is curious, we agree. Rightly or wrongly, however, it is a number that has been widely used as a fixed cutoff for source differentiation by many glass examiners for roughly 20 years (4). RI differs from other parameters in our study, in that RI measurement uncertainty (bin width) does not vary with RI measurements (bin center locations). Therefore, we chose 0.0002 as a constant bin width, recognizing that it is smaller than the  $12\sigma$  widths of the other bins. For purposes of casework assessment of glass fragments, we agree that a fixed cutoff of 0.0002 is generally inappropriate and it is preferable to use a statistical test criterion based on repeated measures of the glass fragments in question. Curran et al.’s reference to other published RI density distributions is curious, as these are clearly inappropriate for case-specific situations. For example, a frequency distribution given in a 1978 article about glass in England is certainly not applicable to a 1999 case in the United States. However, this does not matter for our approach, since the choice of element concentration bin widths is based on analytical precision and source heterogeneity and has nothing to do with probability density distributions. The selection of  $12\sigma$  bin widths for element concentrations is explained in our paper. That the bins are wide is supported by the fact that two specimens having data at adjacent bin centers are clearly distinguishable by any reasonable statistical test. In fact, two specimens lying near opposite edges within the same bin are readily distinguishable using the match criteria of the FBI Laboratory. The use of a calculated standard deviation measure in setting bin widths does imply some degree of normal distribution to the underlying data. We agree that for some broken glass objects, some or all of the measured parameters do not exhibit a normal distribution. This should have no effect on our selection of bin widths for purposes of assessing the variations in observed compositions. It would, however, adversely affect the commonly used methods of statistical evaluation of the data (i.e., pooled t-test, calculations of LR) from that broken object.

The letter writers state that standard deviations are unknown for our specimens. In fact, as pointed out repeatedly in our paper, the standard deviations are calculated based on measurements from triplicate fragments from each specimen. That the standard deviation

is not constant across samples is the point of our Fig. 1 and the related selection of variable bin widths. The standard deviation is unknown only in the sense that three samples may not be enough to calculate a standard deviation when the distribution is not normal. Generally, a t-test of means is appropriate for comparison of specimens, because, as shown clearly in our Fig. 1, two specimens with similar means will have similar standard deviations. Comparison of two samples with dissimilar means, where the standard deviations are different, is a trivial exercise because widely different means are readily distinguished by any statistical test.

Curran et al. make several comments concerning the state of casework samples and the assumption that our data were obtained from “perfect” samples. All of the samples in this study were derived from casework samples, either as specimens of known broken windows, fragments recovered from clothing and other sources, or comparison exemplars, such as alibi sources. Approximately one-fourth of our specimens were recovered fragments and three-fourths were from known broken glass objects. Samples were cleaned with concentrated nitric acid prior to analysis (5), a procedure that removes contamination and results in consistent element concentration measurements. Whether or not questioned samples exhibit a preponderance of fragments containing an original surface is a moot point. No one has reported and we have seen no evidence of measurable differences between the concentrations of the measured elements in surface and bulk samples of cleaned glass. The claim that the samples are too small is not true, in that they all meet the size requirements for elemental analysis according to the FBI Laboratory protocols in effect at the time of their examination. The claim that this “error” is potentially serious is untrue, because no error of the type Curran et al. describe exists. The comment that because the samples were weighed, they are atypical of recovered glass fragments reveals a lack of analytical experience of the letter writers. Samples as small as 100  $\mu\text{g}$  are routinely weighed and analyzed in many analytical laboratories. Microbalances are capable of weighing samples with a precision of 0.1  $\mu\text{g}$ , which equates to a relative precision of 0.1% for a 100  $\mu\text{g}$  fragment.

We do not understand why the processing method of the data in our Fig. 1 is unclear, since it is described in the text. Figure 1 is a plot for each element of the RSD of the triplicate samples for each specimen versus the mean for that specimen. To convert this data to bin widths, a smooth curve was drawn through the points and the value for each  $12\sigma$  bin width was calculated by multiplying the standard deviation value corresponding to the mean concentration at each bin center by 12. The comments about serious data editing to eliminate duplicate samples seem unwarranted to us. Since these are case-derived samples, many of which are of unknown ultimate source, we limited the number of samples to include equal weighting (3 replicates) from each source. The number of samples was not reduced from 1504 to 204, as Curran et al. suggest. Rather, removal of samples with less than three replicates and those duplicate samples from the same case reduced the data set from 1504 samples to 612 (triplicate samples from 204 specimens). Limiting the number of samples in this way will not diminish the correlation coefficients, but rather would increase them. For example, if we were to include 100 samples from the same source it would generate a symmetric cluster of points about a mean value, the size of the cluster dictated by the combined analytical precision and sample variation. Such a cluster of points in a regression plot would lessen the value of any calculated correlation coefficient.

We do not agree with the comment that casework samples are an odd set that is not as useful as samples collected from persons not

associated with crime. There is no evidence we can find in the literature or in our considerable past experience that there is any difference in the distribution of any of the measured parameters between glass recovered from people suspected of crimes and from those not suspected of being associated with crime. The data set of glass from people unassociated with crime would be an interesting one for comparison with other existing databases. However, such a database does not exist, because it would be impossible to collect. In various comments throughout their letter, Curran et al. suggest that to interpret our data in a Bayesian context we would need something on the order of  $10^{11}$  specimens collected from random individuals unassociated with crime. Further, we would need perhaps 10 analyses of each specimen to correctly assess standard deviations, normality of parameter distributions, and to use multivariate versions of the t-test, such as Hotelling's  $T^2$ . Collection of such a database is impossible because people unassociated with crime involving broken glass typically do not have many fragments from the same source on their persons (6).

The calculations of coefficients of linear regression were based upon raw data, not binned data, because binning first would have decreased the information content of the data, as stated by Curran et al. We apologize for not making this clearer in the text of the article. The caption of Fig. 3 should have read, "The distribution of Al and Mn among glass specimens." The statement that the skewed distributions shown in our Figs. 1 and 2 would result in nonlinear correlations between pairs of variables is not correct. Figure 1 displays precision of measurements, which effectively has no bearing on correlation coefficients. The fact that samples are not evenly distributed across Fig. 2 cannot be directly translated into correlation coefficients, because it cannot be discerned from Fig. 2 which point in one element plot corresponds with a point in another element plot. Thus nothing can be said about the linearity of correlations by observing Fig. 2, despite the claim of Curran et al. The elements Al and Mn were selected for the scatter plot shown as our Fig. 3 because this is the pair of variables with the *best* correlation. No nonlinear relationships are apparent from visual observation of this figure or similar figures of every other pairwise combination of variables. In summary, we find no evidence of strong correlations between pairs of variables, either linearly or nonlinearly. Curran et al. use the fact that we observe no correlation between RI and composition as evidence of our inability to detect correlations between variables. In fact, there should be no direct correlation between RI and the concentrations of any single element. The sum of all measured elements in our analytical protocol is roughly 18% of the total mass of the glass fragment. The RI is more profoundly influenced by the elements not determined in our protocol (such as silicon, lithium, potassium, and lead) than by the elements determined. If we had measured the concentration of every element and the RI for each sample, then two dimensions of redundancy would exist in our data (the sum of all oxides must be 100% and the RI is roughly calculable from the composition). At any rate, the lack of correlation is not caused by the "serious data editing" which Curran et al. purport to exist in our database.

The comment concerning the inability to prove lack of dependence among 11 variables is a good point. It is possible that there is an interdependence of element concentrations such that the data could be rotated in 11-dimensional space to form linear combinations of variables without *significant* loss of discrimination among samples. If such a dependence exists, then multiplying probabilities together as we did would result in some overestimation of discrimination capability. We have seen no indication of this intervariable correlation, but because it is possible, we suggest that our calculations give reasonable, but not exact estimates of the probability of matches among ran-

domly collected glass fragments. As Curran et al. point out, the "curse of dimensionality" is a consideration in using multivariate databases. The number of samples required to form a probability density function in 11 dimensions is unrealistically large. Variable reduction, such as by factor analysis, can be used to reduce the dimensionality to facilitate classification decisions and for convenient plotting of results. However, any variable reduction method results in loss of information. In the comparison of evidentiary specimens, it is of paramount importance to avoid false associations in that these could lead to incorrect consequences for an innocent accused. Therefore, all measured variables must be indistinguishable to result in a conclusion of two fragments of glass (or hair, soil, fibers, or any other transfer evidence) having come from a single source. Reduction of dimensionality to make the data fit a simple statistical model for purposes of calculating probability statistics does not justify the loss of information and consequent increase in the number of false associations. Another practical consequence of variable reduction methods is that the new factors formed by linear combinations of variables do not have readily discernable physical sense. That is, a factor that is a linear combination of 10 element concentrations and RI cannot be explained to the participants of a criminal proceeding in a manner that they will understand and whose significance they will appreciate.

In summary, we are not against the use of statistics in the evaluation of forensic evidence. Rather, we are proponents of it and believe that the Bayesian approach has considerable merit in the appropriate applications. However, we are stronger advocates of the use of good analytical methods to provide accurate, precise analytical data with as much discrimination capability as possible. The statistical evaluation of such data is much more difficult, particularly for manufactured items such as glass, than it is for data such as RI alone. It is apparent without calculating any probability statistics that a chance matching of randomly selected samples is extremely small and, as a result, the exact calculation of statistic figures is not important to the trier of fact. The Bayesian approach is useful in that factors other than population frequency can be considered in evaluating the significance of the evidence under several alternate hypotheses. We believe that it is more important to use highly discriminating and reliable analytical methods, even if they cannot be used to calculate an exact probability number, than it is to use poorer analytical methods or data reduction in order that statistics can be calculated.

## References

1. Kaiser H. Foundations for the critical discussion of analytical methods. *Spectrochim Acta B* 1978;33B:551-76.
2. Walsh KAJ, Buckleton JS, Triggs CM. A practical example of glass interpretation. *Sci Just* 1996;36:213-8.
3. Curran JM, Triggs CM, Almirall JR, Buckleton JS, Walsh KAJ. The interpretation of elemental composition measurements from forensic glass evidence II. *Sci Just* 1997;37:245-9.
4. Miller E. Forensic glass comparisons. In: Saferstein R, editor. *Forensic science handbook*. Englewood Cliffs (NJ): Prentice-Hall 1982;139-83.
5. Koons RD, Fiedler C, Rawalt, RC. Classification and discrimination of sheet and container glasses by inductively coupled plasma-atomic emission spectrometry and pattern recognition. *J Forensic Sci* 1988;33:49-67.
6. Lau L, Beveridge AD, et al. The frequency of occurrence of paint and glass on the clothing of high school students. *Can Soc Forensic Sci J* 1997;30:233-40.

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**Commentary on** Linch CA, Smith SL, Prahlow JA. Evaluation of the human hair root for DNA typing subsequent to microscopic comparison. *J Forensic Sci.* 1998; 43(2):305–14.

Sir:

It was with some dismay that we read the above-cited article by Linch et al., who reported that, in their experience, the technique of Fluorescence In Situ Hybridization (FISH) was unsuitable for gender determination of hair.

Linch et al., reported that they failed to attain hybridization of commercially (VYSIS) available X- and Y-chromosome-specific alpha-satellite FISH probes to both archived and fresh hair samples. We had previously reported that FISH, using these probes, could correctly identify the gender of hair (1). In addition, we have reported using FISH successfully to identify the gender of cells in a number of different sample types as it could be applicable in forensic analysis (2–7). As a result of this discrepancy, we reviewed their methodology. The technique used was essentially that reported in our article (1) with one major exception. The cells were heat fixed to the slides. In our original report, cells from the hair bulb were attached using liquid nitrogen (2). The step of heating cells represents a critical error in their FISH methodology. In our experience with FISH, those of other colleagues, reports and recommendations in the literature (8,9) and “trouble shooting” recommendations by commercial companies (VYSIS, Venatana-Oncor), heating, baking, or flaming a slide prior to the hybridization step severely inhibits the efficient hybridization of DNA probes to the cells. Hence, probes do not hybridize well, if at all, and may result in inconclusive results and/or cause false hybridization signals. This appears to be the case with the observation made by Linch et al., wherein they report seeing either no signals (i.e., no hybridization), some hybridization or false hybridization. A simple change in the way they made slides would have solved their lack of FISH hybridization.

Linch et al. attempted to justify their negative results by stating that “FISH probes have inherent problems even when used with fresh viable cells. Loss of target DNA, poor penetration of probe, and incomplete or non-specific hybridization are problems associated with apoptotic, necrotic, and keratinizing cells. FISH requires examination of a large number of cells, the use of control cells on the same microscope slide as the evidence slide (due to critical temperature requirement) and sophisticated statistical analysis” (10). Those statements may have had validity some years ago, however they are no longer of critical concern with newer techniques and commercial probes. Techniques have been so well standardized that FISH is now used routinely for prenatal, postnatal and leukemia diagnosis (11–15). In fact, in microdeletion syndrome cases such as DiGeorge or William syndromes, FISH is the only truly confirmatory test. A number of the currently available probes have been FDA approved for clinical testing.

It is our recommendation that Linch et al. or any other investigator planning to use FISH, first thoroughly familiarize themselves with the technique and its potential pitfalls, before reporting conflicting information in the literature.

## References

1. Prahlow J, Lantz PE, Cox-Jones K, Rao PN, Pettenati MJ. Gender identification of human hair using fluorescence in situ hybridization. *J Forensic Sci* 1996;41:1035–7.
2. Collins K, Rao PN, Hayworth R, Geisinger K, Pettenati MJ. Identification of male cells using fish with Y-probes in cervicovaginal smears in alleged sexual assault cases. *Am J Hum Genet* 1993;53:A535.

3. Collins KA, Rao PN, Hayworth R, Schnell S, Tap M, Lantz PE, et al. Identification of sperm and nonsperm cells in cervicovaginal smears using FISH: Applications in alleged sexual assault cases. *J Forensic Sci* 1994;39:1347–55.
4. Pettenati MJ, Rao PN, Collins KA, Parsons LH, Lantz PE, Geisinger KR. Identification of male epithelial cells in cervicovaginal smears using fluorescence in situ hybridization: Application in sexual assault and molestation. *Am J Hum Genet* 1994;55:A115.
5. Collins K, Geisinger K, Rao PN, Lantz PE, Pettenati MJ, Parsons L, et al. Application of molecular cytogenetics to cervicovaginal smears in alleged sexual assault cases. *Acta Cytologica* 1994;38:795–6.
6. Rao PN, Collins KA, Geisinger KR, Parsons LH, Schnell S, Hayworth-Hodge R, et al. Identification of male epithelial cells in postcoital cervicovaginal smears using fluorescence in situ hybridization: Application in sexual assault and molestation. *Am J Clin Path* 1995;104:32–5.
7. Pettenati MJ, Rao PN, Schnell S, Hayworth-Hodge R, Lantz PE, Geisinger KR. Gender identification of dried human bloodstains using fluorescence in situ hybridization. *J Forensic Sci* 1995;40:883–5.
8. Reiley PA. Fluorescent in situ hybridization. *The Cytogenetic Symposia*, 1994. Ed. BJ Kaplan and KS Dale.
9. The AGT Cytogenetic Laboratory Manual. 3rd edition. Ed. MJ Garch, T Knutsen, JL Spurbach. Lippincott-Raven 1997.
10. Studzinski GP. Cell growth and apoptosis. Oxford: Oxford University Press. 1995.
11. Jalal SM, Law ME, Carlson RO, Dewald GW. Prenatal detection of aneuploidy by directly labeled multicolored probes and interphase fluorescence in situ hybridization. *Mayo Clin Proc* 1998;73(2):132–7.
12. Dewald GW, Brothman AR, Butler MG, Cooley LD, Patil SR, Saikovich IA, et al. Pilot studies for proficiency testing using fluorescence in situ hybridization with chromosome-specific DNA probes: a College of American Pathologists/American College of Medical Genetics Program. *Arch Pathol Lab Med.* 1997;121(4):359–6.
13. Schad CR, Kuffel DG, Wyatt WA, Zinsmeister AR, Jenkins RB, Dewald GW, et al. Application of fluorescent in situ hybridization with X and Y chromosome specific probes to buccal smear analysis. *Am J Med Genet* 1992;66(2):187–92.
14. Leana-Cox J, Jenkins L, Palmer CG, Plattner R, Sheppard L, Flejter WL, et al. Molecular cytogenetic analysis of inv dup(15) chromosomes, using probes specific for the Prader-Willi/Angelman syndrome region: clinical implications. *Am J Hum Genet* 1994;54(5):748–56.
15. Philip J, Bryndorf T, Christensen B. Prenatal aneuploidy detection in interphase cells by fluorescence in situ hybridization (FISH). *Prenat Diagn* 1994;14(13):1203–15.

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## Authors' Response

Sir:

Early reviewers and colleagues suggested we separate the paper into three articles: (1) Fluorescence in situ hybridization (FISH) gender typing of telogen hair club material, (2) Transmission electron microscopy (TEM) of telogen hair club material and anagen hair bulb material, and, (3) Polymerase Chain Reaction (PCR) nuclear DNA typing of all hair root stages. We protested however because we hoped the reader would appreciate the relationship between hair root morphology and expected DNA typing results if the three parts were taken as a whole. One of the main goals of the paper was to urge the reader to microscopically evaluate hair roots prior to attempting biotechnical methods.

A complete read of the paper will show that we had FISH X-Y probe failure with telogen hair club material (trichilemmal keratin) and not with anagen hair bulb cells. Telogen hair clubs have no intact nuclei and anagen hair bulbs do, as revealed by the TEM part of the study. In 1997 FISH X-Y probes required interphase nuclei or metaphase chromosomes for success. We did not attempt FISH gender typing of the anagen hair bulb material because the practicing forensic community prefers the STR, amelogenin typing of such material for obvious reasons. FISH gender typing of trichilemmal keratin would be similar to FISH gender typing of fingernails absent soft tissue. There is a 1993 report of successful FISH gender typing in which the slides containing "sheath cells from the shaft of the hair roots" were heated to 80 degrees C for 20 minutes prior to the dehydration steps (1). It was refreshing to see investigators actually identify the material they were testing but, again, these types of hairs (anagen) are a waste of time for FISH X-Y forensic analysis since more informative methods exist for such cell rich materials (STR, amelogenin).

The commentators' use of the term "hair bulb" indicates their focus on anagen phase hairs which we did not use. Investigators not experienced with hair root microscopy do not know if they are testing clubs or bulbs, each of which may, or may not, also have follicular tissue present. In Prahlow et al., (2), Dr. Pettenati, Dr. Rao, and Dr. Prahlow reported successful FISH typing of "pulled" and "combed" hairs from autopsy patients without benefit of microscopic examination of the hair roots prior to typing. It is extremely difficult to comb the hair of an autopsy patient without obtaining some hairs that contain either sheath cells or bulb cells (not telogen clubs).

Forensic scientists do not have the luxury of testing clinical diagnostic material. Our brief touch of the micro slide to the hot plate to evaporate the acetic acid, as complained about, was a minor tissue insult compared to that suffered by hairs left at crime scenes. Forensic validation guidelines require that degradative environmental and matrix studies be performed on specimens prior to implementation of such biotechnologies for crime lab use (3-5). In other words, subject the telogen club (trichilemmal keratin) material to extreme temperatures, humidity, direct sunlight, dyes, soils, and foreign blood/semen/saliva contaminants; wash with an appropriate method (5), and then, attempt FISH gender typing if one expects to find interphase nuclei in keratin material. We did contact Vysis technical support about our results, March 1997, and they recommended purchase of their FISH apoptosis detection kit. (The telogen club is the final product of an apoptosis process that shrinks the hair root stem from the active (anagen) growth stage to the resting (telogen) stage). At that time the Vysis technical staff was not concerned about our brief specimen heat fixation method.

The focus of the FISH portion of the study was the telogen hair club since its exploitation for gender typing would be an addition to comparison microscopy and mitochondrial DNA D-loop sequence analysis, the only currently useful techniques for forensic comparison of such. Biomedical and forensic investigators should take the time to learn proper hair histogenic micro structure and language. "Shed", "combed", "pulled", and "plucked" hair specimen categories only add to the confusing data that have been published using FISH, nuclear DNA PCR, and mitochondrial DNA PCR sequence methods. One must know the nature of the material actually being tested and account for the potential environmental insults the material may have had prior to arriving at the sterile laboratory.

We have no doubt that FISH is a useful methodology for clinical specimens. We have no doubt that FISH X-Y probes work on anagen hairs. FISH X-Y probes will not work on telogen hair clubs

(absent attached follicular cells) no matter what methodology is used.

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**Commentary on Willey P, Scott DD. Who's buried in Custer's grave? J Forensic Sci 1999;44(3):656-65.**

Sir:

The excellent article, referenced above, was absolutely fascinating!

As a forensic dentist and a clinical dentist, I have the following comments. The suggestion that skull (Burial 8B) was a tobacco user and specifically a pipe smoker, due to "pipestem abrasion" on the left mandibular premolar teeth may not be perfectly accurate for the following reasons:

1. All of the left posterior teeth depict a degree of occlusal abrasion, but I believe that this abrasion was the result of bruxism. (I am sure that soldiers over 125 years ago had plenty of problems over which to clench and grind their teeth.)
2. I am not sure what pipestems were made of in the 1870's, but I cannot think of many materials suitable for pipestems harder than enamel, thus, I would expect the stem to yield before the enamel structure of the teeth.
3. If the individual were a pipe smoker, and clenched the stem in a chronic fashion, more than likely the stem would have caused a vertical downward movement of the involved tooth or teeth, much like an orthodontic appliance.

The bottom line: I would not think that one of the elements in eliminating Custer should be the fact that he was disdainful of smoking, simply because I don't believe there is ample evidence that the abrasion came from a pipestem in the first place! Eliminate him on other factors if you will, but not on that particular one.

Again, I thank the authors for a meticulous and interesting account of the events surrounding the death of Gen. Custer. The photographs, sketches and maps were very illustrative and engrossing.

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#### Authors' Response

Sir:

We appreciate Dr. Norman Sperber's comments and insights concerning our assessment of Burial 8B. We concur with many of his statements, particularly those concerning the exceptional service that the *Journal of Forensic Sciences's* editor and

staff performed when arranging and reproducing our article's illustrations.

We take to heart his comments concerning the possibility that Burial 8B was not a smoker, thus further supporting the possible identification of those skeletal remains as being George Armstrong Custer. Dr. Sperber puts us in the enviable position of arguing, at least in part, against our own thesis—that the remains may be those of Custer. For that and the opportunity to expand our discussion on the matter of pipe smoking, we owe him a debt of thanks.

The first point Dr. Sperber makes is that some or all of the occlusal attrition on the left posterior teeth may be due to bruxism. We did assess the teeth for bruxism in an earlier paper, where we reported being unable to arrive at a definitive conclusion on the matter (1). As Sperber notes, nineteenth century soldiers had plenty of reasons to grit their teeth—and perhaps the Seventh Cavalry troopers had even more reasons than others. It is certainly possible that the individual represented by Burial 8B was prone to bruxism, but bruxism alone does not explain the groove in the left mandibular premolars (no. 20 and 21).

Dr. Sperber's second point is that present-day pipestems are made of materials far softer than dental enamel and do not abrade the teeth. Nineteenth century pipestem materials were different than those of today. In the 1870s pipestem bits were of three types. The first type was a reed stem. This pipestem was made from a dried reed and was detachable from the pipe bowl. The stem was hard, contained abrasive plant silicates, and usually lasted until it "burned out" (2–4). The second type was a fired, white Kaolin clay pipe, the most common pipe of the era and usually manufactured in Great Britain or Holland. The stem and the bowl were a single unit, and the bit was either round or slightly flattened in cross section. The integral fired-clay bit was hard and had a gritty feel when held in the mouth. Although the clay itself was softer than dental enamel, the quartz crystals it contained were hard (5) and being angular were extremely abrasive. We suspect this kind of stem bit was the one responsible for most of the pipe abrasions in the archeological record of the period. The third type was the "new fangled" hard rubber bit and stem which were attached to a wooden or briar bowl. It came into vogue during the Civil War (2,4) and is essentially the same shape we use today, although the materials employed have changed. The vulcanized rubber stem was hard in contrast with today's plastic stems, although less abrasive than either of the other two bits of the day. Smoking pipes, although not yet recovered from the Little Bighorn Battlefield site, are common artifacts found in military archaeological sites throughout the United States.

Dr. Sperber's third point is that today's chronic pipe smokers typically experience orthodontic-like movement of the teeth employed in clenching a pipe, thus seeming to reject our identification of pipe use based on the abraded grooves. Nevertheless, similar abrasions have been reported in the historic archaeological literature with little or no tooth movement. Grooves similar to that of Burial 8B have been presented, illustrated and attributed to pipestems in several recent summaries (6–8). Incidentally, all three of the grooves illustrated in these sources are grooved on the left side, similar to Burial 8B, although all three show the grooves being between canines and first premolars, unlike Burial 8B's groove which is between the first and second premolars.

In conclusion, we thank Dr. Sperber for his insights concerning bruxism, and this opportunity to expand and clarify our interpretations related to Burial 8B's pipe smoking. Although pipe smoking is an apparent contraindication to Burial 8B being a portion

of Custer's skeleton, fairness to the remains and the potential identification demand its note.

Finally and unrelated to the present topic, an unfortunate typographical error crept into the final sentence of the article's text. It was embedded in a quotation, making the error doubly bad. Misquoting Snow and Fitzpatrick (9), it reads, "there exists the possibility, at least, that one or more unknown troopers may be perpetually doomed to the commission of that most cardinal of military sins: impersonating an office' (sic.)" Few enlisted men—or officers, for that matter—would be capable of impersonating a copying machine, let alone a whole office. The word should be "officer." Our apologies to Snow, Fitzpatrick and the troopers of the Seventh Cavalry.

## References

1. Willey P, Glenner RA, Scott DD. Oral health of Seventh Cavalry troopers: Dentitions from the Custer National Cemetery. *J Hist Dentistry* 1996;44(1):12, table 14.
2. Pfeiffer MA. Clay tobacco pipes and the fur trade of the Pacific Northwest and the Northern Plains [master's thesis]. Lincoln, NE: University of Nebraska, 1982.
3. Thomas BB, Burnett RM. A study of clay smoking pipes produced at a nineteenth century kiln at Point Pleasant, Ohio. 1971 Conf Historic Site Arch Papers 1972;6(1–2):1–31.
4. Wilson RL. Clay tobacco pipes from Fort Laramie. *Annals Wyoming* 1961;33(2):120–34.
5. Snow R. Evidence of pipe stem grooving from Monserrat, West Indies and South Ravine, Iowa. 1986. Manuscript in author's possession.
6. Alt KW, Pichler SL. Artificial modifications of human teeth. In: Alt KW, Rosing FW, Teschler-Nicola, M, editors. *Dental anthropology: Fundamentals, limits, and prospects*. New York: Springer Wien 1998; 399, fig. 13.
7. Hillson, S. *Dental anthropology*. Cambridge: Cambridge University Press 1996;252–3, fig. 11.12.
8. Scott GR, Turner CG. The anthropology of modern teeth: Dental morphology and its variation in recent human populations. Cambridge: Cambridge University Press 1997; xix, fig. P.2A.
9. Snow CC, Fitzpatrick, J. Human osteological remains from the Battle of the Little Bighorn. In: Scott DD, Fox RA, Connor MA, Harmon D, editors. *Archaeological perspectives on the Battle of the Little Bighorn*. Norman: University of Oklahoma Press 1989; 247.

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## Partisan Expert Witness Testimony

Sir:

Partisan, as a characterization of a forensic expert, has become a term of derision in legal parlance. The word "partisan" has acquired the suggestion that the expert is less than honest when giving opinion testimony in a court of law. In reality, the word "partisan" means taking sides. An expert who takes the witness stand has in fact taken sides; otherwise he or she would not be called as a witness. Unlike the material witness, the professional who testifies did not just happen to have observed a relevant fact and is compelled to give testimony. The professional, a chemist or a psychiatrist, testifies after being retained by one side in a controversy to assist in a specific case. He or she is asked to interpret (give opinion) data available to both sides. The expert's opinion may be helpful in which case the

expert will be called upon to give testimony. If the opinion does not support the view of the retaining lawyer, the expert will not testify, which does not mean that his or her work was not useful.

Charles Simkins, a nationally known personal injury lawyer specializing in brain injury, has repeatedly said in public forums that he found my opinions that he does not have a case very useful. "It saves me wasting time and money," he said.

A forensic expert would be self-destructive if he or she falsified the data in order to arrive at a tailor made opinion. This would not be partisanship but deception. Unethical professionals would be ill advised to go into forensic work. It is much easier to falsify data and offer unfounded opinions outside of the scrutiny of the adversary proceedings. It would be much easier for dishonest physicians to misrepresent clinical data to patients than to opposing lawyers

and their experts; it would also, in the long run, be financially more rewarding.

The notion that being paid for professional services given in connection with litigation makes one's ethics suspect is self-serving. Lawyers, unable to undermine an adverse opinion on the merits, resort to ad hominem attacks. Expert testimony is not simply a matter of facts that can be true or false. The opposite of opinion testimony, unlike that of a material witness, is not a falsehood but another opinion. The divergence of opinions of appellate judges is rarely the result of bias or corruption. The same holds true for professionals who give opinion testimony in the courts of law.

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