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 specifity 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 specifity 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.

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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,

$$\mathrm{LR} = \frac{\Pr(Evidence \mid Contact)}{\Pr(Evidence \mid \overline{Contact})}$$

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

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

where $\frac{T_L P_0}{S_1 P_L}$ represents expert knowledge about the number of fragments that might been 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 12σ 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.

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