Letters to the Editor

Discussion of "Positive Identification of Torn Paper Matches with Emphasis on Crosscut and Torn Fiber Comparisons"

Dear Sir:

I have read with interest the paper "Positive Identification of Torn Paper Matches with Emphasis on Crosscut and Torn Fiber Comparisons" by Kent C. Dixon as published in the Vol. 28, No. 2, April 1983 issue of the *Journal*. Mr. Dixon's paper illustrates nicely the various aspects of paper match comparison and will be useful to those not already familiar with the techniques. It is unfortunate however that his research did not apparently extend to this *Journal*. Although he states that "Paper covering these types of comparisons have not been located, although it is possible that there has been work done and something written," the Vol. 13, No. 1, January 1968 issue contained a paper "Comparison of Paper Matches" by H. J. Funk. This paper focused on the same two features of paper matches which Mr. Dixon "has shown to have major significance." It also described a technique for staining paper fibers that may be helpful to Mr. Dixon in his continuing research.

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Author's Response

Dear Sir:

Thank you for calling to my attention the January 1968 article in the *Journal* by H. J. Funk, "Comparison of Paper Matches," of which until now I was unaware. This may seem to be an example of not "seeing the forest for the trees." However, I found no reference to this article in our files, or any other texts which I have read.

I have now obtained a copy of this article from the Justice Department Library and found it quite interesting, particularly the use of phloroglucinol in hydrochloric acid as a staining agent. I will now do some experimenting with this agent in the hopes of facilitating my future match comparisons. Thank you again.

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Discussion of "The Trapping, Storing, and Subsequent Analysis of Ethanol in In-Vitro Samples Previously Analyzed by a Nondestructive Technique"

Dear Sir:

In their paper, "The Trapping, Storing, and Subsequent Analysis of Ethanol in In-Vitro Samples Previously Analyzed by a Nondestructive Technique," in the Vol. 26, No. 4, Oct. 1981 issue of the *Journal*, authors Wilkinson et al have apparently made a significant error which leads to erroneous conclusions about the rate of release of ethanol from silica gel adsorbents and the overall recovery of the process.

Wilkinson et al report (Fig. 1, p. 673) that the release of ethanol from silica gel increases

with time to a more or less constant value after 60 min. If the samples are allowed to stand the requisite time before analysis, according to the authors, the recovery of ethanol approaches 100%; that is, the release of ethanol is quantitative. Considering the usual distribution of a solute between both phases of a two-phase system according to a partition constant, this result must be considered surprising. Accordingly, we undertook to examine this result.

Ethanol-in-air samples generated by a Mark IIA Simulator (Smith and Wesson) were analyzed by a 4011A Intoxilyzer (CMI), then trapped in, respectively, SM-10 tubes (Luckey Laboratories), Tox-Traps (Tox-Traps, Inc., Smyrna DE), and Molecular Sieve 3A prepared in our laboratory. The trapped samples were then analyzed by two different methods as follows:

Method 1—The procedure reported by Wilkinson et al, with quantitation by direct-injection gas chromatography (GC), using *n*-propanol added with eluant water. (Used with SM-10 tubes only.)

Method 2—The same as Method 1, except that only water is added as eluant, and *n*-propanol added immediately before GC analysis.

When Method 1 is applied to the analysis of samples trapped on SM-10 tubes, indicated recoveries are found to range from 90 to 110% and to increase with time, as reported by Wilkinson et al. We deduced that this result is caused by adsorption of the *n*-propanol internal standard by silica gel. The equation by which ethanol is quantitated by the internal standard technique is:

$$\% \text{ ethanol in sample} = R_x/R_s \times C_s$$

where

R_x = ratio ethanol/*n*-propanol in sample,
R_s = ratio ethanol/*n*-propanol in standard, and
C_s = % ethanol in standard.

An increase in the value of a fraction may be the result of an increase in the numerator, a decrease in the denominator, or both. Since *n*-propanol is a larger molecule than ethanol, it would be expected that *n*-propanol would be adsorbed to a larger extent than ethanol, and since the denominator of the internal standard fraction includes a term which is proportional to the *n*-propanol concentration, any decrease in this term will cause an increase in the overall value of the expression and will be reflected as an apparent increase in recovery. That this is true is shown by the results of Method 2, where the *n*-propanol is added immediately before analysis, thereby preventing its adsorption by silica gel, as shown in Table 1.

These data show that recoveries are lower than stated by Wilkinson et al and also that the anomalously high recoveries are caused by removal of *n*-propanol from the eluant by adsorption on silica gel.

We find experimentally that many factors, including temperature and degree of agitation, influence the recovery of alcohol from all trapping media tested. These and other variables are

TABLE 1—*Recoveries obtained with Method 2.*

	% Recovery		
Trapping Medium	Low	High	Average
Molecular sieve 3A	9.9	44.8	27.1
SM-10	37.6	40.5	39.1
Tox-Trap	43.8	96.4	74.3

being examined and will be reported in a more detailed communication when data have been collected and assimilated.

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Authors' Response

Dear Sir:

Since the publication of our paper on the trapping and reanalysis of ethanol in breath samples, we have spent considerable time in studying the percent recovery of ethanol from various absorbing materials. We have found that since changing to a 35–60 mesh silica gel size (Tox-Trap samples similar to those tested by the Albuquerque Police Department Laboratory):

1. Our average percentage of recovery of ethanol is 82.3%.
2. Our range in percentage of recovery is very small when using our recommended method.
3. *n*-Propanol does indeed adsorb on the silica gel (Table 1).
4. When the analyst keeps conditions fairly constant, that is, constant temperature, constant time factor, and so forth, many of the variables are held constant and if standards are run under the same conditions, results are very reproducible (Table 2).
5. A slight increase in temperature during the 60-min standing time decreases adsorption of the *n*-propanol and ethanol on the silica gel and brings about a slight increase in reproducibility.
6. A major problem involved is leaks in the system. When a trap is attached to the instrument, it creates a back pressure, which can vary slightly from trap to trap. This leak will cause a loss of sample or standard resulting in high or low values depending on the amount of sample or standard lost. We have tested all instruments in Delaware and have found leaks of various size in 26% of them. All leaks were sealed without much difficulty and trapping has been acceptable in both precision and accuracy.
7. Molecular sieve will not work as an adsorption material when instruments with rapid pumping rates (such as the Intoxilyzer 4011AS) are used. The alcohol will "blow through" the packing.
8. When pulling (rather than pumping) the sample from the chamber through the trap, leaks (even major leaks) will have no effect on the final results, because of a positive flow *into* the system rather than a leak *out* of the system. The Model 4011AS can be easily converted to perform this task with an additional hose attached to the pump inlet.
9. Samples can be heated (to 35 to 40°C) to increase % recovery if desired.

TABLE 1—*Recovery of* n-*propanol from silica gel.*

Sample No.	Time, Min	Ratio of *n*-Propanol/Ethanol	Percent of *n*-Propanol Recovered
1	0	3.03	...
2	8	2.18	71.9
3	15	2.60	85.8
4	25	2.82	93.0
5	30	2.77	91.4
6	35	2.59	85.4
7	40	2.57	84.8
8	60	2.56	84.4
9	70	2.73	89.1
10	80	2.60	84.8

TABLE 2—*Sample quality control data Tox-Trap Lots 11 and 12.*

Intoxilyzer Reading, %	Tox-Trap Results, %	Deviation, %
0.102	0.101	−0.001
0.102	0.101	−0.001 Av. Dev. = 0.001%
0.101	0.099	−0.002 Av. % Dev. = 0.9%
0.101	0.100	−0.002 Range
0.101	0.103	+0.002 % Dev. = (0−2.4%)
0.158	0.152	−0.006
0.158	0.152	−0.006 Av. Dev. = 0.005%
0.157	0.158	+0.001 Av. % Dev. = 3.2%
0.157	0.156	−0.001 Range
0.152	0.164	−0.012 % Dev. = (0−7.6%)
0.291	0.284	−0.007
0.291	0.293	+0.002 Av. Dev. = 0.003%
0.294	0.296	+0.002 Av. *T* Dev. = 1.0%
0.294	0.292	−0.002 Range
0.290	0.287	−0.003 % Dev. = (0−2.4%)

In our original paper we used incorrect instrument volume and liquid standards in determining the percentage of recovery. We since have recommended as standard procedure that either one must know the exact instrument volume (which can be readily found relative to an instrument of known volume), or one must trap standards (calibrators) as one would trap samples. Using this technique we have found a percentage of recovery of approximately 82.3% from the silica gel traps.

O'Donnel et al state that "since *n*-PrOH is a larger molecule than EtOH, it would be expected that *n*-PrOH would be adsorbed to a larger extent than EtOH...." Grace Chemicals, silica gel manufacturers, report in their information booklet on silica gel that "in a given homologous series (methyl alcohol, ethyl alcohol, propyl alcohol and butyl alcohol) the extent of adsorbatility decreases in order of decreasing polarity. For instance, in progressing from methyl alcohol (highly polar) to butyl alcohol (less polar) the adsortivity decreases." Therefore, *n*-propanol adsorption is less than ethanol. (Our results indicate 17.7% of the ethanol is adsorbed and 14.3% of the *n*-propanol is adsorbed.) We have found a quick adsorption of *n*-propanol followed by a release and leveling off as a state of equilibrium is reached. Once again within 1 h the silica gel:water:ethanol:*n*-propanol system is in a state of equilibrium and whatever distribution constants are in effect have been established. If after this time period the analyst allows the silica gel to settle and does not change the temperature of the system, she or he will obtain *very* reproducible results.

The wide range in recoveries on Tox-Traps (43.8 to 96.4%) reported by O'Donnell could indicate that the analysts took samples before the minimum 1-h waiting period, they varied conditions, they have a leak, or they have problems with their procedures.

We have recently completed a study of silica gel traps on a 4011AS. The analyses were performed by an inexperienced chemist following our specific instructions (Table 3). It would be impossible to achieve this level of precision and accuracy if such a wide range in recovery of ethanol as suggested by O'Donnell occurred.

Dr. Dubowski at Oklahoma University has long recommended trapping of breath samples and has published papers on trapping using molecular sieve and calcium sulfate. It is apparent that trapping of alcohol in breath samples is both possible and economical. We have trapped and reanalyzed samples using S & W models 900, 900A, 1000, and 2000 and Intoxilyzer Models 4011AS and 5000. In all cases, it is important that the system be free of leaks, that conditions (time, temperature, allowing silica gel to settle just before injection, use of an internal standard, and so forth) be held constant.

TABLE 3—*Delaware State College (results by inexperienced chemist).*[a]

Mean Intoxilyzer Reading, %	Mean Tox-Trap Reading, %	Average Deviation, %	Average Percent of Deviation
0.101	0.101	0.002	2.5
0.154	0.154	0.005	3.2
0.292	0.290	0.003	1.0

[a]Summary of ten samples in each concentration.

Courts have ruled that arresting agencies should trap and store breath alcohol samples, if it is possible and economical. Results such as those indicated in our paper and those by Dr. Dubowski show that it is possible and economical. One does have a responsibility to the accused to see that his/her rights are protected. As scientists, we have a responsibility to constantly improve the system. We should not set out to show that something cannot be done, but to prove that it can. If we can confirm, by scientific means, that the original breath test was correct or in a few cases indicate that there *may* be a problem, then justice is better served. As scientists, we cannot say that the courts should or should not rule that we must trap samples, but we *must* be able to say that we are or are not capable of trapping the samples.

Colorado, Arizona, Vermont, and New Hampshire have gone to trapping. Florida, California, and New York have had court decisions in this direction. Oklahoma will shortly begin trapping. Whether our state traps or not, we must prepare for this requirement. If it comes, we must do the best possible analytical work we can.

Trapped samples offer a secondary confirming test, but are not primary evidential breath tests. It is scientifically impossible to achieve the same degree of precision and accuracy in samples that are trapped, desorbed, and reanalyzed that one achieves using frequently calibrated, stationary primary evidential breath testing equipment. Dr. Dubowski, J. R. Zettl, and Dr. Wilkinson anticipate writing a letter to the *Journal of Forensic Sciences* concerning recommended acceptable deviations in reanalysis of trapped samples.

It might be noted that trapping will (in the vast majority of cases) confirm the original results and in a small number of cases indicate that there is a problem with the instrument used, with the traps, or with procedures used in trapping or in reanalysis. The traps also offer an answer to the acetone question (they also trap acetone and other organic compounds) and to the radio frequency interference (RFI) question (they are not effected by RFI).

We, the authors of the paper in question, are not recommending that police agencies should trap breath samples (this is for the courts to decide), but we are stating, without question, that it is currently scientifically and economically possible to trap alcohol in breath samples.

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The Misapplication of Genetic Analysis in Forensic Science

Sir:

Electrophoresis has long been used to study genetically determined differences between both individuals and populations. Under controlled laboratory conditions this tool has proven invaluable and contributed an enormous volume of data that has elucidated population genetics theory and allowed for the very rapid progress of somatic cell genetics. Its recent introduction into the field of forensic science, however, is of dubious value. To understand why electrophoresis is not as valuable in forensic science as many think it is, it is necessary to take a close look at what it is and how it is used in the research laboratory as compared to how it is used in what might be called the real world.

In the research laboratory electrophoresis is used to distinguish among genetic variants, called isozymes, of specific enzymes. These variants are the result of minor changes in the genetic code and consist of minor changes in the amino acid composition of the proteinaceous enzymes. Because each amino acid has a characteristic electrical charge, two proteins which differ by only one amino acid can be separated from each other when placed in an electrical field. Since the separation by charge of two proteins is counteracted by diffusion, it is necessary to have this separation take place in a medium that minimizes diffusion but allows for the movement of proteins caused by charge differences. Initially, the proteins were applied to paper strips which were bathed by a solution that transmitted the electrical current. Now, however, a gel is used as the medium through which the proteins move.

The composition of the solution bathing the gel and the composition of the gel itself are of vital importance in effecting separation of proteins. The net electrical charge and the conformation of a protein change with the pH of the solution in which it is dissolved. Charge and conformational changes in turn affect the speed with which a protein moves through the gel in which it is placed. Gel composition also affects this migration rate because the gel acts as a barrier to the free flow of protein molecules: the thicker the gel, the slower the molecules move through it. Electrical charges on the molecules making up the gel also affect protein flow through it. Finally, minor impurities in the gel can drastically alter protein migration.

In the research laboratory it is standard procedure to run known samples of all possible variants in the same gel in which an unknown sample is being run. In addition, the known samples are treated identically to the unknown samples in all respects. Without the use of such internal controls, the identification of an unknown sample is tentative at best. It is not sufficient to utilize a subset of the known variants and guess as to the identity of the unknown sample if it matches none of the controls.

The identification of a specific isozyme depends on several factors, all of which are subject to error. Most of the enzymes that are used in forensic science laboratories do not appear as one band on the gel. Rather, they are often composed of many bands, and it is the relative distribution, or pattern, of these bands that allows for the identification of specific isozymes. Localized irregularities in the gel can alter the pattern of an isozyme and lead to misidentification of it. For this reason, it is quite common to repeat the test for an important sample. Because some isozymes are identified by relative intensity of staining of the band pattern, rather than the band pattern per se, the amount of enzyme loaded onto the gel is of crucial importance in isozyme identification. Frequently, it is not possible to distinguish among isozymes without repeating the test. Finally, most isozymes are identified by a chemical reaction which may be altered as a result of minor contamination of the sample, the gel and the buffer solution bathing it, or the chemicals used for the reaction. Such contamination may not be detected if it resides in the sample. In research laboratories a picture of the results is routinely taken. This allows for leisurely examination of the results and independent verification of conclusions if necessary.

In contrast to the use of electrophoresis in the research laboratory, where all samples are fresh and in plentiful supply for a repeat analysis should there be any question of identification, the electrophoretic identification of samples in forensic science laboratories most fre-

quently is conducted on old, nonsterile samples that are in limited supply and have had an unknown history before their collection. As will become evident in the discussion of specific enzymes, there are serious doubts as to the possibility of obtaining correct identification with such samples. There are aging effects, oxidation effects, heating effects, and both biological and chemical contamination effects that must be considered but cannot be, simply because the samples have had an unknown history. Even if the exact history of a sample were known, controls could not be treated in a similar fashion with the expectation that correct identification would then be achieved because identical samples treated in the same manner have been found to react differently to such treatment [*1,2*].

The enzyme phosphoglucomutase-1 (PGM) illustrates clearly the risks associated with using old, nonsterile blood and tissue samples. There are a total of 36 forms, or isozymes, that exist for this enzyme although only 3 (Type 1, Type 2, and Type 2-1) are common. Routine forensic science work employs, at most, the three common forms as known controls [*3*], thus making the identification of other forms somewhat of a guess even with fresh samples. In a starch gel, which best separates the PGM isozymes [*4*] although it is not routinely used for separation, fresh PGM Type 3 and Type 7-1 can be read as Type 1 if proper controls are eliminated [*4*]. Other misidentification of types could also be cited. As a blood or tissue sample ages, the possibility of misidentification increases even when starch gels and all proper controls are utilized. This is because aging causes a gradual increase in one band, a gradual decrease and eventual disappearance of another band [*5*], and a gradual appearance of yet a third band [*1*]. In starch, Type 1 becomes easily confused with Type 3-1 [*1*]. If, instead of starch, agar or agarose gels are used, two bands in fresh Type 2-1 run as one band [*4*]. Combined with the aging effects, this could result in Type 1 being confused with either Type 2-1 or Type 2 when run with fresh controls. In addition to aging effects, PGM is very sensitive to bacterial contamination. Many bacteria have been found to have Type 2 PGM, for example. Thus, Type 1 blood when contaminated by bacteria that have Type 2 PGM would be identified as Type 2-1 despite the use of starch gels and proper controls. Finally, sodium fluoride is routinely used as a preservative when old blood stains are collected. This additive distorts bands and causes the false appearance of a new band [*1*], which would result in Type 1 being read as Type 2-1 if the gel is composed of agar or agarose. In summary, then, the use of at most three control types, the use of gels other than starch, the use of sodium fluoride as a preservative, and the use of fresh controls for aged samples quarantees a high rate of error in the identification of PGM-1 isozymes. When the possibility of bacterial contamination of a sample is considered, and it must be considered in every blood stain and in every sample taken from decomposing tissue, the probability of error in identification of type approaches 100%. It should be noted that the manual put out by the National Institute of Law Enforcement and Criminal Justice [*1*] recommends the addition of sodium fluoride, the use of agar gels, and no controls for routine forensic science work with this enzyme.

The enzyme erythrocyte acid phosphatase (EAP) illustrates other sources of error in identifying isozymes. There are five common isozymes of the enzyme, and identification in part is by the relative intensity of the bands [*2*]. Zajac and Grunbaum [*6*] took four different known samples, allowed a portion of each to dry under ambient conditions and heated the remainder for up to 48 h at 37°C. All samples were then subjected to electrophoresis and identified by four skilled technicians. Of the dried samples, two were correctly identified by all four technicians, a third sample was correctly identified by three technicians, with the fourth technician reaching no conclusion as to the isozyme. The fourth sample was correctly identified by one technician. Two were unable to identify it, and the fourth technician misidentified it. Only one of the heated blood samples was correctly identified by all four technicians. One sample was correctly identified twice, misidentified twice, and not identified twice. The last sample was misidentified by three technicians and not identified by one. As the authors pointed out, the treatment of neither the blood stains nor the heated liquid blood was extreme as compared to real life situations. The technicians were highly trained, yet they misidentified isozymes 7 out of 32 times, for an error rate of 21.8%. Furthermore, they correctly identified the isozyme only

53.1% of the time. The authors concluded that "... determinations based on partial or altered patterns are questionable and unreliable and could, and should, be challenged in a court of law."

While the effects of heating, aging, and consistency of identification have yet to be as systematically investigated for other enzymes that are routinely identified in forensic science laboratories, it has been noted that changes in enzymes do occur over time even when fresh blood is stored at 4°C. Thus, adenosine deaminase-1 shows alteration of bands, movement of bands, and the appearance of a new band when stored for ten days at 4°C before electrophoresis [*7,8*]. Type 2 and Type 2-1 could be read as Type 1 when compared to fresh controls. This enzyme also reacts to the addition of sodium fluoride as a preservative by the loss of one band and the appearance of another band [*1*], which further confuses identification. Esterase D also shows storage changes in just a few days at 4°C [*9–11*] which, again, could lead to the misidentification of isozymes.

The enzyme adenylate kinase-1 (AK-1) demonstrates further possible sources of error in isozyme identification. In addition to storage changes [*5*], this enzyme is inhibited by the preservative sodium fluoride [*1*]. The use of a phosphate buffer causes Type 2-1 to be read as Type 1 [*1*]. Also, Type 2-1 may be read as Type 1 unless histidine or succinic buffer is used with aged samples. Despite the sensitivity of this enzyme to phosphate buffer, Wrede et al [*12*] reported typing PGM, ADA, and AK-1 on the same gel, using phosphate buffer. Since forensic science laboratories work primarily with bloodstains and samples taken from decomposing corpses, only histidine or succinic buffer should be used. Neither buffer is the buffer of choice for most of the other enzymes that are typed. Thus, AK-1 should never be typed on the same gel as other enzymes. Increasingly, however, there is a tendency on the part of forensic science laboratories to combine several different enzyme tests on the same gel to save time and money [*13,14,1,3*]. This could easily result in the use of nonoptimum buffers, as practiced by Wrede et al, which if not thoroughly tested would cause misidentification of isozymes under some conditions.

Although not an enzyme and not examined by electrophoresis, the ABO system also clearly illustrates some of the dangers associated with using old, contaminated samples with an unknown history. Using fresh sterile blood, the rate of misidentification is 1.6% [*15*]. Pereira [*16*] found that in decomposing tissues there can be a loss of antigenic activity, a gain of antigenic activity, or a substitution of one antigenic activity for another. Bacterial contamination of samples resulted in false positives in 137 of 282 samples in a study by Springer et al [*17*]. Kind and Lang [*18*] observed false positives in 22 of 30 samples of house dust, 3 of 24 samples of dirt, 24 of 42 samples of wood shavings, and 80 of 257 unclassified microbiological colonies. False positives can also be caused by plant material, viruses, snails, worms, and other invertebrates [*19–21*]. Finally, sweat has been found to eliminate all antigenic activity [*22*].

Special problems arise with the ABO system when dealing with either fresh semen or saliva specimens, especially when the blood of the suspect is compared to the findings. A separate gene, called the secretor gene, determines whether or not the ABO group and the Lewis antigen, controlled by yet another gene, are expressed in semen and saliva. When the genotypes *SeSe* or *Sese* are present for the secretor gene, samples from all three sources will tend to coincide for the ABO group antigens. In addition, the Lewis a and b antigens will be present in semen and saliva but in blood samples only Lewis b antigen will be detected. If, however, the genotype for the secretor locus is *sese*, then blood, semen, and saliva may have the a antigen.

A further complication involves those people who, although of Type A or O, routinely show B activity in semen and saliva. Also, some people in groups B and O show A activity in both types of samples. These spurious results are thought to be caused by bacterial activity although that has yet to be proven. Vaginal secretions are subject to the same ABO group difficulties that are found with saliva and semen samples, including bacterial contamination [*1*].

Dried stains of saliva and seminal or vaginal secretions are much more difficult to type than fresh specimens because the extent to which these antigens can be resolubilized varies considerably [*1*]. In addition, all the problems of typing old, nonsterile contaminated blood for enzymes apply to these specimens. Finally, Rees and Rothwell [*23*] found only a 78% correspon-

dence between blood and semen samples from the same persons with regard to PGM types. This strongly suggests that screening by blood samples should not be done when the evidence is a semen sample.

An added complication in typing saliva, semen, and vaginal secretions was recently raised by Sensabaugh et al [*24*]. They found that contamination of semen samples by saliva results in altered PGM patterns, leading to misidentification. The a and b bands gradually disappear, while other bands are gradually generated. Since the possibility of altered results for any enzyme exists, because of a similar process, and since any of these three secretions might be contaminated by one of the other two, the results of such typing are completely suspect at present.

Any bloodstain will undoubtedly be exposed to dust and dirt and most likely be contaminated by bacteria, contact with wood, plant material, sweat if on clothing, and many more, as yet untested, substances. Depending on season and climate, it may be exposed to very high temperatures. In the attempt to classify blood stains and tissues from decomposing bodies, methods that were originally developed for fresh sterile samples have been used. Unfortunately, the application of these methods, which are very accurate when used under proper conditions, is inappropriate for the classification of old, contaminated samples. Their use lends an aura of accuracy to the identification of such samples that has proven to be not justified upon closer examination. If the results of such testing were either correct or inconclusive, then ABO determination and the application of electrophoresis to enzyme typing would be justified. Regretably, misidentifications are possible and have been reported whenever such a possibility has been studied. Misidentification is a much more serious error than nonidentification, especially when the result is generally regarded as accurate.

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Response to "The Misapplication of Genetic Analysis in Forensic Science"

Dr. Juricek's letter "The Misapplication of Genetic Analysis in Forensic Science" presents a naive and often erroneous characterization of genetic typing analysis in both the research and forensic science contexts. This is most charitably explained by presuming that Dr. Juricek has no real familiarity with the actual practice of genetic typing. One's charity may be strained by the disregard of pertinent literature, the distortion of context of cited works, and the polemical tone of the writing, but explanation and charity are not really important issues here. What is important is that the misconceptions and errors contained within the letter are exposed lest they gain authority from a lack of challenge. Hence this response.

Juricek's central argument against the genetic typing of biological evidence reduces to the following syllogism:

1. Biological evidence samples are generally not fresh when they enter the laboratory; they may be contaminated and have uncertain histories.
2. In such samples, anything can happen; the genetic markers might be altered in unaccountable different ways.
3. Therefore, the genetic markers in these samples cannot be typed with any reliability.

The critical statement in this argument is (2), the proposition that "anything can happen." This proposition can be challenged on two counts. First, one can question whether "anything" can happen. The answer is no; in fact what can happen is constrained by the chemistry of the markers and their environment. One can catalog the possible kinds of changes that can affect a marker and can assess the consequences of these changes with regard to typing error potential. The result of this somewhat theoretical exercise is that the changes most markers undergo

introduce no significant risk of typing error; either marker activity is lost or the change signals itself.

The second objection to the "anything can happen" proposition is that there is considerable empirical evidence that markers undergo only a limited array of changes and that these changes are recognizible. Accordingly, altered markers can be picked out and judged untypeable. Thus errors are avoided. For each of the few select markers used in forensic science casework, there is a substantial body of experience affirming typing reliability. If "anything" in the form of random and unpredictible alterations happened with any frequency, our experience would be different than it is.

In this regard, it is appropriate to review the evaluation process each marker must pass before being accepted for use in evidence analysis. The first phase involves testing samples of known type prepared in ways simulating typical case conditions. These trials demonstrate whether a marker can survive the environmental insults typically encountered by evidence samples and, more importantly, indicate whether the marker has tendencies to undergo changes which make one type look like another or which otherwise introduce a significant risk of typing error. These tests also establish the "rules" for making typing judgements; these rules provide the basis for distinguishing between typeable and untypeable test results. The second phase of evaluation is blind trial testing. Samples of known type are again prepared under a variety of typical case conditions and are tested blind. The only acceptable outcome of these trials is that the samples are correctly typed or are classed as untypeable. The next step towards acceptance is the unreported testing of actual case samples; this indicates whether the marker behaves differently in case and prepared samples. Only after a marker has successfully passed each of these evaluation phases does it finally find its way into casework. Even then, acceptance is conditional; a marker accepted with regard to bloodstain analysis would undergo independent evaluation with regard to, say, semen stain analysis or cadaver blood analysis.

In summary, the evaluation process serves both to screen out potentially unreliable markers and to provide the analyst with a body of experience with each accepted marker. Of the hundred or so known genetic markers, only about a dozen have passed muster and are commonly accepted for use in casework.

It should be noted that analysts as well as markers undergo evaluation. The quality assurance programs operating in most crime laboratories prevent analysts from employing a marker until they have developed the critical judgement to work with that marker. In addition, many laboratories have an established policy that typing judgments require independent assessments by two or more analysts. It is also the rule in most laboratories that typing gels are documented photographically. These safeguards are in effect to minimize risk of analyst error.

The proof of the pudding in terms of typing reliability has been demonstrated in proficiency trials. For the commonly used markers, the typing error rate is usually less than 1%. This figure compares favorably to the error level in routine clinical laboratory practice where samples and procedures are highly standardized. Put another way, if there were basic deficiencies either in the markers used or in the analysts using them, the proficiency test results would not be nearly so good.

What, then, is to be made of Juricek's "evidence" of rampant unreliability? Let us look critically at this "evidence" paragraph by paragraph beginning with her discussion of phosphoglucomutase (PGM).

PGM is cited as an object enzyme to illustrate typing unreliability. She feels the problems with this marker are so severe that "the probability of error in identification of type approaches 100%." However most of the problems described are imaginary and there are a number of otherwise misleading statements:

1. PGM is stated to have 36 isozymes of which 3 are common. Juricek here appears to have confused the terms "isozyme" and "type." There are three common types, not three common isozymes; each type is represented by several isozymes.

2. It is stated that the use of the three common types as standards is inadequate, "making the identification of other forms [types] somewhat of a guess." Neither geneticists nor forensic scientists "guess" types for the record. If a sample yields an unusual electrophoretic pattern, judgement is suspended until the sample can be compared against a standard of the suspected rare type.

3. It is stated that although starch gel electrophoresis best separates the PGM isozymes, it is not routinely used. In fact, starch gel methods were the standard in forensic science laboratories for many years and are still used in many. Indeed the Culliford manual (her Ref *1*) describes a starch gel technique. It is not clear how she comes to the later statement that this manual recommends an agar gel technique unless she has confused the agar staining overlay for the electrophoresis gel. This is either a failure to read carefully or an ignorance of technique or both.

4. Agar and agarose gels are condemned as giving inferior resolution resulting in a confusion of types; the paper cited dates from 1968. In fact, recent agarose gel techniques offer resolution superior to the conventional starch gel techniques.

5. Aging effects are stated to cause confusion of types. In fact this should not occur if the analyst is adequately trained; this is why aging effects are described in the training manual. Learning to recognize aging effects and to make appropriate judgemental adjustments is part of the analyst's baggage of experience.

6. It is stated without citation that many bacteria possess PGM isozymes with mobilities corresponding to the human PGM Type 2 isozymes. This is contrary to our experience; all the bacterial PGMs we have seen have mobilities quite distinct from any of the human isozymes. In any case, bacterial contamination is something of a straw man. In order for enzymes from contaminating microorganisms to be a problem, contamination levels must be substantial. Dry stains are not good media for microorganisms; this is one reason why we preserve foodstuffs and other biologicals by dehydration. Evidence samples that are overgrown are very often not analyzed; when they are, they are interpreted with considerable caution. Again, knowing how to recognize and deal with contaminated samples is part of the analyst's baggage of experience.

7. It is stated that "sodium fluoride is routinely used as a preservative when old blood stains are collected." It is not clear what this means since blood stains are not "collected" in any preservative, much less fluoride solutions. Fluoride is only used as a preservative in freshly drawn blood samples but, as stated in the Culliford manual, causes no typing problems if red cells are adequately washed.

Erythrocyte acid phosphatase is also held up as an object lesson in typing mistakes. Juricek describes in detail the 1978 experiment of Zajac and Grunbaum which demonstrated that unwary analysts can make mistakes with this marker. Wary analysts seem to have a better track record however; recent proficiency trials involving many laboratories indicate a mistyping rate of less than 1%.

The statement that the "effects of heating, aging, and consistency of identification have yet to be systematically investigated" is simply incorrect. As indicated earlier in the communication, there is a good sized body of such information. Juricek should look at Denault et al [*1*] as an example of one such study. She would also be advised to reread the Culliford manual from which she has drawn so many of her problem situations; this manual is nothing if not a collection of observations on factors that affect typing reliability. The problem of anodal band development with adenosine deaminase, for example, is not only described in the manual, but the solution to the problem is also given; Juricek cites the problem but seems to have overlooked the solution. Similarly, Culliford's discussion of what should be done to get adenylate kinase right has been transmuted to imply that forensic serologists routinely do what he says they should not. In this regard, it is not clear why the electrophoretic system described by Wrede et al, is held up to derision since this system is not used in forensic science laboratories; it seems to be simply another straw man set up to be knocked down. With regard to the comment on the combination

of enzyme tests on single gels, it need only be said that whatever combination systems are in use have been extensively tested and found not to compromise typing reliability.

Juricek fares no better in discussing the ABO antigens. Her discussion not only contains misleading statements, it also reveals near total ignorance of ABO testing method in the crime laboratory.

1. The stated figure of 1.6% misidentification of fresh blood may well be correct but it does not originate from the cited reference (her Ref *15*). For what it is worth, the national proficiency trials on ABO typing of dried bloodstains show error rates between 1 and 2%. If the figure for fresh blood is correct, there is no significant difference in error rate between fresh and dried blood typing.
2. ABO typing of decomposed tissue is universally recognized as a problem by forensic analysts. Few analysts attempt to type badly decomposed tissue. Otherwise one simply has to include the degree of decomposition in the evaluation of the typing result. This problem has prompted considerable research to find tissue sites protected from rapid decomposition processes.
3. Springer's study on blood group activity on bacterial and plant material was conducted directly on those materials. The text incorrectly implies that tests were conducted on contaminated blood samples.
4. The presence of ABO blood group activity on microbial, plant, and animal material has been recognized in the forensic science community for many years. For this reason it is routine to test the background materials from which a stain is taken as well as the stain itself. These serve as negative controls to protect against mistyping as a result of background contamination. Juricek either did not bother to read about negative controls in the Culliford manual or feels no need to talk about them.
5. It is stated that there are "special problems" associated with ABO typing fresh semen and saliva samples. It is not clear what these problems are. Juricek seems to imply that the differences in ABO and Lewis antigen expression between secretors and nonsecretors confounds meaningful typing. In fact, however, these differences are well known by serologists to be systematically associated with secretor status and are used by them to delineate secretors and nonsecretors. Juricek simply seems to be confused by the intricacies of blood group expression.
6. The Culliford manual is cited as stating that some people show spurious antigens in their secretions. Juricek does not continue, as does the manual, to point out that on the rare occasions when this does occur, it is readily recognizible.

For her parting shot, Juricek returns to PGM typing, this time in semen. She cites Rees and Rothwell as stating only 78% correspondence between blood and semen samples. In fact, Rees and Rothwell state "No examples of non-correlation in PGM typing between corresponding samples (of blood and semen) have been found." The 78% figure in their paper refers to the proportion of semen stains that could be typed, a very different matter. Finally, work of ours describing the accelerated decay of PGM in semen samples contaminated with saliva is cited as indicating that the typing of any enzyme in semen, saliva, and vaginal fluids is suspect. Nonsense. The paper describes a phenomenon so that analysts can recognize it when it occurs and make their typing judgements (or their judgement not to type) accordingly.

The final paragraph shows that Juricek has missed or ignored the point of so much of the literature she cites. She states that if the results of testing were either correct or inconclusive, the application of typing tests would be justified. This is exactly the point of the literature: to provide guideposts for the recognition of problem situations so that typing results that otherwise might be erroneously interpreted are instead relegated to the inconclusive category. In fact, a significant proportion of typing tests conducted in forensic science laboratories yield inconclusive results. This simply goes with the territory.

In summary, Juricek has through naiveté or distortion or both generated a fictitious caricature of the practice of forensic serology. It is distressing that a person trained to do research

would make no apparent effort to check facts or to test supposition against reality. It is also distressing that a scientist would make such sweeping pronouncements based on so little apparent expertise. One hopes Dr. Juricek follows a more rigorous path in pursuing her own research work.

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Discussion of "The Use of Marihuana, Ethanol, and Other Drugs Among Drivers Killed in Single Vehicle Crashes"

Dear Sir:

The study reported by Owens et al, "The Use of Marihuana, Ethanol, and Other Drugs Among Drivers Killed in Single Vehicle Crashes" (Vol. 28, No. 2, April 1983, pp. 372-379) is a valuable addition to our knowledge of traffic fatalities, and especially single vehicle fatal accidents. Relatively few studies have addressed the problem of the single vehicle accident, and fewer yet have considered it as a phenomenon somewhat different from that of vehicular collision accidents.

The authors found that 66.9% of their drivers' bloods contained in excess of 0.09% ethanol, and comment that this figure is "greater than most published figures for ethanol in operator fatalities."

I believe this figure is reasonable, and may well be the norm for drivers in single vehicle fatal accidents. A study of single vehicle fatal accidents in Clark County, Nevada, in 1977 and 1978, showed 65% of drivers (dead within 1 h of the fatal accident) to have blood alcohol concentrations of 0.100% or more [1].

I urge medical legal investigators throughout the country to examine and report data from their areas. It would be well to know whether or not two thirds of the driver victims of single vehicle accidents are intoxicated nationwide, or if the experiences of North Carolina and Nevada are unique. I doubt it!

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Procedures and Responsibilities in Forensic Toxicology—To What Extent Are They the Results of Laboratory Facilities?

Sir:

In a letter to the editor of this journal [1], Dr. R. A. de Zeeuw reported three cases to illustrate some issues connected with casework in forensic toxicology. Among the problems he

raised were the analyst's use of inadequate chemical methods and the toxicologist's lack of impartiality in interpreting various findings. The purpose of our letter is to elaborate on the same topic by relating a case recently proceeded in Sweden. We shall also discuss the disadvantage for toxicology work of using small, local laboratories with insufficient facilities, a circumstance that we believe contributed to the creation of the problems in the referred case.

Parts of the investigation of the death which we here describe in whole received some professional interest not only in Sweden, but also in Germany, and reached the headlines of some Swedish newspapers. As the investigators in charge of the case also reported on parts of it in a scientific journal, we feel free to recount it and present additional data.

The autopsy of a one-year-old boy who had been found dead in his home on 16 Nov. 1979, revealed that the victim had deceased from suffocation by aspiration of gastric contents. It was suspected that the aspiration was precipitated after the child had ingested some chemicals; before his death he had had access to a variety of chemical products stored in his home.

The toxicological analyses were performed at a local unit of the National Laboratory of Forensic Chemistry. Signed by a laboratory technician and countersigned by a consultant physician acting as toxicologist at the branch laboratory, the chemical report issued on 21 Jan. 1980, read: "traces of ethanol and methanol were detectable in the blood; 0.28‰ and 5.2‰ of ethanol were found in the urine and in the gastric contents, respectively; 0.18‰ of methanol were found in the gastric contents; and barbiturates, salicylic acid, or carbamates were not detectable in the liver or gastric contents."

Based on these data along with some other analytical results not included in the chemical report, but obviously to the effect that one of the suspected chemical products—a detergent—contained ethanol and methanol, as did the gastric contents, the medical examiner concluded in his final report to the police on 28 Jan. 1980 that: the detergent was the cause of the intoxication, the intoxication in turn precipitated the aspiration of gastric contents, and the aspirated materials in turn suffocated the child.

On behalf of the multinational corporation responsible for manufacturing and marketing the detergent, the central unit of the National Laboratory of Forensic Toxicology in Linköping began reinvestigating the case in September 1980. In short our report issued on 1 Dec. 1980, read: no significant amounts of the surface active agent, linear alkyl benzene sulphonate (LAS) (which constitutes about 15% of the detergent), were detectable in the gastric contents of the deceased; and propanol, butanol, *iso*butanol, 3-methylbutanol, hydroxybutanon, methylacetate, and methylethylketon were identified in the gastric contents or in the urine of the deceased, but not in the detergent from the bottle to which he had had access prior to his death. Being one of the key issues, the presence of the LAS in the gastric contents was also investigated by two other and independent laboratories. One of these reported negative findings while the other laboratory, belonging to the company, reported "... that very little LAS, if any, was ingested and that the overall analytical results available are not consistent with an assumption of the ingestion of (product name)."

Based on these chemical data the medical examiner in charge of the case withdrew his earlier protocol by issuing on 23 Dec. 1980, a new report to the effect that the detergent was not involved in the death of the child.

In our minds the toxicologist's handling of this case illustrates several of the areas of concern raised by Dr. de Zeeuw, such as the analyst's use of inadequate analytical procedures. The lack of sufficient chemical methods certainly contributed to the scanty data presented by the toxicologist in the first run. This is, however, a problem that may impinge on small laboratory units of forensic chemistry. Being a technically demanding and multidimensional profession, forensic toxicology needs a sufficient body of resources concentrated to one laboratory. Even seemingly trivial cases require that a variety of often sophisticated analytical methods be used and, also, that the toxicologist be supported with associates in forensic toxicology to discuss professional issues on a daily basis. As neither of these conditions prevailed at the local laboratory that investigated the above referred case, the toxicologist had no simple means to corrob-

orate with other experts in the field on the adequacy of his analytical methods, his chemical findings, or what implications on the investigations these might generate.

His interpretation of the analytical results also indicated that the toxicologist might have gotten too involved with certain aspects of the case and that he was no longer impartial in its other areas. This suspicion of ours has indeed been somewhat substantiated by a paper on the case which the toxicologist and the medical examiner in charge of the investigation wrote. In this communication submitted to the journal on 21 Jan. 1982, they seem to yield to their first standpoint taken on the legal matter: "The reason why the child aspirated is not clear, but the history supported by the chemical analysis suggests an involvement of the detergent" [*2*].

In many European countries some of the work in forensic toxicology seems to be performed at rather small laboratory units; and it can be anticipated that the procedures for the case investigations vary to some extent from one branch laboratory to another, as may the toxicologists' responsibilities. In addition to the aforementioned disadvantages such organization brings, scattering forensic toxicologists into small laboratories also renders it difficult to convey information of mutual professional interest. In overcoming this situation, scientific journals in the forensic sciences may play an important role as a communication medium. Whatever the reason may be, letters to the editor dealing with various problems, for example, scientific ones, issues similar to those raised by Dr. de Zeeuw, or case report commentaries are as yet rarely seen in European journals for forensic medicine.

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