Letters to the Editor

The Technical Working Group on DNA Analysis Methods (TWGDAM) Consensus Approach for Applying the "Ceiling Principle" to Derive Conservative Estimates of DNA Profile Frequencies

Dear Sir:

An alternative method, termed the "ceiling principle," has been devised by the National Research Council (NRC) of the National Academy of Sciences [*1*] for calculating DNA profile frequency estimates. This method provides an even more conservative estimate of the likelihood of occurrence of a DNA profile than the fixed bin or floating bin methods currently employed by the forensic community. The need for the ceiling principle approach was based on a "for the sake of discussion" (see Ref *1*, p. 80) premise that population substructure might affect the ability to obtain valid estimates of the likelihood of occurrence of a DNA profile when using general population databases.

The "interim" ceiling principle method for deriving very conservative DNA profile frequency estimates is the current approach described in the NRC Report [*1*]. In the interim ceiling principle, the 95% upper confidence limit of allele frequencies in at least three major United States population groups are tabulated. In other countries the three databases could differ from that required in the United States. The ceiling frequency for each band in a DNA profile is defined as the maximum of the 95% upper confidence limits of the fixed or floating bin frequencies in each general database, or a minimum of 0.100. The frequency of a single locus and/or multiple locus profile is obtained by the product of these interim ceiling frequencies. At such time when data from 15 to 20 "genetically homogeneous" population subgroups become available, the minimum frequency will become 0.050.

TWGDAM cannot recommend the application of the ceiling principle. The basis for the need for a ceiling principle approach is flawed [*2–15*]. The need for the ceiling principle is based upon the faulty premise that there is more genetic variation among subgroups within a major population group than between major population groups [*1,16,17*]; the extant data demonstrate the opposite and that the application of the ceiling principle is unnecessary. The current methods employed by forensic scientists have been demonstrated to be robust scientifically [*3–5,18–28*].

While the ceiling principle approach has been criticized severely [*2,6,10,14,15,22,29,30*], it is generally accepted that the interim ceiling principle approach for estimating DNA profile frequency estimates produces conservative results [*1,2,29,31–33*]. This is supported by the observation that ceiling principle estimates of DNA profile frequencies generally are more common estimates than those derived by the already conservative fixed bin approach [*2,25,34–36*]; and for those few situations where the ceiling principle estimate is less common than a fixed bin estimate the differences in the frequencies are so small as to have no consequence on the inference of the rarity of the DNA profile [*2*].

However, for those courts that still would desire DNA profile frequency estimates derived using the very conservative interim ceiling principle approach, TWGDAM has developed a consensus approach for the ceiling principle. The attempt is to interpret the intent of the NRC Report [*1*] for the use of the interim ceiling principle and to eliminate confusion that some courts may encounter when considering alternate interpretations of the ceiling principle. There are two basic approaches to establishing ceiling principle frequencies. These are

based on using either fixed or floating bins to assign allele frequencies. Since both the fixed bin and floating bin ceiling principle approaches, described below, yield similar DNA profile frequency estimates, either can be employed. It is unnecessary to provide estimates for both approaches. The choice is up to the laboratory.

Fixed Bin Ceiling Approach

1. Fixed bin frequencies will be generated for the appropriate data set as described by Budowle et al. [*34*]. The rebinned format will be used. The data sets to be used are at least three of the four major population groups. These are Caucasians, African Americans, Hispanics, and Orientals (this requirement applies to US laboratories).

2. Global tests for equilibrium will be performed on each population data set [for example, see *23,24,28*]. If all the loci meet Hardy-Weinberg and pair-wise linkage equilibrium expectations (that is, two locus independence tests), proceed with calculating the ceiling principle estimate (step 5).

3. If a locus is not in equilibrium, based on a global test, in one of the data sets, a local test [*28*] for equilibrium will be performed only on the alleles in the particular population sample (this step is required only if the criteria for step 2 is not met). If the loci meet Hardy-Weinberg and linkage equilibrium expectations at the local test level, proceed with calculating the ceiling principle estimate (step 5).

4. When the criterion in step 3 is not met, the counting method will be used for that locus. The observed number of genotypes with the particular combination of alleles will be used. For the situation where there are no observed genotypes, the 95% upper confidence limit on no observations for those databases with no observed genotypes will be employed. For a database of size n the formula [*28*] for calculating the 95% upper confidence limit for no observed genotypes is

$$1 - 0.05^{1/n}$$

The highest genotype frequency or a minimum of 0.02 (which is the minimum interim ceiling single locus genotype frequency) will be used.

4*a*. The ceiling principle approach described by the NRC Report [*1*] did not describe how to proceed when the alleles in one population are in disequilibrium based on a local test, yet the second and third populations meet equilibrium expectations, and the alleles used in the ceiling principle estimate derive from the second and third populations. It is obvious that there are no tests for determining independence across populations and using the allele frequencies with the product rule should present no problem. But to avoid confusion, it is recommended to use the genotype counting method for the two alleles in all databases and use the most conservative estimate or a minimum frequency of 0.02.

4*b*. The same approach as in 4*a* would apply when two loci are found to be in disequilibrium by the local test. The counting method will be used for the two loci. The observed number of genotypes with the particular combination of alleles at the two loci will be used to determine a genotype frequency. For the situation where there are no observed genotypes, the 95% upper confidence limit on no observations for each of the databases will be estimated. The highest frequency or a minimum of 0.0004 (which is the minimum interim ceiling two-locus genotype frequency) will be used.

5. The putative bands for estimating a DNA profile frequency generally derive from the evidentiary sample. It also is acceptable to use either the band(s) from the evidentiary sample or the known sample, as long as the more common bin frequency is used. The appropriate bin frequency for the putative band, or bands, will be determined by establishing a measurement error window (e.g., for the FBI that would be ±2.5%). When the measure-

ment error correction window spans a fixed bin boundary, the larger bin frequency will be used [*35,36*].

6. The 95% upper confidence limit will be calculated for each of the fixed bin frequencies in each population data set. The 95% upper confidence limit will be derived using the following formula, where p is the bin frequency and n is the number of alleles in the data set:

$$p + 1.645 \sqrt{p(1 - p)/n}$$

7. The largest 95% upper confidence limit value across the data sets at each bin or a minimum of 0.100 will be selected for a ceiling allele frequency estimate.

8. The product rule will be applied. The single-locus estimates for a two-band pattern will be derived using $2p_1p_2$, where p_1 and p_2 are the respective allele frequencies; for single band patterns $2p$ will be used. The multi-locus DNA profile frequency estimate will be calculated as the product of the individual locus frequencies.

NOTE: It should be noted that the fixed bin ceiling approach recommended by TWGDAM differs from the NRC Report (1) recommendations in two aspects. First, when estimating an allele frequency for a target profile the TWGDAM fixed bin ceiling approach selects the bin with the higher frequency when the measurement error window spans a bin boundary, instead of summing adjacent bins. Chakraborty et al. [*35*] and Monson and Budowle [*36*] have demonstrated that selecting the higher frequency of the adjacent bins is sufficiently conservative and there is no demonstrated need for summing the bin frequencies. Moreover, the floating bin approach, described below, yields very similar ceiling principle estimates to the fixed bin ceiling approach. Second, no interim ceiling frequency estimates will be determined using a Native American database, because each database represents a subgroup and not a major race or population category. The NRC Report [*1*] did not describe a method for generating "*general* Native American" databases when applying the interim ceiling principle.

Floating Bin Ceiling Approach

The floating bin ceiling approach recommended by TWGDAM essentially is carried out in the same manner as the fixed bin ceiling approach described above. There are only two points to consider for floating bins.

1. The size of the floating window should be twice the laboratory's quantitative match criterion. For example, for a match criterion of ±2.5%, the floating window for ceiling principle allele frequencies will be ±5.0%, which is a total width of 10%.

2. The tests for independence can not be done globally. They must be done locally for each case.

It is the opinion of TWGDAM members that previous interpretations for applying the ceiling principle (such as, for example, using ±2.5% floating window instead of a ±5.0% floating window for establishing ceiling principle allele frequencies) also yield conservative DNA profile frequencies. The attempt here is to provide a consensus approach, which does not suggest that previous interpretations of the ceiling principle yielded non-conservative estimates.

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An Antistatic Device for Use When Sampling with a Diamond Anvil Cell

Dear Sir:

The high pressure diamond anvil cell (DAC) has been used in forensic science laboratories since 1965 [*1*] for the microsampling of a wide variety of materials using infrared spectroscopy. In 1984, a low pressure version of the DAC was introduced for use with either a beam condenser or an infrared microscope. With the infrared microscope, the use of a single diamond anvil for sampling [*2*] provides a more durable substrate than salt windows, and samples considerably smaller than those possible using a beam condenser system can be analyzed. One problem that occasionally occurs when mounting these smaller samples is static electricity. This static electricity can be produced when the anvil faces are wiped or scraped to clean them, often causing subsequent samples to be repelled from the anvil faces. Not only is this annoying when attempting to mount a particle which may have required considerable effort to locate or retrieve, but evidence may be lost as these particles travel through the air.

Previously, it was suggested [*2*] that this static electricity could be minimized by wiping the anvil face with a damp piece of tissue paper. We report here a simpler and more effective method using a commercial product. For the last year, we have been using a Staticmaster

ionizing unit (Model number 2U500, NRD Inc., Grand Island, NY; available through VWR Scientific) to neutralize static electricity when sampling with our DACs. This device, which sells for around $20, has a polonium ($^{210}_{84}$Po) radioactive source which produces a weak flux of alpha particles. These alpha particles cause the air around the device to ionize, neutralizing any static charge. One simply holds the unit—a 1″ by 3″ strip—with its face directed towards the anvil, at a distance of approximately 3/4″; a few seconds of exposure is usually all that is required. In certain cases it may also be necessary to treat samples in this manner, and we have found this device to be useful for some other trace evidence manipulations involving small objects as well. According to the manufacturer of DACs (High Pressure Diamond Optics, Inc., Tucson, AZ), this process should have no adverse effects on the diamonds.

Because of safety concerns, we tested one of the units using a radiation monitoring badge (normally worn by analysts using our XRF spectrometer and those in our DNA section). The badge was stored with the unit when not in use (often placed directly under it) for a period of three months. Only minimal radiation, almost all of it nonpenetrating, was detected when the units were new, and none was detected after several months. The life of an ionizing unit is one year. Old units are returned to the manufacturer and should not be discarded.

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Discussion of "A Comparative Study of Erotomanic and Obsessional Subjects in a Forensic Sample" (J. Forensic Sci., Vol. 38, No. 4, July 1993, pp. 894-903)

Dear Sir:

Dr. Zona and his associates are to be commended for their excellent group study of erotomanic and obsessional forensic subjects [1]. They also record the extraordinarily high frequency of foreign born subjects (43%) in their erotomanic subgroup when compared to the love obsessional (3%) and simple obsessional (5%) subgroups.

The relationship between erotomania and acculturation problems remains empirically unexplored, but the link appears to be clinically sensible. Males immigrating from heterosexually repressive cultures, to more sexually open, westernized cultures may be particularly inclined to misconstrue the social behaviors of females, and attach romantic meaning when there is none. This acculturation process could go awry in a borderline or psychotic individual and lead to the development of erotomania.

This link is theoretically made in the psychoanalytic literature, where both culture shock and erotomania have been described as variants of pathological mourning [*2,3*]. The role of paranoid thinking has also been discussed in both erotomania and acculturation [*4,5*]. The former involves a functional illness while the latter is an adaptive mechanism toward a new environment in which the acculturated individual is more vulnerable to distrust and suspicion.

The following case study is one of several that have been observed by us in forensic mental health settings in Southern California. This case is consistent with recent research that suggests erotomania affects a larger percentage of males than was originally thought [*6,7*] and is a precipitating factor in some forensic cases [*8*].

When traditional Islamic men are diagnosed with delusional (paranoid) disorder, erotomanic type (DSM III-R; 9), a failed cross-cultural adjustment may be an important precipitant of mental illness. Case history information usually reveals early losses and sexual repression. The move to the new culture is characterized by a loss of status and difficulty integrating into a meaningful new social context. The erotomanic symptoms reflect deficits in personality organization that impair ability to deal with the multiple losses inherent in culture shock. The frantic attachment to the love object is a defense against mourning and a loss of identity. Paranoid reactions to the new culture, often a feature of the disorganization phase of culture shock, take on delusional proportions. The more disparate the customs and social cues between the two cultures, the more vulnerable the individual:

> Patient A[1] was a 37 year old Libyan male evaluated to determine his violence risk toward a young American woman. He had never personally known her, but she had sat in front of him during a college course. He had subsequently pursued her for five years, sending her gifts and flowers, several letters, and, on one occasion, a blood-soaked feather. He had telephoned her, her mother, and her employer, and intermittently approached her in public places. She became anxious and fearful, and entered psychotherapy. Prior to our evaluation patient A violated five temporary restraining orders issued by the Superior Court.

The patient was born in Libya to an intact family with two older brothers and eight younger sisters. He finished high school at seventeen, worked in the family clothing store, and became engaged to his first cousin, ten years his junior. She terminated their relationship two years later. Patient A immigrated to the United States at age 27. He worked at menial tasks, took some college courses, and lived alone in a large American city.

During the clinical evaluation he denied any approach behavior toward the victim or intent to harm her, but did acknowledge that she encouraged him: "the way she looked at me, the way she did her hair, she gave a smile from a distance like she wanted to engage in a puzzle . . . the challenge is what keeps me going." Psychological testing confirmed an individual organized at a psychotic level of personality who had difficulties with reality testing, identity diffusion, and modulation of affect. His self-esteem was low, but an idealized grandiosity meant his aspirations far outstripped his abilities. Formal thought disorder was apparent, and content analysis of his Rorschach indicated a plethora of symbiotic merging

[1]data has been altered to adequately disguise the identity of the patient.

responses. His IQ was in the bright normal range (110–119). Our diagnosis was delusional (paranoid) disorder, erotomanic type (DSM-IIIR). We opined that he did not present an imminent risk of physical harm to the victim, treatment would have little effect, and if the behavior persisted he should be deported.

In this case, the patient's misperception of social friendliness as serious romantic intent was a partial product of his attempt to integrate two widely divergent heterosexual cultures. But it was also exacerbated by a psychotic personality organization which seriously impaired his reality testing. The motivational dynamics involved pathological mourning for the lost culture [*10*], threats of loss of identity [*3*], inability to process the complexities of the new culture [*11*], and an affectional hunger for a new object that would defend against his loss [*4*].

In cases such as this, we recommend taking a careful history about the culture of origin and the circumstances around the patient's transition to the new culture. We also explore the individual's beliefs regarding sexual roles and mores and his perceptions of his current niche in the new culture.

We would urge that acculturation problems be considered an important precipitant in the development of erotomania, particularly in traditional Islamic male immigrants. Grossly different patterns of heterosexual behavior in the two cultures and borderline or psychotic personality organization would be the respective predisposing social and psychobiological risks.

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The Effect of Killing and Preservative Solutions on Estimates of Maggot Age in Forensic Cases

Dear Sir:

A recent informative article which discusses an oversight by forensic entomologist of possible maggot shrinkage artefact suggests that "*boiling water should be used as a standard killing solution for maggots of forensic importance*" [*1*]. The larvae can be subsequently preserved in 70% ethanol, since maggots killed in boiling water and then placed in the preservative solution did not shrink [*1*].

It would seem contradictory to what has been previously emphasised that the method of killing maggots in hot water was "*definitely not recommended*" [*2*]. This was a response to a complimentary "letter to the editor," welcoming the spotlight on forensic entomology [*3*], in which I had referred to killing maggot larvae at the scene by immersion in almost boiling water as a feasible alternative. My comments generally pertained to a preliminary entomological examination by the forensic pathologist in routine or non-contentious cases and where a decomposed body together with its infestation may have been removed to the mortuary.

It is said that boiling appears to greatly lessen maggot autolysis and it has been found that larvae undergo maximum extension in boiling water (which is comparable to maximum extension of a live larva) and become rigid, thus further improving precision [*1*]. The availability of hot water at a crime scene has been discussed [*1*] and generally it is no more difficult than at a "non-crime" scene, especially indoors.

As hot water can cause adverse changes to the internal maggot structures, an often important determination of the age of the larva from a dissection of the gut, especially in the post-feeding larvae stage, may be counterproductive.[1] Where practicable, the problem may be decreased by collecting samples in duplicates, one to be killed in hot water for length measurement and the other kept alive or preserved for dissection if required.[2] However, for a valid and consistent interpretation of the larval length, standardization in the entomological treatment is obviously necessary to reduce or eliminate potential errors (as much as 24 hours or more) in estimates of the postmortem interval.[1] It would be good forensic practice if an interpretation of the entomological evidence is made less inaccurate [*4*].

It can be argued that the use of boiling water would give misleading results as a consequence of inappropriate comparative analysis between different killing or preservative solutions. The study of the effect on estimates of maggot age from shrinkage of larvae in various killing solutions, which can lead to miscalculation of the postmortem interval [*1*] may be interpreted to be simply reflecting this point. If it is not the choice of the killing medium itself that matters but the awareness of the shrinkage artefact, then there ought not to be a conflict with the hydrous method of killing maggots!

At a time when there is an editorial call for expansion of forensic entomology [*5*], it may be popular to resolve in a non-hostile manner what may be puzzling to a forensic practitioner who indulges in "do-it-yourself" entomology at a "non-crime" scene where an on-site entomologist may not be necessary.

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[2] Erzinclioglu, Y. Z., "Protocol for Collecting Entomological Evidence," (Letter), *Forensic Science International,* Vol. 45, 1990, pp. 191–192.

[1]Greenberg, personal communication.

[2]Greenberg, personal communication.

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[4] Patel, F., "On the Interpretation of Maggot Evidence in Forensic Cases," (Letter), *Medicine, Science and the Law,* Vol. 30, 1990, p. 184.
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A Discussion of "Method Comparison of EMIT II and OnLine with RIA for Drug Screening," (J. Forensic Sci., Vol. 38, No. 6, Nov. 1993, pp. 1326–1341)

Dear Sir:

Forensic toxicologists rely on immunoassay evaluations for choosing initial tests appropriate to their needs. Immunoassays used in forensic urine drug testing are difficult to compare not only because laboratories use a variety of instruments but, complicating matters, there are differences in calibrator concentrations assigned by manufacturers. Even with the same calibrator concentrations assay responses differ widely.

On this subject I call the readers' attention to the above-referenced article by Armbruster (*et al.* The authors' use of raw data (absorbance units or AU) to compare precision of OnLine® with EMIT II® is in question. Due to differences in the slopes of the standard curves, a more uniform method for evaluating precision is conversion of raw AU data to response per ng/mL.

Copied verbatim from their Table 3 are data shown here in Table 1. Dividing the standard deviation by the mean AU at 50 ng/mL they conclude that the OnLine marijuana assay

TABLE 1—*Raw absorbance data from marijuana assays compared with ng/mL units applied to calculating percent coefficient of variation (%CV). The last two columns show transformed numbers (based on AU change from zero to 50 ng/mL[1] and zero to 100 ng/mL[2]).*

Calibrator, ng/mL	Raw Absorbance Data: Mean	Raw Absorbance Data: "SD" from raw AU	"%CV" from raw AU	corrected SD using ng/mL	corrected %CV using ng/mL
EMIT II					
Marijuana 0	0.292	0.007	2.4	—	—
50	0.347	0.018	5.6	16.4[1] ng/mL	32.7[1]
100	0.420	0.008	1.9	6.2[2] ng/mL	12.5[2]
zero-to-50 AU change	0.055				
AU change per ng/mL	0.00110				
zero-to-100 AU change	0.128				
AU change per ng/mL	0.00128				
OnLine					
Marijuana 0	1.129	0.057	5.0	—	—
50	0.723	0.051	7.0	6.3[1] ng/mL	12.6[1]
100	0.614	0.032	6.6	5.0[2] ng/mL	5.0[2]
zero-to-50 AU change	0.406				
AU change per ng/mL	0.00812				
zero-to-100 AU change	0.639				
AU change per ng/mL	0.00639				

has a coefficient of variation (CV) of 7.0% versus 5.6% for EMIT II. Similarly, at 100 ng/mL they find OnLine's CV is 6.6% when EMIT II is 1.9%.

Armbruster's conclusions are reversed when corrected for concentration. At 50 ng/mL, Online's CV computes to 12.6% with EMIT II at 32.7%. At 100 ng/mL, Online's CV calculates to 5.0% when EMIT II is 12.5%. The same point can be made for each of the assays that appear in Table 3 of the article.

With today's forensic toxicologist having such a broad range of choices in instrument types, assay formats (for example, kinetic or end-point chemistries, homo- or heterogeneous microparticle, radioimmune, or fluorescence polarization), the most technically correct approach to methods comparisons is based on the forensically germane unit of measure. The yardstick in the present case is concentration units (ng/mL). Comparisons of unnormalized measurements are like comparing apples to oranges; they don't mix.

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Author's Reply

Smith's letter compares the standard approach for assessing precision that we followed in our article with his unique, if unorthodox, method. The SDs and CVs listed in the article's Table 3 (reproduced in Smith's Table 1) for the EMIT II and OnLine marijuana assays were derived from replicate measurements (n = 20) of the negative, cutoff, and high calibrators for both assays. The values were calculated using the traditional equations: $SD = [\Sigma (Xi - \bar{X})^2/N - 1]^{1/2}$, and $CV = (SD/\bar{X})\ 100$. (1.2) Smith argues that using concentration instead of absorbance data is preferable to gauge assay precision. Using data presented in the paper, he proceeds to "convert" absorbance SDs to concentration SDs. He begins by calculating the analytical sensitivity (change in analytical signal per change in analyte concentration) from 0–50 μg/L and 0–100 μg/L for both assays. Smith proceeds to "transform" absorbance SDs by dividing them by the absorbance change per μg/L of marijuana, for example, for the EMIT II assay at the 50 μg/L cutoff, .007 AU/.00110 AU/(μg/L) = 16.4 μg/L. The CV is then calculated using this "corrected SD." We are dubious of the validity of this type of conversion for several reasons. A precision study requires making replicate measurements at a stated analyte concentration and the absorbance SDs represent the product of this type of experiment. Dividing the absorbance SDs by the analytical sensitivity of the assay *is not* a direct conversion of raw absorbance data into the corresponding concentration data. Our article's Table 3 provides an estimate of precision for the zero μg/L, or negative, calibrators using absorbance data. Precision values for the zero calibrators are conspicuously missing from Smith's Table 1. Using Smith's method, it is impossible to convert the zero calibrator absorbance SD into a concentration SD. Yet every analytical procedure measures a zero calibrator with some demonstrable degree of precision and the ability to determine precision is important. Smith performs his "conversion" using two different analytical sensitivity factors: one for the change from 0 to 50 μg/L and one for the change from 0 to 100 μg/L. Smith does not explain how he determined which value was appropriate for the "conversions." Why not use the 0 to 100 μg/L value for both conversions, or perhaps use the analytical sensitivity for 50 to 100 μg/L for the second conversion? The SDs and CVs calculated using Smith's approach will vary depending on which analytical sensitivity is used in his "transformation." We contend that the precision of an analytical procedure at a given analyte concentration is not subject to such vagaries. The need to pick and choose among two or more conversion factors is obviated by simply

measuring replicates of a sample and performing the well accepted calculations referenced above.

We still feel that Table 3 accurately reflects the reproducibility of the assays' analytical signals at the calibrator concentrations, despite Smith's interesting manipulation of the data. Our experience, gained through participating in many inspections of Department of Health and Human Services certified drug testing labs, is that the stability and precision of these types of immunoassays are carefully monitored on a daily basis. The data used are typically either raw absorbance values or the corresponding "machine numbers" (as described in our paper). Labs usually establish limits corresponding to +/− 2 SD or 20% of the mean values for calibrators and controls to assess assay acceptability and also evaluate the separation between calibrators on the basis of absorbance data. Labs *do not* typically convert absorbance data into concentration units for calibrators, controls, or samples.

At a minimum, it is good practice to monitor the performance of the zero and cutoff calibrators, often accomplished by establishing the kind of statistical acceptance window described above. The zero calibrator demonstrates the practicality of using absorbance values or "machine numbers" for this purpose. Obviously, some scatter of absorbance readings will be obtained for the zero calibrator from day to day. The mean absorbance value corresponds to a concentration of 0 μg/L. To construct an acceptance window for the zero calibrator using concentration data requires converting absorbance readings falling on either side of the mean value into concentration units. This means that absorbance values on one side or the other of the mean value will be converted to *negative numbers (− μg/L)*. The use of *negative drug concentrations* is scientifically unappealing, if not outright silly. Smith feels that the most technically correct basis for method comparison is the use of the most forensically germane unit of measure (μg/L). We suggest that for these immunoassays, used for drugs of abuse screening, raw absorbance data are the forensically germane unit of measure.

We think that the point Smith is really trying to make is that the analytical sensitivity of the OnLine assays is better than that of the EMIT II tests. He demonstrates this in his Table 1, as did we in Table 2 and the associated text in our paper. Since the OnLine assays enjoy a wider separation between the zero, cutoff, and high calibrators, they can probably afford to exhibit somewhat less precision than the EMIT II assays and still not be in any danger of having calibrator absorbance readings overlap or compromise their ability to distinguish positives from negatives. While increased analytical sensitivity is obviously desirable, it appears that the ultimate effectiveness of a drugs of abuse immunoassay, that is, how many confirmable positive samples it can detect, is significantly affected by other factors. The characteristics of the antibody(ies) used, for example, antibody specificity, and the actual composition of the samples tested (mix of drug/drug metabolites and relative concentrations) undoubtedly influence screen immunoassay performance in the real world setting.

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