Correspondence

Drug Usage in San Diego County 1990–1997

Sir:

These comments are based on urine drug testing at the San Diego County Sheriff's Crime Laboratory 1990–1994, Poison Laboratory, San Diego (Ref. Lab 1) 1995–1996, and National Toxicology Laboratory, Bakersfield, CA (Ref. Lab 2) 1996–1997. A total of more than 24,000 samples were analyzed for Methamphetamine (MEA), Cannaboids (POT), Cocaine Metabolites (COC), Morphine Metabolites (MOR), and Phencyclidine (PCP). All samples were tested at least in duplicate by differential procedures that include GC/MS, immunoassay, GC and TLC. A positive is defined as: MEA 500 ng/mL., POT 100 ng/mL., COC and MOR 300 ng/mL., PCP 25 ng/mL. At these levels we feel confident that the arrestee has: 1) used the drug(s) recently, and 2) his behavior is consistent with impairment.

You will observe (Fig. 1) the relative consistency of percentages of the various drugs found in urine. This is true even when the actual testing was subcontracted to off-site reference laboratories in 1995–1997. The high percent of methamphetamine is consistent with the dubious distinction of San Diego as the "Meth capitol of the world", and the many meth lab seizures over this period of time.

Our court experience has been very positive. With the increasing number of qualified DRE officers in the field, together with positive drug levels, a drug profile is presented to the prosecutor. I have given DUI-Drug orientations to all interested City Attorneys and District Attorneys last year. The heart of my testimony is: 1) positive drug level, 2) proper probable cause, and 3) complete DRE evaluation. This is consistent with mental confusion and uncoordinated behavior and recent drug use by the arrestee.

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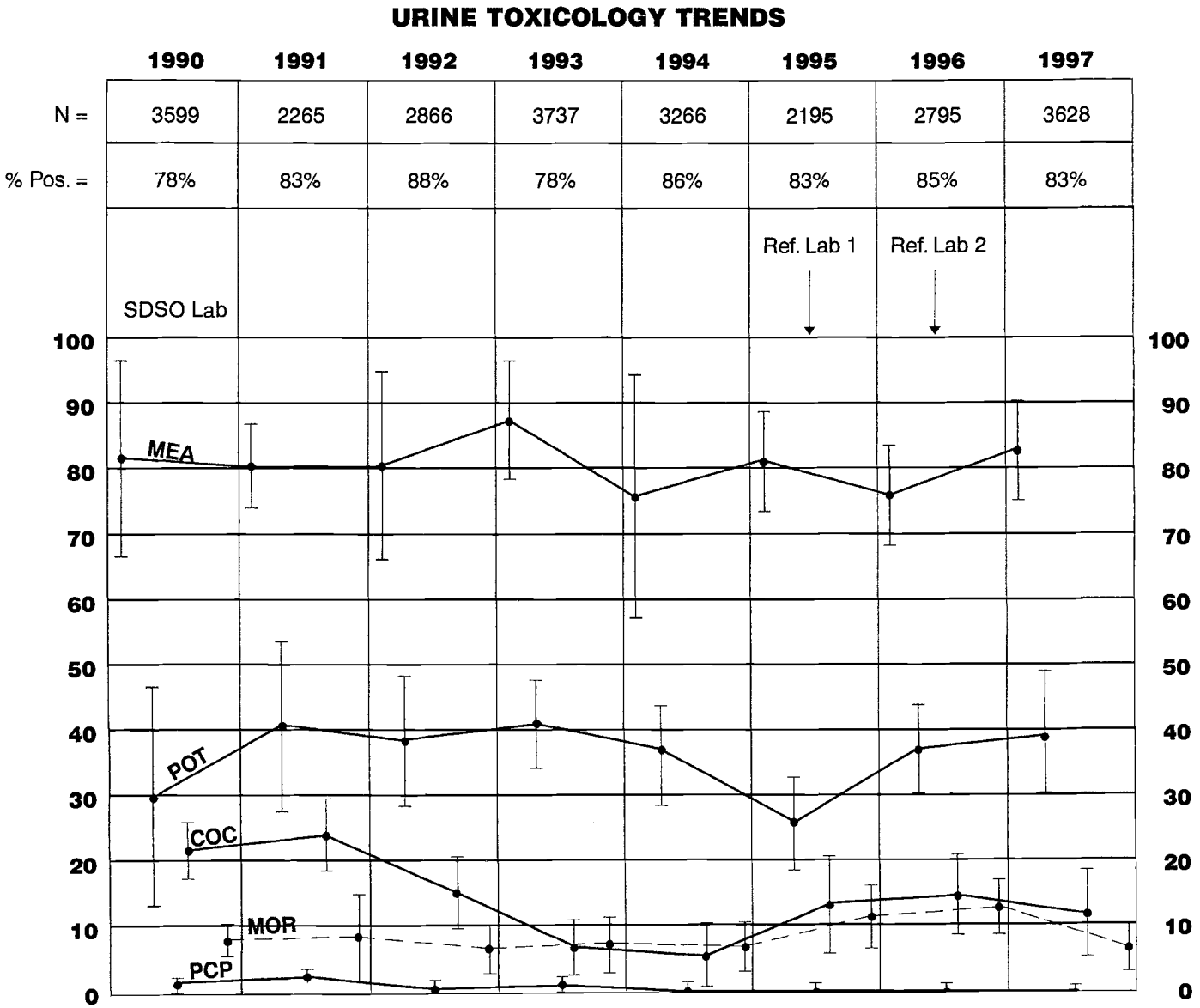

FIG. 1—*Urine toxicology trends.*

Commentary on Kline MC, Jenkins B, Rogers S. Non-Amplification of a vWA allele. J Forensic Sci 1998 Jan;43(1):250.

Sir:

Two population database samples were provided to us by laboratories that observed non-amplification of a vWA allele using an AmpFISTR® PCR Amplification Kit. These samples were observed to have homozygous vWA genotypes with the AmpFISTR kit, but heterozygous genotypes when amplified with alternative vWA primer sets, including the Promega PowerPlex™ and PE Applied Biosystem's ABI PRISM® Primer Set vWA Kit. One sample, provided by the National Institute of Standards and Technology (NIST), was an African American sample where the vWA 19 allele did not amplify using the AmpFISTR kit. The other sample, provided by the Office of Chief Medical Examiner, New York City (OCME), was a U.S. Hispanic sample where the vWA 17 allele did not amplify using the AmpFISTR kit. Upon receipt of these samples, PE Applied Biosystems scientists amplified them using vWA primers that anneal to sequences outside of the standard AmpFISTR vWA primers. Each of the individual alleles was then isolated and sequenced.

Sequencing results indicated a point mutation in the upstream sequence flanking the tandem repeat region; the mutation was the same for both the vWA 17 and 19 alleles. The mutation is a T to A transversion in the DNA template, at the second position from the 3′ end of the AmpFISTR vWA forward primer binding region. This mutation destabilizes primer binding and prevents amplification of an allele carrying the mutation.

One way to estimate the frequency of this mutation is through concordance studies that compare the vWA genotypes for samples amplified using AmpFISTR vWA primers versus alternative vWA primers. The NIST laboratory found the one discordant sample noted above by analyzing 600 database samples (200 U.S. Caucasian, 200 African American, 200 U.S. Hispanic) with both the AmpFISTR and Promega PowerPlex kits. The OCME laboratory similarly analyzed 200 samples (50 African American, 150 U.S. Hispanic) and found the one discordant sample noted above. Our laboratory (PE Applied Biosystems) has additionally performed concordance studies using the vWA primers found in the SGM (Second Generation Multiplex, Forensic Science Service, England). The SGM forward vWA primer has no sequence overlap with the AmpFISTR forward vWA primer. This data set indicated 100% concordance for the 683 tested samples (200 Caucasian, 194 African American, 189 U.S. Hispanic, and 100 samples not identified by ethnic origin). Thus the total frequency of vWA discordance in all studies reported to date is two alleles in 1,483 individuals, yielding an allele frequency estimate for the mutant vWA allele of 0.00067.

Primer binding site mutations present a challenge in the development of PCR systems. Particularly challenging are the development of: 1) 'universal' primers for closely related microbial sequences, 2) primers for the amplification of highly polymorphic loci such as HLA class I and class II, and 3) primers for noncoding sequences such as STR loci, where mutations can accumulate that are nondeleterious to the organism. As far as the human genome is concerned, we fully expect to find a tremendous degree of genetic variation in the world's population. Indeed, this is the basis of genetic typing for human identification applications.

Most laboratories that have reported on length and sequence polymorphism at STR loci have focused on the tandem repeat region. However, sequence polymorphism in the sequences flanking the tandem repeat region is also expected at some frequency for any STR locus. Our laboratory has observed flanking sequence mutations in several STR loci, including vWA (reported here), D16S539, and TPOX. Other laboratories have recently reported flanking sequence mutations at D13S317 and D7S820. Thus, we expect mutations to exist in the flanking sequences, and so the challenge is to choose primer binding sequences that are as highly conserved as possible.

In developing primers to amplify STR loci, our laboratory invokes several means of selecting conserved regions and then assessing candidate primers to avoid primer binding site mutations, as follows: 1) sequence analysis of multiple alleles, 2) family (inheritance) studies, 3) examination of within-locus peak signal ratios in population database samples, 4) examination of peak signal balance between loci in population database samples, 5) re-amplification of apparent homozygous samples at low stringency, and/or with alternative primer sets, and 6) statistical analysis of observed versus expected homozygosity in the population database. This approach is necessary to facilitate detection of any mutations present in the samples examined; sequence regions containing a mutation can then be avoided in primer selection. However, no reasonable methods are available for 'early' detection of extremely rare or population specific mutations. The result of this is that we fully expect to find primer binding site mutations at any STR locus as more and more samples are examined, particularly as samples from a much greater variety of population groups are examined.

When a mutation is present in the primer binding region for any particular allele, the three possible PCR outcomes are: 1) no effect on amplification, 2) weak amplification of the mutant sequence, resulting in peak signal imbalance in a heterozygote, 3) non-amplification of the mutant allele, resulting in a homozygous genotype in a true heterozygote. The particular outcome for any given mutation depends on several factors, including the nature and position of the mutation relative to the 3′ end of the primer. Primer mismatch at or near the 3′ end is likely to result in non-amplification. Other relevant factors include the overall length of the primer, as well as conditions related to the stringency of the PCR, such as the annealing temperature and salt concentrations.

A fundamental expectation that follows from these observations is that full concordance is expected when the same primer set is always used for all analyses. This is the typical situation in forensic casework applications, where a laboratory or laboratory system validates and implements a particular system for casework, such that evidence samples and suspect reference samples are amplified with identical PCR primers. Thus, full concordance in casework is expected; primer binding site mutations are of no practical consequence in making determinations of inclusion or exclusion of casework specimens.

However, non-concordant results will always be a possibility when the same DNA sample is amplified using different pairs of PCR primers. This situation is a possibility in the case of forensic DNA databanks, where a laboratory in one region of the country may be using PCR primers that differ from a laboratory in another region of the country. Thus, different primers may be used to amplify the evidence sample versus the convicted offender sample that is on the database. Appropriate database search algorithms should be implemented that do not overlook convicted offender samples differing by only a single allele from the evidence sample. For example, the database can be searched for convicted offender samples that match the evidence sample at 25 out of 26 alleles.

This type of search criterion is also recommended to account for the possibility of human or experimental error in the genotyping process. Broadening the search criterion should not significantly increase the number of 'adventitious hits', considering that the combined probability of identity for the 13 STR loci recently selected for the CODIS system is approximately $1/10^{14}$. In any case, convicted offender samples identified as potential matches by the search process will always be reanalyzed using the same STR primers used to amplify the evidence sample, thus providing full concordance for the final conclusion.

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