INTEROFFICE MEMORANDUM

Date: September 10, 2012

TO: Anthony DeMaria

FROM: Michelle Hassler

CC: Steve Guroff, Connie Milton, and Shelley Webster

SUBJECT: **Response to 2012 Forensic Biology Section Audit Findings**

I have reviewed the external DNA audit report. Below is my response to the findings.

Standard 3.2: "Does the laboratory maintain and follow a procedure regarding document retention..."

The Laboratory's Quality Manual was revised (revision 6) to address this finding. See the attached manual revision section 9.21.

Standard 8.1 / 8.3.2: "Have quality assurance parameters and interpretation guidelines, including, as applicable, guidelines for mixture interpretation, been defined pursuant to internal validation?"

We respectfully disagree with this finding. We cannot find any support for the auditors' position. Standard 8.3.2 requires us to define quality assurance parameters and interpretation guidelines pursuant to our internal validation. We have done this with our quantitation and amplification kits. It does not require us to reevaluate our guidelines in light of subsequent experience. Whether this standard is construed as a quality assurance parameter or an interpretation guideline, it is clearly defined pursuant to internal validation, exactly as required. The auditors agreed that our guidelines were justified by our validations, and therefore they could not give us a finding based on standard 9.5.

Per our protocol, analysts can amplify less than 250 pg of DNA with extreme caution. The analysts take into account the sample type (if it is likely to yield a single source profile), whether or not other evidence in the case exists, and if they have any reason to think the quantitation is underestimating DNA concentrations. Our lab uses "Quantifiler" kits from Life Technologies for human DNA quantitation. Like all quantitation products, these kits vary somewhat from lot to lot. As part of our quality control process, if a specific lot of kits consistently underestimates the amount of DNA in a sample, the fact is communicated to the analysts so they can use this information in determining which

samples to amplify. It would not be practical or appropriate to re-evaluate our cut off with each new kit lot we receive. Additionally, analysts can use typing data from other samples quantified at the same time, such as a positive extraction control, to determine if the quantitation underestimated the amount of DNA present in the samples.

Whether or not the auditors' position is justified by the language of the standard, the two cases that were cited in the finding do not support the auditors' conclusion that full profiles can be obtained from less than 250 pg input DNA. In both cases, the data indicate that the amount of DNA in the samples was significantly underestimated. The amount of DNA from case 01061001 was estimated to be 163pg; however, the amplified product produced peak heights as high as 3400 RFU. Similarly, in case 9407679, the amounts of DNA amplified were estimated to be 150pg and 200pg but the resulting profiles showed saturated data. It is obvious that the actual amount of DNA amplified from each sample was far greater than 250 pg. In both cases most samples from the batch, including a positive extraction control, produced profiles that clearly indicate more DNA was amplified than the original estimates. This information would alert any analyst that the quantitation was underestimating the amount of DNA present in the samples. The result from these cases are atypical and our cut off appropriately allowed the amplification of these samples.

This is not a noncompliance with the cited standard. There is not a requirement for amplifying samples with very little or no DNA.

Standard 9.6: - "Does the laboratory have and follow written guidelines for the interpretation of data?"

Formulae and a reference were added to the Forensic Biology Technical Manual to address this finding. See attached section 4.5.5.1 for the revisions.

Standard 10.3: "Does the laboratory have a schedule and follow a documented program to ensure that instruments and equipment are maintained properly?"

A procedure was added to the Forensic Biology Technical Manual to address this finding. See attached section 8.2.3 for the revisions.

09/10/12

Michelle Y. Hassler
DNA Technical Manager

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QUALITY ASSURANCE RECORDS RETENTION	9.21
All quality assurance records shall be maintained for a minimum of five years, or as required by Sheriff's Department policy and procedure. Quality assurance records consist of proficiency tests, corrective actions, audits, training records, continuing education and court testimony monitoring. Some quality assurance records may be retained for longer periods, or indefinitely, as deemed necessary by the Quality Assurance Manager. Quality assurance records are stored in a secured room controlled by an electronic card reader. Access to this room is restricted to individuals authorized by the Crime Lab Director.	

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4.5.5 Statistical Calculations

4.5.5.1 Statistics for Identifiler DNA Results

Several legitimate methods exist to assess the strength of DNA evidence. The approach taken in any particular case will be determined by a combination of: the question that needs to be answered, the available data, any requisite assumptions, and the practicality of the approach. The strength of the relationship between any two samples may be expressed using various statistical tools including: a random match probability/frequency of occurrence, a probability of inclusion, or a likelihood ratio. These three calculations assume that the randomly selected individual from the population is not closely related to the included individual (for example, the statistic given for an evidence sample in which a suspect is included as a possible contributor does not represent the probability of the suspect's sibling also being included in that sample). The Popstats statistical program should be used for these calculations. Manual calculations may also be performed as necessary. All allele frequencies shall be calculated using a minimum allele count of 5.

All statistical calculations shall be performed using the following population databases:

-Budowle, B. et al. Population data on the thirteen CODIS core short tandem repeat loci in African Americans, U.S. Caucasians, Hispanics, Bahamanians, Jamaicans, and Trinidadians. *JFS* (1999) (for CODIS core loci)

-Budowle, B. et al. Population Data on the STR Loci D2S1338 and D19S433. *Forensic Science Communications*, July 2001 vol 3 (3).

Statistical analysis of an evidence DNA profile (or a portion of the evidence DNA profile) shall be provided when a probative inclusion is reported. An inclusion of an individual who is assumed to be in a sample (victim in her own intimate sample, elimination reference in a sample, etc.) does not require a statistic. If the probative value of a sample is unclear, generally the analyst should report a statistic on the evidence. In situations where there are several samplings of one item, it is at the analyst's discretion to report the statistic on the most complete or probative sample; the analyst may report statistics on all the associated samples. Similarly, if a probative inclusion of the same individual is made in a sperm and non-sperm fraction of a single sample, the analyst will generally only need to report a statistic for the most probative fraction.

In some situations, when the presence of one of the contributors (e.g., the victim) can be assumed based on the nature of the sample, the information contained in the profile from the known source can be used to assist in mixture deconvolution and subsequent statistical analysis. Depending on the profiles in the specific

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instance, this can be accomplished by subtracting the contribution of the known donor from the mixed profile. It may be possible to infer the foreign profile by examining peak height ratios (see FB manual section 4.5.3). A deduced foreign profile may be treated as a single source sample for statistical analysis.

Single-source samples

- For single-source samples, frequency of occurrence (random match probability) can be calculated using the following formulae for each locus:
  $f = 2pq$ (heterozygote)
  $f = p2 + p(1 - p)\theta$ (homozygote)
  $\theta = 0.01$
  The calculated frequency at each locus shall be multiplied together to obtained a frequency of the entire profile.

- Alternatively, a Likelihood Ratio (LR) can be performed on single source samples. LR= 1 / RMP (random match probability). The numerator of this calculation assumes the individual in question's contribution. The denominator would assume that the individual in question is not the contributor. The probability of a randomly selected person having the evidence profile is represented by the RMP.

- Loci from apparent single source samples exhibiting three alleles at a single locus will not be used for this calculation.

Mixtures

- Generally, mixture calculations include the Combined Probability of Inclusion (CPI) or a Likelihood Ratio (LR).

- For mixtures in which a major profile can be distinguished and is attributed to a single source, the frequency of occurrence of the major profile can be calculated using the same formulae as for single-source samples using those loci at which the major profile is distinguishable.

Combined Probability of Inclusion for Mixtures

- For mixtures in which a major contributor cannot be distinguished or in mixtures of DNA from three or more individuals, the combined probability of inclusion (CPI) may be used. The probabilities of all possible genotype combinations at each locus are calculated and summed to generate a probability of inclusion (PI). The homozygote correction factor of θ will not be used for the PI calculations.

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The probability of inclusion for each of the acceptable loci are multiplied together to calculate the CPI.

$$PI = (p + q + r + \ldots)^2$$
$$CPI = PI_1 * PI_2 * \ldots PI_n$$

- The CPI calculation estimates the frequency that a randomly selected person of a given population would be included as a possible contributor to an observed mixture.

- If a dual major is distinguished in a mixture of three or more individuals, the restricted CPI may be used on the dual major.

- For calculation purposes it may be inappropriate to use those loci which do not show evidence of a mixture in a sample which is clearly a mixture or where it is reasonable to assume the presence of dropout.

- In mixed samples where the average peak height is 200 RFU or less, the inclusion and exclusion of a contributor should be made with caution as expected genetic information may be absent in the sample. In such instances, a determination of no conclusion may be justified.

Likelihood Ratio for Mixtures (unrestricted)

- Generally, if a mixture of DNA can be assumed to be from only two contributors, it may be appropriate to employ a likelihood ratio (LR). Likelihood ratios may be used in particular with mixtures where competing hypotheses regarding the origin of the evidence shall be weighed.

- The LR is the probability of the evidence given one hypothesis compared to the probability of the evidence given the alternative hypothesis. These hypotheses are mutually exclusive.

$$LR = [P(E \mid H_1)] / [P(E \mid H_2)]$$

H_1 = hypothesis 1
H_2 = hypothesis 2

The homozygote correction factor of θ will not be used for the likelihood ratio calculations.

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- The LR is typically used in scenarios where one of two contributors can be assumed (like in an intimate sample or similar) although it can be used in other scenarios.

  The numerator in this scenario assumes the two included individuals' contribution, meaning the probability of observing results consistent with their genotypes would be 1.0. The denominator of the LR calculation assumes that the mixture is a combination of the assumed contributor and an unknown individual. The LR denominator is the sum of the possible combinations of genotypes at a locus. The results from each locus are multiplied together to determine the LR for the entire profile.

- For additional LR formulas and assistance with manual calculations, refer to Chapter 7 of Evett, I.W. and Weir, B.S., Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists, Sinauer Associates, Sunderland, MA, 1998.

Partial profiles

- Single source –

  If a particular donor can be included as a possible source of an apparent single source sample for which only a partial profile has been obtained, the statistical calculation shall only include the alleles detected in the evidence sample.

  In instances where a single allele is present below the stochastic threshold, there is a possibility that there is dropout of the second allele of a heterozygote. Therefore, the locus shall be dropped for statistical analysis.

- Mixture –

  If a particular donor can be included as one of two or more possible sources of a sample for which only a partial profile has apparently been obtained, the statistical calculation shall include only the alleles detected in the evidence sample.

Parentage

The laboratory performs parentage analysis in certain situations. These may include identification of remains, cases where an evidentiary sample is compared to relatives of a victim for whom no reference sample is available, and criminal paternity cases. In these instances, the analyst should use the likelihood ratio approach and calculate a parentage (or paternity/maternity) index. For example, in a paternity case, this is the ratio comparing the probability that the tested man

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passed the obligatory paternal alleles to the probability that a random man passed the obligatory alleles.

A Parentage Trio is when there is a question of paternity or maternity when the DNA types of the child, the known parent, and the alleged parent are available. Popstats can be used to calculate the parentage trio. When the known parent's DNA profile is unknown, the calculations can be performed by Popstats within the kinship tools.

A Reverse Parentage Index will be used in cases where a man and woman are thought to be the biological parents of the donor of an evidence sample. This likelihood ratio compares the probability that the tested couple produced a child with the observed profile to the probability that a random couple produced a child with this profile.

The Popstats statistical program shall be used for these calculations (Parentage Trio calculation or the Reverse Parentage calculation). If an exclusion is noted at a given locus, the average mutation rate for that locus should be used based on the following formula:

$$LR = P(E|H_1)/P(E|H_2)$$

$= u$ (mutation rate for locus)/ (average exclusion probability for locus)

This term can be calculated by hand using the published mutation rate for the given locus from the STRBase website. This calculation assumes that the allele has mutated to either one repeat larger or smaller than the putative parental allele. In the event that the putative parental has mutated 2 repeats larger or smaller then use u^2. The incorporation of the mutation rate in the calculation will serve to lower that likelihood ratio.

Relatives

- If possible contributors of the evidence sample include relatives of the suspect, DNA profiles of those relatives should be obtained. If these profiles cannot be obtained, the likelihood of finding the evidence profile in those relatives can be calculated with formulae 4.8a, 4.8b, 4.9a or 4.9b from the National Research Council report "The Evaluation of Forensic DNA Evidence" (1996). Popstats kinship program can be used for this calculation.

4.5.5.2 Statistics for Y-STR DNA Results

Y-STR loci are linked on the Y-chromosome. Therefore, the Y-STR statistics shall be calculated differently than autosomal STR analysis. The significance of a haplotype match in casework should be expressed in terms of the observed

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8.2.3 Temperature-Controlled Equipment Monitoring

All temperature-controlled equipment used to store critical DNA reagents (refrigerators and freezers), and heat blocks used for digestion procedures, shall be monitored weekly. The automated system uses NIST certified probes to monitor temperature in the heat blocks and other equipment. The probes used to monitor critical equipment are replaced annually with NIST certified probes. Monitoring shall be performed either by automatic system or manually, as described below.

Procedure for automatic monitoring:

1. Log in to Tempsys CheckPoint software (see Tempsys User Manual). The 'All Equipment Status' window will open.

2. Examine the entry for each monitored piece of equipment. Verify that the temperature is within the acceptable range.

3. If the average temperature recorded for a monitored piece of equipment falls outside the acceptable range, remediate the situation as described below. Note any remediative action in the CheckPoint software (see Tempsys User Manual).

Typically, the temperature monitoring system will alert designated individuals (via a pop-up alert on their computer and/or email) that a piece of equipment is outside the acceptable temperature range.

Procedure for manual monitoring:

1. Read the temperature on the thermometer mounted on the equipment, and record it on the temperature monitoring log for the equipment.

2. If the temperature recorded falls outside the acceptable range noted on the temperature monitoring log, take appropriate action to correct it, such as adjusting thermostatic controls. Note any actions of this type on the temperature monitoring log.

After completing monitoring procedures for each monitored piece of equipment, initial the appropriate box on the weekly cleaning and maintenance log. This serves as documentation that each monitored piece of equipment has been checked.

Procedure for remediation if temperature is out of range (for critical equipment):

1. Examine the monitored equipment and evaluate whether it is operating properly. If it appears the equipment is not operating properly, inform the DNA Technical Manager or designee, or a supervisor.

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2. If the monitored equipment appears to be operating properly, adjust the equipment's thermostatic controls as needed to bring the temperature into the acceptable range. Wait approximately one hour after adjusting the controls and check the temperature again. Repeat this process until the temperature falls into the acceptable range.

3. If the temperature cannot be brought into the acceptable range by adjusting the controls, inform the DNA Technical Manager or designee, or a supervisor.

4. If a piece of monitored equipment is not operating properly, or cannot be brought into the acceptable range by adjusting the controls, the person informed is responsible for taking any further corrective action needed. Depending on the situation, this may consist of repairing or replacing the equipment, removing samples or reagents to other locations, and initiating quality concerns or formal corrective actions as appropriate,

8.2.3.1 Replacement of the Temperature Probes

Order new, NIST-traceable temperature probes from the manufacturer once a year (TempSys item #63005-SS for stainless steel probes used in heat blocks, and item #63005-A for air probes used in other equipment). NIST-traceable probes are only needed for instruments in which the temperature is considered critical (refrigerators and freezers containing DNA reagents that need to be kept at a specific temperature and the heat blocks used to digest samples for DNA analysis).

To change probes:

1. Print out the current equipment list from the TempSys CheckPoint system, as follows: log into the system, and select **Settings**, then **Equipment**, to open the current equipment list. Right-click any item on the list and select **Print All Equipment**.

2. For each probe to be replaced, disconnect the old probe from the yellow transmitting sensor (Keep the old probes, these will be sent back to the manufacturer for credit).

3. Connect the new probe, and note the new serial number on the printed equipment list.

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