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DEPARTMENT OF JUSTICE

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GREGORY S. MCLEOD
DIRECTOR

December 15, 2010

Dr. Douglas Hares
NDIS Custodian
FBI Laboratory
Room 1120
2501 Investigation Parkway
FBI Academy Complex
Quantico, VA 22135

21DEC 10 2:21PM

Dear Dr. Hares:

I am writing to notify you that an external DAB QAS audit was conducted of the North Carolina State Bureau of Investigation on October 26-28, 2010. The audit of the North Carolina State Bureau of Investigation revealed the findings listed below with our corresponding responses. The audit report document is enclosed for your review.

The FBI Quality Assurance Standards Audit for DNA Databasing Laboratories:

9.2/9.2.2b Are commercial reagents labeled with the expiration date as provided by the manufacturer or as determined by the laboratory?

Finding: The laboratory has no policy for setting expiration dates for reagents without a manufacturer-provided expiration date. They track reagent lot numbers in their casework, but have not assigned expiration dates to reagents such as phenol/chloroform, formamide, and other commercial reagents for which no manufacturer expiration date has been assigned.

Response: See Exhibit #1 (Forensic Biology Administrative Order 10-PRO-17). This order sets expiration dates for commercial reagents within the Forensic Biology Section which do not have a manufacturer provided expiration date. Reagents listed in this administrative order are now labeled with the appropriate expiration date.



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The FBI Quality Assurance Standards Audit for DNA Forensic DNA Testing Laboratories:

9.2/9.2.2b Are commercial reagents labeled with the expiration date as provided by the manufacturer or as determined by the laboratory?

Finding: The laboratory has no policy for setting expiration dates for reagents without a manufacturer-provided expiration date. They track reagent lot numbers in their casework, but have not assigned expiration dates to reagents such as phenol/chloroform, formamide, and other commercial reagents for which no manufacturer expiration date has been assigned.

Response: See Exhibit #1 (Forensic Biology Administrative Order 10-PRO-17). This order sets expiration dates for commercial reagents within the Forensic Biology Section which do not have a manufacturer provided expiration date. Reagents listed in this administrative order are now labeled with the appropriate expiration date.

9.6 Does the laboratory have and follow written guidelines for the interpretation of data?

Finding: The laboratory has written interpretation guidelines, but is not consistently following them with respect to their STR interpretation guidelines 3.2.7.5 (overblown samples may be rerun with lesser amounts of DNA) and section 3.3 (definition of an artifact). Auditors noted many sample electropherograms exhibiting off-scale/overblown data yet few of the "overblown" samples were re-run (or re-amplified) using a lower amount of amplified product (or reduced template) as indicated by 3.2.7.5. It was also noted that the "artifact" labeled was overused and often did not meet the definition of an artifact as defined in the interpretation guidelines.

In reviewing recent corrective actions it was noted that there had been two corrective actions involving erroneous interpretation of artifacts. In one, an artifact peak was labeled as an allele, and in the other an allele peak was removed as an artifact.

Response: The NC SBI respectfully disagrees with this finding.

See Exhibit #2 (Forensic Biology Quality Assurance Manual Appendix E – Interpretational Guidelines). Analysts consistently follow the STR interpretational guideline 3.2.7.5. This guideline reads as follows:

Samples that are overblown **MAY** need to be re-run with a lower amount of amplified product (a dilution) or re-amplified using a lower DNA template, depending on the overall quality of the electropherogram.

Section 3.1 of the Forensic Biology STR Interpretational Guidelines additionally states "It is the responsibility of the analyst to use these guidelines in conjunction with their training and experience to provide a solid scientific interpretation of the results." Both of these guidelines clearly show when dealing with a potentially overblown sample, analysts (based on their training and experience) have the option of re-running the sample if they deem it necessary. Instances of analysts not re-running potentially overblown samples is merely a result of the analyst's assessment of the overall quality of the electropherogram as being sufficient based on their training and experience. It should also be noted that the words must, will, or shall do not appear in interpretational guideline 3.2.7.5.

In regards to "artifacts" being overused and often not meeting the definition of an artifact as defined in the interpretation guidelines, it should be noted that auditors relied exclusively on electropherogram printouts during their assessment. In this laboratory, case working analysts make their determinations on "artifacts" while looking at the raw electronic data in the Genemapper ID software program and do not rely solely on paper printouts. Auditors were given the option of reviewing the raw electronic data of case samples by laboratory personnel and declined this offer. Additionally, no specific cases were cited or listed by auditors to laboratory personnel. It is the opinion of this laboratory that proper assessments of "artifacts" in casework should be performed electronically and not exclusively from paper printouts. While "artifacts" are properly defined in section 3.3 of the STR Interpretational Guidelines, Section 3.1 (as quoted above) does not require the analyst to solely use these guidelines, but allows them to also use their training and experience in making solid scientific interpretations. In other words, if an analyst refers to a peak as an "artifact" this is not just solely based on what is defined in the interpretational guidelines, but also based on their training and experience.

Considering the two instances cited by auditors involving erroneous interpretation of "artifacts," these instances have been clearly documented (in memoranda as mentioned by the auditors) and preventative steps were taken with the analysts at the time to ensure proper assessment of "artifacts" in the future. These instances are clearly analyst specific and are not systemic problems involving analysts failing to follow set guidelines for data interpretation.

12.2/12.2.2.4 Does the laboratory conduct a review of all controls, internal lane standards, and allelic ladders to verify that the expected results were obtained?

Finding: The laboratory does not require review of internal lane standards in their entirety, but only a review of the ~250bp peak within the internal lane standard. When questioned, some analysts said that they only looked at the 250 peak and not the rest of the size standard.

Response: See Exhibit #3 (Forensic Biology Administrative Order 10-PRO-18). This order changes the technical review process to include a visual inspection and review of all ILS (LIZ) including the 250 base pair peak. DNA case working analysts have also been instructed by the DNA technical leader to print out the LIZ size standard with every sample ran in casework to ensure a proper review by the assigned technical reviewer.

Please let me know if you have any questions. I can be reached at 919-662-4509 ext. 2643.

Sincerely,



S/A Amanda Fox Overman
North Carolina State CODIS Administrator
Forensic Scientist Supervisor
North Carolina State Bureau of Investigation

(Enclosures)

Document Reviewed by Chris Parker, Technical Leader/Forensic Scientist Supervisor:





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GREGORY S. MCLEOD
DIRECTOR

ADMINISTRATIVE ORDER

EFFECTIVE DATE: December 01, 2010

10-PRO-17

TO: Forensic Biology Section

FROM: Special Agent in Charge *MOA* Michael J. Budzynski/ DNA TL *ACP* Chris Parker

SUBJECT: Assignment of expiration dates for reagents without manufacturer-provided dates

The following reagents shall have a DNA section mandated expiration date set 2 years from date of receipt into the section:

- Phenol/Chloroform (or equivalent)
- Hi-Di Formamide (stock supply)
- LIZ sizing standard
- 10x Buffer
- ATL Buffer for Qiagen BioRobot Universal

The following reagents shall have an expiration date set 3 years from d

- Proteinase K (stock supply)
- Dithiothreitol (stock supply)
- Bovine Serum Albumin (stock supply)

The following reagents shall have an expiration date set of 2 weeks aft

Carrier RNA from the Qiagen MDx Media Kit

Exhibit # 1
9.2/9.2.2b
Finding



Any aliquots made from stock supply reagents (formamide, proteinase K, DTT) will expire 1 year from date of preparation and must be noted on the container. If the reagent container is too small for individual notation of expiration dates, it must be noted on the container (box, bag, bottle or equivalent) storing the supply of reagents. Reagent expiration dates must be noted in FA by the QC Officer or designee. Expired reagents may be retained in the section for future validation or training purposes at the discretion and approval of the DNA Technical Leader.

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Title: Quality Assurance Manual Appendix E – STR Interpretation Guidelines		Revision 11

3 PCR STR Interpretation

3.1_ Introduction: The interpretation of results in casework is a matter of professional judgment and expertise. These criteria are based on our validation studies, literature, and over 15 years of forensic DNA casework experience by this laboratory. However, it is not possible to address every situation with a pre-set rule. It is the responsibility of the analyst to use these guidelines in conjunction with their training and experience to provide a solid scientific interpretation of the results.

3.2_ Preliminary Evaluation of Data

3.2.1 General

3.2.1.1 The Peak/Height cut off in the GeneMapper™ ID software will be set at 75 RFU for Casework and 100 RFU for Convicted Offender Testing.

3.2.1.2 "Activity" shall not be designated as alleles for Convicted Offender samples or for determining matches for forensic cases. However, any information obtained from activity may be used for qualitative data interpretation (including, but not limited to, evidence for a mixture within a given sample or exclusions). "Activity" below threshold will be used for exclusion purposes only. The analyst must take great care and take into consideration possible artifacts, high noise, and the general quality of data when making the decision of use data below threshold for this purpose. Section 3.2.1.3 below must also be considered.

3.2.1.3 A general rule is that the Peak/Height to Noise (background) ratio should be 3:1. In other words, the Peak/Height should be at least 3 times greater than the average background for a peak to be called.

3.2.2 Positive Amplification Control: The Positive Amplification Control must have peaks that are in the proper location relative to the allelic markers. If these characteristic peaks are not in their correct position or are not present (too weak to interpret), that particular locus must be considered inconclusive for all samples and must be

Exhibit #2

96 Friday

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3.2.6. Internal Lane Standards (ILS)/250 bp peak: GeneMapper ID will not size the samples/ladders/controls unless all ILS are present and above threshold. Therefore, the analyst will examine the 250 base pair (bp) ILS peak of each sample. The 250 bp peaks should fall within a size window of $< \pm 0.5$ bp. If a sample or control is $> \pm 0.5$ bp, then that sample should be re-run unless the sample or control does not exhibit amplified product (peaks). For example, if the ILS fails for a negative control, that negative control may be used for analysis if no peaks are observed when viewing the raw data. In this instance, the raw data must be printed instead of the electropherogram.

3.2.7. Samples

3.2.7.1. Visually inspect the known and questioned samples. Assess the quality of the peaks including RFU values and if artifacts are present. The peaks must be equal to or greater than 75 RFU for alleles to be called. The size standards within each sample must be present and correctly called.

3.2.7.2. Examine the electropherogram of the mixture. Note any inhibition, allelic dropout, and/or artifacts.

3.2.7.3. If the question sample(s) contain more than two peaks at the same locus, then the results may indicate a mixture. NOTE: If three peaks are observed at only one locus, then there may not be a mixture; the individual contributor may have a tri-allelic pattern at that locus. Both sample and standard should express the tri-allelic pattern in cases of a MATCH.

3.2.7.4. Failure of any loci to amplify for a multiplex STR system will not preclude the analysts from reporting those loci that are present, even if only one locus amplifies.

3.2.7.5. Samples that are overblown may need to be

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re-run with a lower amount of amplified product (a dilution) or re-amplified using a lower DNA template, depending on the overall quality of the electropherogram.

3.2.7.6. It is permissible to combine results from different injections (including dilutions) of the same sample when determining a final DNA profile.

3.3. Artifacts: The PCR process produces artifacts that are known and well characterized. All by-products of PCR and/or capillary electrophoresis will be labeled on the electropherograms as an "artifact" in the case notes.

3.3.1 Stutter

3.3.1.1 The STR results should not be considered to be inconclusive if stutter peaks are present in single source samples. Care must be taken when interpreting samples where mixtures are present in regards to peaks in the stutter position.

3.3.1.2 The GeneMapper™ ID software from ABI contains designated cutoff for peaks in stutter positions and will be used for designating stutter. Based upon analyst discretion, a minor peak in the stutter position that is called by the GeneMapper™ ID software may be disregarded as stutter, if the peak in question is 1) not in a mixed sample and 2) when compared to the predominant sister allele, it is close to the percent stutter cutoff for that particular locus. If a mixture is observed, then great care must be used in interpreting weaker peaks in the stutter position.

3.3.2 Pull up: Generally, pull-up can be noted when all the alleles are overlapped using the software and the "pull-up" is observed as a relatively small peak located directly under the larger peak. Analysts should be aware of this phenomenon and use the computer software to aid them in discerning actual alleles from pull-up.

3.3.3 Unincorporated Dye: Analyst should not call "dye-blobs" as an

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actual allele. "Dye-blobs" shall not be considered for interpretation.

3.3.4 Assigning Values to Microvariants and Off Ladder Alleles

- 3.3.4.1. The GeneMapper™ID determines the base pair sizes of all peaks. The analyst may add the allele call by determining the correct allele size typing in the correct allele designation based on the base pair size.
- 3.3.4.2. Variant alleles that vary by less than the consensus repeat unit will be designated as an integer of that variation (for example TH01 9.3 allele), as per CODIS recommendations.
- 3.3.4.3. When the sample contains an off-ladder allele, the analyst must assign the off-ladder allele to the correct locus, if possible. The peak in this situation lies between two loci, so the analyst should first determine if the patterns of the two loci are heterozygous or homozygous. If the pattern is heterozygous at one locus and homozygous at the other, then the off-ladder allele is assigned to the homozygous locus (making it heterozygous). If both loci show homozygous patterns, the analyst will document that the allele was observed between the two loci (a locus will not be designated for that allele).
- 3.3.4.4. If an allele falls above the largest value or below the smallest value of the allelic ladder for a locus, the allele will be designated as either greater than (>) or less than (<) their respective allelic ladder, as per CODIS recommendations.
- 3.3.4.5. Microvariants must be documented in the case notes by including either the Sizing Table or the Allele Plots in GeneMapper ID that show the base pair size of the Microvariants and corresponding Ladder alleles. Microvariants do not have to be re-amplified or rerun.
- 3.3.5. Split peaks. Samples with alleles displayed as split peaks may be called. However, if a potential split peak is observed at a single homozygote locus (and not across the profile), the analyst should

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rerun the sample to ensure it is not actually a heterozygote with a microvariant with a 1 base pair difference between the two alleles.

3.3.6. Shoulder and Tail: Shoulders and tails will not prevent the analyst from assigning the specific peak an allelic value.

3.3.7. n+4 peaks: Analysts should also keep in mind that an artifact peak may appear in the n+4 position. When an n+4 peak is suspected, this should be documented on the allele call sheets or on the electropherograms.

3.4. Comparison of Profiles

3.4.1 The comparison and interpretation of DNA profiles is primarily a qualitative judgment based on careful review by a qualified analyst, utilizing all information pertinent to the tests undertaken.

3.4.2 Matches and non-matches are determined by careful, objective, qualitative and quantitative evaluation of the entire profile produced by the various loci tested. It is scientifically acceptable for a match or non-match to be determined for a case when one or more of the loci yield inconclusive results. A match will be based only on loci which yield conclusive results.

3.4.3 Match: DNA profiles are considered to match if their patterns are the same and after taking into consideration the properties of the substrate tested and limitations of the specific techniques used.

3.4.4 Non-Match: Assuming a single source from a forensic sample, two DNA profiles are considered to be a non-match if there is a difference of even one allele after taking into consideration the circumstances of collection and preparation of samples and knowledge of the properties of the substrate tested and limitations of the specific techniques used.

3.4.5 Consistent With/Cannot Be Excluded: In a mixed DNA sample, a person cannot be excluded if the individuals entire profile is present in the mixture or if part of the individuals profile is in the mixture, allelic dropout is present, and the individual cannot be scientifically excluded from the mixture.

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STR Interpretation Guidelines

1. Definitions

Activity: A point on an electropherogram that is on-ladder, has a maximum height < 75 RFU, and is three times greater than the average background.

Allele: An alternative form of a gene; the allele designation is used to designate a specific size fragment of DNA for a specific locus in STR analysis.

Allelic Dropout: An occurrence where one or more alleles from an individual's DNA profile fails to amplify and is not observed in the profile. Allelic dropout may be detected by severe imbalance of loci where the smaller fragments are observed and the larger fragments are not observed and/or observance of activity as defined above.

Artifact: Erroneous peaks or activity that are byproducts of PCR technology and capillary electrophoresis.

DNA Profile: The genotype obtained from a completed DNA analysis tested at multiple loci.

Injection: When a DNA sample is electrokinetically introduced into a capillary for electrophoretic separation.

Inhibition: The total or partial suppression of the PCR process that would result in partial or no DNA profile being obtained.

Intimate Sample: A sample collected from a person's body or an object collected from a person that has made close physical contact with that person. There is an expectation that the person's DNA profile from whom the sample or object was collected may be observed when analyzing that sample or object. The analyst may use discretion when determining whether or not an item is considered "intimate".

Locus (plural=Loci): The chromosomal location or location of a gene or DNA marker.

Microvariant: An allele that varies by less than the consensus repeat unit and is not defined by a ladder allele. Microvariants are observed "in-between" the ladder alleles for a specific locus.

Mixture: If a DNA profile is observed to have more than two peaks at more than one locus, then there is a high possibility that there is a mixture of two or more

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individual's DNA profiles. **NOTE:** If three peaks are observed at only one locus, then there may not be mixture; the individual contributor may have a tri-allelic pattern at that locus. Both sample and standard should express the tri-allelic pattern in cases of a MATCH.

Off-Ladder Allele: An allele observed outside the region covered by the allelic ladder at a given locus.

Partial DNA Profile: A DNA profile that exhibits probable allelic dropout, degradation, and/or preferential amplification at one or more loci. The analyst may not be able to make conclusions as to the individual(s) that can be potentially included or excluded.

Partial Predominant DNA Profile: A Predominant DNA Profile (see definition below) that exhibits probable allelic dropout, degradation, and/or preferential amplification at one or more loci OR mixture that contains a Predominant DNA Profile (see definition below) at most of the loci.

Peak: A well defined point on an electropherogram that is on-ladder and has a minimum height of ≥ 75 RFU. Peaks should be considered alleles unless the analyst justifies not doing so. See "Microvariant" and "Off-Ladder Alleles" for exceptions to the "on-ladder" requirement.

Predominant Alleles/Predominance: Given a mixture of two or more contributors at a specific locus, one or two alleles have an RFU value greater than approximately 50% of all other alleles at that locus and a single source may be readily inferred by the observed data.

Predominant DNA Profile: Given a mixture or two or more contributors, one or two alleles at every locus typed has an RFU value greater than 50% of all other alleles and a single source may be readily inferred by the observed data.

Pull-up: A signal from an allele labeled with one dye-set may show up as a peak or Off-Ladder Allele in another dye-set.

Run: Each set of 16 samples that are separated electrophoretically on the Capillary Electrophoresis Unit (ABI 3100 or equivalent).

Shoulder and Tail: A "Shoulder" and "Tail" will be defined as an elongated or raised area to the immediate left and right of a main peak but is not separated from the main peak.

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Spike/electrical Spike: An artifact believed to be caused by a spike in the current within a capillary that causes a sharp increase in signal. This artifact lacks the defined morphology of a peak.

Split peaks: A split peak will be defined as one allele that is represented by two peaks. Lack of full A nucleotide addition may be observed when the amount of input DNA is greater than the recommended protocol. In this case, more time is needed for Taq Polymerase to add the A nucleotide to all molecules. Amplification of too much input DNA will also result in off-scale data (saturation of signal) and will be manifested as split peaks.

Stutter: An artifact of PCR amplification that is one repeat unit less than the corresponding main allele peak.

Tri-allelic Pattern: Three peaks observed at a single locus and is not the result of a mixture. These peaks may or may not be of equal intensity.

Unincorporated Dye: Unincorporated dye (a.k.a. "dye-blobs") can be observed in an electropherogram and are distinct morphologically from a labeled DNA fragment. A Dye-blob does not exhibit the typical sharp, distinct peak that is produced by actual alleles and is observed as a wider, thicker peak and may be lacking the sharply defined slope to the apex of a peak.



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GREGORY S. MCLEOD
DIRECTOR

ADMINISTRATIVE ORDER

EFFECTIVE DATE: December 7, 2010

10-PRO-18

TO: Forensic Biology Section

FROM: Forensic Scientist Manager Michael J. Budzynski/ Chris Parker DNA TL *MBG* *AP*

SUBJECT: Change to Forensic Biology Policy and Procedure Manual Technical Review
Section 12.1.1.2.11

The Forensic Biology Policy and Procedure Section regarding DNA technical reviews currently reads as follows:

12.1.1.2. The Technical Reviewer for DNA Cases should verify the following for the notes and/or report if applicable:

Exhibit # 3
12.2/12.2.2.4
Finding

- 12.1.1.2.1 Communication Log Present (if applicable)
- 12.1.1.2.2 SBI-5 Evidence Submission Forms Present (if applicable)
- 12.1.1.2.3 Extraction Forms Completed and Reviewed
- 12.1.1.2.4 Quantitation Forms Present and Reviewed
- 12.1.1.2.5 Amplification Forms Completed and Reviewed
- 12.1.1.2.6 Electrophoresis Forms Completed and Reviewed
- 12.1.1.2.7 Allelic Values Correct for positive controls.



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- 12.1.1.2.8 Statistical Analysis Completed and Reviewed
- 12.1.1.2.9 All scanned pages reviewed, numbered, and initialed
- 12.1.1.2.10 Careful Visual Inspection of the Electropherograms
 - 12.1.1.2.10.1 Negative Controls
 - 12.1.1.2.10.2 Positive Controls
 - 12.1.1.2.10.3 Ladders
- 12.1.1.2.11 ILS 250 bp peaks examined
- 12.1.1.2.12 Verify Alleles for Each Locus
- 12.1.1.2.13 Results are accurate and complete
- 12.1.1.2.14 Data marked for CODIS entry checked prior to entry

12.1.1.2.11 will be modified as follows:

Visual inspection and review of all ILS (LIZ) printouts including the 250 bp peaks

Reason for Administrative Order

The purpose of the internal lane standard is to provide information to the analyst as to the migration of DNA during electrophoresis. The most important part of the internal lane standard is the 250 base pair peak. Upon completing their analysis, the examiner performs a review of all internal lane standards to ensure migration has occurred properly. Currently, analysts only print out the 250 base pair peak and will now be required to also print out all internal lane standards for their case notes. This administrative order will ensure the technical reviewer is able to review all internal lane standards present in the samples being analyzed.