Deviation Request Form (DRF)

Directions: The Initiator will complete Sections A through C. Additional continuation pages can be included if necessary.

Initiator	MJ DeHaan	**Date**	12/22/2020

A. Requested deviation applies to (Technical Procedure – include specific section):

Forensic Biology Section Procedure for Kinship Statistics Section 5.7

B. Requested deviation:

Update wording: 5.7 PopStats calculations cannot account for the possible linkage of D12S391 and vWA in cases with increased recombination potential possibilities (incest-type cases). For these cases, the locus with the strongest statistical weight will be used in the calculations.
5.7.1 Initial calculations with both loci present will be performed. The results will be evaluated and the statistics will be recalculated using only the locus with the stronger weight. Both sets of calculations will be included in the case documentation.

C. Necessity for the deviation:

To correct wording discepancy discovered during 2020 external QAS audit.

D. Technical review and Authorization (to be completed by the Quality Manager and/or Technical Leader)
Comments(to include merits and impacts):

Case files currently in review and previously released will be amended to be consistent with procedural wording in place at the time of reporting.

Approved	☑	Yes	☐	No	Duration	until next version
Signature	Mackenzie DeHaan Digitally signed by Mackenzie DeHaan DN: C=US, OU=Forensic Biology, O=NCSCL, CN=Mackenzie DeHaan, E=mdehaan@ncdoj.gov Reason: I am approving this document Location: Date: 2021-01-07 08:10:52 Foxit PhantomPDF Version: 9.3.0				Date	1/7/2021

E. Quality Assurance Authorization (to be completed by the Quality Manager, Forensic Scientist Manager or designee)

Acceptable within general QA guidelines and good laboratory practice?	☑	Yes	☐	No
Significant negative impact to Crime Laboratory Quality System?	☐	Yes	☑	No

Restrictions/limitations:

☑	Authorized	☐	Rejected	Signature	Jody H. West Digitally signed by Jody H. West DN: cn=Jody H. West, o=DOJ, ou=State Crime Laboratory, email=jwest@ncdoj.gov, c=US Date: 2021.01.07 08:57:29 -05'00'	Date	1/7/21

Procedure for Kinship Statistics

1.0 Purpose - This procedure specifies the method for performing comparisons and statistical analysis for kinship analysis within the Forensic Biology Section.

2.0 Scope – This procedure applies to those Forensic Scientists authorized to perform kinship analysis.

3.0 Definitions – See Section Definitions List

4.0 Equipment, Materials and Reagents

- CODIS Popstats software (and associated computer)

5.0 Procedure

5.1 Kinship analysis tests alternate or competing hypotheses of kinship. It may be useful in determining familial relationships, identifying unknown bodies, and identifying samples when the donor in unavailable (missing person cases), and the identification of mother/father of offspring.

5.1.1 Case Acceptance – Samples processed will include bloodstains/bone samples in missing person cases, standards from individuals in the form of blood standards or cheek swabbings, or submitted alternate standards. The Forensic Biology section will not process products of conception.

5.2 Samples will be analyzed following the current procedures in use in the Forensic Biology Section.

5.3 Parentage – For use of Parentage Trio and Reverse Parentage PopStats Statistics, two reference STR DNA profiles are required along with a third unknown sample.

5.3.1 Parentage Trio – This mode uses a known parent along with the known biological child and a third person to assess the relative probability of the assumption that the third person is the biological parent of the child. This mode allows mutations to exist between the alleged parent and child.

5.3.2 Reverse Parentage – Two known parents (not biologically related to each other) and an alleged child are tested in this mode to assess the relative probability of the assumption that the child is the biological child of the two known parents. This mode does not allow mutations to exist between the alleged parent and child.

5.4 Single parentage/kinship – This uses a known and unknown sample to determine if they are a parent-offspring relationship pairing. This mode gives the relative probability of the relationship of the alleged parent and child. This mode allows mutations to exist between the alleged parent and child.

5.5 Comparisons

5.5.1 For parent(s)/child comparisons, the loci are first evaluated to determine whether the individual in question can be excluded as a biological relative of the other individuals. If the individual cannot be excluded or for comparisons not involving a parent(s)/child relationship, a Parentage Index (PI) is calculated using the CODIS PopStats program.

5.5.2 Examine the data from the known parent and child to determine the obligate alleles contributed by the unknown parent. Absent mutation, the known parent and child should share one allele at all loci.

5.5.2.1 For heterozygote loci in the child, the non-shared allele shall be attributed to the unknown parent.

5.5.2.2 For heterozygote loci where the known parent could share either allele, both alleles could be attributed to the unknown parent.

5.5.2.3 For homozygote loci, the allele (above stochastic) shall be attributed to both parents.

5.5.2.4 For single alleles below the stochastic threshold, if the obligate allele could be missing by drop-out then the loci will not be used in the comparison or calculations.

5.5.3 The obligate alleles should be compared to the data from the alleged parent to see if the obligate alleles are present. If they are not present at more than 2, then the alleged parent can be excluded as being the parent of the child. Due to mutation rates present in the STR loci exclusion at more than 2 loci is required before an exclusion conclusion can be made.

5.5.3.1 It is possible to encounter a multi-step mutations during comparison. The CODIS Popstats software cannot account for this mutation in the calculations, therefore a locus will be marked inconclusive for the comparison if this mutation is believed to have occurred.

5.5.4 If the alleged parent cannot be excluded, use the PopStats software to calculate the PI. PI is a likelihood ratio based on two conditional probabilities: the probability of the child's genotype given the genotypes of the known and alleged parents, on the condition that the alleged parent is the other biological parent against the probability of the child's genotype given the known parent on the condition that the alleged parent is biologically unrelated to the child.

$$\text{PI} = \frac{\text{P of genotypes observed given known and alleged parent}}{\text{P of genotypes observed given known and unknown parent}}$$

5.6 For Kinship analysis, the two profiles in question will be entered into the PopStats software. Reporting shall be based on the PI obtained from the comparison. For a more informative kinship relationship result, additional standards may be requested.

5.6.1 For kinship analysis without an extensive pedigree, the results for relatedness will be qualified as either inclusion or inconclusive. This is based on inheritance patterns of siblings from their parents and the potential for full siblings to share 0 alleles at a locus.

5.6.2 The qualification is based upon the verbal scale qualifiers as reported by the Scientific Working Group for DNA Analysis. A likelihood ratio (PI) of >100 gives moderate support for the probability that the two analyzed samples are related. A PI <100 will be reported as inconclusive.

***LR* for H_p Support and 1/*LR* for H_d Support**	**Verbal Qualifier**
1	Uninformative
2 – 99	Limited Support
100 – 9,999	Moderate Support
10,000 – 999,999	Strong Support
≥1,000,000	Very Strong Support

5.7 PopStats calculations cannot account for the possible linkage of D12S391 and vWA, the locus with the strongest statistical weight will be used in the calculations.

5.8 Popstats – The CODIS Popstats software will be used for all calculations.

5.8.1 Trio/Reverse Parentage

5.8.1.1 Select Parentage in the PopStats Calculations menu.

5.8.1.2 Ensure that the prior probability is equal to 0.5.

5.8.1.3 Select trio for alleged parent scenario and reverse for missing child scenario. Enter all the alleles for each locus to be used in the calculations.

5.8.1.3.1 Any locus with a single allele observed at or below the stochastic threshold will be marked as inconclusive and not used for the calculations.

5.8.1.3.2 Kinship Parent-Offspring child profile is entered on left side and parent is entered on right to allow proper calculations for Single Parentage and Kinship.

5.8.1.4 Select Calculate. Use the default mutation rate and mean power of exclusion.

5.8.1.5 Print the parent/reverse parentage statistics report.

5.8.2 Kinship/Single Parentage

5.8.2.1 Select Kinship in the PopStats Calculations menu.

5.8.2.2 Enter all the alleles for each locus to be used in the calculations.

5.8.2.3 Select the appropriate kinship relationships to test and select Calculate from the menu. Use the default mutation rate and mean power of exclusion.

5.8.2.4 Select print report for the Kinship statistics summary or single parentage.

5.9 **Conclusions** – The three possible conclusions of the tested hypothesis are exclusion, inconclusive, or inclusion.

5.9.1 Exclusions occur when obligate alleles at more than 2 loci are not consistent between a parent/child combinations.

5.9.2 Inconclusive loci occur when there are other peaks visible which could potentially exclude or include but could not be typed by the software (partial/degraded profiles). Inconclusive loci may occur when data is below the stochastic and dropout of alleles may have occurred.

5.9.3 Inclusions occur when obligate alleles at 2 loci or less are not consistent between parent/child combinations. The analyst should bear in mind the strength of the inclusion based upon the PI result.

5.10 Reporting

5.10.1 DNA extractions were performed on Item __, as well as on the known DNA standards from __ and ___(Items ___). These extracts were then quantitated, amplified and tested with DNA genetic markers CSF1PO, FGA, TH01, TPOX, vWA, D1S1656, D2S1338, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, Penta D, Penta E and SE33 as well as Amelogenin, DYS391, DYS570, and DYS576.

5.10.2 Statistical calculations were computed by CODIS PopStats.

5.10.3 The following wording shall be used as appropriate for the report wording results:

The ALLEGED FATHER/MOTHER (Item __) cannot be eliminated/ is eliminated as the biological parent of the CHILD/EVIDENCE SAMPLE (Item __).

The DONOR/EVIDENCE (Item __) cannot be eliminated/is eliminated as a biological child of the known reference samples from ___ (Items ___).

5.10.4 PI should be reported from the combined population group PI. PI statistics will be reported to 3 significant figures.

5.10.5 Paternity/Maternity Statistical Statements

5.10.5.1 Inclusion of alleged parent with no mismatch – "It is X times more likely that the genetic testing results obtained from child (Item __) would be observed if the donor of the known sample ___ (Item __) is/was the biological father/mother of the child (Item __) rather thanan unrelated individual selected at random from the U.S. population."

5.10.5.2 Inclusion of alleged parent with a mismatch – The following statement is to be added in addition to the above statement in the case of a one or two locus mismatch. "To determine a non-paternity relationship by STR analysis, exclusion at more than two loci is necessary. In this case, there was a mismatch at (locus) that could be due to a mutation. The mutation rate and mean power of exclusion from this locus were included in calculating the parentage index."

5.10.5.3 Exclusion – "The donor of the known sample ___ (Item __) is/was not the biological father/mother of the donor of Item ___ (child)."

5.10.6 Reverse Parentage Statistical Statements

5.10.6.1 Inclusion of an unknown child – "It is X times more likely that the genetic testing results obtained would be observed if the donor of Item __ (alleged child) is/was the biological child of the donors of the known samples Item ___ (mother) and Item __ (father) rather than an unrelated individual selected at random from the U.S. population."

5.10.6.2 Inclusion with a mismatch - The following statement is to be added in addition to the above statement in the case of a one or two locus mismatch. "To determine a non-parentage relationship by STR analysis, exclusion at more than two loci is necessary. In this case, there was a mismatch at (locus) that could be due to a mutation."

5.10.6.3 Exclusion – "The donor of the known sample Item __ (alleged child) is/was not the biological child of the donors of Items ___."

5.10.7 Single Parentage

5.10.7.1 Inclusion of missing person relative – "It is X times more likely that the genetic testing results obtained from Item __ (evidence) would be observed if the source of the profile is/was a biological child of the donor of the known sample (Item __) rather than an unrelated individual selected at random from the U.S. population."

5.10.7.2 Inclusion with a mismatch - The following statement is to be added in addition to the above statement in the case of a one or two locus mismatch. "To determine a non-parentage relationship by STR analysis, exclusion at more than two loci is necessary. In this case, there was a mismatch at (locus) that could be due to a mutation. The mutation rate and mean power of exclusion from this locus were included in calculating the likelihood of parentage."

5.10.7.3 Exclusion – "The source of the profile (Evidence Item __) is/was not the biological child of the donor of Item __."

5.10.8 Kinship

5.10.8.1 "It is X times more likely that the DNA profile obtained from ____ (Item __) would be observed if the source of the profile is/was a full sibling (or other relationship) of ___ rather than an unrelated individual selected at random from the U.S. population."

5.10.8.2 If values are below 100, then the results will be reported as inconclusive- "The reported values do not support inclusion as siblings. However, based on inheritance patterns these results support a finding of inconclusive regarding the question of sibling relatedness between ___ (Item __) and ___ (Item __)."

5.11 Standards and Controls – N/A

5.12 Calibrations – N/A

5.13 Maintenance – N/A

5.14 Sampling – N/A

5.15 Calculations – Calculations are performed using the formulas in the CODIS PopStats Software.

5.16 Uncertainty of Measurement – N/A

6.0 Limitations – N/A

7.0 Safety - N/A

8.0 References – Recommendations of the SWGDAM Ad Hoc Working Groups on Genotyping Results Reported as Likelihood Ratios. www.swgdam.org.

9.0 Records – N/A

10.0 Attachments –N/A

Revision History

Effective Date	Version Number	Reason
03/09/2020	2	5.3.1 – remove biologically related; 5.5.4 – reword to clarify; 5.5.3.1 – added to address multi-step mutations; 5.10.5.1, 5.10.6.2, 5.10.7.2 – update wording