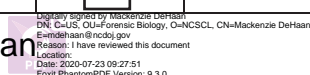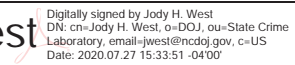# Deviation Request Form (DRF)

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

Initiator	MJ DeHaan	**Date**	07/23/2020

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

Forensic Biology Procedure for Use of ArmedXpert for Mixture Interpretation_multiple sections

B. Requested deviation:

See attached

C. Necessity for the deviation:

To update the procedure following implementation of the STRmix software.

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

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 have reviewed this document Location: Date: 2020-07-23 09:27:51 Foxit PhantomPDF Version: 9.3.0				Date	07/23/2020

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:

effective date 7/29/2020

✓	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: 2020.07.27 15:33:51 -04'00'	Date	7/27/20

DRF_Armed Xpert Procedure_07232020 Section B- Requested Deviation:

1.0 add wording "for cases being reviewed during the ownership review of data generated at vendor laboratories."

2.0 Add wording "from cases being reviewed for ownership of data from a vendor laboratory."

5.9.3 Add wording: The formulas used in the calculation of the frequency of a DNA profile shall be in accordance with those published in the NRC II guidelines and in PopStats. Additional information of the calculations may be found in the software user's manual.

5.9.3.1 Heterozygote frequencies: 2pq (p and q are the frequencies of the alleles observed.)

5.9.3.2 Homozygote frequencies: $p^2 + p(1-p)\theta$, where $\theta = 0.01$ (p is the frequency of the allele observed and θ is a correction factor for expected levels of population substructure in a given population).

5.9.3.2.1 If a locus contains a homozygote allele which falls below the stochastic threshold, that locus shall not be used without utilizing the mRMP function of ArmedXpert.

5.9.3.3 Multi-locus frequencies: the product rule shall be used.

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Procedure for Use of ArmedXpert™ for Mixture Interpretation

1.0   **Purpose** – The purpose of this document is to provide guidelines for interpreting DNA mixture results when using the ArmedXpert™ software.

2.0   **Scope** – This document applies to analysts and trainees in the Forensic Biology Section who are qualified to interpret mixtures in DNA casework.

3.0   **Definitions** - See Section Definition list

4.0   **Equipment, Materials and Reagents** – Computer with installed ArmedXpert™ software

5.0   **Procedure**

   5.1   **Introduction** – The ArmedXpert™ software is a tool to assist qualified Forensic Scientists in the interpretation of DNA mixtures. It is not an expert system. The RMP in ArmedXpert™ can be used to calculate statistics for inclusions to a mixture one of three ways: as unresolved for multiple contributors (the unrestricted RMP), deduced for one or more contributors (the restricted RMP), or as a combination of both handled on a locus by locus basis. This approach may be used for mixtures from both 2 and 3 contributors. In situations where dropout of an allele or alleles is suspected at a given locus for a particular contributor or contributors, the software can also calculate a modified RMP, either for a deduced contributor or for all contributors.

   5.2   ArmedXpert™ can also combine RMP approaches within a profile. This allows the most informative statistic to be calculated for a profile. These statistical approaches are all in accordance with the 2010 SWGDAM guidelines and can be used for both 2 and 3 contributors. The formulas appropriately remove combinations to avoid double-counting.

   5.3   The data generated from analysis using the ArmedXpert™ software is used to support the interpretation of the generated DNA profiles. When using the ArmedXpert™ software, the analyst must decide how the data generated is utilized during the interpretation. More than one data analysis may be necessary to support the interpretation.

   5.4   The ArmedXpert™ program provides tools for the following:

      5.4.1   Single contributor samples - RMP statistic

      5.4.2   Multiple contributor samples (2 and 3 person)

         ● Mixture Interpretation/De-convolving
         ● Stutter filter to aid in the evaluation of stutter
         ● Contributor proportion calculations based on validated peak height ratio values

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- Determination of a major profile
- Statistics
- Condition on a known contributor - Use of references (owner/wearer, elimination) to aid in proportion ratios, genotype certainty, and exclusion

5.4.3   QC checks

5.5   Data Import

5.5.1   After evaluation of the GeneMapper project is complete, select 'ArmedXpert' Table Setting for the data set. Ensure that the appropriate analysis method has been applied to the samples prior to export. OL allele variants require relabeling to the most appropriate numerical allele naming designation before importing into ArmedXpert™.

5.5.2   Go to the File menu, select 'Export Combined Table', and ensure that 'One line per sample' is selected in the export window. Save the file as 'Tab-delimited Text (*.txt)' in an appropriate location for retrieval.

5.5.3   Click the Import command under the ArmedXpert™ Icon (DNA helix) in the open program. Select Other. Select the desired text file and open it.

5.5.4   Select the Interpretation tab to reveal the mixture interpretation and frequency calculations options.

- Single contributor sample, select the appropriate Frequency Calculations button.
- Multiple contributor sample, select the "Begin Mixture Interpretation" button.

5.5.5   If returning to an ArmedXpert™ analysis, click on the Mixture Interpretation or Frequency Analysis from the main drop down menu to access previously saved mixture interpretations or frequency reports.

5.5.6   Control and sample profiles can be filtered using the options in the Views tab.

5.6   Mixture Interpretation

5.6.1   These tools shall be used in conjunction with the guidance in the section procedures for Interpretation using Fusion 6C.

5.6.2   Click on Begin Mixture Interpretation and select the desired sample from the table to start a mixture interpretation.

5.6.3   Verify the following settings in the Mixture Interpretation window are appropriately set:

- Peak Height Ratio – PHr = 60%

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- Minimum Peak Height – set by AT threshold levels
- Minimum Proportion – mP = 0
- Heterozygote (stochastic) threshold – HT = 300
- Verify Ignore allele below mPH is checked and Multi PHr is not checked

5.6.4 The global stutter filter may be used to assist in the interpretation of the mixture. As a starting point, ensure that the Apply Stutter button is set at 50%. Check the Apply Globally checkbox to apply the stutter filter to all loci. If a peak falls below the mPH setting when filtered at 50% stutter, the peak is generally considered to be stutter.

5.6.5 Set the number of contributors in the Contributor # box based on previous analysis in GMID-X. This number may be changed as necessary.

    5.6.5.1 Analysis may be performed using a number of contributors less than the overall mixture interpretation. The reason shall be documented within the notes in a manner that can be reviewed by a qualified analyst.

    5.6.5.2 The analyst needs to consider how the data is being used in interpretation to decide which scenario is supported. (E.g. If the analyst has stated the assumption that the 3rd contributor has degraded, then it may be acceptable to analyze a mixture as 2 contributors.)

    5.6.5.3 It may be necessary to analyze a mixture (or specific loci in a mixture) as both a 2 and 3 person contributor mixture. This analysis method would allow the analyst to document the potential impact of a 3rd contributor on the mixture being analyzed. If performed, the analyst shall export the data used from both analyses to include in the case file.

5.6.6 The various possible genotype combinations, peak height ratios, and proportions are displayed under Mixture Information in the Interpretations window. This information can assist in deconvoluting the mixture.

    5.6.6.1 The data is used to support the interpretation decisions made by the analyst. The analyst shall include any documentation necessary to support the mixture interpretation. (E.g. If mixture/multiple major is interpreted as indistinguishable, then the 2 person mixture data that supports a mixture ratio of ~1:1 should be documented, or if an analyst is interpreting a major and a 2nd contributor in a 3 person mixture, then the 3 person mixture data the supports sufficient separation between the 3 contributors should be documented.)

    5.6.6.2 If the mixture is being interpreted as a 2 contributor mixture, the allele call table columns shall be labeled as major/assumed and minor (not minor alleles). If the minor is not being interpreted due to limited information/amount of dropout in the profile, then documentation shall be made to support this interpretation. Documentation must include denoting the "any" alleles that would be a portion

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of the profile to ensure the delineation between loci with dropout and homozygous loci.

5.6.7 The view call report button opens a window in which contributor profiles and proportions are displayed as the sample is analyzed.

5.6.8 If a genotype can be determined for a contributor at a locus, associate the genotype with the contributor using one of the following methods:

5.6.8.1 Select the combination from the Mixture Information section. A pop out window with "Send to..." buttons appears. Select the appropriate button; the determined genotype will be sent to the contributor profile associated with the selected button.

5.6.8.2 Use the Popout Calls button. Mark the checkboxes under the appropriate alleles to designate the genotype for the contributor profile.

5.6.8.3 Use the Add Profile drop down box (right of the stutter filter) to select the desired contributor profile. Mark the checkboxes under the appropriate alleles to designate the genotype.

5.6.9 Special Allele Designation – Any: a designation for alleles under the stochastic threshold if drop out is a possibility. Check the box under the allele and select Any. Do not use this designation when all the alleles from all the contributors are accounted for.

5.6.10 The Add Profile drop down box can be used to rename, add, or remove contributor profiles.

5.7 In mixtures where a contributor is known or expected to be present (e.g. intimate samples), subtracting out the known contributor's alleles may allow for determination of the remaining profile. Assumptions shall be stated in the report.

5.7.1 To apply a reference, select the References button in the Mixture Interpretation window.

5.7.2 In the pop-up window, choose Select a reference to apply, and choose the reference in the drop-down menu. The reference may be within the project, in a related case, or one that has been manually input.

5.8 Accounting for Stutter

5.8.1 When applied, a stutter filter of 50% is multiplied against the allele peak heights and the resulting values are subtracted from the peaks in the stutter positions. Any stutter peak below mPH after the stutter threshold is applied will appear grayed out and will not be considered by the software.

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5.8.2 If a peak labeled as stutter is to be considered based on the interpretation of the mixture, a stutter filter can be applied to see if stutter peaks should be considered as hidden alleles using the criteria of peak height ratio and contributor proportion estimates. If the remaining peak in stutter position cannot be paired with any other peak (i.e. using 60% or 30% as appropriate) or all the alleles can be accounted for by the number of contributors assumed, then treat it as stutter. When a peak is determined to be stutter and is still above mPH after application of an appropriate stutter filter, use the allele designation drop down menu window and select the "ignore" allele option.

5.9 Calculating Random Match Probability

5.9.1 For single contributor samples and derived major profiles from mixtures, the Single Source or RMP buttons under the Interpretation tab may be selected to calculate a random match probability. The NIST population database shall be used to calculate frequencies. For mixtures, globally restricted RMP is not being utilized since it uses peak heights in an indistinguishable mixture to limit genotype options in a profile.

5.9.2 If the allele, any designation has been applied, use the RMP button to generate the statistic for that profile. This allows the user to see that the appropriate statistical formula was applied.

5.9.3 The Random Match Probability shall be printed using print to .pdf to add to the case record.

5.10 Documentation – Any comments or information used in statistical calculations or in the interpretation of samples shall be added to the case record.

6.0 Limitations – N/A

7.0 Safety – N/A

8.0 References

Forensic Biology Section Procedure for Analysis and Interpretation using Fusion 6C

Forensic Biology Section Procedure for Casework Report Writing

9.0 Records – N/A

10.0 Attachments – N/A

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Revision History		
Effective Date	Version Number	Reason
12/20/2016	1	Original Document
01/25/2019	2	5.3 – clarified wording for AX analysis generation; 5.6.4 – changed wording to allow other options; 5.6.5 – added interpretation explanation; 5.6.6 – added data use explanation; 5.9.1 – removed global restricted as stat option

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