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 ArmedXpertTM 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 ArmedXpertTM 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 ArmedXpertTM program provides tools for the following:
 - **5.3.1** Single contributor samples RMP statistic
 - **5.3.2** 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
 - 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

5.3.3 QC checks

5.4 Data Import

- **5.4.1** 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 ArmedXpertTM.
- **5.4.2** 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.
- **5.4.3** Click the Import command under the ArmedXpert[™] Icon (DNA helix) in the open program. Select Other. Select the desired text file and open it.
- **5.4.4** 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.
- **5.4.5** 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.
- **5.4.6** Control and sample profiles can be filtered using the options in the Views tab.

5.5 Mixture Interpretation

- **5.5.1** These tools shall be used in conjunction with the guidance in the section procedures for Interpretation using Fusion 6C.
- **5.5.2** Click on Begin Mixture Interpretation and select the desired sample from the table to start a mixture interpretation.
- **5.5.3** Verify the following settings in the Mixture Interpretation window are appropriately set:
 - Peak Height Ratio PHr = 60%
 - 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

- **5.5.4** The global stutter filter may be used to assist in the interpretation of the mixture. If used, 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.
- **5.5.5** Set the number of contributors in the Contributor # box based on previous analysis in GMID-X. This number may be changed as necessary.
- **5.5.6** 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.
- **5.5.7** The view call report button opens a window in which contributor profiles and proportions are displayed as the sample is analyzed.
- **5.5.8** If a genotype can be determined for a contributor at a locus, associate the genotype with the contributor using one of the following methods:
 - **5.5.8.1** 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.
 - **5.5.8.2** Use the Popout Calls button. Mark the checkboxes under the appropriate alleles to designate the genotype for the contributor profile.
 - **5.5.8.3** 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.
- **5.5.9** 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.
- **5.5.10** The Add Profile drop down box can be used to rename, add, or remove contributor profiles.
- **5.6** 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.
 - **5.6.1** To apply a reference, select the References button in the Mixture Interpretation window.
 - **5.6.2** 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.

- **5.7** Accounting for Stutter
 - **5.7.1** 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.
 - **5.7.2** 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.
- **5.8** Calculating Random Match Probability
 - **5.8.1** 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.
 - **5.8.2** 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.
 - **5.8.3** The Random Match Probability shall be printed using print to .pdf to add to the case record.
- **5.9** 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

Revision History		
Effective Date	Version Number	Reason
12/20/2016	1	Original Document