



CMPD Crime Laboratory Biology
Standard Operating Procedures

Approved by: _____ Date: _____
(DNA Technical Leader)

_____ Date: _____
(Laboratory Director)

Provisions for Modification and Update of This Manual

Any updates, modifications, additions, or deletions to this manual prepared after the issue date on the cover sheet will have the following information and an updated issue date located at the bottom of each page.

Summary of Revisions

Date Issued	Summary of Changes Made
3/23/00	Intro pg 7, Sec 1 pg 4-6, Sec 5 all
5/31/00	Sec 5 pg 5, 9-11
6/6/00	Sec 5 all
10/19/00	TOC, Sec 1 pg 4-5,16,22, Sec 3 pg 3-4, Sec 5 all
12/5/00	Sec 3 pg 3,5
7/26/01	Sec 1 pg 4,17,23,25
4/29/02	Sec 1 pg 2,16,18,20,22,24,26, Sec 2 pg 3, Sec 3 pg 2-4, Sec 4 pg 5,6, Sec 5 all
8/26/02	Sec 2 pg 3, Sec 4, pg 6
11/21/02	Sec3 pg 1-3, Sec 5
7/10/03	TOC, Secs 1, 2, 3, 4, 5 (all)
2/26/04	Sec 2 all
5/17/04	Sec 3 pg 1 and 8
1/13/06	TOC, Secs 1, 2, 3, 4, 5 (all)
5/12/06	Sec 1 pg. 15, 16
10/25/06	Sec 3 all
1/30/07	Sec 4 pg. 2, 6, 7
6/18/07	Sec 2 pg. 7, 8
1/30/08	Sec 3 all and Sec 4 pg. 2, 4-7
8/26/08	Sec 1 added 1.5
4/9/09	Sec 2 remove 2.1; Sec 4 change dye colors
11/30/09	Sec 1, add LEV, changes to Reagent Blank treatment
4/13/2010	Sec 1, change out Mini kit for Investigator kit; Sec 2, change out 7000 for 7500
7/30/2010	Sec 1, add suggested amounts and rpms; Sec 3, additional information on 3130 samples; Sec 4, addition of 3130;
10/12/2010	Sec 5, added/changed report wording
1/1/2011	Sec. 1, references added; Sec 2 clarification added; Sec 3 option added; Sec 4 310 removed;
4/1/2011	Sec 5. 310 reference removed, wordings added
7/18/2011	Changes made for ASCLD International and combination of both the Serology SOP's and the DNA SOP's, all but SOP 8
7/20/2011	SOP 8- changes made and SOP split into 8 and 9
10/12/2011	Changes made for the 7/1/2011 QAS document, 3, 4 & 8
2/7/2012	Change to SOP 1 Abacard procedure; SOP 2 remove

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	2.5.3 and 2.5.4 LEV protocols
2/22/2012	Change to statistics calculations and references, creation of Appendix A
6/4/2012	Change interpretation of SOP 1, change wording in SOP 8 and 9
8/28/2012	Added PrepFiler/Automate procedure to SOP 2 following validation, removed Qiagen for blood and saliva
11/14/13	Added profiles for EE, KMA, MA & DS to Appendix A
2/2014	Added QIAgility additions to SOP 3 and 4
4/4/2014	Changes made to all SOP's due to PLIMS additions and review
10/5/2014	SOP 1 added IR and Hematrace, remove Tak and Ouch. SOP 2 added sample size for tip. SOP 3 added Trio, removed Quantifiler Human and Male. SOP 4 new target amount. SOP 6 clarify re-injections. SOP 7 clarifications. SOP 8 clarifications. SOP 9 added wording
4/7/2015	SOP 4, added 96 well plate usage, SOP 5 added 3130 worksheet usage; SOP 6 added worksheet usage and new software instructions, SOP 7 created for GMID-X
6/22/2015	SOP 1- no sperm verification, controls and dates, wireless and light; SOP 2- no notation needed for consuming other than *, handling of penile swabs, notations for samples incubated longer; SOP 3- notations on amplifying samples; SOP 7- injection list added, changes made to accommodate using copies of e-grams; SOP 8- changes in stats; SOP 9- changes in report wording
8/4/15	Volume in 2.6.2.9 changed from 300 µl to 150 µl to correspond with the manufacturer's protocols.
2/24/2016	Removed "n-21" from SOP 4. Updated SOP 6 and 7 to reflect workbook. Created a new SOP 8 for Armed Xpert, changed mixture interpretation for SOP 9 and added report wordings to SOP 10
5/2/2016	SOP 2- remove separate reagent blanks from the Automate, only one is needed; SOP 5 – remove optional LIZ set up; SOP 6 – add when spectral is needed; SOP 7 – clarify what to print when; SOP 8 – clarify when statistics is needed; SOP 9 –add combined allele tables and remove match and comparison; SOP 10- add CODIS and DEMS report wording

Foreword

General

This manual is designed to ensure that the CMPD Biology Laboratory produces scientifically valid and professionally acceptable casework analysis. It is designed to be

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used in conjunction with other safety, standard operating procedure, and training manuals to achieve this goal. The CMPD Crime Laboratory Quality Manual and Policy Manuals are our parent manuals and will be followed along with this manual.

The CMPD Biology SOP Manual is a compilation primarily of guidelines provided by Applied Biosystems, Promega, Qiagen, professional publications and presentations, and CMPD validation studies.

Throughout this document, references are made to other CMPD manuals in the laboratory and anyone consulting this manual for information regarding the function of the lab should have access to the other manuals. The other manuals include but are not limited to:

1. Biology QA Manual
2. Biology Training Manual
3. CMPD Crime Laboratory Safety Manual
4. CMPD Crime Laboratory Quality Manual (QM)
5. CMPD Crime Laboratory Operating Manual (OM)
6. CMPD Policy and Procedures and Field Volumes

If there are any questions regarding the contents of this or any Biology manual, they may be directed to the Chief Criminalist.

Non-Routine Procedures

This manual covers a wide variety of situations that will be encountered frequently in the analysis of forensic Biology evidence. However, not every situation that will be encountered can be addressed in this manual. Therefore, non-routine procedures will need to be performed occasionally. In the event this occurs only published protocols will be used. A small validation study may be required in some circumstances. The DNA analyst, the DNA Technical Leader, and the Chief Criminalist will discuss the situation and develop an appropriate approach. All non-routine procedures will be documented in the case file and, following the validation, will be added to the manual. Any deviations from approved SOP's must be approved by the DNA Technical Leader prior to moving on to the next step in the process.

Critical Reagents

The analyst must perform quality control on the reagents that are critical to the process (see Biology Quality Assurance Manual Section 8 for instructions).

References

References to the basic publications or validation studies are given at the end of each section. The reference list is maintained separately in the section. The articles listed at the end of each section are presented as the fundamental articles to the procedure.

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1. Stain Identification Procedures and Techniques

The analyst who breaks the initial seal is responsible for preservation and documentation of not only biological stains, but any possibly probative evidence present on an item. The examiner must rely on their expertise, training, experience and knowledge of the case to determine which evidence is potentially probative. Consultation with other analysts is encouraged. Such consultations are required when a potential analysis is outside the analyst's own expertise. The results of consultations with experts outside the Biology section are to be included in the case notes.

High quality lighting is required for the examination of all items of evidence. It is recognized that some evidence, such as spermatozoa and hair, is too small to be studied with the naked eye; therefore, a magnifying glass, stereoscope, and microscope with magnification of at least 400 X should be readily available to the analyst.

The initial examination of any item of evidence for stains is a visual one. A thorough visual examination is the primary method used to locate suspected blood stains on most items. However, since it is often difficult to visualize blood stains on red, dark, or textured surfaces, additional examinations should be considered. The Foster and Freeman 82S near- IR and violet light sources may aid in the location of hidden blood without consuming sample or distorting patterns. Presumptive testing of the area where blood might be hidden may be conducted when sample size or patterns are not considerations or when the substrate causes the light sources to be ineffective. The wireless connection on the laptop that is connected to the light source needs to be turned off while using the light. Once the analyst has finished using it, it can be reconnected to the wireless for saving photos to the R Drive.

Bodily fluids such as semen and saliva are not easily detected by visual examination. Location of these stains on clothing or other items may be aided by the use of an alternate light source (ALS), such as the Mini-Crimescope, Luma-Lite or Omniprint 1000. When semen or saliva stains fluoresce under the UV light it is due to the presence of the bacteria *Pseudomonas fluorescens* in those stains. Because the level of bacteria varies with each stain, an absence of fluorescence does not necessarily indicate that no stain is present. It is also important to note that certain synthetic fibers are highly reflective in the presence of UV light and this reflectivity may mask a potential stain. Therefore, presumptive testing for the presence of semen or saliva or microscopic testing for the presence of spermatozoa may follow negative visual and UV examinations.

Caution should be used when using an alternate light source to locate stains. Since the light is in the UV part of the spectrum it will break down DNA. Any searches for biological material with the light should be of very short duration. It is also important to note that blood stains, even when mixed with other fluids, do not fluoresce under

UV light. Unless it is set on “white light” the alternate light source should not be used to search for blood stains.

If the QC portion of PLIMS is used, a separate QC form needs to be created each day that testing is performed.

1.1 Bloodstain Identification

A list of the procedures available to perform analysis on blood evidence is provided. If more than one type of test is performed on the same stain, each result must be recorded.

1.1.1 Phenolphthalin - (PHT or KM)

A. Purpose and Introduction

Phenolphthalin testing is a chemical test that is used as a presumptive test for the presence of blood. It is a very sensitive test and will detect small traces of blood. If this test is negative, the testing on that stain generally ceases. However, it is acceptable to perform further testing if the analyst has a reason to believe that a substance may be interfering with the peroxidase-like reaction.

Due to its sensitivity, the phenolphthalin test is an acceptable method for determining that blood is not likely present on a given item.

B. Materials

- Phenolphthalin or Kastle-Meyer Reagent
- 3% Hydrogen Peroxide
- Deionized water

C. Procedures

1. Test controls prior to performing tests on case samples. The positive control will be a known blood standard, while the negative control will be the reagents. The passing of the controls will be noted on the worksheet by the date. If multiple dates are needed for analysis, all dates will be noted in the control blank on the worksheet.

2. Acceptable methods of sample collection:

- A. Swab-to-swab (for use with visible staining)--Dampen a swab(s) with deionized water. The number of swabs prepared will depend on the number of sample swabs. Press one of the sterile swab(s) against

each sample swab. Hold for a minimum of 10 seconds or until an obvious transfer can be seen.

- B.** Filter paper (for use with visible or non-visible staining)--Dampen the filter paper then either rub the sample swab(s) against the filter paper for a minimum of 10 seconds or until a visible transfer of stain is seen (for visible staining) or for non-visible staining press the filter paper around the sample swab(s) for a minimum of 10 seconds.
- C.** Cutting (visible or non-visible staining)--A small cutting of sample may be obtained. Place the cutting on a piece of filter paper and place a drop of deionized water on the cutting.
- D.** Apply one or two drops of phenolphthalin (PHT/KM) reagent to each test sample.
- E.** Count 10 seconds then apply an equal number of drops of 3% hydrogen peroxide solution to each test sample.
- F.** ***Interpretation:*** A visible change of color (bright pink) within 10 seconds is considered positive. No change of color to pink within the 10 seconds *or* any change of color that is not consistent with that on the controls is considered negative.
- G.** If the remaining (original) stain appears to contain sufficient sample for DNA testing, dispose of the test swab. If little or no original stain remains, dry and package the test swab as a sub item.
- H.** Dry the evidence swabs before returning them to their original package.

D. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

Warning: The following are considered hazardous reagents:

Phenolphthalin is a known carcinogen if ingested in large amounts. It may cause irritation of the lungs if inhaled. If inhaled move the subject to fresh air. Avoid contact with the skin. If exposed wash with soap and water.

Zinc should not be exposed to an open flame, or heated with sulfur. Zinc waste should always be disposed of in a sealed glass container.

E. Limitations

- This test gives a chemical indication for the presence of blood. It is not a confirmatory test for blood.
- Only an immediate pink color change should be deemed a positive reaction. The reagents will turn pink in time even in the absence of blood.
- Color change following the addition of the phenolphthalin but prior to the addition of the hydrogen peroxide suggests the presence of a chemical oxidant.
- Plant peroxidases may cause a false positive reaction. These can be deactivated with heat or time. The pH of the phenolphthalin reagent however is not optimal for plant peroxidases.
- For items that are pink or may leach a pink color when swabbed, leucomalachite green (LMG) may be used.

F. Validation

This procedure is generally accepted in the forensic community. See blood references (Section 1.1.6)

1.1.2 Leucomalachite Green - (LMG) - Two Step Method

A. Purpose and Introduction

Leucomalachite green testing is a chemical test that is used as a presumptive test for the presence of blood. It is a very sensitive test and will detect small traces of blood. If this test is negative, the testing on that stain generally ceases. However, it is acceptable to perform further testing if the analyst has a reason to believe that a substance may be interfering with the peroxidase-like reaction.

Though phenolphthalin testing is the primary test used for presumptive testing for the presence of blood, LMG is useful when a red or pink color on the test substrate would make accurate reading of the phenolphthalin test impossible.

The LMG test is an acceptable method for determining that blood is not likely present on a given item.

B. Materials

- Leucomalachite Green Reagent (LMG)
- Glacial Acetic Acid
- Deionized water

C. Procedure

1. Test controls prior to performing tests on case sample. The positive control will be a known blood standard, while the negative control will be the reagents. The passing of the controls will be noted on the worksheet by the date. If multiple dates are needed for analysis, all dates will be noted in the control blank on the worksheet.
2. Acceptable methods of sample collection:
 - A. Swab-to-swab (for use with visible staining)--Dampen a swab(s) with deionized water. The number of swabs prepared will depend on the number of sample swabs. Press one of the sterile swab(s) against each sample swab. Hold for a minimum of 10 seconds or until an obvious transfer can be seen.
 - B. Filter paper (for use with visible or non-visible staining)--Dampen the filter paper then either rub the sample swab(s) against the filter paper for a minimum of 10 seconds or until a visible transfer of stain is seen (for visible staining) or for non-visible staining press the filter paper around the sample swab(s) for a minimum of 10 seconds.
 - C. Cutting (visible or non-visible staining)--A small cutting of sample may be obtained. Place the cutting on a piece of filter paper and place a drop of deionized water on the cutting.
1. Apply one or two drops of leucomalachite green (LMG) reagent to each test sample.
2. Count 10 seconds then apply an equal number of drops of 3% hydrogen peroxide solution to each test sample.
3. ***Interpretation:*** A visible change of color (blue-green) within 10 seconds is considered positive. No change of color to green within the 10 seconds **or** any change of color that is not consistent with that on the controls is considered negative.
4. If the remaining (original) stain appears to contain sufficient sample for DNA testing, dispose of the test swab. If little or no original stain remains, dry and package the test swab as a sub item.

5. Dry the evidence swabs before returning them to their original package.

D. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

Warning: The following are considered hazardous reagents:

Leucomalachite green may cause skin, eye and lung irritation. If exposed, wash with soap and copious amounts of water.

Glacial acetic acid may cause severe irritation or burns to the eyes, skin and lungs. Wear proper protective garments and handle the reagent in a ventilated hood. Though it is stored over zinc, the mixed reagent produces a gas that may explode. Always provide proper ventilation and refrigerate at 4°C when not in use.

Zinc should not be exposed to an open flame, or heated with sulfur. Zinc waste should always be disposed of in a sealed glass container.

E. Limitations

- This test gives a chemical indication for the presence of blood. It is not a confirmatory test for blood.
- Only an immediate blue-green color change should be deemed a positive reaction.
- Color change following the addition of the leucomalachite green but prior to the addition of the hydrogen peroxide suggests the presence of a chemical oxidant.

F. Validation

This procedure is generally accepted in the forensic community. See blood references (Section 1.1.6).

1.1.3 Luminol – screening test for blood

A. Purpose and Introduction

Luminol testing is a chemical test used as a presumptive test for the presence of blood. It is a very sensitive test designed to detect small traces of blood. Luminol spraying is used almost exclusively to locate blood stains that cannot be visualized with the naked eye. Due to its chemical properties it is particularly effective on older blood stains. Because it must be applied to a surface or stain in a darkened room its usefulness may be limited. If results are negative, the testing on that stain or area generally ceases. However, it is acceptable to perform further testing if the analyst has a reason to believe that a substance may be interfering with the peroxidase-like reaction.

Due to its sensitivity, spraying an item with Luminol solution is an acceptable method for determining that blood is not likely present on a given item or area.

B. Materials

- Luminol solution
- Spray misting bottle

C. Procedures

1. Test spray bottle to ensure proper misting action.
2. Test controls prior to performing tests on case samples.
3. In a dark room, spray-mist the item of interest with the Luminol solution. Be careful not to saturate stains to avoid unnecessary dilution, especially stains on non-porous surfaces.
4. ***Interpretation:*** A blue-green illumination is indicative of a positive reaction. No blue-green illumination **or** any change of color that is not consistent with that on the controls is considered negative. This is a pre-screening test only. A positive color change has to be followed by LMG or PHT.
5. Delineate any illuminated areas.
6. Using a sterile swab(s), swab the illuminated area for presumptive blood testing.

7. Follow procedures for presumptive blood testing (PHT or LMG) on the collected swabs.
8. If a swab(s) indicates a positive PHT or LMG reaction, the swab will be retained for potential future testing.
9. The item that was spray-misted with Luminol solution must be dried completely before re-packaging.

D. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

Warning: The following are considered hazardous reagents:

Luminol solution causes respiratory tract irritation and should be used only with the appropriate protective equipment. If inhaled, move to fresh air.

E. Limitations

- Luminol is a pre-screening test only.

F. Validation

This procedure is generally accepted in the forensic community. See blood literature references (Section 1.1.6).

1.1.4 Hematrace - ABACard[®]

A. Purpose and Introduction

This procedure provides a method for the probable identification of human blood using a commercially prepared hemoglobin detection card. The ABACard[®] HemaTrace test strips are manufactured by Abacus Diagnostics, Inc. The human hemoglobin present in the extract will combine with a monoclonal antihuman hemoglobin antibody that is labeled with a dye. Any antibody-antigen formed then migrates through an absorbent membrane to the test area of the strip. The test area has an immobilized polyclonal antihuman hemoglobin that will capture the Ag-Ab complex to form an Ab-Ag-Ab sandwich. The pink dye becomes visible as a band in the test region at concentrations of human hemoglobin above about 0.05 µg/ml. An internal control consisting of human hemoglobin

antibody–dye conjugate cannot bind to the antibody in the test area but is captured by an antibody in the control area. A correctly functioning positive test will therefore show two pink bands, one in the test area and one in the control area. A correctly functioning negative test will show only one pink band, in the control area. If there is any problem with the test there will be no visible bands.

B. Materials

- Extraction Buffer (included in the kit)
- OneStep ABACard[®] Hematrace Test Strips

C. Procedures

1. Positive blood and negative controls will be tested on the first run of a given day of use (additional tests the same day may use the results of the first test).
2. Extract $\frac{1}{4}$ to $\frac{1}{2}$ of a swab or a small portion of a stain (approx. 2mm²) in the buffer extraction vial. The size of the cutting depends on the stain intensity; the darker the staining the less sample needed. Incubate at room temperature for 5 minutes.
3. Add approximately 200 uL (or 6-7 drops with the dropper) of the extract to the “S” well of the ABACard and allow the liquid to migrate to the top of the strip.
4. Read the results at 5 minutes. Positive results may be seen as soon as 1 minute. Negative results must wait the full 10 minutes.
5. ***Interpretation:*** Examine the strip for a pink band at the “T” and “C” locations. For a positive result, both locations must exhibit a visible band. If only a “C” band is present, the test is negative. If only the “T” value is present, the test is inconclusive. False negative results may occur when the Hb concentration extremely high, and therefore; when this is a possibility, re-test taking a smaller cutting.

D. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

E. Limitations

- ABACard HemaTrace is only for in-vitro detection of human hemoglobin (primates) for forensic use. It may be used for medical research but is not for diagnostic use. The test must be performed in strict accordance with these instructions to obtain accurate and reproducible results.
- Old and encrusted bloodstains should be properly extracted before use. Storage temperature of the stain may also affect the results.
- Positive results may be obtained from whole blood from the domestic ferret. However in forensic casework, the practical implications of this cross reactivity is minimal, since one can assume that the number of cases where ferret blood and higher primates may be found is low and crime scene investigation can determine if a pet ferret was possibly at the scene. The test results should be interpreted in conjunction with other information.
- Due to the extreme sensitivity of the test, trace levels of hemoglobin might be detected occasionally in body fluid samples other than blood (e.g. urine, semen stool, saliva, vaginal fluid, perspiration). However, knowing this fact, this limitation has no practical impact in the vast majority of cases.

F. Validation

This procedure is generally accepted in the forensic community and was validated by the CMPD laboratory.

1.1.6 Blood References:

- Gaensslen, R.E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*; U.S. Government Printing Office: Washington, DC, 1983.
- Lee, H.C., *Identification and Grouping of Bloodstains, Forensic Science Handbook, Vol. I*, Ed. R. Saferstein, Prentice Hall, Englewood Cliffs, NJ, 1982.
- Cox, M. A study of the sensitivity and specificity of four presumptive tests for blood. *J Forensic Sci*, 36 (5), pp 1503-1511, 1991.
- Tobe, S., Watson, N. & N. Dauid. Evaluation of six presumptive tests for blood, their specificity, sensitivity, and effect on high molecular weight DNA, *J Forensic Sci*, 52 (1), pp. 102-109, 2007.

- Laux, D. Effects of luminol on the subsequent analysis of bloodstains. *J Forensic Sci*, 36 (5), pp 1512-1520, 1991.
- Higaki, RS and WMS Philp. A Study of the Sensitivity, Stability and Specificity of Phenolphthalein as an Indicator Test for Blood. *Can Soc Foren Sci J*. 9:97-102. 1976.
- Atkinson, C., Sileniek, E., Pearman, C. Validation of ABACard[®] HemaTrace[®] Kits to Determine the Presence of Higher Primate Hemoglobin in Bloodstains
- Swander, C.J., Stites, J.G. Evaluation of the ABACard[®] HemaTrace[®] for the forensic identification of human blood. MAFS Annual Meeting.
- Kristaly, A., Smith, D.A.S. Validation of the ABACard[®] HemaTrace[®] for the rapid forensic identification of human blood.
- CMPD Internal Validation of ABACard[®] Hematrace and IR

1.2 Seminal Stain Identification

A list of the procedures available to perform analysis on semen evidence is provided. If more than one type of test is performed on the same stain, each result must be recorded.

1.2.1 Acid Phosphatase - One Step Method

A. Purpose and Introduction

If a stain is identified as possible semen by either visual or UV light examination, testing must be performed to characterize the stain as semen.

An acid phosphatase (AP) test is a biochemical test that is sensitive but not specific for seminal acid phosphatase (SAP), a constituent of semen. Since the presence of SAP is considered highly indicative of semen, the test is used as a screening tool for the location of semen stains.

Since SAP degrades with heat and over time, and the level of initial SAP varies with the individual donor, its absence does not mean that semen is not present. Based on experience and results of the visual and UV exams the analyst may choose to do further testing even in the absence of a positive AP result.

B. Materials

- Acid Phosphatase (AP) Reagent
- Deionized water

C. Procedures

1. Test controls prior to performing tests on case samples. The positive control will be a known semen standard, while the negative control will be the reagents. The passing of the controls will be noted on the worksheet by the date. If multiple dates are needed for analysis, all dates will be noted in the control blank on the worksheet.
2. Acceptable methods of sample collection:
 - A. Swab to swab--Dampen a swab(s) with deionized water. The number of swabs prepared will depend on the number of sample swabs. Press one of the sterile swab(s) against each sample swab. Hold for a minimum of 20 seconds.
 - B. Filter paper--Dampen the filter paper, then wrap around the swab and press for a minimum of 20 seconds.
 - C. Cutting--A small cutting of sample may be obtained. Place the cutting on a piece of filter paper and place a drop of deionized water on the cutting. Once the stain has been thoroughly wet the reagent may be applied.
 - D. Pressout (may be used with underpants & large items)--Obtain sterile filter paper large enough to cover the stain. Dampen the filter paper with deionized water and lay it over the surface to be tested. On the filter paper, mark the location of any landmarks such as seams or leg holes. Press the filter paper onto the sample for a minimum of 30 seconds. Flip the filter paper over onto a clean piece of paper before applying reagent.
3. Add 1 to 2 drops of AP reagent to the filter paper or test swab. Pressouts will require sufficient reagent to cover the test surface of the filter paper.
4. **Interpretation:** Examine the filter paper for the development of a purple color within 60 seconds and grade the reaction as to intensity. The grading scales range from 0 (or negative) to 4+ explained as follows:

Negative: no color change observed after 90 seconds
0: slight color change between 60 and 90 seconds
1+: color change appears between 45 and 60 seconds
2+: color change appears between 30 and 45 seconds
3+: color change appears between 15 and 30 seconds
4+: color change appears before 15 seconds or a deep purple (almost black) color develops on any stain prior to 60 seconds

5. Dry the evidence swabs or clothing items before returning them to their original package.

D. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

E. Limitations

- This is a presumptive test for the presence of semen. Confirmation requires further testing such as sperm identification or p30 detection.
- Purple color changes observed after 60 seconds should be interpreted with caution since reagents may change color over time in the absence of semen.
- Vaginal acid phosphatase may be present which will result in a more pink than purple color change. This reaction is usually much slower (>60 seconds) than that seen with seminal acid phosphatase except in pregnant women and young pre-pubescent girls who may exhibit increased vaginal acid phosphatase levels.
- Fecal stains and certain plants may give false positive reactions.

F. Validation

This procedure is generally accepted in the forensic community. See semen references (Section 1.2.4).

1.2.2 Microscopic Examination using the Christmas Tree Stain

A. Purpose and Introduction

After the presumptive test is performed, tests that confirm the presence of semen are available. Semen may be confirmed by microscopic identification of spermatozoa. Microscopic identification is the ideal test to confirm semen

since the likelihood of obtaining genetic information is much greater if sperm are present.

Confirmed spermatozoa, a positive AP test and a record of the victim's activities and/or the presence of motile spermatozoa may be used to estimate a timeframe for deposit of a sample in certain cases.

B. Materials

- Kernechtrot stain (NFR)
- Picroindigocarmine stain (PICS)
- Microscope with 400X and phase contrast capabilities
- Sonicator
- Centrifuge

C. Procedures

1. Stained Christmas Tree Stain – Kernechtrot (NFR) Stain and Picroindigocarmine (PICS) Stain

A. A portion of the suspected stain or swab(s) should be immersed in deionized water for 15-60 minutes and centrifuged at high speed to collect the sperm cell pellet. A 15 minute sonication step may be inserted before centrifugation if the sample is believed to contain a low level of semen. The excess supernatant may be discarded or retained for additional testing. Re-suspend the cell pellet and place a portion of it on a slide.

Alternatively, a cutting from a sample such as a vaginal swab, may be macerated onto a slide with deionized water, then heated and stained as above. This procedure may be used to confirm a positive result, but not a negative result.

- B. Fix the stain of interest to a glass slide using heat (i.e. candle flame or incubator).
- C. Cover the fixed sample with NFR for at least 5 minutes and then rinse the slide with DI water.
- D. Cover the fixed sample with PICS for 15-60 seconds and then rinse the slide with 95% ethanol. Allow to dry.
- E. Examine the slide at 200 X or 400 X with a light microscope and search for the presence of spermatozoa. Xylene may be used under the cover slip to minimize the viewing of background.

- F. **Interpretation:** Spermatozoa are indicated by a red stained body and dark-outlined head (acrosomal cap). The presence of tails is not necessary to confirm spermatozoa; however, their presence should be recorded in the notes by writing out "tails". The amount of spermatozoa present should be confirmed at 400 X and graded from few to 4+ using the following guidelines:

Few: fewer than 5 sperm

1+: hard to find

2+: some in same fields, easy to find

3+: many or some in most fields

4+: too numerous to count (TNTC)

D. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

Warning: The following are considered hazardous reagents:

Picric acid crystals are highly explosive. Picric acid is a solid that must be submerged in water at all times and care should be taken that none of the slurry sticks to the inside of the jar above the water. Before storing swirl the liquid around until all of the slurry is again beneath the water in the jar. Should any of the slurry get on the outside of the bottle it should be washed off with a large volume of water. Picric acid is toxic if ingested. Obtain emergency first aid.

Aluminum sulfate is a lung and skin irritant. If skin is exposed wash with large amounts of water. If inhaled move to fresh air.

E. Limitations

- This is a confirmatory test for the presence of semen. However, the absence of sperm cells does not mean semen is not present.
- Cellular material, including epithelial cells as well as yeast and bacteria, may also stain. These, however, may be easily distinguished from sperm due to the characteristic shape of the sperm cell and differential staining of the sperm heads.
- This stain is not specific for human sperm. Sperm from other species will also be stained. However, sperm from different species are relatively distinct from human sperm.

F. Validation

This procedure is generally accepted in the forensic community. See semen references (Section 1.2.4).

1.2.3 P30 Analysis - ABACard[®]

A. Purpose and Introduction

While the presence of spermatozoa is the primary confirmatory test, semen may also be confirmed by the presence of p30 by the ABACard[®] method.

For medical or behavioral reasons, some individuals have a low sperm count (azoospermic) while others produce no sperm (aspermic). A test for the prostate specific antigen also known as p30, is a viable means of confirming semen in cases where no spermatozoa were located upon microscopic examination and presumptive testing for AP was positive.

B. Materials

- OneStep ABACard[®] p30 Test Strips
- Extraction Buffer
- Deionized water
- Spin baskets
- Centrifuge

C. Procedures

1. Positive semen and negative controls will be tested on the first run of a given day of use (additional tests the same day may use the results of the first test).
2. Extract $\frac{1}{4}$ to $\frac{1}{2}$ of a swab or a small portion of a stain in 500-750 uL extraction buffer or deionized water (record what is used) in a microcentrifuge tube and place in a 2-8° C refrigerator for 30 minutes. Place the sample in a spin basket and centrifuge for three minutes at 13,000 RPM.

Alternatively, the sample may be sonicated for fifteen minutes and the pellet removed for slide preparation before the sample is placed in the refrigerator.

3. Add 200 uL (or 6-7 drops with the dropper) of the extract to the “S” well of the ABACard and allow the liquid to migrate to the top of the strip.
4. Read the results at 5 minutes. Positive results may be seen as soon as 1 minute. Negative results must wait the full 10 minutes.
5. ***Interpretation:*** Examine the strip for a pink band at the “T” and “C” locations. For a positive result, both locations must exhibit a visible band. If only a “C” band is present, the test is negative. If only the “T” value is present, the test is inconclusive. If only the “T” or “C” band is present, the test may need to be repeated using dilutions of the original extract.

D. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

E. Limitations

- p30 degrades with heat and over time
- If the concentration of the extract is extremely high, a high dose hook effect may be observed thus yielding a false negative. Extracts of stains that yield high acid phosphatase activity and negative p30 results, should be diluted approximately 1:100 and re-tested.
- This test is a confirmation for the presence of semen in conjunction with a positive AP test.
- There have been reports of PSA at low levels in some female body fluids. However these reported levels are significantly lower than those observed in semen (less than 1 ng per ml versus 1-2 mg/ml).

F. Validation

This procedure is generally accepted in the forensic community. See semen references (Section 1.2.4).

1.2.4 Semen References

- Gaensslen, R.E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*; U.S. Government Printing Office: Washington, DC, 1983

- Baechtel, F.S. The Identification and Individualization of Semen Stains, *Forensic Science Handbook Vol. II* Ed. R. Saferstein, Prentice Hall, Englewood Cliffs, NJ, 1988
- Kind, S.S., The use of acid phosphatase in searching for seminal stains, *J. of Crim. Law Criminology and Police Science*, Vol No 5, Jan. – Feb 1957
- Sensabaugh, G.F. The quantitative acid phosphatase test. A statistical analysis of endogenous and postcoital acid phosphatase levels in the vagina. *J Forensic Sci*, 24(2), pp 346-365, 1979
- Sensabaugh, G.F., Isolation and characterization of a semen-specific protein from human seminal plasma. A potential new marker for semen identification. *J. Forensic Sci.*, Vol 23, pp. 106-115, 1978
- Collins, K.A. and A.T. Bennett. Persistence of spermatozoa and prostatic acid phosphatase in specimens from deceased individuals during varied post-mortem intervals. *The American Journal of Forensic Medicine and Pathology*, 23(3). pp 228-232, 2001
- Allard, J.E. The collection of data from findings in cases of sexual assault and the significance of spermatozoa on vaginal, anal and oral swabs. *Science and Justice*, 37. pp 99-108, 1997
- Randall, B. Persistence of vaginal spermatozoa as assessed by routine cervicovaginal (Pap) smears. *J Forensic Sci*, 32 (3) pp 678-683, 1987
- Abacus Diagnostics, *One Step ABACard® p30 Test for the Forensic Identification of Semen*, Revision 8/98, Abacus Diagnostics, West Hills, CA, 1998
- Poyntz, F.M. and Martin, P.D., *Comparison of p30 and Acid Phosphatase Levels in Post-Coital Vaginal Swabs from Donor and Casework Studies*, *For. Sci. Int.*, Vol 24, 17-25, 1984
- Kristaly, A et al., *Validation of the OneStep ABACard® PSA Test for the Rapid Forensic Identification of Semen*, presented at the Spring 1999 Meeting of the Southern Association of Forensic Scientists, Decatur, GA.
- Hochmeister, MN et al., Evaluation of prostate-specific antigen (PSA) membrane test assays for the forensic identification of seminal fluid, *J. For. Sci.*, Vol 44, No.5, Sept 1999
- Davies, A & E. Wilson. The persistence of seminal constituents in the human vagina. *Forensic Science* (30) pp 45-55, 1974

- Schmidt, S et al., Prostate-specific antigen in female urine: A prospective study involving 217 women. *Elsevier Science* pp 717-720, 2001
- CMPD Internal Procedure Modification Study

1.3. Saliva Stain Examination

A procedure is available to perform analysis on **suspected** saliva evidence.

1.3.1 Phadebas[®] test

A. Purpose and Introduction

If a potential saliva stain is visualized or located with the alternate light source or if saliva is suspected to be present on a sample based on case information, a presumptive test for the presence of saliva may be performed. If a positive result is obtained the stains may be forwarded to DNA analysis.

α -amylase is a component of the saliva of certain mammals, including humans. Because it is present in much higher quantities in human saliva than any other bodily fluids and is rarely detectable in domestic dogs and cats, testing for α -amylase may be used as a presumptive test for the presence of saliva on crime scene samples.

B. Materials

- Phadebas[®] tablets
- 0.5N NaOH
- Sterile deionized water
- Oven
- Centrifuge

C. Procedure

1. Test controls in conjunction with case samples. The positive control will be a known saliva standard while the negative control will be the reagents. The passing of the controls will be noted on the worksheet by the date. If multiple dates are needed for analysis, all dates will be noted in the control blank on the worksheet.
2. Label clear test tubes with separate identifiers for each questioned sample to be tested. In addition, label one tube as the negative control and one tube as the positive control.

3. Place a 5 mm² piece of the stained fabric (or equivalent size) **or** 1/3 of a stained swab (or equivalent total amount from several swabs) into a test tube.
4. Place a 5 mm² piece of filter paper (or 1/3 of a swab) saturated with human saliva from a donor known to have high amylase activity in the tube labeled positive control.
5. Place ¼ of a Phadebas[®] tablet into each tube.
6. Add 1 mL of deionized water to each tube.
7. The negative control will contain only the ¼ Phadebas[®] tablet and deionized water. If blood is suspected on the sample also prepare a blood negative control using human blood from a known source.
8. Vortex all samples and place in a 37°C incubator.
9. After 30 minutes, add 1 mL of .5 N NaOH to the tubes and vortex.
10. Centrifuge the tubes for 5 minutes.
11. Read result immediately using the color chart.
12. ***Interpretation:*** A blue supernatant that meets or exceeds the intensity depicted by the 1+ example on the color chart is indicative of a positive reaction. Positive samples will be graded on a 1+-4+ scale as depicted by the examples on the color chart. Samples with colorless supernatant (as compared to the negative control) or a color that does not at least match the intensity of the 1+ sample are considered negative. The analyst may choose to go forward with DNA analysis with the samples, however, any DNA found present cannot be attributed to saliva. The positive control serves to test the function of the Phadebas tablets, and does not serve as a basis of comparison for any test sample.

D. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

E. Limitations

- The Phadebas[®] tablet test, or other amylase tests, are sensitive, but not specific, tests for saliva. The theoretical basis relies on the fact that alpha amylase is present in much higher quantities in saliva than other bodily fluids.
- While it is often a good indicator of saliva, other bodily fluids including vaginal secretions contain a weak amount of amylase and may react positively with the test.
- This test gives a chemical indication for the presence of amylase. It is not confirmatory for the presence of saliva.
- When testing for amylase in the presence of semen, positive results should be interpreted with caution.
- This test is not specific for human amylase. It can also be found in higher primates, pigs, rodents, and elephants.

F. Validation

This procedure is generally accepted in the forensic community and was validated internally at CMPD.

G. References:

- Gaensslen, R.E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*; U.S. Government Printing Office: Washington, DC, 1983
- Willott, G.M., An improved method for detection of salivary amylase in stains. *J. Forens. Sci. Soc.*, 14:341-344, 1974
- Magle Scientific, 2006. http://www.Phadebas.com/areas_of_use, Accessed June 13, 2011
- Myers, J. R. & W. K. Atkins. Comparison of modern techniques for saliva screening. *J. For. Sci.* 53:4, pp 862-867, 2008
- Magle Life Sciences (Magle AB), 2007. Phadebas amylase test. Directions for use. Package insert.
- Keating, S.M., & D.F. Higgs. The detection of amylase on swabs from sexual assault cases. *J Forensic Sc. Soc.*, 34 (2), pp. 89-93, 1993
- CMPD Internal Procedure Modification Study

2. Extraction of DNA Using the QIAamp, DNA IQ, and PrepFiler Express Methods

A. Purpose and Introduction:

DNA profiles obtained from biological evidence provide information as to the source of the sample. The goal of DNA isolation is to recover/extract high molecular weight DNA in sufficient quantities. Through the use of standard procedures, DNA is isolated from nucleated cells from various biological specimens.

The QIAamp extraction method involves cell lysis by the addition of Proteinase K and DTT. Following lysis, the sample is purified by passing it through a spin column where the DNA is adsorbed onto the silica-gel membrane. Residual contaminants are washed through the column and the purified DNA is then eluted from the column.

The DNA IQ extraction method involves cell lysis using either a Proteinase K containing buffer or the addition of the DNA IQ Lysis Buffer. DNA is then bound to a silica-coated paramagnetic resin, washed, then eluted from the resin into a buffer for long-term storage.

The PrepFiler Express method involves the lysis of DNA followed by the automated extraction and wash using the Automate Express. The cartridge provides the lysis medium, the magnetic particles for DNA binding, wash buffers to purify and buffer for elution.

B. Materials and Instrumentation:

- QIAamp DNA Investigator Kit
- DNA IQ™ Kit
- Maxwell 16® Kits
- PrepFiler® Express Forensic DNA Extraction Kit
- PrepFiler® Express BTA Forensic DNA Extraction Kit
- QIAamp (DNA Investigator Kit)
- Digest Buffer
- Proteinase K
- DTT
- Thermal mixer
- Microcentrifuge
- Oven
- Maxwell 16®
- Automate Express™
- Hoods
- Pipettes (2, 10, 20, 100, 200 and 1000 µl)

C. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

Warning: The following are considered hazardous reagents:

Sodium Dodecyl Sulfate (SDS) potentially causes burns when in contact with the skin or eyes. It causes irritation if inhaled and may cause severe digestive tract irritation if ingested. Accidental skin or eye contact will be treated according to routine laboratory safety procedures (give milk or water if ingested and seek medical assistance). When preparing solutions using this chemical, proper personal protective equipment, including a dust mask or respirator, should be employed.

Sodium Hydroxide is a corrosive chemical, which may be fatal if absorbed through the skin. It causes severe eye and skin burns, gastrointestinal burns if ingested and severe irritation if inhaled. Accidental skin or eye contact will be treated with routine laboratory safety practices. Do not induce vomiting if ingested, seek immediate medical aid. Wear appropriate personal protective equipment and use the fume hood when using this chemical. It generates a lot of heat when put into solution so add this chemical slowly and carefully.

D. Minimum Standards and Controls

A reagent blank should be processed as the last sample of a set for each extraction protocol followed. The purpose of this control is to ensure that contamination has not occurred due to the manipulation of the sample or the reagents used in the procedure. All reagent blanks will be processed under the same conditions as required for the sample with the least amount of DNA. Even if not amplifying the full amount of the sample, always amplify 10µl of the corresponding reagent blank.

E. Other Considerations

Separate stains will be treated independently of one another. Under some circumstances an analyst may choose to combine DNA extracts from separate stains, usually due to small size and close proximity on the substrate. Pooling or combining of DNA extracts from separate stains will only be done after the individual DNA extracts have been quantified, documented, and approved by the DNA Technical Leader. As a general consideration, pooling of DNA extracts from separate stains is not recommended as a standard procedure and should be avoided.

If a sample is re-extracted, the reason will be noted at the bottom of the original extraction sheet and a new DNA # will be created.

If the original sample has been consumed, the cutting will be kept in a tube labeled with the DNA # and M and created as a sub item in PLIMS. This will be noted with a * on the extraction sheet by the quantity sampled. The * indicates sample saved so no further notation needs to be made. For purposes of quantity, a tip is approximately 1/8 of a swab.

Penile swabs will be screened using the AP test found in SOP 1.2.1. If positive, the differential extraction procedure found in SOP 2.2.2 will be performed on penile swabs. At step #7 all 350 uL will be saved from the non-sperm cell fraction.

F. Validation

These procedures are generally accepted in the forensic community.

These procedures have undergone internal validation at the CMPD.

2.1 QIAamp Extraction (DNA Investigator Kit)

2.1.1 QIAamp Extraction from Hair

Before beginning:

- If Buffer ATL or Buffer AL contains precipitates, dissolve by heating to 70° C with gentle agitation.
 - Be certain that 95% ethanol has been added to the AW1 and AW2 buffers.
1. Examine the hair for the presence of sheath material. Note the possible presence of body fluids on the hair.
 2. Wash the hairs:

For unmounted hairs: (this step is optional)

- i. Fill a clean 50 ml beaker with 95-100% ethanol.
- ii. Pick up a single hair and immerse the hair in the ethanol.
- iii. Fill a clean 50 ml beaker with sterile dl water.
- iv. Transfer the hair and immerse it in the dl water.
- v. Repeat steps i-iv for each hair to be analyzed. **Proceed to step 3.**

For mounted hairs:

- i. Freeze the slide in a -15°C to -20°C freezer for 20 minutes.
- ii. Remove the cover slip by prying it off using a scalpel. Alternatively, the cover slip may be removed by soaking the slide in xylene for several hours after cracking the cover slip with a diamond scribe.
- iii. Using a Pasteur pipette, wash away the mounting medium by squirting the slide with xylene.
- iv. Pick up the hair and immerse in 95-100% ethanol.
- v. Wash the hair in sterile deionized water. **Proceed to step 3.**

Note: Because hair may contain cellular material on the surface which may or may not originate from the hair donor; cut off about 0.5 – 1.0 cm of the shaft adjacent to the root portion for separate analysis as a control. It should be noted that this shaft is a substrate control and is used as an interpretation aid, not a negative control. If the hairs are not long enough, then no shaft control needs to be run, but this should be noted in the notes.

3. Place a 0.5 – 1.0 cm portion of hair in a 1.5 ml microcentrifuge tube.
4. Add 20 μl Proteinase K, 20 μl 1 M DTT and 300 μl of Buffer ATL, close lid and mix by vortexing for approximately 10 seconds.
5. Place sample in a thermal mixer (250 to 900 rpm) and incubate at 56°C for at least 1 hour.
6. Briefly centrifuge sample to remove drops from the inside of the lid.
7. Add 300 μl Buffer AL, close lid and vortex for approximately 10 seconds. (A white precipitate may form when the buffer is added. The precipitate will dissolve during incubation and does not affect the extraction process.)
8. Place sample in a thermal mixer (250 to 900 rpm), or heat block and incubate at 70°C for 10 minutes.
9. Briefly centrifuge sample to remove drops from the inside of the lid.
10. Add 150 μl of ethanol, close lid and mix by vortexing for approximately 15 seconds.
11. Briefly centrifuge sample to remove drops from the inside of the lid.

12. Transfer all lysate (700µl at a time) to a QIAamp MinElute column (in a 2 ml collection tube) without wetting the rim, close the lid and centrifuge at 6,000 x g (8,000 rpm) for 1 minute. Place column in a clean 2 ml collection tube and discard collection tube containing the flow-through. Step may be repeated.
13. Add 500µl of Buffer AW1 to the column without wetting the rim. Close the lid and centrifuge at 6,000 x g (8,000 rpm) for 1 minute. Place column in a clean 2 ml collection tube and discard collection tube containing the flow-through.
14. Add 700µl of Buffer AW2 to the column without wetting the rim. Close the lid and centrifuge at 6,000 x g (8,000 rpm) for 1 minute. Place column in a clean 2 ml collection tube and discard collection tube containing the flow-through.
15. Add 700µl of ethanol to the column without wetting the rim. Close the lid and centrifuge at 6,000 x g (8,000 rpm) for 1 minute. Place column in a clean 2 ml collection tube and discard collection tube containing the flow-through.
16. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 minutes to completely dry the membrane.
17. Place column in a clean 1.5 ml microcentrifuge tube and discard collection tube containing the flow-through. Open the lid of the column and incubate at room temperature for 10 minutes.
18. Add 30-100µl of Buffer ATE to the center of the membrane.
19. Close lid and incubate at room temperature for 1 minute. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 1 minute. This is your purified DNA.

Store the samples at 2-6° C (short term storage) or freeze at -15 to -20° C (long term storage). Vortex briefly and spin in a microcentrifuge for 5 seconds prior to quantification or amplification. If desired, the sample may be concentrated with the Microcon unit.

2.1.2 QIAamp Extraction from Bone or Teeth

Before beginning:

- If Buffer ATL or Buffer AL contains precipitates, dissolve by heating to 70° C with gentle agitation.
- Be certain that 95% ethanol has been added to the AW1 and AW2 buffers.

Bone specimens should be stored at -20° C to -70° C until processed. Steps 1-4 of the bone processing procedure should be performed in a vented hood.

To clean: Clean Dremel bits, cutting disc and Micro-Mill grinding chamber thoroughly using 10% bleach, followed by a deionized (dl) water rinse and then 95% ethanol.

Allow the surfaces of the Micro-Mill to dry thoroughly before use. Use a new sanding disc for each sample.

Bone:

1. Using a separating disc affixed to a Dremel tool, or a Stryker saw, cut the bone specimen to a size of approximately 2 cm x 5 cm.
2. Using the Dremel tool with an emery disk affixed, sand the outer surface of the bone specimen such that the outer surface of the bone appears visibly free of dirt, blood, marrow and debris.
3. Place the sanded bone specimen in a cold water cooled Micro-Mill Grinder, set the timer for two one minute increments. **Proceed to step 4.**

Tooth:

1. Using a separating disc affixed to a Dremel tool, or a Stryker saw, cut out any filling material present on the teeth. Soak the teeth in 5.25% bleach for 20 minutes.
2. Rinse the teeth with sterile dl water, soak in sterile dl water for 20 minutes, then rinse with 95% ethanol and air dry under shortwave (256nm) UV light for 20 minutes.
3. Place the specimen in a cold water cooled Micro-Mill Grinder, set the timer for two one minute increments.
4. Place ~100 mg of ground bone or tooth in a 1.5 ml microcentrifuge tube.
5. Add 20µl Proteinase K and 360µl of Buffer ATL, close lid and mix by vortexing for approximately 10 seconds.
6. Incubate sample at 56°C in thermal mixer (250 to 900 rpm) overnight.
7. Briefly centrifuge sample to remove drops from the inside of the lid.
8. Add 300µl Buffer AL, close lid and vortex for approximately 10 seconds. (A white precipitate may form when the buffer is added. The precipitate will dissolve during incubation and does not affect the extraction process.)
9. Place sample in a thermal mixer (250 to 900 rpm), or heat block and incubate at 70°C for 10 minutes.
10. Centrifuge sample at full speed (20,000 x g; 14,000 rpm) for 1 minute. Transfer supernatant to a new 1.5 ml tube.
11. Add 150µl of ethanol, close lid and mix by vortexing for approximately 15 seconds.

12. Briefly centrifuge sample to remove drops from the inside of the lid.
13. Transfer all lysate (700µl at a time) to a QIAamp MinElute column (in a 2 ml collection tube) without wetting the rim, close the lid and centrifuge at 6,000 x g (8,000 rpm) for 1 minute. Place column in a clean 2 ml collection tube and discard collection tube containing the flow-through. Step may be repeated.
14. Add 600µl of Buffer AW1 to the column without wetting the rim. Close the lid and centrifuge at 6,000 x g (8,000 rpm) for 1 minute. Place column in a clean 2 ml collection tube and discard collection tube containing the flow-through.
15. Add 700µl of Buffer AW2 to the column without wetting the rim. Close the lid and centrifuge at 6,000 x g (8,000 rpm) for 1 minute. Place column in a clean 2 ml collection tube and discard collection tube containing the flow-through.
16. Add 700µl of ethanol to the column without wetting the rim. Close the lid and centrifuge at 6,000 x g (8,000 rpm) for 1 minute. Place column in a clean 2 ml collection tube and discard collection tube containing the flow-through.
17. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 minutes to completely dry the membrane.
18. Place column in a clean 1.5 ml microcentrifuge tube and discard collection tube containing the flow-through. Open the lid of the column and incubate at room temperature for 10 minutes (or at 56°C for 3 minutes).
19. Add 30-100 µl of Buffer ATE to the center of the membrane.
20. Close lid and incubate at room temperature for 1 minute. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 1 minute. This is your purified DNA.

Store the samples at 2-6° C (short term storage) or freeze at -15 to -20° C (long term storage). Vortex briefly and spin in a microcentrifuge for 5 seconds prior to quantification or amplification. If desired, the sample may be concentrated with the Microcon unit.

2.1.3 QIAamp Extraction from Cigarette Butt Filters

Before beginning:

- If Buffer ATL or Buffer AL contains precipitates, dissolve by heating to 70° C with gentle agitation.
 - Be certain that 95% ethanol has been added to the AW1 and AW2 buffers.
1. Place a $\approx 1 \text{ cm}^2$ portion of cigarette filter in a 1.5 ml microcentrifuge tube.

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2. Add 20µl proteinase K and 300µl of Buffer ATL, close lid and mix by vortexing for approximately 10 seconds.
3. Place sample in a thermal mixer (250 to 900 rpm) and incubate at 56°C for at least 1 hour.
4. Briefly centrifuge sample to remove drops from the inside of the lid.
5. Remove cutting, place in a spin basket and centrifuge at full speed (20,000 x g; 14,000 rpm) for 1 minute. Discard Spin basket.
6. Add 300µl Buffer AL, close lid and vortex for approximately 10 seconds. (A white precipitate may form when the buffer is added. The precipitate will dissolve during incubation and does not affect the extraction process.)
7. Place sample in a thermal mixer (250 to 900 rpm), or heat block and incubate at 70°C for 10 minutes.
8. Briefly centrifuge sample to remove drops from the inside of the lid.
9. Add 150µl of ethanol, close lid and mix by vortexing for approximately 15 seconds.
10. Briefly centrifuge sample to remove drops from the inside of the lid.
11. Transfer all lysate (700µl at a time) to a QIAmp MinElute column (in a 2 ml collection tube) without wetting the rim, close the lid and centrifuge at 6,000 x g (8,000 rpm) for 1 minutes. Place column in a clean 2 ml collection tube and discard collection tube containing the flow-through. Step may be repeated.
12. Add 500µl of Buffer AW1 to the column without wetting the rim. Close the lid and centrifuge at 6,000 x g (8,000 rpm) for 1 minute. Place column in a clean 2 ml collection tube and discard collection tube containing the flow-through.
13. Add 700µl of Buffer AW2 to the column without wetting the rim. Close the lid and centrifuge at 6,000 x g (8,000 rpm) for 1 minute. Place column in a clean 2 ml collection tube and discard collection tube containing the flow-through.
14. Add 700µl of ethanol to the column without wetting the rim. Close the lid and centrifuge at 6,000 x g (8,000 rpm) for 1 minute. Place column in a clean 2 ml collection tube and discard collection tube containing the flow-through.
15. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 minutes to completely dry the membrane.

16. Place column in a clean 1.5 ml microcentrifuge tube and discard collection tube containing the flow-through. Open the lid of the column and incubate at room temperature for 10 minutes (or at 56°C for 3 minutes).

17. Add 30-100µl of Buffer ATE to the center of the membrane.

18. Close lid and incubate at room temperature for 1 minute. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 1 minute. This is your purified DNA.

Store the samples at 2-6° C (short term storage) or freeze at -15 to -20° C (long term storage). Vortex briefly and spin in a microcentrifuge for 5 seconds prior to quantification or amplification. If desired, the sample may be concentrated with the Microcon unit.

QIAamp References:

- Melzak, Kathryn A. et al. "Driving Forces for DNA Adsorption to Silica in Perchlorate Solutions". *J of Colloid and Interface Sci.* 1996. Vol 181. Article 0421. Pp 635-644.
- Montpetit, Shawn A. et al. "A Simple Automated Instrument for DNA Extraction in Forensic Casework". *J Forensic Sci.* May 2005. Vol 50. No 3.
- "EZ1 DNA Investigator Kit STR Analyses Data". Qiagen.com
- "Product Profile: EZ1 DNA Investigator Kit". Qiagen.com.
- CMPD Internal Validation

2.2 DNA IQ Extraction

2.2.1 DNA IQ Extraction from Blood, Saliva, and Epithelial Cells/Tissue

Before beginning:

- prepare a 56° C bath/oven for use in step 3 and a 65° C bath for use in step 17.
 - ensure that the Lysis Buffer is in solution; if not, warm solution at 37-60° C.
 - be certain that 35 ml 95% ethanol and 35 ml Propanol (2-) have been added to the Wash Buffer.
1. Determine the total amount of Lysis Buffer to be used (based on the following table) and add 1 µl 1M DTT for every 100 µl Lysis Buffer. (**NOTE:** These are the approximate amounts of material to be used for extraction.)

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Material (see NOTE above)	Lysis Buffer (extraction)	Lysis Buffer (wash)
3 mm ² FTA paper	200 µl	100 µl
¼ - ½ cotton swab	200 µl	100 µl
Buccal scrubber (one tooth)	200 µl	100 µl
Cotton swab	400 µl	100 µl
Stain cutting	Variable	100 µl

- Place the sample in a 1.5 ml microcentrifuge tubes.

OPTIONAL: If necessary, large cuttings may be soaked in Digest Buffer for a minimum of 30 minutes. The sample matrix should then be placed in a spin basket and centrifuged at maximum speed for 3-5 minutes to pellet the cells. Remove all but 100 µl of the supernatant and pellet.

OPTIONAL: The sample may be pretreated with Digest Buffer/Proteinase K (13.5 mg/ml) in a ratio of 1 µl Proteinase K: 49 µl Digest Buffer (for example, 4 µl Pro K + 196 µl Digest Buffer, 10 µl Pro K + 490 µl Digest Buffer, etc). Add sufficient volume to cover the sample and digest at 56° C for 1-2 hours, then proceed to step 4.

- Add the appropriate amount of Lysis Buffer/DTT. Sufficient volume should be added to cover the entire sample. Close the tube lid and incubate at 56° C for 1 to 2 hours at 250 to 900 rpm.

OPTIONAL: 200 µl of Lysis Buffer/DTT should be added if the optional Digest Buffer soak was performed in step 2. Incubate as above.

- Remove the tube from the heat block/oven and spot spin to collect all liquid at the bottom of the tube. Use a sterile wood applicator stick or pipette tip to place the cutting in a spin basket. (Spin basket is optional if extracting known standards).
- Centrifuge at maximum speed for 2 minutes. Discard Spin basket.

NOTE: If the sample was digested with Proteinase K/Digest Buffer, add two volumes of Lysis Buffer/DTT. It may be necessary to utilize additional tubes at this point.

- Vortex the resin bottle until the resin is thoroughly mixed. Add 7 µl of resin to the sample. If multiple tubes are being used, add resin to one tube only.
- Vortex the sample/Lysis Buffer/resin mix for 5-10 seconds at moderate speed and avoid getting liquid on the tube cap. Incubate at room temperature for 5-10 minutes.
- Vortex again for 5-10 seconds at moderate speed, then place the tube in the magnetic stand.
- Remove and discard all of the solution without disturbing the resin pellet on the side of the tube.

NOTE: If multiple tubes were used due to large volume, add the remaining liquid extract to the tube containing the resin and repeat steps 7-9.

10. Add 100 µl of Lysis Buffer/DTT. Remove the tube from the stand and vortex for 5-10 seconds at moderate speed. Return the tube to the magnetic stand.
11. Remove and discard the liquid without disturbing the pellet.
12. Add 100 µl of 1X Wash Buffer. Remove the tube from the stand and vortex for 5-10 seconds at moderate speed. Return the tube to the magnetic stand.
13. Remove and discard the liquid without disturbing the pellet.
14. Repeat steps 12 and 13 two more times for a total of three washes. Make sure that all liquid has been removed from the tube after the third wash.
15. With the lid open, air-dry the resin in the magnetic stand for 5-10 minutes. Do not dry the resin for any longer than 20 minutes, as this may inhibit removal of DNA.
16. Add 30-100 µl of Elution Buffer, close the lid, and vortex at moderate speed for 5-10 seconds.
17. Place the tube at 65° C for 5 minutes at 250 to 900 rpm.
18. Remove the tube from the heat block, vortex for 5 seconds at moderate speed, then place the tube immediately on the magnetic stand. The tube must remain hot until placed on the stand or the yield will decrease.
19. Transfer the liquid to a new container. This is your purified DNA.

Store the samples at 2-6° C (short term storage) or freeze at -15 to -20° C (long term storage). Vortex briefly and spin in a microcentrifuge for 5 seconds prior to quantification or amplification. If desired, the sample may be concentrated with the Microcon unit.

2.2.2 DNA IQ Extraction from Semen Stains

Before beginning:

- prepare a 56°C bath/oven for use in step 4 and a 65°C bath for use in step 30.
- ensure that the Lysis Buffer is in solution; if not, warm solution at 37-60° C.
- be certain that 35 ml 95% ethanol and 35 ml Propanol (2-) have been added to the Wash Buffer.

Note: If extracting from a slide, refer to ***DNA Extraction from Sexual Assault Slides*** (section 2.3). For extraction of penile swabs, see “E. Other Considerations” at beginning of SOP 2.

1. Determine the total volume to be used in the extractions and prepare the Digest Buffer/Proteinase K master mix by adding 20 µl of Proteinase K (13.5 mg/ml) for every 980 µl of Digest Buffer. The total master mix volume will depend upon the number of samples, but the ratio of Proteinase K to Digest Buffer will always be 1:49. As an alternative, the Digest Buffer and Proteinase K can be added separately to each sample.
2. Place the swab/cutting in a 1.5 ml microcentrifuge tube.
OPTIONAL: If necessary, large cuttings may be soaked in Digest Buffer for a minimum of 30 minutes. The sample matrix should then be placed in a spin basket and centrifuged at maximum speed for 3-5 minutes to pellet the cells. Remove all but 50 µl of the supernatant and pellet.
3. Add to each sample either:
 - (a) 400-500 µl of the Digest Buffer/Proteinase K master mix, or
 - (b) 490 µl Digest buffer + 10 µl Proteinase K
4. Incubate at 56° C for 1-2 hours at 250 to 900 rpm.
5. Remove the tube from the heat block/oven and briefly spin to collect the liquid at the bottom of the tube. Use a sterile wood applicator stick or pipette tip to place the cutting in a spin basket.
6. Centrifuge at maximum speed for 10 minutes. Discard Spin basket.
7. Remove at least 100 µl of the supernatant and transfer to a new tube. This is your epithelial cell fraction. Up to 350 µl of the supernatant can be reserved, but reserving >100 µl will require an adjustment of the volume of Lysis Buffer/DTT added at step 17.
8. Remove and discard the remainder of the supernatant without disturbing the sperm pellet.

NOTE: If a significant number of epithelial cells are suspected to be present, steps 3-6 and step 8 may be repeated as long as the total incubation time does not exceed 2 hours. There is no need to repeat step 7.
9. Add 1000 µl of Digest Buffer (not Digest Buffer/Proteinase K) to wash the sperm pellet. Gently mix the pellet and the buffer and centrifuge for 5 minutes at maximum speed.
10. Remove all but 50 µl of the supernatant.

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11. Repeat steps 9 and 10 at least once for a minimum of two Digest Buffer washes. Steps 9 and 10 may be repeated as many times as desired if the ratio of sperm to epithelial cells is suspected to be low.
12. Add 1000 μ l of sterile H₂O to the pellet, mix gently, and centrifuge for 5 minutes at maximum speed.
13. Remove all but 100 μ l of the supernatant.
14. Resuspend the pellet in the remaining 100 μ l.
15. Spot 2-5 μ l of the resuspended pellet on a microscope slide and examine for the presence of sperm using either the Christmas tree stain or phase contrast. This step may be skipped if the examiner has previously identified sperm on this sample. The purpose for making this slide is to ensure that the examiner has taken the appropriate cutting according to the screener's notes. If spermatozoa was not confirmed in the screeners report, then it will be reported in the DNA report.
16. Determine the total amount of Lysis Buffer to be used based on the following formula:

(2 times the [volume of sample] + 100) x number of samples = total μ l

Add 4 μ l 1M DTT for every 100 μ l Lysis Buffer.
17. Add 200 μ l or at least two volumes of Lysis Buffer/DTT to the sperm and epithelial fractions.
18. Vortex the resin bottle until the resin is thoroughly mixed. Add 7 μ l of resin to the sample.
19. Vortex the sample/Lysis Buffer/resin mix for 5-10 seconds at moderate speed and avoid getting liquid on the tube cap. Incubate at room temperature for 5-10 minutes.
20. Vortex again for 5-10 seconds at moderate speed, then place the tube in the magnetic stand.
21. Remove and discard all of the solution without disturbing the resin pellet on the side of the tube.
22. Add 100 μ l of prepared Lysis Buffer. Remove the tube from the stand and vortex for 5-10 seconds at moderate speed. Return the tube to the magnetic stand.
23. Remove and discard the Lysis Buffer without disturbing the pellet.

24. Add 100 µl of 1X Wash Buffer. Remove the tube from the stand and vortex for 5-10 seconds at moderate speed. Return the tube to the magnetic stand.
25. Remove and discard the 1X Wash Buffer without disturbing the pellet.
26. Repeat steps 24 and 25 two more times for a total of three washes. Make sure that all liquid has been removed from the tube after the third wash.
27. With the lid open, air-dry the resin in the magnetic stand for 5-10 minutes. Do not dry the resin for any longer than 20 minutes, as this may inhibit removal of DNA.
28. Add 30-100 µl of Elution Buffer, close the lid, and vortex at moderate speed for 5-10 seconds.
29. Place the tube at 65° C for 5 minutes at 250 to 900 rpm.
30. Remove the tube from the heat block, vortex for 5 seconds at moderate speed, then place the tube immediately on the magnetic stand. The tube must remain hot until placed on the stand or the yield will decrease.
31. Transfer the liquid to a new container. This is your purified DNA.

Store the samples at 2-6° C (short term storage) or freeze at -15 to -20° C (long term storage). Vortex briefly and spin in a microcentrifuge for 5 seconds prior to quantification or amplification. If desired, the sample may be concentrated with the Microcon unit.

2.3 DNA Extraction from Sexual Assault Slides

1. Gently slide the coverslip off the slide. Moisten a sterile swab with dl water and swab the coverslip. Using a clean surface for each sample, cut (or pull) the swab material from the applicator stick and place it into a 1.5 ml labeled centrifuge tube.
2. Moisten a sterile swab with dl water and remove any material on the slide with the swab. Using a clean surface for each sample, cut (or pull) the swab material from the applicator stick and place it into the same 1.5 ml tube as the swab of the coverslip.

Proceed to:

DNA IQ Extraction from Semen Stains (section 2.2.2).

DNA IQ References:

- DNA IQ Manual

- DNA Purification from Various Sample Types Using the DNA IQ™ System, Profiles in DNA Vol. 5(2) p. 14 2002
- Genomic DNA Purification from Cigarette Butts and Buccal Swabs Using the DNA IQ™ System, By Caroline Turner, Rita Weispenning, Eric Vincent, Kimberly Huston and Joseph Bessetti , Promega Corporation
- CMPD Internal Validation

2.4 Microcon Cleanup/Concentration

Introduction: The Microcon unit can be used to remove salts and other PCR inhibitors from the DNA extract through multiple washings and elutions. In addition, the Microcon unit may be used to concentrate samples previously extracted through either the QIAamp or DNA IQ methods. The corresponding reagent blank has to be concentrated/cleaned up at the same time. Elution in TE at the last step should be no less than 30 µl.

1. Assemble a Microcon unit. Irradiate the assembled unit and the filtrate tubes with ultraviolet light for a minimum of 20 minutes, then add 200 µl TE to the top of the microconcentrator. Spin at maximum speed for 3 minutes. Discard the filtrate and place the microconcentrator into a new pre-sterilized filtrate tube.
2. Approximate the amount of eluant remaining from your extract and record on the worksheet.
3. Transfer the DNA eluant to the top of the microconcentrator. If the entire volume will not fit into the microconcentrator, steps 1-3 may be repeated.
4. Place a spin cap on the microconcentrator and spin in a microcentrifuge at 500 x g (2475 rpm) for 5 minutes. If liquid is still visible in the concentrator, spin for additional time. Caution: Do not spin to dryness. (NOTE: If used for concentration after Qiagen extraction or quantitation, spin until concentrated to the desired volume, then proceed to step 7).
5. Carefully remove the microconcentrator unit from the assembly and discard the filtrate fluid from the filtrate cup. Return the microconcentrator to the top of the filtrate cup.
6. Remove the spin cap and add 400 µl TE to the microconcentrator. Replace the spin cap and spin the assembly in a microcentrifuge at 500 x g (2475 rpm) for 10 minutes. If liquid is still visible in the concentrator, spin for additional time. Caution: Do not spin to dryness.
7. Remove the spin cap and add a measured volume of TE or appropriate solution that is between 30µl and 200 µl to the microconcentrator.

8. Remove the microconcentrator from the filtrate cup and carefully invert the microconcentrator onto a labeled retentate cup (i.e. 1.5 ml tube). Discard the filtrate cup.
9. Spin the assembly in a microcentrifuge at 500 x g (2475 rpm) for 5 minutes.
10. Discard the microconcentrator. Make sure that the volume is no less than 30 µl. Cap the retentate cup or transfer to a new tube for long term storage.
11. The samples are now ready for DNA quantitation and PCR. These samples need to be labelled DNA # and C.

Store the samples at 2-6⁰ C (short term storage) or freeze at -15 to -20⁰C (long term storage). Prior to the use of samples after storage, vortex briefly and spin in a microcentrifuge for 5 seconds.

Microcon References:

- Anderson T, Comey CT, Fisher DL, Medintz I, Scherczinger 1997, Greenspoon S; VS Extraction, VS Mock Samples, VS Bone Extraction, VS Buccal FTA, VS PowerPlex 16, VS DNA IQ
- Microcon Operators Manual, Millipore 2000.
- Forensic DNA Typing: Biology and Technology behind the STR Markers. John M. Butler. Academic Press 2001.

2.5 Maxwell 16 Robot

Introduction: The Maxwell 16 Robot uses the DNA IQ chemistry.

2.5.1 Preparation of DNA standards for the Maxwell 16

Note: If precipitate forms, warm the Lysis Buffer at 60⁰C until it clears (no more than 5 minutes)

1. Cut sample depending on type (these are minimal amounts):
 - Blood standard – two 3mm² cuttings
 - Buccal swab – ½ swab
 - Buccal scrubber – 1 tooth
 - Buccal foam – 1/8 sponge
2. Add 500µl of Lysis Buffer and 5µl of 1M DTT to a 1.5mL or 2.0 mL tube.
3. For blood samples: place in 70⁰C heat block or thermal mixer at 250 to 900 rpm for one hour.

For buccals: place in 70°C heat block or thermal mixer at 250 to 900 rpm for 30 minutes.

- When extracting blood and buccal swabs in the same set, the reagent blank will remain in the thermal mixer for the entire hour. On the extraction sheet a *** will be made by the sample(s) and the corresponding reagent blank that this has been done.
4. Quick spin the tube to remove any precipitation from the lid.
 5. Carefully open the lid, transfer the sample to a Spin basket, replace in tube.
 6. Centrifuge at room temperature for 2 minutes at maximum speed.
 7. Carefully remove the Spin Basket. Close lid and save for Maxwell.

2.5.2 Maxwell 16 Automated DNA purification

1. Place the number of cartridges to be used into the cartridge preparation rack. Place each cartridge into the holder with the ridged side of the cartridge facing towards the numbered side of the rack. Hold the cartridge firmly and remove the seal.
2. Place the plunger into well #7 of each cartridge (the well closest to you).
3. Transfer your sample into well #1 (the well furthest away).
4. Turn the Maxwell 16 Instrument on.
5. Open the door, press the “Run/Stop” button to extend platform.
6. Transfer cartridges from the cartridge preparation rack onto the Maxwell 16 platform. Ensure that the cartridges are placed into the Maxwell 16 Instrument with the ridged side of the cartridge closest to the door.
 - If you are processing fewer than 16 samples, center the reagent cartridges on the platform, spacing them evenly outwards from the center.
7. Place blue Elution Tubes into the elution tube slots at the front of the platform. Add 300 µl of Elution Buffer to each elution Tube
8. Press the “Run/Stop” button. The platform will retract. Close the door.
9. The Maxwell 16 instrument will immediately begin the purification run.
 - Pressing the “Run/Stop” button or opening the door will cause the run to stop. Close the door (if open), and select whether to continue or terminate the run.

- If the program is terminated before completion, the instrument will wash the particles off the plungers and eject the plungers into well #1 of the cartridge.
10. When purification is complete, the LCD screen will display a message that the method has ended. Upon method completion, open the instrument door. Check to make sure that all of the plungers have been removed from the magnetic rod assembly. If the plungers have not been removed, push them down gently by hand to remove them from the magnetic rod assembly.
 11. Press the “Run/Stop” button to extend the platform.
 12. Remove the Elution tubes from the heated elution tube slots, and place them into the Magnetic Elution Tube Rack. Any residual DNA IQ resin will be captured along the back of the blue elution tube. Transfer the eluted samples into the storage tubes by pipetting.
 13. Remove cartridges and plungers from the instrument platform and discard.
 14. Use one of the scroll (up/down) buttons to move the cursor to select “Yes” or “No” to run the purification method again.

Store the samples at 2-6° C (short term storage) or freeze at -15 to -20° C (long term storage). Vortex briefly and spin in a microcentrifuge for 5 seconds prior to quantification or amplification. If desired, the sample may be concentrated with the Microcon unit.

Maxwell References:

- Kephart PhD, Dan; Kruefer, Steve; Grunst, Terri; and Sheno, PhD Hemanth. A Maximum Instrument at a Minimum Size, Introducing the Maxwell 16 Instrument: A Simple, Robust and Flexible Tool for DNA Purification.
- Mandrekar, Paraj V; Bessetti, Joseph; Newton, Christine; Krueger, Julia; and Cowan, Cris. Improved Performance of the Maxwell 12 Low Elution Volume System for Forensic Casework. Promega DNA Profiles.
- CMPD Internal Validation

2.6 PrepFiler Express™ with the AutoMate

Introduction: This extraction involves the lysis of samples using the PrepFiler Express™ Forensic DNA Extraction Kit or the PrepFiler Express™ BTA Forensic DNA Extraction Kit and the extraction and clean up of those samples using the AutoMate Express™. A mask must be worn when using this instrument.

2.6.1 Preparation of DNA samples using PrepFiler Express™ Forensic DNA Extraction Kit (body fluids)

Suggested amounts added:

Liquid blood/saliva	up to 5 µL
Blood on substrate	up to 5mm ²
Saliva on fabric	up to 5mm ²
BF on swabs	up to 1/8 swab (more can be added if dilute)
Touch DNA swabs	up to 1 swab
Hair root	up to 5mm

1. If buffer contains precipitate, heat the solution to 37°C, then vortex the bottle for 5 seconds.
2. Bring the thermal shaker temperature to 70°C.
3. Prepare a fresh 1.0 M DTT solution by dissolving 1.54 g of Dithiothreitol (DTT) in 10 mL of molecular-biology grade DNA-free water/ or thaw an aliquot of the desired volume that you previously prepared fresh, then stored at -20 °C for up to a year.
4. Prepare a fresh PrepFiler lysis solution. Each sample requires: 500 µL PrepFiler Lysis buffer and 5 µL freshly-prepared 1 M DTT.
5. Insert a PrepFiler LySep column into a hingeless PrepFiler sample tube, then carefully transfer the sample into the PrepFiler LySep column.
6. Add 500 µL of freshly prepared PrepFiler lysis solution to the column/tube assembly.
7. Tightly close the lid of the column/tube assembly.
8. Place the column/tube assembly in a thermal shaker, then incubate it at 70 °C and 750 rpm for 40 minutes.
9. Centrifuge the column/tube assembly for 2 minutes at 10,000 x g to transfer the lysate to the sample tube.
10. If the volume of sample lysate collected in the sample tube is less than 300 µL:
 - a. Centrifuge the column/tube assembly for an additional 5 minutes.
 - b. If the volume is still less than 300 µL, then add enough PrepFiler Lysis Buffer to bring the lysate volume up to 300 µL.

NOTE: a 300 µL lysate volume is necessary for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the

tip during the automated extraction run. Lower lysate volume may cause liquid handling problems.

11. Complete substrate removal as follows:

- a. Carefully remove the PrepFiler LySep column from the samples tube. If there is clear lysate remaining in the PrepFiler LySep column, transfer the lysate to the sample tube.
- b. Properly dispose of the PrepFiler LySep column. Used LySep columns are potentially hazardous.
- c. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler Sample Tube (sediment may cause liquid handling problems during the automate run).
- d. If you observe any salt precipitation, heat the lysate to 37 °C until the precipitate goes back into solution, then use a pipette to mix the sample lysate. Do not load any sample tube that contains precipitate onto the AutoMate Express.

12. Proceed to 2.6.3.

2.6.2 Preparation of DNA samples using PrepFiler Express™ BTA Forensic DNA Extraction Kit (for bone, teeth and adhesives)

Approximate amounts added:

Bone	up to 50mg powdered
Teeth	up to 50mg powdered
Chewing gum	1 swab
Cigarette Butt	up to 25 mm ² (paper only, no filters)

1. Add sample to PrepFiler tube.
2. Bring the thermal shaker temperature to 56°C.
3. A fresh 1.0 M DTT solution by dissolving 1.54 g of Dithiothreitol (DTT) in 10 mL of molecular-biology grade DNA-free water/ or thaw an aliquot of the desired volume that you previously prepared fresh, then stored at -20 °C for up to one year.
4. Prepare a fresh PrepFiler BTA lysis solution. Each sample requires: 220 µL PrepFiler Lysis buffer, 3 µL freshly-prepared 1 M DTT, and 7 µL Proteinase K.

5. Add 230 μL of freshly prepared PrepFiler BTA lysis solution to the column/tube assembly.
6. Tightly close the lid of the column/tube assembly, and vortex briefly.
7. Place the column/tube assembly in a thermal shaker, then incubate it according to sample type:
 - For bone and teeth: at 56°C , 1100 rpm for at least 2 hours up to 18 hours.
 - For adhesive substrates: at 56°C , 750 rpm for 40 minutes.
8. Centrifuge the column/tube assembly according to sample type:
 - For bone and teeth: 90 seconds at 10,000 x g to transfer the lysate to the sample tube.
 - For adhesive substrates: 2 minutes at 10,000 x g to transfer the lysate to the sample tube.
9. If the volume of sample lysate collected in the sample tube is less than 150 μL :
 - a. Centrifuge the column/tube assembly for an additional 5 minutes.
 - b. If the volume is still less than 150 μL , then add enough PrepFiler BTA Lysis Buffer to bring the lysate volume up to 150 μL .

NOTE: a 150 μL lysate volume is necessary for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated extraction run. Lower lysate volume may cause liquid handling problems.

10. Prepare sample to load:
 - a. Carefully remove the PrepFiler LySep column from the samples tube. If there is clear lysate remaining in the PrepFiler LySep column, transfer the lysate to the sample tube.
 - b. Properly dispose of the PrepFiler LySep column. Used LySep columns are potentially hazardous.
 - c. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler Sample Tube (sediment may cause liquid handling problems during the automate run).
 - d. If you observe any salt precipitation, heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the sample

lysate. Do not load any sample tube that contains precipitate on the AutoMate Express.

2.6.3 AutoMate Express Set up and Sample Loading

1. Open the instrument door by pushing up, then remove the cartridge rack and tip and tube rack from the instrument.
2. From the main menu, press **1** to display the “manual” menu. Press **3** for Clean, and then press **1** to lower the piercing unit. Open the instrument door. Wipe all prongs with Ethanol on a Low-lint Wipe. Press **Esc** to get back to the main menu.
3. Shake and tap the reagent cartridges to resuspend the magnetic particles and to deposit any particles or liquid from the foil.
4. Load the cartridges into the rack by sliding each reagent cartridge along the groove towards the back of the rack until it clicks into place. Make sure that the notches in the cartridges align with those in the rack.
5. Insert the loaded cartridge rack into the instrument.
6. Load the tip & tube rack:
 - Row S (fourth row): Load with PrepFiler sample tubes containing the lysate. This tube must be placed in the rack gently, or the Lysep column left on until the tube is in place. Avoid splashing of the contents.
 - Row T2 (third row): Load with AutoMate express Tips inserted into tip holders.
 - Row T1 (second row): Leave empty
 - Row E (first row): Load with labeled PrepFiler elution tubes with the caps open and secured.
7. Insert the loaded tip & tube rack into Row E in the front of the instrument and close the door.
8. Press Start, then press the “enter” button through the prompts
 - For PrepFiler Express kit, press 1
 - For PrepFiler Express BTA kit, press 2
9. Press “START”. *A screen should show the steps and the approximate run time remaining.*

DO NOT OPEN THE DOORS ONCE THE RUN HAS STARTED.

10. At the end of the run, press the “enter” button to continue and to return to the main menu and open the instrument door.

11. Remove the cartridge rack and the tip & tube rack.
12. Remove and cap the elution tubes containing your purified DNA.
13. Close the instrument door. After each run clean the tip and tube rack.

Store the samples at 2-6° C (short term storage) or freeze at -15 to -20° C (long term storage). Vortex briefly and spin in a microcentrifuge for 5 seconds prior to quantification or amplification. If desired, the sample may be concentrated with the Microcon-100 unit.

PrepFiler/AutoMate References:

- JFS, 7/2012, Vol. 57 No. 4 pp.1022-1030 Liu, et al.
- Applied Biosystems by life technologies, PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits, User Guide
- Applied Biosystems by life technologies, AutoMate Express™ Instrument, User Guide
- CMPD Internal Validation Study

3. Quantitation Procedure

A. Purpose and Introduction

DNA analysis of forensic samples is dependent upon the quantity and quality of the DNA present in an evidentiary sample. The purpose of quantitation is to determine the amount of human DNA present in a sample. The Quantifiler Trio Kit quantifies human DNA at a small autosomal target (80bp), a large autosomal target (214bp) and a Y-chromosome target (75bp). Each target consists of PCR primers and dye-labeled TaqMan® probes for the amplification of multicopy genomic loci. The reporter dye (VIC, ABY, and FAM, respectively) is linked to the 5' end of the probe and a non-fluorescent quencher at the 3' end of the probe. The 5' nuclease assay process occurs during PCR amplification, during every cycle. During PCR, the TaqMan probe anneals specifically to a complementary sequence between the forward and reverse primer sites. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. AmpliTaq Gold DNA polymerase cleaves probes that are hybridized to the target. Cleavage separates the reporter dye from the quencher dye. This results in increased fluorescence by the reporter. The increase in fluorescence signal occurs only if the target sequence is complementary to the probe and is amplified during PCR. Therefore, non-specific amplification is not detected.

B. Materials and Instrumentation

- Quantifiler™ Trio Kit (Life Technologies 4482910)
- TE-4 Buffer
- Desktop computer with Life Technologies HID Software, Version 1.2
- ABI Prism 7500 SDS instrument
- Hoods
- Laptop computer with Qiagility Software, Version 4.17.1.03
- Qiagility instrument
- Consumables as described in the procedure

C. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

D. Minimum Standards and Controls

The Quantifiler Trio kit contains a pooled male DNA standard that is diluted to prepare five DNA standards. These standards represent the following quantities: 50, 5, 0.5, 0.05 and 0.005. Results are obtained through PCR amplification of the size standards and samples. A size standard curve is generated by the 7500 HID

1.2 software, after which the threshold cycle values (C_t) for the case samples are compared to the size curve. By comparison to the C_t values of the size curve, the software can determine the approximate concentration of the case sample DNA.

In addition, the Quantifiler Trio kit contains an internal positive control (IPC) of a defined quantity that acts to confirm the efficiency of the PCR amplification. Normal detection of the IPC allows for confirmation that the amplification occurred as expected, while abnormal or no detection is an indicator of an inefficient or failed amplification and suggests inhibition.

E. Other Considerations

When the IPC is normal and the case sample quantity is less than or equal to 2 picograms, then no amplification has to occur on unknown samples. No notation needs to be made. If however, an analyst continues on to amplification a note will be made as to why.

An elevated (more than 2 points) IPC suggests that inhibition has occurred and the sample should be first diluted then cleaned using the Microcon.

If the degradation index is >3 , the amplification target should be increased. Target should not exceed 2 ng. If the analyst believes that targeting more than 2 ng is necessary, then they should consult a DNA Team Leader or the Technical Leader.

F. Validation

The Quantifiler technology is generally accepted in the forensic community.

These procedures have undergone internal validation at the CMPD.

3.1 Manual Quantification of DNA Using Quantifiler Trio

Preparation in pre-amplification area:

1. If necessary, thaw the primer mix, standards, and case samples. Gently vortex and pulse-spin each before opening. Fill out the "7500 Setup" worksheet throughout the procedure, as appropriate.
2. Prepare the human DNA standards by labeling 5 tubes (1-5). Add 90 ul Dilution Buffer to tubes 2-5 and 10 ul Dilution Buffer to tube 1.
 - a. Add 10 ul of Quantifiler Trio Standard to tube 1 and mix thoroughly.
 - b. Add 10ul from the tube containing standard that was just prepared to the next tube in the series and mix thoroughly.

- c. Repeat step “b” for tubes 3-5.
3. Set up a 96 well optical reaction plate. The DNA Standards may be placed anywhere on the plate. The default template will have the Standards as follows: Standards 1-5 in A1-E1, respectively, and Standards 1-3 in F1-H1, respectively, and Standards 4-5 in A2-B2, respectively
4. Prepare at least the following volume of master mix:

PCR Reaction mix = 10 µl x (number of samples +1)
Primer mix = 8 µl x (number of samples +1)
5. Vortex the master mix thoroughly, then briefly centrifuge.
6. Dispense 18 µl of the master mix in the appropriate wells. Make certain to avoid pipetting bubbles into the well.
7. Add 2 µl of the standards or samples to the appropriate wells. Make certain to avoid pipetting bubbles into the well.
8. Seal the plate with an Optical Adhesive Cover. Spin the plate in the amp room.

Run preparation in amplification room:

9. Press the tray door to open it.
10. Load the plate into the plate holder in the instrument. Ensure that the plate is correctly aligned in the holder.
11. Load standard 96-well plates with the notched A12 position at the top-right of the tray.
12. Close the tray door.

Preparation of plate document:

13. Start the ABI Prism 7500 HID v1.2 software and select the Trio icon from the Home screen.
14. Name the plate using the format “initials”_”MMDDYY” and save.
15. Either import the sample set-up electronically, or list each sample into the correct well in the View Plate Layout tab.
16. Click the green **Start Run** button.
17. When the run is completed, turn off the instrument.

Analysis and Interpretation:

18. The analysis settings for Trio can be located by clicking the **Analysis Settings** button. The settings should be as follows:

Threshold: 0.2 (IPC only, 0.1)
 Baseline Start (cycle): 3
 Baseline End (cycle): 15

19. To analyze the samples, select the green **Analyze** button.
20. View the Standard Curve for the small target. Confirm that the standard curve conforms to the following values:

<u>Slope, Quantifiler</u>	<u>Y-intercept, Quantifiler</u>	<u>R² value</u>
-3.1 to -3.5	26.5 to 27.5	>0.98

- a) If necessary to delete replicates to bring the curve into compliance, first obtain the Technical Leader or their designee's approval. Otherwise, the run will be deemed invalid. If a standard is omitted, it will import into the Trio results worksheet as a "0". No other notation needs to be made.
- b) If the Y-intercept value is outside the noted range, it will be noted on the slope as Y-low or Y-high.
21. View the calculated values for the case samples by selecting the **View Well Table** tab.
22. Examine all samples for an IPC C_T flag.
23. Interpretation of the results will be based on the following table:

Quantifiler Trio result	IPC result	Interpretation
< or = 2.0 pg	OK	No STR amplification
No amplification	No amplification	Invalid QPCR result – possible inhibition
Amplification (low C _t value)	No amplification	Disregard IPC
Amplification (high Ct value)	No amplification	Partial PCR inhibition
Amplification, >5 ng/uL	Amplification appears reduced	High sample concentration may suppress IPC amplification

Note: If the sample(s) that are $<$ or $=$ to 2.0 pg are from a Cold Case or the only sample within a case then they can be amplified, if deemed necessary based on the degradation index. If PCR inhibition is suspected, after the sample has been diluted or cleaned using the Microcon procedure it will be re-quantified before amplification. Invalid results will require a re-quantification.

24. Each case file will contain at least a copy of the small target standard curve values and the quantity values for the standards and the samples for each case.

3.2 Qiagility Quantification of DNA Using Quantifiler Trio

Preparation in pre-amplification area:

1. Set up samples to be quantitated from the DNA extraction worksheets.
 - a. Every sample in the batch must have a DNA number and the DNA numbers within a batch must be in alpha-numeric order.
 - b. In the worksheet "7500-Prep," click the "Quant Setup" button. DNA extracts MUST be in either PrepFiler Express 1.5ml tubes (catalog number 4443226) or USA Scientific 0.5ml tubes (catalog number 1605-000#, where # = can be any integer).
 - i. Right click the tab and choose "move or copy." Create a new copy and then move that copy "To Book: (new book)" (in the upper drop-down list).
 - ii. Save the new worksheet book as a *.csv file on a flash drive.
 - c. Log into the Qiagility as "Qiagility User" and insert the flash drive into the Qiagility computer.
 - d. If necessary, thaw the primer mix, standards, and case samples. Gently vortex and pulse spin each.
2. Open the appropriate QT template being used by double-clicking on the desktop icon. Confirm the deck lay-out for the available tips is the same layout as on the Qiagility software and adjust as necessary. Qiagility 50 μ l Tips are Qiagen catalog number 990512. If adding a new set of tips to the tip holder, first clean the tip holder with purified water and wipe dry.
3. From the Qiagility software, import the samples from the files created in Step 1. Click on the appropriate block (selected block will have a red border). From the right hand window, click "Import."
4. Open the Pre-Run Report and set up the DNA extracts, reagents and tubes on the Qiagility deck as specified in the report. Open and fill out the "7500 Setup" worksheet as appropriate.

- a. Enter the total master mix needed (from the Pre-Run Report) to the 7500 Setup Worksheet. Prepare the master mix in a Fitzco 2.0ml tube according to the volumes listed on the worksheet. Vortex and pulse-spin the master mix.
 - b. If pre-made Quantifiler Trio Standard dilutions are not already made, then choose the template to make standards and add 25ul Dilution Buffer and 25ul Quantifiler Trio Standard to the first Standard tube.
 - c. The consumables to be used are:
 - i. The **1.5ml tapered screw-cap tube** in block **M1** is Promega 1.5ml catalog number V1231.
 - ii. The **200 μ l PCR tube** in block **R1** is Life Technologies 0.2ml catalog number N8010540.
 - iii. The **2.0ml self-standing screw cap tube** in block **R1** is a Fitzco 2ml tube, catalog number 02065.
 - iv. The **200 μ l PCR tube** in block **C1** is Life Technologies 96-well plate catalog number 4306737.
 - v. If adding tips to a block, select the wells in the block, right click, and select "Available."
 - d. The volumes in the Pre-Run Report are minimum values – the listed tubes *must* have *at least* the volumes displayed. They may have larger volumes, but unnecessary waste of reagents is to be avoided.
 - e. Save the Pre-Run Report to **C:\Program Files\QIAGEN\QIAGEN Software\QIAgenius\QIAgenius Data\Pre-Run Reports** and then close it. Use the default name but add your initials to the end. Pre-Run Reports are not printed or saved to the case file.
 - f. Ensure all the tubes are uncapped (& hinged caps are out of the way) and seated firmly.
5. Close the QIAGEN lid. Click the green arrow to start the run. Save the template with the default name but add your initials to the end. Check off the boxes as appropriate and click OK.
 6. After the QIAGEN run has completed, seal the plate with an Optical Adhesive Cover. The plate may be spun in the amp room if bubbles are present.
 7. Save the Post-Run Report to **C:\Program Files\QIAGEN\QIAGEN Software\QIAgenius\QIAgenius Data\Post-Run Reports**.
 - a. Paste-special (html) the first four rows of the first table in the Post-Run Report to cell A1 in the Post-Run worksheet.
 - b. If there are exceptions, then copy from the beginning of the phrase "Exceptions occurred during this run" to the last cell under "User Response." If there are

more than three rows of exceptions, you must stop and wait for the assistance of the Technical Leader or their designee. After copying, paste-special (html) into cell A7 in the Post-Run worksheet.

- c. In the Qiagility Post-Run Report “Reaction Results” table, copy from the word “Well” to the last cell in the “Status” column and paste-special (html) into cell A18 in the Post-Run Worksheet.
 - d. Print the 7500 Setup worksheet to PDF and store it on the flash drive. The 7500 Setup Report is included in the case file to document this procedure.
8. Click on block C1 (in the Qiagility software) and then select **Export**. Select “Custom, tab separated” from the drop-down menu in the pop-up window. Click **Export** and save the file to the desktop.
- a. Right click on the *.txt file and choose “Open With ► scalc.” In the resulting pop-up window, make sure the import is using the Western Europe character set, is importing from row 1, and is separated by Tab (green check in the box). Delete the “character in the “Text Delimiter” box. Select “OK.”
 - b. Click the green “X” to activate the import macro.
 - c. Save the file as a “Text CSV (.csv)” on your flash drive. If you receive a pop-up window while saving, choose “Keep Current Format.”

Run preparation in amplification room:

9. Bring the 7500 reaction plate and flash drive into the amplification room. Insert the flash drive into the 7500 computer and pulse-spin the plate.
 - a. Press the tray door on the 7500 to open it. Load the plate into the plate holder in the instrument. Ensure that the plate is correctly aligned in the holder. Load standard 96-well plates with the notched A12 position at the top-right of the tray. Close the tray door.
 - b. Find the *.csv file and change the “csv” in the file name to “txt.”
10. Turn on the 7500 if it’s not already on. If not opened, open the HID v1.2 and select the Trio icon on the Home Screen.
11. Name the plate using the format “initials”_”MMDDYY” and save.
12. Either import the sample set-up electronically, or list each sample into the correct well in the View Plate Layout tab.
13. Click the green **Start Run** button.

14. When the run is completed, turn off the instrument.

Analysis and Interpretation:

15. The analysis settings for Trio can be located by clicking the **Analysis Settings** button. The settings should be as follows:

Threshold: 0.2 (IPC only, 0.1)
 Baseline Start (cycle): 3
 Baseline End (cycle): 15

16. To analyze the samples, select the green **Analyze** button.

17. View the Standard Curve for the small target. Confirm that the standard curve conforms to the following values:

<u>Slope</u>	<u>Y-intercept</u>	<u>R² value</u>
-3.1 to -3.5	26.5 to 27.5	>0.98

a) If necessary to delete replicates to bring the curve into compliance, first obtain the Technical Leader or their designee's approval. Otherwise, the run will be deemed invalid. **If a standard is omitted, it will import into the Trio results worksheet as a "0". No other notation needs to be made.**

b) If the Y-intercept value is outside the noted range, it will be noted on the slope as Y-low or Y-high.

18. View the calculated values for the case samples by selecting the **View Well Table** tab.

19. Examine all samples for an IPC C_T flag.

20. Interpretation of the results will be based on the following table:

Quantifiler Trio result	IPC result	Interpretation
< or = 2.0 pg	OK	No STR amplification
No amplification	No amplification	Invalid QPCR result – possible inhibition
Amplification (low C _t value)	No amplification	Disregard IPC
Amplification (high C _t value)	No amplification	Partial PCR inhibition
Amplification, >5 ng/uL	Amplification appears reduced	High sample concentration may suppress IPC amplification

Note: If the sample(s) that are \leq to 2.0 pg are from a Cold Case or the only sample within a case then they can be amplified, if deemed necessary based on the degradation index. If PCR inhibition is suspected, after the sample has been diluted or cleaned using the Microcon procedure it will be re-quantified before amplification. Invalid results will require a re-quantification.

21. Each case file will contain at least a copy of the small target standard curve values and the quantity values for the standards and the samples for each case.

References

- Quantifiler User's manual, Life Technologies
- CMPD Validation or Performance Check
- Qiagility Validation

4. Amplification Procedures

A. Purpose and Introduction

Short tandem repeat (STR) genetic markers are polymorphic DNA loci that contain a repeated nucleotide sequence. The STR repeat unit can be from two to seven nucleotides in length. The number of repeat units at an STR locus differs from individual to individual, so alleles of many different lengths are possible. This high power of discrimination makes STRs useful for human identification purposes.

STR loci are amplified using the polymerase chain reaction (PCR). The AmpFSTR Identifiler kit amplifies all 13 of the CODIS core loci (TPOX, D3S1358, FGA, D5S818, CSF1PO, D7S820, D8S1179, TH01, vWA, D13S317, D16S539, D18S51, and D21S11) in addition to the gender marker Amelogenin and the loci: D2S1338 and D19S433. The Identifiler allelic ladder contains 205 alleles but does not include all known alleles, such as rare microvariants.

B. Materials and Instrumentation

- AmpFSTR Identifiler Kit
- TE⁻⁴ Buffer
- Perkin-Elmer thermal cycler model 9700
- Hood
- Laptop computer with Qiagility Software, Version 4.17.1.03
- Qiagility instrument
- Consumables as described in the procedure

C. Safety Considerations

Standard Laboratory Practices.

Warning: Potential biohazard.

D. Minimum Standards and Control

Positive Amplification Control: add 4µl Control DNA 9947A (supplied in kit) and 6 µl TE⁻⁴ to the reaction. An amplification positive control must be run with each amplification and will be labeled with the date of the amplification. If the amplification fails or the known type is not obtained, the results from the entire amplification are inconclusive. The Positive Control will be labeled on the electropherogram as "Positive Control" and the corresponding date of the amplification.

Reagent Blank: Add 10 µl of the reagent blank control to the reaction. Refer to DNA QA Manual section 15.5 (Contamination Contingency Plan) if a pattern of peaks are present in the reagent blank.

Amplification Blank: Add 10 µl of TE⁻⁴ to the reaction. Refer to DNA QA Manual section 15.5 (Contamination Contingency Plan) if peaks are present in the amplification blank. An amplification blank must be run with each amplification, and will be labeled with the date of the amplification. The Negative Control will be labeled on the electropherogram as "Negative Control" and the corresponding date of the amplification.

Positive and Negative controls associated with each sample set will be amplified using the same kit lot used for the forensic samples and will be concurrently run in the same thermal cycler, and on the same genetic analyzer.

All samples within a case that are amplified together will be run on the same 3130. If a sample is re-amplified or re-injected it will be run on the same 3130 as the original sample.

E. Other Considerations

Not applicable

F. Validation

This procedure is generally accepted in the forensic community.

This procedure has undergone internal validation at the CMPD.

4.1 MANUAL PROCEDURE

1. Label appropriate number of 0.2 ml MicroAmp tubes. Alternately, a 96 well plate can be used for amplification, plates wells need to be left empty for ladders and well positions are reflected on the 3130 Set up sheet.
2. Add the following reagents to a 0.5 ml or 1.5 ml pre-sterilized microcentrifuge tube in order:

- 10.5 µl AmpFSTR PCR Reaction Mix X number of samples
- 0.5 µl AmpliTaq Gold DNA Polymerase X number of samples
- 5.5 µl AmpFSTR Identifiler Primer Set X number of samples

Vortex gently in order to avoid drops of liquid on the sides and cap of the tube.

3. Add 15 µl of Master Mix to labeled 0.2 ml amplification tubes or to appropriate plate wells.
4. Add the appropriate amount of TE⁻⁴ and DNA to each tube (or to appropriate plate wells) to a final volume of 25 µl. Close the tubes or seal the plate with caps. Plate Samples diluted and amplified at 10 µl unless otherwise noted. The optimal template quantity for samples quantified with Quant Trio is 0.5 ng, although the analyst may add more if the quantitation results deem necessary. Note on the worksheet the approximate amount targeted for both Standards and Unknowns.
5. Place tubes or 96 well plate in thermal cycler, select file for Identifiler amplification parameters and start amplification.

Identifiler Amplification Parameters for 9700 Thermal Cycler (in 9600 emulation mode):

- 95° C for 11 minutes.
- 94° C for 1 minute.
- 29 cycles, each consisting of ramp to 94° C, 1 minute hold; ramp to 59° C, 1 minute hold; ramp to 72° C, 1 minute hold.
- 1 cycle at 60° C for 60 minutes.
- Hold at 4° C.

After amplification, store tubes protected from light in an amplified DNA dedicated refrigerator for up to 2 weeks or until completion of a technical review.

4.2 QIAGILITY PROCEDURE

1. Open the DNA Workbook and then open the "ID Setup" worksheet. If amplifying samples from multiple QPCR runs, a separate workbook for each quantitation is needed, and the results are merged into "ID Setup."
 - a. The Qiagility will ignore any samples that don't meet the normalization criteria. Samples that will not be amplified remain in order in their batch, but are given either "PFE_Skip" or "USA_Skip" banks.
 - b. Change the tube type in the "ID Setup" worksheet to match the tube types being loaded.
 - c. Confirm that all the tubes in a "skip" bank are to be skipped. If there are some that aren't marked "skip" that you want to skip, replace whatever is in the "concentration" column with an "x". For reagent blanks and samples that will be amplified even though the DNA concentration is zero, replace "No Data" or "Undet." with "0" (i.e., zero).
 - d. Right-click on the worksheet tab, select "Move or Copy...", and then select "Create a Copy." Right click on the new copy (now named "ID Setup (2)") and

then move it to a new book. Save as a *.csv file on a flash drive and take the flash drive to the Qiagility computer.

2. Log in to the PC-Qiagility and open the STR amplification template being used by selecting the shortcut on the desktop. Confirm the deck lay-out for the available tips is the same layout as on the PC-Qiagility and adjust as necessary. If adding a new set of tips to the tip holder, first clean the tip holder with purified water and dry with a low-lint wipe.
 - a. Identifiler only: The Qiagility will make dilutions of extracted DNA sample to the desired concentration of DNA in 10µl. The concentration of extracted DNA is determined from the QPCR procedure (Human target).
 - i. The amplification target is 0.50ng, although the analyst may add more if the quantitation results deem necessary. The amplification minimum for a single DNA donor is >0ng.
 - ii. The amplification threshold is 0ng.
 - iii. For ii and iii will those values change once threshold is established for Trio?
3. Insert the flash drive into the Qiagility computer. Select the block for each tube type being amplified, and then click "Import" on the right-hand menu. Import the sample names for the tubes (organized by tube size) containing extracted DNA to be amplified from the *.csv file.
 - a. Select the appropriate block for the samples to be added. Import sample names and IDs by clicking **Import** in the window on the right side of the screen.
 - b. Samples that are added to the dilution plate must be added to the appropriate dilution bank. Do not add any blank wells to the Dilution Bank.
4. Open the Pre-Run Report and open and fill out the "9700 Setup" worksheet as appropriate. Vortex and pulse-spin all sample tubes prior to adding to the Qiagility. Set up the DNA extracts, reagents and tubes on the Qiagility deck as specified in the report.
 - a. The consumables used are critical to the Qiagility's calibration.
 - i. In block **M1**, the **5.0ml QIAGEN QIAgility tapered tube** is a 5ml Tube (Qiagen catalog number 990552) and the diluent is TE.
 - ii. In block **R1**, the **2.0ml self standing screw cap tube** is the positive control tube from the Life Technologies kit.
 - iii. In block **R1**, the **2.0ml self standing screw cap tube** is a Fitzco (catalog number 02065) 2ml Tube and its contents are the amplification master mix to be made in the next step.
 - iv. The **200µl PCR tube** in blocks **B1** and **C1** is an Amplification Plate (Life Technologies catalog number 4306737).

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- v. Sample tube (1.5ml & 500µl) in block **B2** or **C2** is either a PFE elution tube or an 0.5ml tube from USA Scientific (Catalog number 1605-000#), respectively.
 - vi. Qiagility 50µl Tips are Qiagen catalog number 990512.
 - b. Enter the total master mix needed to the 9700 Setup Worksheet. Prepare the master mix in a Fitzco 2.0ml tube according to the volumes listed on the worksheet. Vortex and pulse-spin the master mix.
 - c. The volumes in the Pre-Run Report are minimum values – the listed tubes *must* have *at least* the volumes displayed. With the exception of the amplification master mix, they may have larger volumes, but unnecessary waste of reagents is to be avoided.
 - d. Save the Pre-Run report to **C:/QiagilityDocs/Pre-Run Reports** and then close it. Pre-Run reports are not printed or saved in the case file.
 - e. Ensure all the tubes are uncapped (& hinged caps are out of the way) and seated firmly.
5. Click on the plate in block **C1** and rename the positive and negative controls in the ID column (in the **Sample Data** window to the right). The names must contain at least “pos” and “neg,” respectively, somewhere in the name.
6. Close the lid and click the green arrow to start the run.
 - a. Save the template with the date stamp in the name when prompted.
 - b. In the resulting pop-up window, check off the boxes that everything is ready (as appropriate). Depending on the concentration of samples being amplified, the software may indicate a problem in the normalized samples (volume <1.0µl). This will not affect the run, so this box can be “checked.” Click OK.
7. Save the Post-Run Report to the Post-Run Reports folder. Also select the “Post-Run” tab in the 9700 Setup worksheet.
 - a. Paste-special (html) the first four rows of the first table in the Post-Run Report to cell A1 in the Post-Run worksheet.
 - b. If there are exceptions, then copy from the beginning of the phrase “Exceptions occurred during this run” to the last cell under “User Response.” If there are more than three rows of exceptions, you must stop and wait for the assistance of the Technical Leader or their designee. Is this still true? I remember an email about it but don’t recall the details. After copying, paste-special (html) into cell A7 in the Post-Run worksheet.

- c. In the Qiagility Post-Run Report “Reaction Results” table for block **C1**, copy from the word “Well” to the last cell in the “Status” column and paste-special (html) into cell A18 in the Post-Run Worksheet.
 - d. In the 9700 Setup Worksheet, select the thermalcycler to be used.
 - e. Print the 9700 Setup worksheet to PDF and store it on the flash drive. The 9700 Setup Report is included in the case file to document this procedure.
8. Click on block **B1** and name the plate “dil” if it’s not already so named. Then select **Export**. Select “**Custom, tab separated**” from the drop-down menu. Click **Export** and then copy the resulting *.txt file to the flash drive. Repeat for block **C1**.
 9. Seal the plate with caps. Close and store the DNA extract tubes. Transfer the plate to the model 9700 thermalcycler in the PAR as specified on the 9700 Setup Worksheet.
 - a. ID Only: Choose “**identifiler**” on the thermalcycler, which amplifies according to the following parameters:
 - 95°C for 11 minutes
 - 29 cycles of
 - 94°C for 1 minute
 - 59°C for 1 minute
 - 72°C for 1 minute
 - 60°C for 60 minutes
 - 4°C until the samples are removed from the thermalcycler
10. At a networked computer, open the DNA workbook. For each exported plate,
 - a. From the workbook, open the .txt file; the file is delimited by space.
 - b. Click on the upper-left hand corner to select the entire sheet and **copy**.
 - c. Paste into the respective tab in the DNA workbook (**dil** for the dilution plate) by selecting cell A1.
 11. Load samples in the QiaAmp worksheet by selecting the first button. The second button ensures that a sample was not removed from the batch after QPCR but before amplification. Click the third button to finish preparing the injection list.
 - a. Adjust the 3130 import worksheet by adding plate name, initials, the ladder (including plate well), etc.
 - b. Create a copy of the 3130 worksheet, move the copy to its own book, and then save it to the flash drive as a .txt file.

12. After amplification, store the amplification plate protected from light in a refrigerator dedicated to amplified DNA for up to 2 weeks or until completion of a technical review.

References

- JFS Nov. 2004 Vol. 49, No. 6 Collins, Hennessy, Leibert, Roby, Reeder, and Foxall
- AmpFISTR Identifiler PCR Amplification Kit User's Manual.
- Characterization of Human Lymphoid Cell Lines GM9947 and GM9948 as Intra- and Interlaboratory Reference Standards for DNA Typing. C.J. Fregeau, et al. (1995) Genomics 28:184-197
- GeneAmp PCR Thermal Cycler, Perkin-Elmer 9600/2400/9700 Users Manual.
- Forensic DNA Typing: Biology and Technology behind the STR Markers. John M. Butler. Academic Press 2001.
- National Institute of Standards & Technology, Certificate of Analysis for Standard Reference Material 2391, PCR-based DNA Profiling Standard (May 26, 1995).
- CMPD Internal Validation Study
- Qiagility Validation

5. Sample Preparation for the 3130 Genetic Analyzer

A. Purpose and Introduction:

This procedure provides a method for preparation of amplified samples for STR analysis by capillary electrophoresis on the 3130xl Genetic Analyzer.

B. Materials and Instrumentation

- GeneScan-LIZ Internal Size Standard (GS500)
- Hi-Di Formamide
- Hood

C. Safety Considerations

- Appropriate safety precautions should be taken and proper personal protective equipment worn when working with hazardous or potentially hazardous materials.
- **WARNING! CHEMICAL HAZARD.** Formamide is a teratogen and is harmful by inhalation, skin contact, and ingestion. This should be used in a biological safety cabinet or under a chemical hood.

D. Minimum Standards and Controls

Not applicable

E. Other Considerations

- Formamide is needed to help the amplified product stay in the denatured state. However, over time formamide will degrade into formate. This will lead to preferential injection of formate ions over DNA resulting in decreased sensitivity. The GS500 will also take on a "Golden Gate" appearance.
- Ionized formamide (containing formate ions) does not appear frozen when removed from the freezer and should be discarded.
- Diluted amplified product will lose sensitivity over time. Samples in formamide greater than one week old should be used with caution or discarded and fresh dilutions made.

F. Validation

- The use of formamide to denature DNA is generally accepted in the scientific community (See References 8.0).

- Capillary electrophoresis for the analysis of DNA fragments is generally accepted in the scientific community (See References 8.0).
- The use of the 3130 Genetic Analyzer was validated internally at the CMPD.

Procedure:

Prepare the Formamide: Size Standard Mixture

- GeneScan -500 LIZ 0.3 μ L x number of sample
 - Formamide 8.7 μ L x number of samples
1. Add 9 μ L of the Formamide: Size Standard Mixture to the wells being used on the 96 well reaction plate. The 3130 injects samples in sets of 4. If the number of samples does not make a complete set of 4, fill the remaining wells with formamide. If the capillary is going into a well it needs liquid in it so air is not injected into the capillary.
 2. Add 1 μ L of sample, controls, or allelic ladder to the appropriate well. An allelic ladder will be placed approximately every 16 samples, and each run will contain two ladders.
 3. Cover the reaction plate with a 96 well plate septa.
 4. Briefly spin the reaction plate in the centrifuge to ensure that the contents of each well are mixed, collected at the bottom, and all bubbles removed.
 5. To denature, heat the reaction plate in the thermal cycler at 95° C for 3 minutes.
 6. Place the reaction plate immediately in the freezer or on ice for 3 minutes.
 7. Place the sample plate into the plate base.
 8. Snap the plate retainer onto the plate and the plate base.
 9. Verify that the holes of the plate retainer and the plate septa are aligned. Damage to the array tips will occur if not aligned properly.
 10. Place plate on instrument.
 11. Import sample list using the 3130 set up sheet described in 6.1.

References:

- CMPD Internal Validation
- AmpFISTR Identifiler PCR Amplification Kit User's Manual. Life Technologies.
- Forensic DNA Typing: Biology and Technology behind the STR Markers. John M. Butler. Academic Press 2001.
- ABI Prism 3100 and 3100 Genetic Analyzers – Protocols for the Processing of AmpFISTR PCR Amplification Kit PCR Products. Users Bulletin.

6. ABI 3130 Genetic Analysis

A. Purpose and Introduction:

This procedure provides a method for DNA typing of amplified product using the Applied Biosystems 3130 Genetic Analyzer. It covers the capillary electrophoresis technique and the collection software.

Spatial: The 3130 genetic analyzer Data Collection software uses images collected during the spatial calibration to establish a relationship between the signal emitted by each capillary and the position where that signal falls on and is detected by the CCD camera.

Spectral: A spectral calibration creates a matrix that is used during a run to reduce raw data from the instrument to the 5-dye data stored in sample files.

B. Materials and Instrumentation:

- Applied Biosystem 3130 Genetic Analyzer
- PC computer running Life Technologies 3130 Series Data Collection Software v. 4.0
- 1X Genetic Analyzer Buffer
- Capillary Array, 36cm
- 3130 POP-4 Performance Optimized Polymer (POP-4)
- Nuclease Free Water
- Hi-Di Formamide
- Multi-Capillary DS-33 (Dye Set G5) Matrix Std. Kit

C. Safety Considerations:

- **Electrical Shock Hazard:** The ABI Prism 3130 capillary electrophoresis unit contains a high voltage power supply. Handle with caution. Under no circumstances should any safety system be bypassed. Arcing may result from incomplete drying of instrument components.
- **Laser Hazard:** The ABI Prism 3130 contains a laser. Operate only with doors closed. This unit should be serviced by Life Technologies personnel only.
- Formamide is an irritant and suspected teratogen that causes irritation to the eyes, skin and mucous membranes. Do not inhale or ingest. Use only in a ventilation hood. Wear appropriate protective equipment. Dispose of properly.
- Only Nuclease –Free water (NF) will be used on the instruments and in buffers.

D. Minimum Standards and Controls:

Spectral Calibration: Is required once a year unless needed before due to the following:

- a new dye set is used on the instrument
- a capillary array is installed or replaced
- the capillary length or polymer type is changed
- after the laser or CCD camera has been realigned/replaced by a service engineer
- when a decrease in spectral separation (pull-up and or/pull down peaks) is observed in the raw or analyzed data

Spatial Calibration: Is required when:

- a capillary array is installed or replaced
- the capillary array is temporarily removed from the detection block
- the instrument is moved

Autosampler Calibration: done only as needed.

Symptoms of autosampler alignment problems may include: poor injection for a small number of capillaries, low signal strength, or no evidence of sample.

Changing the Capillary Array: as needed.

E. Other Considerations:

The following problems may indicate that a new capillary array is required:

- poor sizing precision or allele calling
- poor resolution and/or decreased signal intensity

Maintenance:

- Daily (when instrument is being used): Add buffer to the reservoir in position 1 and (NF) water to the reservoirs in position 2 and 4 (position 3 is left empty), and to the vial near the polymer bottle
- Bi-weekly: Warm water wash using the Water Wash Wizard, replace the polymer using the Replenish Polymer Wizard (can be replaced sooner, if needed), flush the water trap and restart the computer.
- Monthly: Defragment the computer hard drive
- Every six months: Clean the Database

Manager Wizards:

- Install Array
- Change Polymer Type
- Replenish Polymer
- Bubble Remove
- Water Wash
- Instrument Shutdown
- Autosampler Calibration

- Update Capillary Array Info

Re-injections from original plate:

If the plate is still on the instrument, then samples can be re-injected as long as it has not been 24 hours past the completion of the run.

If the plate has been unlinked or removed from the instrument, the plate cannot be re-injected and will need to be re-setup. On the new 3130 setup sheet the date will be of the actual run. All reinjections should contain a ladder and a positive control. In the case file there will be a 3130 setup sheet for each GMID-X project made.

All raw data must be saved to the R Drive. If re-setting up a plate the same day, then add _B, etc to the plate name.

F. Validation:

These procedures are generally accepted in the forensic community.

These procedures have undergone internal validation at the CMPD.

Notes:

1X Genetic Analyzer Buffer

- Preparing 50mL (for single run): Add 5 mL of 10X Genetic Analyzer Buffer to 45 mL of NF water; Mix Well.
- Preparing 250mL: Add 25 mL of 10X Genetic Analyzer Buffer to 225 mL of NF water; Mix Well.
- Once the 1X Genetic Analyzer Buffer has been made, it can be stored for up to 1 month at 2 to 8° C or at Room Temperature for 1 week.
- If Buffer was stored in the refrigerator, allow to come to room temperature before placing it on the instrument.

Performance Optimizing Polymer 4 (POP-4):

- Should be stored at 2 to 8° C until the expiration date printed on the bottle.
- Should be changed bi-weekly. The polymer is good at 25° C for approximately 14 days.
- The polymer should be allowed to reach room temperature before placing on the instrument.

6.1 Creating 3130 Setup Sheet and Injection List:

1. On the 3130 Prep tab: Click “Prepare for 3130” button.
2. Ensure that the Complaint #, DNA # or ID (Ladder, Positive Control, Negative Control), Item # and description are populated. Remove Ladders from portion of plate not used, and add a Ladder to the end of your samples (if

3. In the yellow fields populate: Plate Record (using format - Initials_MMDDYY), Initials, Date, Instrument #, and Number of wells that need the Formamide/Liz mix.
4. Click "Send to 3130 Input File" button.
5. Save the Injection List as a text tab delimited file (.txt). This injection list will be imported on to the 3130 as a Plate Record.
6. Print out worksheet on the "3130 Setup" tab for the case file.

6.2 Running Samples:

1. Import an injection list and use the naming convention "initials_mmddyy". Verify the contents of the plate record and remove unused wells. The default injection time for the 3130 is 5 seconds. Allelic ladders, positive controls, and negative controls must be injected at 5 seconds. All samples must be injected at 5 seconds first, before the injection time can be lowered to 2 seconds or raised to 10 seconds depending on the strength of the sample. If a case sample is injected at 10 seconds, the corresponding reagent blank must also be injected at 10 seconds. Reagent blanks should never be injected at 2 seconds.
2. Go to the Run Scheduler and find your injection list.
3. Click on your plate name and then click on the yellow plate in the lower right corner to "link" your plate. The plate will turn green once it has been linked. Your sample plate must be on the instrument in order to link it.
4. Click on the green arrow at the top of the screen to start your run.
5. At any time while the instrument is running, you can add additional runs. The additional injections that are added will be recorded on the 3130 setup sheet. If you need multiple injections of the same time, the additional injection time should be recorded in on the 3130 setup sheet.
6. Once the plate has been processed go to Plate View and then click "unlink". The plate in the corner will turn back to yellow and then you can remove the plate from the instrument.

6.3 .FSA Files (Raw Data files), .SER Files (GMID-X project files), and R Drive:

On the R drive there is a GMID-X folder. Each Analyst will need to create a shortcut on their desktop to this folder. The pathway to get to the GMID-X folder is: R: drive → Department→ Crimelab→ Sensitive→ Micro→ GMID-X

Each Analyst has a folder in the GMID-X folder. Within each analyst folder there will be folders for Raw Data (.fsa files) and Completed GMID-X projects (.ser files). These folders will be organized and labeled by year and quarter. For example “Projects 2015 Q1” and “Raw Data 2015 Q1”. Any data that is on the R drive will be automatically backed up by the city and will not require any additional data backup steps by the Biology Section.

During data collection the resultant .fsa files will be named: Plate record_well.fsa

After the run is complete all raw data files must be transferred from the 3130 instrument computer to the R drive. Once all raw data has been moved to the R drive, the samples can be added to GMID-X to create a project.

References:

- Applied Biosystems 3130/3130xl Genetic Analyzers: 3130 Series Data Collection Software 4: Getting Started Guide
- Applied Biosystems 3130/3130xl Genetic Analyzers: 3130 Series Data Collection Software 4: Maintenance, Troubleshooting, and Reference Guide

7. GeneMapper® ID-X Analysis

A. Purpose and Introduction

GeneMapper® ID-X (GMID-X) software is used to apply the Analysis Method and Size Standard to raw data generated by the 3130xl Genetic Analyzer thus allowing for automatic conversion of the allele sizes (in base pairs) into allele designations. It also generates electropherograms and builds tables containing the genotype information. Genotypes are assigned by comparing the sizes obtained from the unknown sample files with the sizes obtained for the alleles in the allelic ladder(s). Use of this software corrects for spectral overlap within samples, corrects for differences in baseline, and defines the ranges of interest within the data. Note: This section describes how to perform a GeneMapper® ID-X analysis starting with a new project.

B. Materials and Instrumentation

- PC computer running GMID-X approved operating system
- Life Technologies GeneMapper® ID-X Analysis Software, version 1.4

C. Safety Considerations

Not Applicable

D. Limitations

- The software is designed to help designate allele calls to true peaks by assessing data that has been collected utilizing the AB 3130xl collection software, making calls based on base pair size and then making allele calls by comparing the analyzed data to known allelic ladders. This, however, does not replace the need for analyst interpretation. All data requires analyst review as well as second reader review to ensure that all true peaks have been accurately labeled and called by the software.
- Although GeneMapper® ID-X can flag certain aspects in a sample, it cannot distinguish between a true peak, a stutter peak, a spike or an anomaly. These can only be identified by a knowledgeable analyst.
- All allele calls are dependent on an appropriate ladder and internal size standard.

E. Other Considerations

Not Applicable

F. Validation

- This procedure is generally accepted in the forensic community.
- These procedures have undergone internal validation at the CMPD.

Procedure:

7.1 Creating a GeneMapper® ID-X project

7.1.1 Open GeneMapper® ID-X version 1.4

All analysis will be performed under analyst's name.

7.1.2 Add Samples: select "Edit" → "Add Samples to Project"

7.1.3 Locate the run folder containing the samples to be added to the project: select My Computer → (R:) → Department → Crimelab → Sensitive → Micro → GMIDX → Analysts Initials. Then select the raw data run folder of the samples you want to analyze. Select "Add to List" and then "Add".

7.2 Make sure that the columns are filled out as follows:

7.2.1 Sample Type: select sample, allelic ladder, positive control, or negative control for each sample. All reagent blanks will be labeled as negative control.

7.2.2 Settings:

- Specimen Category: no export
- Analysis Method: CMPD analysis method
- Panel: Identifiler_v1.2x
- Size Standard: CE_G5_HID_GS500
- Custom Control: None
- Matrix: None
- Other fields automatically filled in by the software and instrument

7.3 After columns have been filled out you can then analyze the samples by selecting Analysis → Analyze All. You will be asked to create a name for your project. The project should be labeled with the analyst initials, date of run, and instrument # used. {Example: cse MM-DD-YY #1} If a reinjection is from the original plate, then add "RI" after the 3130 number. If a project needs to be changed after submitted for technical review, the ladders and the samples that need reanalysis or changing need to be put in a separate project and renamed by adding "TRC" after the 3130 number.

The security group should be set to: GeneMapper ID-X Security Group.

7.4 Analysis:

Once all samples have been analyzed, the Analysis Summary tab will appear. This tab shows an overview of all samples, allelic ladders, and controls for the run.

In order to proceed with the analysis of the project there must be a passing positive control and at least one allelic ladder. If there are any failing allelic ladders in the project the analyst must change the sample type from allelic ladder to sample and then re-analyze the project.

A printed copy of all injected samples will be included in the case file. A brief notation on the 3130 Injection List Worksheet and on the electropherogram will be made for injections that failed or are not used for interpretation. If more than one injection of the same Reagent Blank, Negative Control, or Positive Control is performed on the same run and all injections pass the quality control requirements, only one copy needs to be printed for the case file. For Reagent Blanks injected at both a 5 second injection time and a 10 second injection time on the same run and both injections pass the quality control requirements, the 10 second injection will be printed for the case file. All ladders must be printed. If the same Reagent Blank, Negative Control, or Positive Control is injected as a re-injection or re-setup, it must be printed for the case file. A brief notation on the on the 3130 Injection List Worksheet will be made to indicate which injection of the control was printed for the case file. All failed injections will be printed.



















All editing of stutter, minus A, off-ladder alleles or other anomalies will be done in the project under "Rename Allele Label". After the allele label has been changed a screen will prompt you to enter the reason for the change.

7.5 PQV (Process Quality Value) Flags:

GeneMapper ID-X Software uses PQV flags to help aid the analyst in their analysis of sample data.

The GQ (Genotype Quality) PQV Flag indicates the individual loci's genotype quality. The CGQ (Composite Genotype Quality) PQV Flag indicates the overall genotype quality.








CMPD Crime Laboratory Biology Section Standard Operating Procedures

Flag Indicator	Description
Pass 	All individual marker GQ values are  .
<u>Manually overridden</u> 	Status was originally  ,  ,  ,  ,  , or  but was set to  by the user.
Check 	One or more individual marker GQ values are  and no marker GQ values are  .
Low Quality 	One or more marker GQ values are  (affected by marker-level PQVs).
 ,  , or 	At least one marker in the sample was <u>edited</u> .

Any locus that is suitable for comparison must have a green locus header. It may be necessary to change the locus header to green by overriding the GQ of the marker. The locus header does not have to be changed to green if the locus is not suitable for comparison. If an overall profile is determined to be non-interpretable (ex. minimal, too complex), the locus headers do not need to be changed. Once a flag has been overridden and changed to green, it cannot be changed back. If an analyst then decides not to use a locus, a note will be added to the bottom of the printed e-gram.

Overriding GQ (Manually Accepting a Marker Profile)

Note: Access to this function may be restricted by your user account.

1. [Display the Genotypes table for the sample plot](#), then select the [GQ](#) to override by doing any of the following:
 - o Click a line in the Genotypes table.
 - o Click the marker header.
 - o Click a peak.
2. Right-click the , , or  GQ in the Genotypes table to override, then click **Yes**. Note the following:
 - o If all GQ PQV flags are  for the sample, a message asks if you want to [override CGQ](#).
 - o The GQ PQV flag changes to .
 - o All other PQV flags for the marker retain their original symbols, but turn gray (, ) to indicate that the marker has been edited.
 - o The marker header turns green D19S433.

Once all GQ PQV flags are changed to green, you will be asked if you want to override the CGQ for the sample. Click "Yes". The CGQ has to be green if a profile is being used for comparison. If an edit has been made at a locus within the profile the CGQ will appear green in the project; however, in the printed copy the CGQ will appear gray. Once the CGQ has been overridden and changed to green, it cannot be changed back. If an analyst then decides not to use the profile, a note will be added to the bottom of the printed e-gram.

If you want to override the CGQ but leave some flags left to indicate you are not using those loci, from the sample plot view of the sample, right click the symbol in the CGQ box and it will ask you if you want to override the CGQ even though some

CMPD Crime Laboratory Biology Section Standard Operating Procedures
loci are still flagged.

- 7.6 A copy of the printed 3130 setup sheet from each run will be placed in the case file. The electronic copies of the injection list will be moved to the GMIDX folder on the R drive quarterly for backup prior to deleting them from the instrument computer.
- 7.7 All negative controls, reagent blanks, and profiles with no DNA are to be printed at 75 RFU's.
- 7.8 If injection time is different than 5 seconds, the time will be placed on the electropherograms in the Sample Name column when printing.
- 7.9 All sample electropherograms containing DNA will be printed in color, and sized so that the tops of each peak is visible. The header of each electropherogram will contain the sample file (plate record_well), sample name (Complaint #, DNA #, Item #, Description, and Injection Time if it has been changed), Panel, and symbols for the SQO, SQ, and CGQ PQV flags.

It is acceptable to use photocopies of previously analyzed samples, positive controls, negative controls, and reagent blanks. It may be necessary to adjust the copy brightness settings in order to see the locus names on the electropherograms clearly.

- 7.10 If an electropherogram needs to be re-printed after submission to Technical Review, then the original will be maintained in the case file at the end of the technical portion of the case file. If an electropherogram needs to be added, an explanation will be placed on the added page.
- 7.11 Exporting/ Importing/ Deleting GMID-X Projects:

From Tools→ GeneMapper ID-X Manager the following will be done:

Once the project is created in GeneMapper ID-X and all analysis is complete, using the desktop shortcut, the analyst will export the project to their folder on the R drive for completed projects. Then they will delete that project from the GeneMapper ID-X software.

When an analyst is ready to perform a Technical Review on a GeneMapper ID-X project they will import the completed project from the analyst's GMIDX folder on the R drive, using the desktop shortcut. Once the review is complete, they will delete the imported project from the GeneMapper ID-X software.

- 7.12 Creating a Genotypes Table for Import into Armed Xpert

After completing analysis, saving all changes, and exporting the .ser file, ensure that the Samples Plot window is closed.

CMPD Crime Laboratory Biology Section Standard Operating Procedures
Click the Genotypes tab to bring up the genotypes table – Ensure that the Table Setting is ArmedXpert.

File > Export Table.

Save .txt file – this file does not have to permanently be retained; however, it will be imported into Armed Xpert.

7.13 Analysis Method Editor Parameters:

Analysis Method Editor

General | Allele | Peak Detector | **Peak Quality** | SQ & GQ Settings

Min/Max Peak Height (LPH/MPH)

Homozygous min peak height	75.0
Heterozygous min peak height	75.0
Max Peak Height (MPH)	5000.0

Peak Height Ratio (PHR)

Min peak height ratio	0.0
-----------------------	-----

Broad Peak (BD)

Max peak width (basepairs)	1.5
----------------------------	-----

Allele Number (AN)

Max expected alleles:

For autosomal markers & AMEL	6
For Y markers	1

Allelic Ladder Spike

Spike Detection	Enable
Cut-off Value	0.2

Sample Spike Detection

Spike Detection	Enable
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Factory Defaults

Save As | Save | Cancel | Help

REFERENCES:

- Applied Biosystems, GeneMapper® ID-X Software v1.2: Installation Guide
- Applied Biosystems, GeneMapper® ID-X Software v1.2: Reference Guide
- Applied Biosystems, GeneMapper® ID-X Software v1.2: Quick Reference Guide
- Applied Biosystems, GeneMapper® ID-X Software v1.0: Getting Started Guide
- CMPD Internal Validation
- AmpF/STR Identifiler PCR Amplification Kit User's Manual. Life Technologies.
- Forensic DNA Typing: Biology and Technology behind the STR Markers. John M. Butler. Academic Press 2001.

8. Data Interpretation Using Armed Xpert

A. Purpose and Introduction:

Following GeneMapper ID-X analysis, data will be evaluated using the Armed Xpert software. This program facilitates the interpretation of data. Before Armed Xpert is used by the analyst, the raw data and electropherograms in GMID-X must first be reviewed to determine that all allele calls are correct, any artifacts are annotated, and all samples and controls used for interpretation and comparison pass all quality control measures. Armed Xpert is not an expert system.

B. Materials and Instrumentation:

Armed Xpert 3

C. Safety Considerations:

Not Applicable

D. Limitations:

The software is designed to assist in mixture interpretation of two or three contributors considering all possible combinations at each locus using thresholds determined by validation. This, however, does not replace the need for analyst interpretation. All data requires analyst review as well as second reader review to ensure accuracy.

E. Other Considerations:

In conjunction with notes on the electropherograms that demonstrate the number of contributors, the suitability for comparison, and the interpretation of the profile by noting the type of statistic applied or the allele combinations at the locus, statistics will be generated for all applicable questioned profiles. **Statistics will not be calculated or reported for profiles generated from intimate samples that are consistent with the expected donor, for questioned profiles consistent with an elimination standard profile, or for questioned profiles consistent with a staff profile; however, any interpretation other than a complete single source profile (e.g. partial profile, allele, any, obligate allele calls, or major determination) performed must be conveyed on the electropherogram and in the report.** The statistics include all allele frequencies from the four population sub groups reported (Caucasian, African American, Southeast Hispanic, and Southwest Hispanic) and theta values where applicable will be printed out and placed in the case file.

F. Validation:

These procedures are generally accepted in the forensic community and meet SWGDAM guidelines.

This procedure has undergone internal Validation at the CMPD.

Procedure:

8.1. Import the analyzed data in the form of a GeneMapper table (.txt file) into Armed Xpert. See detailed instructions in SOP 7.12.

Click the Armed Xpert menu button (double helix) in the top left corner → Import → Other → browse to location where Genotypes table created from GeneMapper ID-X was saved → select → Open

If you have previously created and saved an Armed Xpert project, Click the Armed Xpert menu button (double helix) in the top left corner → Import → Other Files: Project → browse to location where Project file was saved → select → Open → select any or all of the Tables you want to resume working with by using the Organize function.

All tables and mixture interpretation documentation will be printed in color; however, it is not required to print in color for the statistics pages.

8.2. As a check of the data imported from GeneMapper ID-X, the ladders and Positive and Negative control samples will be evaluated for correct results. A Green color in the software indicates that all controls and ladders are within the established parameters. If a pink color appears, this is a visual indication that the data may not fall within the established parameters, but can be verified and used once a manual check has been performed. This control check data will be printed in color and in landscape orientation and placed in the case file. A note will be added if a manual check was required. After the QC check is performed and printed reset the table by clicking the Clear Checks button. The technical reviewer will be required to date and initial this page demonstrating that a check of this table has been done in conjunction with a check of the underlying data in the GeneMapper ID-X software.

8.3. Organize your data:

Under the Views Tab → Samples then navigate to Data → Sort Samples

Auto-sort by → Sample Name → OK

Click Armed Xpert menu button

Save → prompt to save Genotypes Table → Yes New Project → NAME (same as .ser)

Data → Combine Sources → Expand Genotypes Table under Samples & Sources →

Select (Control and Shift buttons work here) all samples that are being reported together under same case (Generally all the same Complaint #'s)
Using the single carrot button ">" – send selected
Change Title to Complaint # under which the report is being issued → Combine Repeat → Combine Sources (remembering to use double reverse carrot "<<" to clear previous selections

If no profiles are being further interpreted, then the table will be printed in landscape orientation and placed into the corresponding case file. To Navigate between tables go to Data tab → Organize → expand project → double click table to open.

8.4. Using the "Interpretation" tool:

- Single source profile: Interpretation tab → Single Source → navigate to sample → print statistics page
- Partial single source (drop-out, allele any designations, and obligate allele designations): Interpretation tab → Begin Mixture Interpretation → Pick via mouse → Popout calls → (change name of Profile 01 to DNA # by selecting "Rename Profile" from drop down menu, Profile 02 does not need to be removed) → Select the allele combinations → Save as part of the project (this profile will be added to the Combined table) → View call report → RMP → select profile → change Contributors to 1 → adjust any allele combinations to reflect interpretation → print statistics page
- Major profile: no conclusion on minor(s) – Interpretation tab → Begin Mixture Interpretation → Pick via mouse → Popout calls → (change the name of Profile 01 to "Major and DNA #" by selecting "Rename Profile" from drop down menu, Profile 02 does not need to be removed) → Select the major profile by checking the boxes or clicking the allele combinations → Save as part of the project (this profile will be added to the Combined table) → View call report → RMP → select profile → change Contributors to 1 → adjust any allele combinations to reflect interpretation → print statistics page
- Major profile/ minor profile: Mixture consistent with two or three: Interpretation tab → Begin Mixture Interpretation → Pick via mouse → Popout calls → (change the name of Profile 01 to "Major and DNA #" and change Profile 02 to "Minor and DNA #" and "Middle and DNA #" by selecting "Rename Profile" from drop down menu) → Select the profiles by checking the boxes or clicking the allele combinations → Save as part of the project (these profiles will be added to the Combined table) → View call report → Print Calls page and Mix Interp page if using proportions to establish allele calls → RMP → select profile → change Contributors to 1 → adjust any allele combinations to reflect interpretation → print statistics page

- Major Group: Interpretation tab → **Begin Mixture Interpretation** → Pick via mouse → Popout calls → (change the name of Profile 01 to “Major Group and DNA #” by selecting “Rename Profile” from drop down menu, Profile 02 does not need to be removed → Select the major group by checking the boxes and ignoring alleles attributed to minor(s) → click through each locus to ensure that allele combinations work → **Save as part of the project (this profile will be added to the Combined table)** → Operations → RMP → Restricted RMP → Apply Restricted Globally → Contributors 2 → Open Frequency Report → → print statistics page
- Mixture Restricted: Interpretation tab → Click through each locus → Operations → RMP Restricted RMP → Apply Restricted Globally → make sure number of contributors is correct → Open Frequency Report → adjust any allele combinations to reflect interpretation → print statistics page
- Mixture Unrestricted: Interpretations tab → Click through each locus → Operations → RMP → Modified RMP → make sure number of contributors is correct → Open Frequency Report → → adjust any allele combinations to reflect interpretation → print statistics page
- Conditioned Mixtures: **(Only used when assuming the presence of a known contributor to an intimate sample makes the deduction of the foreign genotype(s) possible)** Interpretation tab → Begin Mixture Interpretation → Pick via mouse → References → Select Reference to apply → Popout calls → (change the name of Profile 01 to “Reference and DNA # and change Profile 02 to “Foreign and DNA #” by selecting “Rename Profile” from drop down menu) → Select the profiles by checking the boxes or clicking the allele combinations → **Save as part of the project (this profile will be added to the Combined table)** → View call report → Print Mix Interpretation page if using proportions to establish allele calls **and note the proportional range used to assign genotypes** → RMP → select foreign profile → change Contributors to 1 → adjust any allele combinations to reflect interpretation → print statistics page

For the **purpose of creating a combined table all interpretations should be saved under** the previously named project. All supporting documentation from the software should be printed out and placed in the electronic case file as applicable (e.g. restricting genotypes based on proportions and peak height ratios). Allele tables, QC checks, and Match and Comparison tables will be printed in landscape orientation. Statistics and Mixture Interpretation pages will be printed in portrait orientation with the minimum number of pages changed to two. Some Statistics pages will not print to two pages, so instead these will be printed in landscape orientation. There is no requirement to

permanently save or retain these (.axp) files, since all supporting documentation will be in the case file.

NOTE: The “Restrict HT Globally” button may be used to include *allele*, any options to a mixture that has a restricted approach applied. Caution; however, should be used when applying this approach as the peaks below the stochastic threshold may be indicative of the overall quality of the mixture. Assessment as to the reliability of PHR and proportions should be evaluated during interpretation and an unrestricted approach may be instead considered.

NOTE: The “Apply Stutter” button may be used to subtract out the contributing influence of stutter so that heterozygote alleles may be within the established PHR of 50%. The application of this button should be minimally used, and if necessary to apply, will always be “Applied Globally” to the interpretation of the mixture.

8.5 **Creating a Combined Table:** Once all interpretation has been performed and saved in the project, a combined table will be made and printed for the case file. Tables are combined by Data → Combine Sources → highlighting and selecting the appropriate tables and or interpreted profiles within a mixture interpretation to combine (View call report must be open in order for the table to be accessible to combine) → Name the table “Complaint # Combined”. The table will be printed and added to the case file.

REFERENCES:

- SWGDAM Interpretation Guidelines for Autosomal STR typing by forensic DNA testing laboratories, 2010, Available at www.swgdam.org
- Evett, et al, Taking account of peak areas when interpreting mixed DNA profiles, *J Forensic Sci.* 1998, 43: 62– 69.
- Clayton, et al, Analysis and interpretation of mixed forensic stains using DNA STR profiling, *FSI* 1998, 91; 55-70.
- Bill, et al, Pendulum – a guideline based approach to the interpretation of STR mixtures, *FSI* 2005, 148: 181-189.
- Bille, et al, Application of Random Match Probability Calculations to Mixed STR Profiles, *J Forensic Sci* 2013, 58 (2): 474-485.
- Kelly, et al, A comparison of statistical models for the analysis of complex forensic DNA profiles, *Science and Justice* 54 (2014): 66-70.

9. Interpretation Guidelines

A. Purpose and Introduction

The purpose of this procedure is to provide interpretation guidelines for DNA and to accurately reflect the scientific weight given to the various situations encountered when interpreting and calculating statistics.

B. Materials

- Case file
- Allele Table (or Combined Table, when applicable) - will list all profiles obtained or deduced from the case. The allele table will be generated in the Armed Xpert software. An allele table will be generated for each Complaint # and all profiles associated with the Complaint # will be included regardless of whether the data is used for interpretation or comparison.
- Mixture Interpretation Summary – This summary document will be printed and included in the case file if proportions are used to establish genotype combinations. This summary sheet is printed from the View Call Report function within the Armed Xpert software.
- CODIS Entry Form – This form will be included if a profile obtained or deduced is being entered into CODIS. The CODIS entry form will include the DNA #, CODIS entry checked, and the allele calls being entered into CODIS.

C. Safety Considerations

Not Applicable

D. Limitations

- Analysis of forensic casework can produce several possible results. This documentation is to standardize the interpretation of profiles obtained.
- Before interpretation can be done, the following controls must be checked: Allelic ladders, Positive Control, Negative Control, Reagent Blanks and internal size standards. If these controls do not meet quality control measures, then the samples cannot be used and must be re-analyzed. The check of the controls is done manually by the analyst, automatically within the GeneMapper ID-X software, and further demonstrated by printing and including a QC check from the Armed Xpert software.

E. Definitions:

- *Allelic Ladder*: An allelic ladder is an artificial mixture of the common alleles present in the human population for a particular STR marker that provides a

reference DNA size for each allele included. Allelic ladders are important for obtaining accurate genotype determinations. Verify that the correct alleles are typed based on the known allele values that are included in the ladder. If a ladder fails or if the allele calls are incomplete or incorrect the ladder may be omitted. If all ladders fail, any DNA specimens run on the same plate are to be considered inconclusive for typing.

- *Internal Size Standard:* An internal size standard contains known sized DNA fragments that are labeled with a different color dye so that it can be spectrally distinguished from the DNA fragments of unknown size. Verify that the LIZ GS500 size standard has sized correctly by ensuring that the fragments ranging in size from 75 to 500 base pairs are properly labelled. If an internal size standard fails (SQ red in GeneMapper ID-X) that particular DNA specimen is considered inconclusive for typing.
- *Reagent Blank:* A reagent blank must be present in each extraction set. The reagent blank serves as a check for possible contamination by adventitious DNA in samples, reagents or supplies during the testing procedure. Adventitious DNA can be non-amplified or amplified. If a reagent blank contains a pattern of peaks which are above the analytical threshold, any DNA specimens co-extracted with the reagent aliquots contained in the reagent blank are to be considered inconclusive for typing.
- *Negative Control:* An amplification blank must be present in each amplification set and serves as a check for sample contamination introduced during amplification set-up. This blank control consists of the PCR Master Mix to which sterile TE Buffer is added instead of DNA. If a negative control contains a pattern of peaks which are above the analytical threshold, any DNA specimens co-extracted with the reagent aliquots contained in the reagent blank are to be considered inconclusive for typing.
- *Positive Control:* A positive amplification control is required for each amplification set and is used to assess the proper performance of amplification and typing procedures. The manufacturer supplies DNA Control 9947A as a kit component. The Control DNA 9947A is human genomic DNA. If the positive control exhibits a discordant result, all co-amplified samples are considered inconclusive for typing.

F. Other Considerations

- The interpretation of results in casework is a matter of professional judgment and expertise. Not every situation can or should be covered by a predetermined rule. These criteria will be based on validation studies, literature references, and casework experience and will be developed with input from the analysts. It is expected that interpretation guidelines will continue to evolve as the collective experience of the laboratory grows. These guidelines ensure that conclusions in casework reports are scientifically supported by the analytical data, including appropriate standards and controls. In addition, interpretations are made as objectively as possible and consistently from analyst to analyst. Intimate samples

are defined as samples that come from the body, underwear that was collected with the sexual assault kit and worn during the assault, and clothing taken from a body by medical personnel or crime scene.

- All standards run in a case will be compared to all evidence submitted in that case.

G. Validation

These procedures are generally accepted in the forensic community, and have undergone internal validation at the CMPD.

9.1 Possible Conclusions

In a DNA analysis evaluation, three conclusions are generally possible: 1) there is an inclusion, 2) there is an exclusion, or 3) the results are not interpretable. It is scientifically acceptable for an inclusion or exclusion to be determined for a case when one or more of the loci yield non-interpretable results. A consistency statement will be based on only those loci that yield conclusive results; therefore, any inclusions/exclusions will be made using interpretable loci.

9.1.1 Inclusions

- The patterns are consistent with having come from the same individual and are determined by careful, objective, qualitative and quantitative evaluation of the entire pattern produced by the various loci tested.
- Inclusion reporting terminology for single source profiles, major profiles, and foreign profiles:
 - Match – full single source, full major, full foreign – all genotypes fully resolved at all loci tested
 - Consistent – partial single source, partial major, partial foreign – genotypes not fully resolved

9.1.2 Exclusions

The patterns are not consistent with having come from the same individual and are determined by careful, objective, qualitative and quantitative evaluation of the entire pattern produced by the various loci tested.

9.1.3 Non interpretable results

If a sample is deemed non interpretable, a notation will be made in the file that clearly states why the sample is non-interpretable.

These can be minimal profiles, complex mixtures, etc, and can result from the following:

- Insufficient amounts of DNA that can result in observation of stochastic peak imbalance or allele dropout.
- Degradation due to environmental or chemical processes.
- Preferential amplification due to the presence of inhibitors or other factors which limit the amplification of larger fragments.
- Or any combination of the above.

9.2 Allele Designation

9.2.1 Stutter Peaks:

- The PCR amplification of STR loci typically produces a minor product peak four bases shorter (n-4) than the corresponding main allele peak. This is a well-known aspect of STR analysis and should not cause concern when detected. On occasion with high level samples, stutter will appear as a peak that is eight bases shorter (n-8) or four bases larger (n+4) than the allele. Peaks at (n-4) follow the percentage noted below.
- For any given locus, minor peaks that are above 75 RFU and are located at a position one repeat unit smaller than a major peak must be evaluated as to whether it represents stutter or a real allele. Peaks may be considered as stutter in Identifiler if their peak height percentages to the larger peaks are equal to or less than approximately:

<u>Locus</u>	<u>Stutter</u>
D8S1179	8.2%
D21S11	9.4%
D7S820	8.2%
CSF1PO	9.2%
D3S1358	10.7%
TH01	5.1%
D13S317	8.0%
D16S539	10.4%
D2S1338	11.1 %
D19S433	13.3%
vWA	12.6%
TPOX	4.8%
D18S51	17.0%
D5S818	6.8%
FGA	14.7%

- Exact percentages can be found in the Identifiler validation study. These stutter percentages have been determined by the manufacturer and were confirmed

during internal validation studies. Occasionally, stutter may exceed these values and an evaluation of the entire profile is necessary. For any given locus, minor peaks that are above 75 RFU and are located at a position one repeat unit smaller than a major peak may be considered as a real allele if their peak heights exceed the stutter percentage established for that locus as indicated above.

9.2.2 Repeat Sequences:

All allele designations will be based on the number of repeat sequences as defined in the Identifiler technical manual and in the GeneMapper software distributed from Life Technologies.

9.2.3 Minor Peaks:

For any given locus, minor peaks above 75 RFU with no major peaks at a position one repeat unit larger to it will be interpreted as a real allele unless it is likely due to a technical reason such as spiking or rendered inconclusive due to lack of reproducibility.

9.2.4 Variants:

The designation of alleles containing an incomplete repeat motif (i.e., an off-ladder allele falling within the range spanned by the ladder alleles) should include the number of complete repeats and, separated by a decimal point, the number of base pairs in the incomplete repeat (e.g., FGA 18.2 allele). If an allele falls above the largest or below the smallest allele of the allelic ladder, the allele should be designated as either greater than (>) or less than (<) the respective ladder allele, or when appropriate interpolation can be used. Variants need to be re-analyzed for confirmation and printed with the allele call, base pair and size. This is accomplished by re-injection of the sample.

9.2.5 Peak Height Ratios:

Stochastic effects causing a departure from the 50% peak height ratio rule, due to limited template of one allele compared to another, may occur. Preferential amplification, due to the propensity of the PCR to provide stronger amplification of shorter fragments, may also occur. Such results may be graded as inconclusive if the deviation is significant or causes the analyst to lack confidence in the profile. As a part of this analysis it is recognized that low template or degraded samples may deviate from the 50% rule, and therefore a broader combination of genotypes should be considered during interpretation. This determination should be based on the amount of template used, the number of contributors, and the presence of degradation and or inhibition. The PHR of 50% is the absolute minimum value that can be tolerated when applying a Restricted approach to the interpretation of a mixture. If viable genotype options are not available by applying a much more conservative 50% PHR, then an Unrestricted approach will be applied to that portion of the mixture that is being interpreted. Caution should be taken with low template contributors since a low PHR could

also be signaling an additional contributor may be present.

9.3 Thresholds:

All peaks must be above 75 RFU to be used for comparison, statistical, and CODIS entry purposes.

9.3.1 Analytical Threshold:

The analytical threshold is defined as the minimum RFU at which detectable peaks can be reliably distinguished from background noise or non-allelic peaks. Allelic peaks above the analytical threshold may be used for reporting purposes to include comparison, statistical calculations, and CODIS entry. Allelic peaks may also be observed below the analytical threshold, and although not reportable should be cautiously considered as part of the overall interpretation when appropriate. The analytical threshold has been established from validation studies at three times the noise for a threshold of 75 RFU.

9.3.2 Stochastic Threshold:

The stochastic threshold is defined as the RFU value where stochastic effects are observed (e.g. allelic dropout and peak height ratio imbalance) making quantitative interpretation more difficult, especially with mixtures. Based on validation results, this threshold is set at 250 RFU for 5 second injections and 325 RFU for 10 second injections. Mixtures with contributors that contain alleles below this threshold must be interpreted with caution since there is an increased potential for peak height imbalance. The possibility of undetected or masked alleles should also be considered for contributors below this threshold when appropriate.

9.3.3 Upper Analytical Threshold:

The upper analytical threshold is defined as the RFU value where allelic peaks display a saturated signal above the linear dynamic range for the instrument and can result in unreliable data from increased artifacts (e.g. pull-up/elevated baseline). This off-scale data should not be used for quantitative analysis in mixture data. The upper analytical threshold is set at 5000 RFU. The minor component from a DNA mixture containing major peaks greater than the upper analytical threshold will not be interpreted without approval from the Technical Leader. These off-scale peaks from the major contributor are known to produce artifacts in other loci which could impact reliable interpretation for the minor component. These samples should instead be re-amplified targeting a lesser template concentration.

9.4 Extraneous Peaks

- Peaks other than the true alleles may be detected on the electropherograms display. It is important to identify the most likely cause for the appearance of extra

peaks.

- These peaks will be labeled on the electropherograms in GeneMapper with the editing function. The designations for each of these will be {ART} for artifact.

9.4.1 Incomplete 3' (A) nucleotide addition:

The DNA polymerase used in STR/PCR amplification catalyzes the addition of a single nucleotide to the 3' ends of double stranded PCR products. This non-template addition results in a PCR product which is one base pair longer than the actual target DNA sequence. STR/PCR amplifications have been optimized to favor the A addition. Incomplete A addition is recognized as "split peaks" (n-1), where a target allele is represented by two peaks one base pair apart.

9.4.2 Spectral failure:

A matrix failure is a result of the instrument's inability to separate the colors (spectral overlap) used to fluorescently label STR/PCR products. A matrix failure is observed as a peak beneath a peak or as an elevation of the baselines for any color and can occur as reagents change slightly over the course of time. A peak of another color is "pulled up" as a minor component beneath the true allele peak.

9.4.3 Stutter:

Defined in 9.2.1. If elevated 1% above the known percentages, the actual percentage will also be noted in the editing function of GMIDX, but does not have to be printed.

9.4.4 Spike:

A spike is an extra peak that can be caused by a polymer crystal, an electrical impulse, or bumping of the instrument.

9.4.5 Mixed samples:

Evidence samples may contain DNA from more than one individual. The possibility of multiple contributors must be considered when interpreting the results and will be noted at the bottom of the e-gram (i.e- assuming X individuals). The following observations should aid the analyst in determining whether a sample is a mixture:

- the presence of more than two alleles at a locus.
- the presence of a peak at a stutter position that is greater than the maximum threshold significantly imbalanced alleles (<50%) for a heterozygous genotype.

By no means does the presence of one of these situations automatically render the sample as a mixture. The likelihood that any sample is a mixture must be

determined by the analyst in the context of each particular case, including information provided at multiple loci.

9.5 Single Source Profiles

At a minimum, there needs to be seven loci (excluding Amelogenin) where at least one allele is detected above the analytical threshold to begin to interpret the partial profile. If the minimum number of loci with data is not met, the partial result will be uninterpretable and reported out as a minimal profile due to a lack of sufficient genetic data.

For a profile to be used for interpretation, comparison, statistical calculation, and CODIS entry there must be at least seven fully represented loci (homozygote peak above stochastic threshold or both heterozygote alleles above the analytical threshold).

Caution should be used with interpretation of partial results due to degradation, inhibition, and/or stochastic effects. Care should be taken when interpreting samples in which less than the optimal amount of template DNA has been amplified, as stochastic peak imbalances may result. However, since occasional departures from the 50 % rule occur, the interpretation of these will be left to the discretion of the analyst and the reviewer. Extreme caution should be taken if a partial profile is recovered from a sample with a concentration of less than 250 pg have been amplified.

If there are indications of a second contributor below the analytical threshold at multiple loci, then the profile must be cautiously evaluated to ensure all called alleles are coming from one contributor. This is especially true if all alleles detected are below the stochastic threshold. If all the called alleles are below the stochastic threshold, and there are indications of a second contributor below the analytical threshold then the profile will be treated as a partial low-level irresolvable mixture and not interpreted further.

If the peak heights of the two alleles are within 50 % of one another, they can usually be considered as a heterozygote pair. In such cases, one would expect to find no other locus in the profile exhibiting more than two alleles. Sample quality and quantity for amplification may occasionally result in deviations from this 50 % model due to stochastic effects or preferential amplification discussed earlier in this manual.

Occasionally a DNA profile will show a three allele pattern at one locus. If a similar three allele pattern at the locus is obtained from profiles in the case, then the tri-allelic will be called and nothing further need to be done. If only one profile exhibits the tri-allelic pattern, then re-extract the sample if possible. If re-extraction is not possible, then the sample will be re-amplified.

If several single source or major profiles are obtained from unknown samples, they need to be compared to each other, or noted that profiles were different or similar. They do not need to be compared to mixtures other than the major profile.

9.6 Mixed Profiles:

All possible casework scenarios cannot be exhaustively described; therefore, extensive training, casework experience, and critical thinking skills are all essential factors in the

proper interpretation and reporting of DNA mixture results. To the extent possible, evidence profiles should be interpreted prior to comparison to the known DNA profile(s) of the individual(s) in question.

A DNA mixture is generally defined as a DNA profile where more than two alleles are detected (except for a tri-allelic individual) at one or more loci, and/or peak height imbalance is observed at one or more loci. Peak height ratios of less than 60% at multiple loci generally indicate the presence of more than one contributor in the evidence sample, especially when contributors are above the stochastic threshold.

At a minimum, there needs to be seven loci (excluding Amelogenin) where at least one allele is detected above the analytical threshold to begin to interpret the mixture profile. If the minimum number of loci with data is not met, the result will be uninterpretable and reported out as a minimal profile due to a lack of sufficient genetic data.

For a profile to be used for interpretation, comparison, statistical calculation, and CODIS entry there must be at least seven loci where all of the contributors are at least partially represented (at least one allele present from each of the contributors based on the assumed number of contributors determined). Caution should be used with interpretation of mixtures that demonstrate degradation, inhibition, and/or stochastic effects.

The entire DNA profile should be evaluated for overall quality before determining whether or not it is a true mixture, especially with low-level profiles and/or partial results.

9.6.1 Determining the number of contributors:

- Evaluate the loci with the most alleles detected. Start with the more discriminating loci (e.g. FGA, D2, and D18) where alleles are less likely to be shared.
- Look for any peak height imbalance (< 50%) at multiple loci. Stutter or low template DNA should be considered with this evaluation.
- Look for possible alleles below analytical threshold. Additionally, assess if the peak heights are comparable to the minor component which is being detected. If so, these indications of an additional contributor could have an impact on interpreting the detected minor alleles as one contributor.
- Evaluate the overall quality of the DNA profile. If the profile is partial and degraded the best approach is not to assume the number of contributors with the interpretation.
- If the mixture can be based on an assumed number of contributors, it will be reported out as being 'consistent with' that number, and all further interpretations to include statistical analysis will be based on this assumption.
- If the number of contributors cannot be reliably determined, then the mixture will be reported out as 'at least' a minimum number based on the assessment. Major profiles may still be determined from these mixtures; however, no interpretation or

comparison will be performed on the minor profile(s). In the major profile, at least seven fully deduced loci must be determined for the major contributor to be used for interpretation, comparison, and CODIS entry. If the seven loci threshold is not met for a major or discrete number of contributors, then the mixture will not be considered for further interpretation.

9.6.2 Proportions:

Generally, a 2:1 proportion is needed to resolve major/minor components in a 2 contributor mixture. When resolving a major contributor from a minor group of contributors, a more conservative 4:1 proportion should be considered. Additionally if the amplification target for the sample does not meet 0.5 ng or if the sample shows indications of degradation from the quantitation data, a 4:1 proportion should be considered.

Proportionate allele sharing: for 2 and 3 contributor mixtures, where alleles are shared at a given locus, the approximate proportions at a given locus will be calculated using the following three rules:

- 1) whenever possible, peak height ratios are assumed = 1.
- 2) whenever possible, alleles will be shared proportionately.
- 3) minimum peak heights (75 rfu) will be maintained, and take precedence over rule 1 and 2. This approach is for loci where proportionate allele sharing using Rule 1 or 2 results in an rfu value < 75 rfu for a particular contributor proportion. The default will be set so that the rfu value never drops below 75 rfu for that contributor. So the proportion will default to 75 rfu.

Stochastic threshold: Caution must be taken when interpreting mixtures with alleles below the ST. Quantitative assessment using peak height ratios and proportions is not always reliable below this threshold.

Mixture Interpretation: first evaluate the overall quality of the profile, and then decide if contributor genotypes at a particular locus can be restricted based on certain criteria, or if they should be unrestricted and/or modified.

9.6.3 Mixture Terminology: definitions for interpreting mixtures

- **Restricted:** genotype combinations for contributors are restricted into different categories (e.g. major/minor) based on reliable data using peak height information (peak height ratios and proportions).
- **Unrestricted:** all detected genotype combinations are possible for all contributors. This is a qualitative approach and does not consider peak height information.

- **Modified:** the genotype combination accounts for an undetected allele where appropriate. This approach could be used when deducing a single contributor or accounting for all contributors.
- **Conditioned:** genotype combinations are restricted based on assuming a known contributor in the mixture. Since it is expected that an intimate sample will contain that individual's DNA, assumptions of ownership for a known contributor may be made for intimate items (from their body or underwear found in a sexual assault kit or clothing removed from their body by medical personnel).

9.6.4 Mixture based on an assumed number of contributors (2 or 3) and distinguishable contributors

- The data is of sufficient quality and/or quantity so that it can be resolved into respective contributors (major/minor). These mixtures can also be conditioned on an assumed known contributor or contributors.
- When a known individual can be assumed, the mixture may be conditioned on the known contributor, and the remaining genotype deduced using alleles foreign to the known contributor.
- If a known contributor cannot be assumed, then peak height ratios (PHR) and proportions may be used to resolve major/minor; or an obligate allele may be used to deduce pair-wise combinations for a particular contributor based on peak heights.
- When interpreting a contributor that is below the stochastic threshold at a given locus, the possibility of undetected alleles (modified approach) should be considered and applied where appropriate.
- If a running proportion (average) across loci is used to deduce contributors, supporting documentation defining the upper and lower bound limits will be provided in the case file.
- It is possible to use the same guidance to resolve a mixture of three individuals; however, keep in mind more allele sharing can take place making proportions more difficult to assess. Proportionate allele sharing may be applied to three contributor mixtures, but there are limitations where peak height ratios and proportions cannot be calculated due to multiple alleles being shared by multiple contributors. This "double sharing" must be considered when deducing mixtures based on proportions. In these instances, it may be more appropriate to deduce a major using proportions and PHR, while treating the remaining minor component as an indistinguishable mixture and apply either a restricted or unrestricted statistic.

9.6.5 Mixture based on an assumed number of contributors (2 or 3) and no distinguishable contributors:

- Restrict genotypes based on 50% peak height ratio for all contributors. All data should be above the stochastic threshold for this interpretation.
- Unrestricted approach to include all possible genotype combinations for all contributors at a given locus.
- Modified approach to account for undetected alleles where appropriate at a given locus.
- Loci which will be interpreted using an unrestricted/modified approach must be able to account for at least a partial representation of all contributors, so where there is uncertainty at a locus, it should be deemed inconclusive.

9.6.6 Mixture where the number of contributors cannot be assumed but major group determined:

- Generally these mixtures are consistent with two main contributors, but there is a low-level contributor or contributors also detected or indicated below analytical threshold in the profile. As a result, this makes interpreting the minor component more difficult, but may have only minimal effects on the major contributors. You need at least seven interpretable loci for the major contributors to use this interpretation, and the alleles used should be consistent with two individuals.
- The major contributors should be distinct from the rest of the group. In general, a 4:1 proportion (major group to the remaining minor group) should be observed at a locus, and the alleles assigned to the major group are all above the stochastic threshold.
- If the major group is not distinct at a locus using the above criteria, the locus should be deemed inconclusive.
- Once the interpretation of a major group of contributors has been determined from the detected mixture a restricted statistic will be applied.

If the profile is a mixture of more than three individuals, and a major profile cannot be determined, this profile may be considered to be a complex mixture and therefore non-interpretable.

9.7 Population Statistics

9.7.1 Formulas:

- The random match probability (RMP) formula as described in NRCII formula 4. b

will be used for a heterozygous locus frequency: $2pq$.

- The random match probability (RMP) formula as described in NRCII recommendation 4.4a will be used for a homozygous locus frequency: $p^2 + p(1-p)\theta$. (using $\theta = 0.01$).
- Statistical assessment of a locus containing a potential undetected allele will be calculated based on NRCII 4.1 recommendations: $[p^2 + p(1-p)\theta] + [2p(1-p)\theta]$ (using $\theta = 0.01$) where p represents the detected allele.
- A conditional probability using the RMP (random match probability) may be used when appropriate to interpret and report genotype frequencies when resolving mixtures. This can be extended to two or three contributor mixtures, and is conditioned on an assumed number of contributors. This type of RMP is simply the sum of the individual genotypes appropriate for a particular contributor at a given locus. All of the formulas are based in accordance with the 2010 SWGDAM guidelines sections 4-5.

9.7.2 General Population Statistics Considerations

- Statistical calculations will be given for an inclusion using allele frequencies based on population data published by the Federal Bureau of Investigation as part of the U.S. STR Population Database. The data set for each locus has been verified independently. The database has been reported to demonstrate little departure from Hardy-Weinberg equilibrium and linkage equilibrium was also mostly observed for the alleles across loci. As a result, the statistical genotype frequencies and product rule will be used in accordance with the NRCII recommendations.
- The Caucasian, African American, and Hispanic databases used are from the following publications:
- *Statistics were calculated using the FBI population database which is published in the July 2015 Journal of Forensic Science Erratum referencing the Journal of Forensic Science, Volume 44(6):1277-1286., and Journal of Forensic Science, Volume 46 (3): 453-489 by Budowle, et al. The reported statistics are an estimation for which a deviation of approximately +/- 10-fold may exist. The loci D2S1338 and D19S433 are not included in the statistical calculations.*
- Off ladder variants once confirmed, will be used in statistical calculations. If the analyst is uncertain to which locus the allele corresponds, the loci in question may be determined to be inconclusive and no interpretation will be given.
- Since in most cases the epithelial cell fraction is merely a result of the differential lysis, no statistics will be **calculated or** reported on the epithelial cell fraction of sexual assault samples unless the sample is probative to the case and/or no documentation exists to support the origin of the sample. **All interpretations will be reported.**

- Statistics will only be performed **and reported** on probative samples that match or are consistent with a known standard, with the exception of buccal standards obtained from Staff. These standards will only be used for comparison and will be noted “cannot be excluded from the mixture”.
- The statistics page in the case file will be initialed and dated by the technical reviewer showing their agreement with the calculations. The most conservative statistic will be highlighted and will be the only statistic that is reported.
- For all mixtures that can be used in analysis, the loci not suitable for statistics will be written on the bottom of the electropherogram during evaluation before comparison to standards.
- Composite profiles will not be used in the CMPD laboratory. Since only the most comprehensive data is used in the report, if an additional injection/ amplification/ extraction occurs on the same DNA sample, then the results of each should be checked against each other. This does not include cuttings made from various places on the same item.
- Statistics will be reported as two significant figures with no rounding
- A restricted RMP will be used when genotype combinations can be restricted based on relative peak heights, peak height ratios, contributor proportions, and/or obligate alleles from a known contributor.
- An unrestricted RMP will be used when genotype combinations cannot be restricted.
- A modified RMP will be used when undetected alleles are a concern as discussed.
- Genotypes will be subtracted out for RMP stats where appropriate to avoid double counting.
- If a locus is considered inconclusive for any reason, a frequency of 1 will be assigned for statistical purposes.

References:

- SWGDAM Interpretation Guidelines for Autosomal STR typing by forensic DNA testing laboratories, 2010, Available at www.swgdam.org
- The Federal Bureau of Investigations Quality Assurance Standards Audit Document for Forensic DNA Testing Laboratories
- NRC II, 1996

- Journal of Forensic Science, Volume 44(6):1277-1286.
- Journal of Forensic Science, Volume 46 (3): 453-489.
- Journal of Forensic Science, Erratum Volume 60 (4): 1114-1116

10. Report Writing Segments

A. Purpose and Introduction

The purpose of this procedure is to standardize the report format for the Biology section by using a standard format with reporting segments.

B. Materials

- Report template
- Case File

C. Safety Considerations

Not Applicable

D. Limitations

- Analysis of forensic casework can produce several possible results. This document is designed to provide general guidelines for proper reporting taking into account the strengths and limitations of the analysis; modifications may be required.

E. Other Considerations

- The following report wording guidelines are designed to establish consistency in reporting results. They are not meant to be all-inclusive and are listed only as a guide for wording. It is hoped that these guidelines will provide uniformity in reporting and assist readers in understanding the reports while still providing the necessary scientifically valid information. Modifications may be necessary and should be made when the case scenario does not fit the statements.

F. Validation

Not applicable

G. Definitions:

Full Match (match) – if all 15 loci are completely deduced – no allele / locus dropout, allele any's, or combos

Partial Match (consistent with) – if less than 15 loci are completely deduced – allele / locus drop out, allele any's, or combos present

Minimal – DNA results cannot be interpreted due to less than seven loci with data

Complex – DNA results cannot be interpreted due to 4 or more contributors (must have at least one loci with 7 or greater called alleles)

Intimate Samples – Swabs collected from the body of an individual or clothing removed directly from an individual by medical personnel or crime scene personnel

General Guidance:

All buccal swabs, regardless of type, will be reported as “buccal standard”.

All items received or being compared will be listed on the report.

Collected indicates it was removed from the item or the original envelope and placed in coin envelope or on tape strips, etc. for preservation.

When a differential extraction is performed, both non-sperm and sperm fractions will be reported. It may not be necessary to provide statistical calculations of a non-exclusion between an individual and a mixture in one fraction of a differential extraction if the individual has been identified in the other fraction of the differential extraction. (EG: if there is a sole source match to suspect in the sperm fraction of victim’s vaginal swab with statistics, no statistics are needed on the non-sperm fraction which is an apparent mixture of only the suspect and victim. In such instances, the sperm donor can be reasonably expected to be present in the non-sperm fraction.)

When a hair extraction is performed, both root and shaft (control) will be reported. If the length of the hair does not allow for the extraction of a shaft (control), then the results will be reported collectively as “apparent hair”.

When an unknown single source, partial single source, or full major DNA profile is obtained and a Y is present at Amelogenin, such profiles will be qualified as male in the report.

If a Y is present at Amelogenin in a mixture or partial result and no inclusionary statement to a male reference standard has been made, the overall profile will be qualified as having a male contributor.

If a Y is present at Amelogenin in a result that is not being used for interpretation or comparison, the overall profile will be qualified as having a male contributor.

For results in which no Y is present at Amelogenin, but male DNA was observed in the quantitation results, the presence of male DNA will be reported as having male DNA detected.

In the report, all inclusionary statements when compared to a reference sample will be accompanied by an appropriate statistic. Exceptions to this requirement occur when an

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assumed contributor is present in an intimate item (e.g., vaginal swab) or an included contributor's DNA profile is generated from an elimination standard.

For mixtures, any inclusionary statement that does not include a reported statistic will be reported as "cannot be excluded".

For single source or partial single source profiles, any inclusionary statement that does not include a reported statistic will be reported as "consistent with".

The most common frequency from the Caucasian, African American, Southwest Hispanic, or Southeast Hispanic populations will be reported.

A source attribution is reported at rarer than 1 in 700 billion and in the absence of a known identical twin. If the rarity of the statistic does not exceed the threshold of 1 in 700 billion or if the statistic reported is in reference to a mixture profile, then the actual numerical value calculated will be reported.

Mixtures or portions of mixtures that are interpreted and are; therefore, able to be used for comparison (both inclusion and exclusion) will be interpreted based on an assumed number of contributors. Assuming a discrete number of contributors will be based on the data, and is done by taking into consideration overall quality of the data, number of peaks at each locus, peak heights, peak height balance, contributory influence of degradation and/or inhibition and total DNA quantity. Assuming a number of contributors does not mean that it is impossible for additional contributors to be present, only that the data presents as such and that the interpretation was performed under that assumption.

For mixtures, if an interpretation is based on an assumed number of contributors, then the wording "...consistent with originating from..." will be applied.

For mixtures, if the number of contributors in a mixture cannot be determined, then the wording "...from at least..." will be applied.

Interpreted results will be used for both inclusion and exclusion. A profile will not be used for exclusion only.

The comparison of a known reference will yield either an exclusionary or inclusionary statement.

The recommendation to do Y-STR testing on a particular sample will be based on evidence type, case scenario, and other reported findings in the case. Y-STR testing will only be recommended for intimate samples, and will not be recommended for guns or environmental samples.

10.1.1 Results:

General:

The swab was consumed in analysis.

This item was not suitable for analysis; therefore, no examination was performed.

Please submit a buccal standard from [*subject*] for comparison purposes.

A buccal standard from [*subject*] must be submitted before DNA testing will be performed.

Staff Match:

No reportable DNA results were obtained from [*item*] due to quality control measures not being achieved.

Staff Match, no buccal standard obtained:

No reportable DNA results were obtained from [*item*] due to quality control measures not being achieved. Please submit a buccal standard from [*Staff member*] for comparison purposes (this will only be done after attempts have been made to obtain the standard).

Related individuals:

Due to biological relatedness, no statistics can be applied with respect to [*Name*] as possible contributors to reported mixtures that are suitable for comparison.

Alternate Reference Samples:

A DNA profile was obtained from [*Item*] that will be used as the alternate known standard for comparison purposes; the use of this DNA profile in making the following comparisons assumes that [*subject*] is the source of the DNA profile obtained from [*Item*].

If not doing stats, but profile is same: The DNA profile obtained from [*item*] is consistent with the DNA profile obtained from [*Subject*].

DNA Methodology:

DNA was extracted, quantified, and when appropriate, amplified by the STR polymerase chain reaction (PCR) using the AmpFISTR Identifiler Kit.

Within the same report – same stats obtained from a previous result:

Refer to results for Item X for statistical calculations.

Before Items received for recalculated stats reports:

This report reflects recalculated statistics based on the Amendment of the FBI's STR Population Data referenced below.

Statistics:

Statistics were calculated using the FBI population database which is published in the July 2015 Journal of Forensic Science Erratum referencing the Journal of Forensic Science, Volume 44(6):1277-1286., and Journal of Forensic Science, Volume 46 (3): 453-489 by Budowle, et al. The reported statistics are an estimation for which a deviation of approximately +/- 10-fold may exist. The loci D2S1338 and D19S433 are not included in the statistical calculations.

The most common frequency from the Caucasian, African American, Southwest Hispanic, or Southeast Hispanic populations is reported. A source attribution is reported at rarer than 1 in 700 billion and in the absence of a known identical twin.

If item received but not processed:

This item was not examined.

If work is stopped per ADA or Detective:

No (further) analysis was needed per instruction from [name].

CODIS input:

The DNA profile obtained from [item] (DNA #) has been entered into the Combined DNA Index System (CODIS) and will be searched on a periodic basis.

Photos:

[Items] were photographed. OR The submitted items with packaging, and the packaging to any unopened items received were photographed. All photographs are contained in or are attached to the case file in PLIMS. Copies of all photographs were saved to DEMS as PLIMS Item ____.

Sub-items:

[Item] was retained in the laboratory as [Item #]. (or returned to the original container). The ____ was labeled as [item #] and was returned to the original package.

Serology / Screening:

No stains of serological value were observed on the [item].

No serological examinations were conducted on [item]; however, the [item] was swabbed for DNA analysis.

No serological examinations were conducted on swabs of [item and or area]; however, the [name of swabs] swabs were retained in the laboratory for DNA analysis.

CMPD Crime Laboratory Biology Section Standard Operating Procedures

Prior to conducting tests the [area(s) and item] were swabbed [together] for DNA analysis. No serological examinations were conducted on the swabs and the swabs were retained in the laboratory.

No serological examinations were conducted on the blood standard from [person]; however, the standard was retained in the laboratory for DNA analysis.

No serological examinations were conducted on the known cheek scraping (buccal standard) from [person]; however, the buccal standard was retained in the laboratory for DNA analysis.

Presumptive testing for the presence of blood was positive/negative on [item].
{PHT/LMG + or neg}

Blood was detected on [item]. {PHT/LMG +, Hematrace +}

Testing for amylase, a non-specific constituent of saliva, was positive/negative on [item].
{Phadebas, at least 1+, or negative}

Due to the nature of the test, no conclusion can be made concerning the presumptive testing for saliva. {Phadebas < 1+}

Presumptive testing for the presence of semen was negative on [item]. {AP time > 60 seconds}

Semen was detected on [item]. {AP+/-, micro +} or {AP+, p30+}

Semen was not detected on [item]. {AP-, micro -} or {AP+, micro-, p30-}

A(n) apparent hair was observed on [item].

A(n) apparent hair possibly suitable for nuclear DNA analysis was observed on [item].

Apparent hairs were collected from [item] and were returned to the original package without further examination.

No apparent hair was observed on [item].

Loose debris was observed on [item] and was returned to the original container or sealed manila envelope without examination.

Semen was detected on the ___ slides. Semen was not detected on the ___ swabs; however the ___ swabs were retained in the laboratory as item # XX. / Semen was detected on the ___ swabs. Semen was not detected on the ___ slides.

Drop at quant with no male DNA detected at quant:

Due to an insufficient detection of human DNA for PCR analysis, [item] was not profiled.

Drop at quant with male DNA detected at quant:

Due to an insufficient detection of human DNA for PCR analysis, [item] was not profiled; however, male DNA was detected.

No DNA profile obtained with no male DNA detected at quant:

No DNA profile was obtained from Item X.

No DNA profile obtained with male DNA detected at quant:

No DNA profile was obtained from Item X; however, male DNA was detected.

Minimal with no male DNA:

Due to the limited value of the results obtained from [item], this data is not used for comparison purposes.

Minimal with male DNA only detected at quant:

Due to the limited value of the results obtained from [item], this data is not used for comparison purposes; however, male DNA was detected.

Minimal with Y at Amelogenin:

Due to the limited value of the results obtained from [item], this data is not used for comparison purposes; however, this profile contains at least one male contributor.

Complex with Y at Amelogenin:

The DNA profile obtained from [item] is a complex mixture and cannot be used for comparison purposes; however, this profile contains at least one male contributor.

Mixture of at least two or three not used for comparison:

A mixture of DNA profiles from at least [two or three] individuals was obtained from Item X. Due to the limited value and/or the complexity of the results obtained, this data is not used for comparison purposes.

Mixture of at least two or three not used for comparison with male DNA only detected at quant:

A mixture of DNA profiles from at least [two or three] individuals was obtained from Item X. Due to the limited value and/or the complexity of the results obtained, this data is not used for comparison purposes; however, male DNA was detected.

Mixture of at least two or three not used for comparison with Y at Amelogenin:

A mixture of DNA profiles from at least [two or three] individuals was obtained from Item X. Due to the limited value and/or the complexity of the results obtained, this data is not used for comparison purposes; however, this profile contains at least one male contributor.

Buccal Standard:

A DNA profile was obtained from the buccal standard from [NAME].

Buccal Standard submitted under different Complaint # than reported:

A DNA profile was obtained from the buccal standard from [NAME], which was submitted under Complaint # XXXX.

Previously run buccal:

A DNA profile was previously obtained from the buccal standard from [NAME], which was analyzed and reported under Complaint # XXXXX.

Single Source:

A DNA profile was obtained from Item X.

Full Single Source Match:

The DNA profile obtained from Item X matches the DNA profile obtained from [NAME]. Given the frequency of occurrence of the DNA profile is rarer than 1 in 700 billion, the evidence supports that [NAME] is the source of the DNA profile obtained from Item X.

Full Single Source Non-Match:

A DNA profile was obtained from Item X. [NAME] is excluded as a possible contributor of this profile.

Partial Single Source:

A partial DNA profile was obtained from Item X.

Partial Single Source Match:

The partial DNA profile obtained from Item X is consistent with the DNA profile obtained from [NAME].

Given the frequency of occurrence of the partial DNA profile is rarer than 1 in 700 billion, the evidence supports that [NAME] is the source of the DNA profile obtained from Item X.

OR

The probability of selecting an unrelated person at random who could be the source of this partial DNA profile is approximately 1 in _____. The loci (include what loci are not used) are not included in the statistical calculations

Partial Single Source Non-Match:

A partial DNA profile was obtained from Item X. [NAME] is excluded as a possible contributor of this profile.

Mixture with major profile and minor inconclusive:

A mixture of DNA profiles from at least [two or three] individuals was obtained from Item X. A [partial] major DNA profile can be determined. No conclusions can be made regarding the minor profile(s); however,

[male DNA was detected.] (quant only)

OR

[this profile contains at least one male contributor.] (Y at Amelogenin minor)

Major Profile Match:

The major DNA profile obtained from Item X matches the DNA profile obtained from [NAME]. Given the frequency of occurrence of the major DNA profile is rarer than 1 in 700 billion, the evidence supports that [NAME] is the source of the DNA profile obtained from Item X.

Partial Major Profile Match:

The partial major DNA profile obtained from Item X is consistent with the DNA profile obtained from [NAME].

Given the frequency of occurrence of the partial major DNA profile is rarer than 1 in 700 billion, the evidence supports that [NAME] is the source of the DNA profile obtained from Item X.

OR

The probability of selecting an unrelated person at random who could be the source of this partial major DNA profile is approximately 1 in _____. The loci (include what loci are not used) are not included in the statistical calculations

Major or Partial Major Profile Non-Match:

[NAME] is excluded as a possible contributor of this major profile.

Mixture with major profile and minor profile(s) interpreted:

A mixture of DNA profiles consistent with originating from [two or three] individuals was obtained from Item X. A major and (partial) minor DNA profile can be determined.

Major Profile Match:

The major DNA profile obtained from Item X matches the DNA profile obtained from [NAME]. Given the frequency of occurrence of the major DNA profile is rarer than 1 in 700 billion, the evidence supports that [NAME] is the source of the DNA profile obtained from Item X.

Partial Major Profile Match:

The partial major DNA profile obtained from Item X is consistent with the DNA profile obtained from [NAME].

Given the frequency of occurrence of the partial major DNA profile is rarer than 1 in 700 billion, the evidence supports that [NAME] is the source of the DNA profile obtained from Item X.

OR

The probability of selecting an unrelated person at random who could be the source of this partial major DNA profile is approximately 1 in _____. The loci (include what loci are not used) are not included in the statistical calculations

Major Profile Non-Match:

[NAME] is excluded as a possible contributor of this major DNA profile.

Partial Major Profile Non-Match:

[NAME] is excluded as a possible contributor of this partial major DNA profile.

Minor Profile Match:

The partial minor DNA profile obtained from Item X is consistent with the DNA profile obtained from [NAME].

Given the frequency of occurrence of the partial minor DNA profile is rarer than 1 in 700 billion, the evidence supports that [NAME] is the source of the DNA profile obtained from Item X.

OR

The probability of selecting an unrelated person at random who could be the source of this partial minor DNA profile is approximately 1 in _____. The loci (include what loci are not used) are not included in the statistical calculations

Minor Profile Non-Match:

[NAME] is excluded as a possible contributor of this partial minor DNA profile.

Major Group Consistent with two in mixture of at least three:

A mixture of DNA profiles from at least three individuals was obtained from Item X. A [partial] major group consistent with originating from two individuals can be determined [at all loci except.....].

Major Group Included:

[NAME(s)] is/are included as [a major contributor or major contributors] to the mixture obtained from Item X. The probability of selecting an unrelated person at random who could be included as a major contributor is approximately 1 in _____. The loci (include what loci are not used) are not included in the statistical calculations. No conclusions can be made regarding the minor profile(s).

Major Group Excluded:

[NAME(s)] is/are excluded as [a major contributor or major contributors] to the mixture obtained from Item X.

Mixture of two or three interpreted – No Major:

A mixture of DNA profiles consistent with originating from [two or three] individuals was obtained from Item X.

Mixture Included:

[NAME(s)] is/are included as [a contributor or contributors] to the mixture obtained from Item X. The probability of selecting an unrelated person at random who could be included as a possible contributor is approximately 1 in _____. The loci (include what loci are not used) are not included in the statistical calculations. No conclusions can be made regarding the minor profile(s).

Mixture Excluded:

[NAME(s)] is/are excluded as [a contributor or contributors] to the mixture obtained from Item X.

Intimate Samples-

Single Source:

A DNA profile foreign to [owner] was obtained from Item X.

Single Source Match:

A DNA profile foreign to [owner] was obtained from Item X that matches the DNA profile obtained from [NAME]. Given the frequency of occurrence of the DNA profile is rarer than 1 in 700 billion, the evidence supports that [NAME] is the source of the DNA profile obtained from Item X.

Full Single Source Non-Match:

A DNA profile foreign to [owner] was obtained from Item X. [NAME] is excluded as a possible contributor of this profile.

Partial Single Source:

A partial DNA profile foreign to [owner] was obtained from Item X.

Partial Single Source Profile Match:

A partial DNA profile foreign to [owner] was obtained from Item X that is consistent with the DNA profile obtained from [NAME].

Given the frequency of occurrence of the partial DNA profile is rarer than 1 in 700 billion, the evidence supports that [NAME] is the source of the DNA profile obtained from Item X.

OR

The probability of selecting an unrelated person at random who could be the source of this partial DNA profile is approximately 1 in _____. The loci (include what loci are not used) are not included in the statistical calculations

Partial Single Source Non-Match:

A partial DNA profile foreign to [owner] was obtained from Item X. [NAME] is excluded as a possible contributor of this profile.

Mixture consistent with two or three – Major determined – minor(s) determined – not conditioned on presence of reference standard(s) – owner dropping out but alleles consistent are present

A mixture of DNA profiles consistent with originating from [two or three] individuals was obtained from Item X.

A major DNA profile can be determined that is foreign to [owner].

[Owner] cannot be excluded as a possible contributor of the minor profile. (no statistics reported) – two person mixture

OR

A partial minor DNA profile can be determined that is foreign to [owner]. [Owner] cannot be excluded as a possible contributor of the remaining minor profile. – three person mixture (victim is low level third)

Mixture consistent with two or three conditioned on presence of reference standard(s) – owner / consensual partner

A mixture of DNA profiles consistent with originating from [two or three] individuals was obtained from Item X.

Assuming the presence of [NAME], a foreign DNA profile can be determined.

Full Foreign Match:

The foreign DNA profile obtained from Item X matches the DNA profile obtained from [NAME]. Given the frequency of occurrence of the foreign DNA profile is rarer than 1 in 700 billion, the evidence supports that [NAME] is the source of the DNA profile obtained from Item X.

Partial Foreign Match:

The partial foreign DNA profile obtained from Item X is consistent with the DNA profile obtained from [NAME].

Given the frequency of occurrence of the partial foreign DNA profile is rarer than 1 in 700 billion, the evidence supports that [NAME] is the source of the DNA profile obtained from Item X.

OR

The probability of selecting an unrelated person at random who could be the source of this partial foreign DNA profile is approximately 1 in _____. The loci (include what loci are not used) are not included in the statistical calculations

Major Group Consistent with two in mixture of at least three – [owner not present]:

A mixture of DNA profiles from at least three individuals was obtained from Item X. A [partial] major group foreign to [owner] and consistent with originating from two individuals can be determined [at all loci except.....].

Major Group Included:

[NAME(s)] is/are included as [a major contributor or major contributors] to the mixture obtained from Item X. The probability of selecting an unrelated person at random who could be included as a major contributor is approximately 1 in _____. The loci (include what loci are not used) are not included in the statistical calculations. No conclusions can be made regarding the minor profile(s).

Major Group Excluded:

[NAME(s)] is/are excluded as [a major contributor or major contributors] to the mixture obtained from Item X.

Foreign Profile Non-Match:

[NAME] is excluded as a possible contributor of this foreign DNA profile.

Partial Foreign Profile Non-Match:

[NAME] is excluded as a possible contributor of this partial foreign DNA profile.

Mixture with victim included (intimate), but not suitable for comparison (remaining portion):

A mixture of DNA profiles from at least [two or three] individuals was obtained from Item X. Victim cannot be excluded as a possible contributor to the mixture; however, no conclusions can be made regarding the remaining contributor(s).

No foreign DNA profile and no male DNA detected (if applicable):

No DNA foreign to [person] was detected on [item].

No foreign DNA profile and male DNA detected at quant (if applicable):

No DNA profile foreign to [person] was identified on [item]; however, male DNA was detected.

Y-STR with no suspect:

If a suspect is developed, it is recommended that this sample be outsourced for Y-STR testing.

Y-STR with suspect:

It is recommended that this sample be outsourced for Y-STR testing.

If sample was processed under old interpretations guidelines, but cannot be used under new guidelines: This profile is not suitable for statistical calculations; therefore, no comparisons can be made to possible contributors.

Disposition statements:

Item # was transferred to [examiner] in the Latent Print/Firearm section following examination on [mm/dd/year].

A request for DNA analysis has been entered.

The submitted items were transferred to the General Biology Cage to be returned to Property Control following examination.

All DNA extracts and any remaining extraction material associated with this case will be maintained in the laboratory and transferred to General Biology Storage.

Any remaining material from Item # will be maintained in the laboratory and transferred to General Biology Storage.

Items listed as retained in the laboratory were transferred to General Biology Storage following examination.

Any remaining material from Item # and all DNA extracts and any remaining extraction material associated with this case will be maintained in the laboratory and transferred to General Biology Storage.

Outsourcing:

The DNA profile obtained from [item] has been reviewed by the Biology section for entry into CODIS.

The DNA profile obtained from [item] (DNA #) has been entered into the Combined DNA Index System (CODIS) and will be searched on a periodic basis.

The evidence will be returned to Property Control following the return receipt of Item # from [outsourcing lab].

Outsourcing Presumptive Positive Blood – Burglary Cases:

Presumptive testing for the presence of blood was positive on Item X. The swabs were repackaged as Item X.1 and retained in the laboratory.

Items listed as retained in the laboratory were transferred to General Biology Storage to be sent to an outsourcing laboratory for DNA analysis which will be the subject of another report. All other items were transferred to the General Biology Cage to be returned to Property Control.

10.2 CODIS Match Reports

10.2.1 Local matches

For a Local case-to-case forensic hit: A search of the Local CODIS database generated a case-to-case match to the DNA profile obtained from [item] (DNA #). The following case information is provided as an investigative lead:

Complaint #:
Investigator:
Sample:
*Source: Unknown

Any connection between these cases must be determined through further investigation.

*The source will be listed as Unknown if the case is unsolved. A case that has been “solved” by an offender hit, but no confirmation sample has been provided will not be considered a known source for match reporting purposes.

If a source is listed, the following will be reported:

Any connection between these cases and/or of this individual to the cases must be determined through further investigation. For comparison between the buccal standard from [Name] and the case sample(s), it will be necessary for you to submit a DNA service request. Please contact the Biology section if you have any questions.

For a Local hit to the suspect database: A search of the Local CODIS database generated a match to the DNA profile obtained from [item] (DNA #). The following case information is provided as an investigative lead:

Complaint #:
Investigator:
Sample: [Buccal standard] from [Name]

Any connection of this individual to the case must be determined through further investigation. For comparison between the buccal standard from [Name] and the case sample(s), it will be necessary for you to submit a DNA service request. Please contact the Biology section if you have any questions.

For a Local hit to a Local case that had previously matched to a convicted offender (for previous matches to an arrestee, only the case-to-case information will be reported): A search of the Local CODIS database generated a match to the DNA profile obtained from [item] (DNA #). The following case information is provided as an investigative lead:

Complaint #:
Investigator:
Sample:

A previous search of the [State/National] CODIS database generated a match between the DNA profile obtained from [item] (DNA #) and [State] convicted offender specimen # []. The DNA database sample was re-examined and confirmed by the [state lab agency]. This individual is identified as:

[Name]
A.K.A.'s:
DOB:
SSN:
[State] SID #:
FBI #:
Race:
Gender:

This information is provided as an investigative lead, and any connection of this individual to the case must be determined through further investigation. In order to confirm this match, it will be necessary for you to submit a known DNA standard from [Name]. Please contact the Biology section if you have any questions.

10.2.2 State and National Hits

For a State or National case-to-case forensic hit: A search of the [State/National] CODIS database generated a case-to-case match to the DNA profile obtained from [item] (DNA #). The following case information is provided as an investigative lead:

Investigator:
Agency:
Address:
Phone:
Agency case #:
Case type:
Sample:

Any connection between these cases must be determined through further investigation.

For a State or National offender/arrestee hit: A search of the [State/National] CODIS database generated a match between the DNA profile obtained from [item] (DNA #) and [State/Federal] convicted offender/arrestee specimen # []. The DNA database sample was re-examined and confirmed by the [state lab agency]. This individual is identified as:

[Name]
A.K.A.'s:

DOB:
SSN:
[State] SID #:
FBI #:
Race:
Gender:

This information is provided as an investigative lead, and any connection of this individual to the case must be determined through further investigation. In order to confirm this match, it will be necessary for you to submit a known DNA standard from [Name]. Please contact the Biology section if you have any questions.

For a State hit to the suspect database: A search of the State CODIS database generated a match to the DNA profile obtained from [item] (DNA #). The following case information is provided as an investigative lead:

Investigator:
Agency:
Address:
Phone:
Agency case #:
Case type:
Sample: [Buccal standard] from [Name]

Any connection of this individual to the case must be determined through further investigation. In order to confirm this match, it will be necessary for you to submit a known DNA standard from [Name]. Please contact the Biology section if you have any questions.

10.2.3 External agency suspect hits

For a Local hit to the suspect database when an outsourcing lab has run a buccal standard submitted to CMPD: A search of the Local CODIS database generated a match to the DNA profile obtained from [item] (DNA #). The following case information is provided as an investigative lead:

Complaint #:
Investigator:
Sample: [Buccal standard] from [Name]

Any connection of this individual to the case must be determined through further investigation. For comparison between the buccal standard from [Name] and the case sample(s), it will be necessary for you to submit a DNA service request; the buccal standard will need to be re-run due to original analysis being conducted

by [outsourcing lab]. Please contact the Biology section if you have any questions.

For a Local hit to the suspect database when an outsourcing lab has run a buccal standard submitted from an agency other than CMPD: A search of the Local CODIS database generated a match to the DNA profile obtained from [item] (DNA #). The following case information is provided as an investigative lead:

Investigator:

Agency:

Address:

Phone:

Agency case #:

Case type:

Sample: [Buccal standard] from [Name]

Any connection of this individual to the case must be determined through further investigation. In order to confirm this match, it will be necessary for you to submit a known DNA standard from [Name]. Please contact the Biology section if you have any questions.

References:

- SWGDAM Interpretation Guidelines for Autosomal STR typing by forensic DNA testing laboratories, 2010, Available at www.swgdam.org
- The Federal Bureau of Investigations Quality Assurance Standards Audit Document for Forensic DNA Testing Laboratories
- CODIS Manual
- Journal of Forensic Science, Volume 44(6):1277-1286.
- Journal of Forensic Science, Volume 46 (3): 453-489.
- Journal of Forensic Science, Erratum Volume 60 (4): 1114-1116