AUSTIN POLICE DEPARTMENT
SEROLOGY/DNA SECTION
TECHNICAL MANUAL

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CHAPTER 1 SEROLOGY

1.1 Screening Guidelines

The following are general guidelines for the screening of evidence. Not every scenario will fit into these guidelines and analysts will use their experience and training to process the evidence in the way that is most appropriate.

Body fluid identification – Blood

A typical analysis scheme for a bloodstain follows.

SUSPECTED BLOODSTAIN
↓
PRESUMPTIVE TEST (TMB/PHT) → NEGATIVE → NO APPARENT BLOOD
↓
POSITIVE
↓
APPARENT BLOOD DETECTED
↓
TEST FOR PRESENCE OF OTHER BODY
FLUIDS IF INDICATED
↓
FREEZE
↓
DNA ANALYSIS

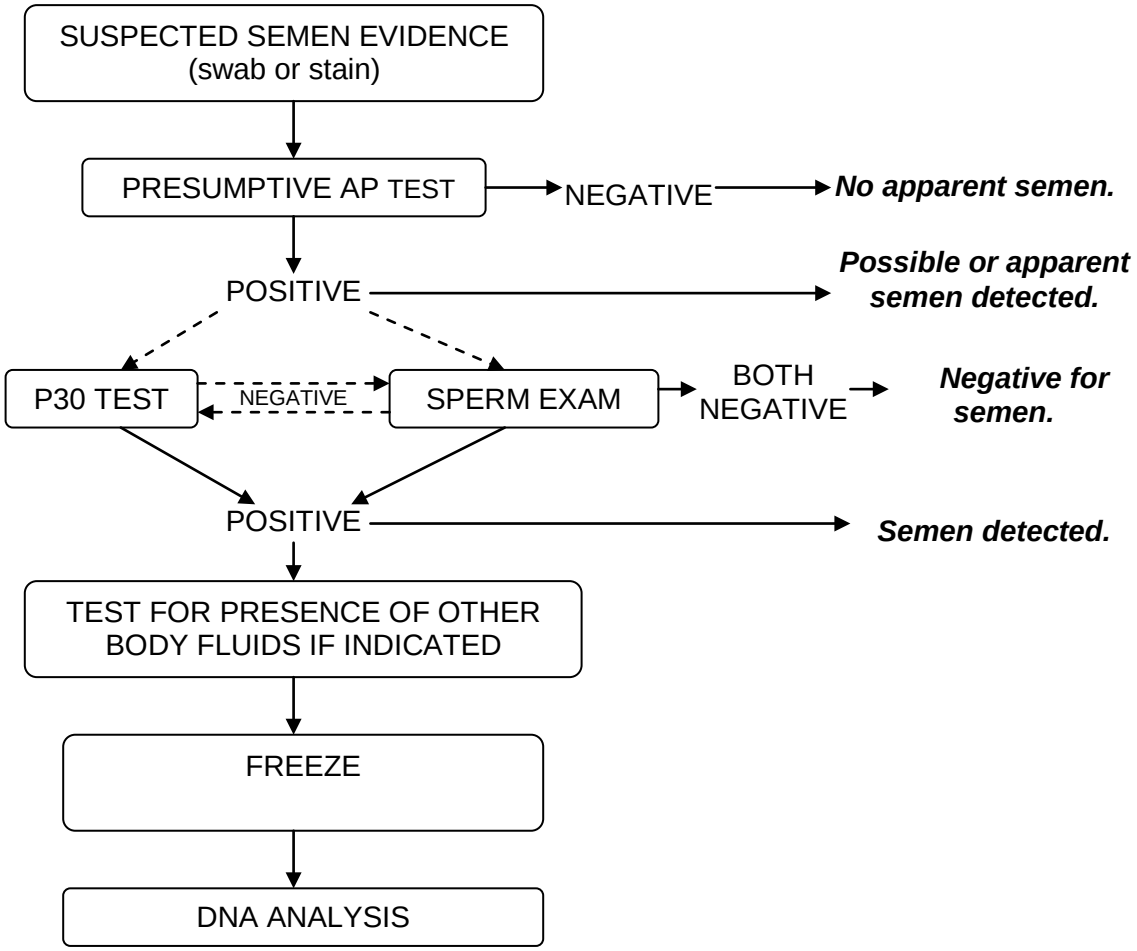
Individual procedures may include additional or fewer steps. How far the examiner proceeds in a particular case will depend on the available sample and on what is necessary to answer the question(s) posed.

Body fluid identification – Semen

Use of an alternate light source can be helpful when screening evidence for semen; especially for clothing or bedding.

Slides will typically be stained with Christmas Tree stain. The magnification typically used by an analyst will be documented on the “Magnification used by Analysts” document on the DNA Unit group drive. If something different is used, the magnification will be documented in the case record. If they are not stained for viewing the analyst will document this in the case notes. If "vaginal", "cervical", "anal", or "oral" swabs are present and to be analyzed without accompanying slides a sperm examination must be performed. Screening a victim’s panties or pants should include some qualitative acid phosphatase test of the crotch area when applicable based on the case scenario.

A typical analysis scheme for a suspected semen stain or swab follows. Note that appropriate outcome is indicated in ***bold italics***.



Individual procedures may include additional or fewer steps. How far the examiner proceeds in a particular case will depend on the available sample and on what is necessary to answer the question(s) posed.

The investigator's report and medical examination report, if submitted, should be reviewed; they may answer questions that could focus the analysis and aid in interpretation of results. Careful review of notes and data should be made.

A negative p30 test alone will not be used with a positive presumptive test result to draw a negative conclusion for the presence of semen. Both a negative p30 and negative spermatozoa exam must be used to conclude that there is no evidence of semen when the presumptive test is positive

Sexual Assault Kits: At analyst discretion, components of a SAFE kit may be documented as receiving no analysis (for example, changing paper, trace evidence or hair collections, body swabs if the victim showered, etc.). If intimate specimens are positive for semen, other body swabs may not need analysis. Intimate specimens may not need analysis based on the scenario (for example, digital penetration cases). All components of the SAFE kit will be documented as existing in the report. If the Sexual Assault Examination Worksheet (DNA 003) is used, the analyst may choose to use that in lieu of the Serology Worksheets (DNA 001 and DNA 002).

1.2 Presumptive blood tests – PHT AND TMB

Catalytic tests for blood are based on the peroxidase-like activity exhibited by the heme group of hemoglobin. For example, colorless phenolphthalein is oxidized to phenolphthalein in the presence of heme and hydrogen peroxide. In a basic solution such as the test reagent, the phenolphthalein is pink. The test is exceedingly sensitive to minute traces of hemoglobin and its derivatives but will produce a false positive reaction in the presence of any of a number of oxidizing substances. Should a color reaction take place, the result only suggests the presence of blood; the test is therefore a presumptive test.

Related Documents
Serology worksheets, Laboratory Information Sheet, Laboratory Information Sheet-Worksheet, and/or sexual assault examination worksheet

Equipment / Materials / Reagents
diH_2O
Cotton swab(s) or filter paper – optional
Test reagent – One of the following (See Reagents section):
Phenolphthalein (PHT) solution
Tetramethylbenzidine (TMB) solution
3-10% hydrogen peroxide – purchased
Alcohol for PHT test

Standards, Controls, and Calibration
A positive and negative control must be tested each day of reagent use. An appropriate positive control is a small bloodstain prepared in-house. Appropriate negative controls include a cotton swab or small fabric swatch with no blood. The results of each control test must be recorded on every applicable Serology Worksheet or Laboratory Information Worksheet. If more than one day of work is on the same page and controls are used, the additional dates will be added to the top of the page by the control results. Reagents will be good as long as the control tests pass.

Procedure

1. Rub a moistened cotton swab or dry folded filter paper over the suspected bloodstain.
2. Apply one to several drops of the test reagent (PHT or TMB).

3. Observe briefly to identify color change
4. Add one to several drops of 3% – 10% hydrogen peroxide.

Interpretation

Appearance of an immediate color change is a presumptive positive result for blood.

Phenolphthalein: colorless → immediate bright pink
Tetramethylbenzidine: colorless → immediate blue-green

Literature / Supporting Documentation
Lee, Henry C. 1982. Identification and grouping of bloodstains. In: Forensic Science Handbook, Volume 1. Richard Saferstein, ed. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. Chapter 7, pp. 272-276.

1.3 AP spot test and map for semen stains

Acid phosphatase is found in relatively large quantities in semen and its detection is reason to suspect the presence of semen in a body fluid stain. In the following procedure, acid phosphatase is detected by a color-change reaction. Acid phosphatase liberates the phosphate from α-naphthyl phosphate, and the released naphthol combines with tetrazotized o-dianisidine to form a purple azo dye.

The AP test is semi-quantitative. A stronger reaction is more likely to indicate semen. However, because acid phosphatase occurs in other body fluids, most notably vaginal secretions, this is only a presumptive test. The presence of semen in the sample can subsequently be confirmed by identifying p30 or spermatozoa.

Related Documents
Serology worksheets, Laboratory Information Sheet, Laboratory Information Sheet-Worksheet, and/or Sexual Assault Examination Worksheet

Equipment / Materials / Reagents
diH_2O
Cotton swab(s) or filter paper
Plastic cutting board and clips (optional, mapping only)
AP Test Reagent

Standards, Controls and Calibration
A positive and negative control must be tested each day of reagent use. An appropriate positive control is a small semen stain. Appropriate negative controls include a cotton swab or small fabric swatch with no semen. The results of each control test must be recorded on every applicable Serology Worksheet or Laboratory Information Worksheet. If more than one day of work is on the same page and controls are used, the additional dates will be added to the top of the page by the control results.

Procedure

AP spot test procedure

1. Take a small cutting, blotting, or swabbing of the unknown stain.
2. Apply one or two drops of the AP Test Reagent.
3. Observe the cutting, blotting, or swabbing for up to 60 seconds for a color change.

AP map procedure

1. Spread the garment, sheet, shirt, panties, towel or other item flat. Fasten the item as necessary to a plastic cutting board with clips.
2. Moisten a sheet or swatch of filter paper with diH_2O. Remove excess water by blotting the moistened paper onto a dry sheet of filter paper.
3. Lay the moist filter paper over the item or area of the item to be tested. Press the paper firmly against the item at all points of the item's surface. Allow approximately 30 seconds to 5 minutes for any possible acid phosphatase to transfer to the paper.
4. Mark the position of the paper on the item.
5. Remove the sheet of filter paper.
6. Spray or cover the surface that was pressed to the item with AP Test Reagent.
7. Observe the treated filter paper for up to 60 seconds for a color change.
8. After interpretation, draw or otherwise document the areas where there is a positive reaction. The filter paper may be placed between plastic sheets to photocopy.

Interpretation
The appearance of a purple color within 60 seconds indicates the presence of acid phosphatase and is a positive presumptive test for semen. Grade the reaction as 4+ (0-15 seconds), 3+ (15-30 seconds), 2+ (30-45 seconds), 1+ (45-60 seconds), or negative. The AP map procedure reflects the size and shape of the acid-phosphatase-containing stains. A negative result indicates acid phosphatase is absent or below the detection threshold.

Literature / Supporting Documentation
Gaensslen, Robert E. 1983. Sourcebook in Forensic Serology, Immunology, and Biochemistry. U.S. Department of Justice, National Institute of Justice. Sections 10.3.2 and 10.3.3

Stevens, J and Thomas, F., Clin Chem. Acta, 37 541 (1972)

1.4 Spermatozoa examination

Spermatozoa (sperm) detected in a stain or on a swab confirm the presence of semen. They can subsequently be differentially extracted from epithelial cells to allow for analysis of the sperm contributor's DNA. For spermatozoa examination, extract (or a

portion of a stain) is fixed to a microscope slide, stained (usually), and examined under the microscope for cells with sperm characteristics. If the slide is not stained for viewing, this will be documented in the case notes. Many staining techniques have been developed; this procedure uses nuclear fast red (also known as Kernechtrot) and picroindigocarmine. This stain is also called “Christmas Tree” because it stains the sperm red and green.

Related Documents
Serology worksheets, Laboratory Information Sheet, Laboratory Information Sheet-Worksheet, and/or Sexual Assault Examination Worksheet

Equipment / Materials / Reagents
Microscope slide
~60°C oven
Nuclear fast red (NFR)
Picroindigocarmine (PIC)
Denatured ethanol or methanol
dIH_20 or p30 extraction buffer

Standards, Controls and Calibration
None

Procedure

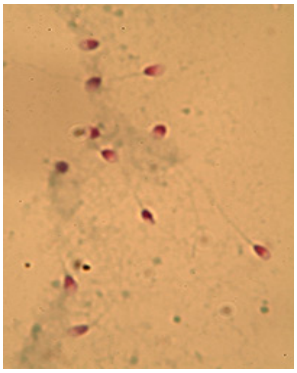
Use a smear provided in the sexual assault evidence kit or prepare a smear from a stain or swab as follows:

1. In a microcentrifuge tube, soak a portion of the dried body fluid stain or swab in ~500ul dIH_2O or p30 extraction buffer for at least 30 minutes to overnight at 2-8°C.
2. Samples may be vortexed to increase dissolution of the stain.
3. Degraded stains or stains fixed to fabrics can become increasingly insoluble and may require longer extraction times, e.g., up to 24 hours.
4. Remove the substrate and place in a spin basket.
5. Collect the cellular debris by centrifugation at top speed (12,700 -14,000) for 2 to 5 minutes. Remove ~ 10ul and spot on a slide. Heat fix the slide in a ~60°C oven.
    a. If a stain or swab produces a strong positive AP reaction, a quick prep slide can be prepared by rubbing either the stain or swab vigorously against a microscope slide that has been moistened with diH2O. The slide may be heat fixed in a 60C oven.
6. Stain the slide. In the event that evidence is extremely limited and the sperm on the slide may be needed for DNA analysis, the slide may be examined without staining.
7. Stain the sample area on the slide with NFR for 10-15 minutes.
8. Wash with dIH_20 water by gentle flooding.
9. Cover the sample area with PIC for up to 15 seconds.
10. Wash and fix by gently flooding the slide with denatured ethanol.
11. Allow to dry.

12. Scan the slide at a minimum magnification of 250X. A log of the magnifications used by each analyst will be maintained on the DNA unit group drive. If an analyst uses a different magnification than what is on the log, the magnification used will be documented in the case record. Record the number of sperm or sperm average per slide or field.

Interpretation

Human sperm are flagellated with a total length of about 50 μm. The sperm cell head generally is oval, flattened at the anterior end, with dimensions about 4.6 μm × 2.6 μm × 1.5μm. After staining, sperm heads are red, mid-pieces blue-green, and tails yellowish green. The acrosome may remain attached to the anterior of the sperm head and be visible as a blue-green to pink cap. Epithelial cells stain blue to blue-green with red nuclei.



Literature / Supporting Documentation

Oppitz, E. 1969. Eine neue Färbemethode zum Nachweis der Spermien bei Sittlichkeitsdelikten. Arch. Kriminol. 144: 145-148.

Stone, I. C. 1972. Staining of spermatozoa with Kernechtrot and picroindigocarmine for microscopical identification. Document CIL No. 2, Southwestern Inst. Forensic Sci., Criminal Investigation Laboratory (USA).

Baechtel, S. F. 1988. The identification and individualization of semen stains. In: Forensic Science Handbook, Volume 2. Richard Saferstein, ed. Prentice-hall, Inc., Englewood Cliffs, New Jersey. Chapter 7, p. 357.

1.5 p30 Identification

The cells that line the ducts of the prostate make a protein known as p30 or prostate-specific antigen (PSA). The protein is secreted into seminal fluid to a concentration of approximately 0.24-5.5 mg/ml, and its detection confirms the presence of semen.
The ABAcard® p30 test is a qualitative detection method specifically designed for forensic identification of semen. Sample is added to a sample well where any detectable p30 present in the sample will bind with mobile p30 antibody. The resultant mobile antigen-antibody complex migrates through an absorbent strip to an area where

immobile p30 antibody is bound. The mobile antigen-antibody complex binds to the immobile antibody creating an antibody-antigen-antibody sandwich. When the p30 concentration in the sample exceeds 4 ng/ml, pink dye particles become visible in the area of immobilized antibody. The resultant pink band indicates a positive result.

Related Documents
Serology worksheets, Laboratory Information Sheet, Laboratory Information Sheet-Worksheet, and/or Sexual Assault Examination Worksheet

Equipment / Materials / Reagents
OneStep ABAcard® p30 Test strips – one per sample or control
Extraction buffer (provided in kit)

Standards, Controls and Calibration
Each card contains a control line that must be visible for each test card.

Procedure

1. Extract a small portion of a swab or cutting in ~500ul extraction buffer for a minimum of 30 minutes at 2-8°C.
2. Allow the sample(s) to warm to room temperature.
3. For each sample and control:
    a. Unwrap an ABAcard device and dropper. Label the card with the item number.
    b. Add 200 μl (8 drops with provided dropper) of sample to the sample well "S" of the device.
4. Read the results up to 10 minutes from the time of application of sample.
5. Samples that produce a negative result but that show strong positive acid phosphatase activity may be diluted 1:100 and re-tested.
6. Complete the Serology worksheet. Optional: photograph, draw, or photocopy the device.

Interpretation
The appearance of a pink line at the "C" (control) area is expected for all tests and must be present. Absence of the "C" line is an inconclusive result, and the test must be performed a second time. The appearance of a pink line at the "T" (test) area is a positive result and indicates that the p30 concentration in the applied solution is at least 4 ng/ml, equivalent to approximately 1:1,000,000 dilution. This confirms the presence of semen in the sample. The absence of a pink line at the "T" area after 10 minutes is a negative result and indicates that semen is absent, below the detection threshold, or above the high dose threshold.

Per the manufacturer, saliva can lead to a third line being present. If this is detected the test will be called inconclusive. A dilution of the sample (recommended 1:1) can be made and the test can then be repeated.

Literature / Supporting Documentation

Gaensslen, Robert E. 1983. Sourcebook in Forensic Serology, Immunology, and Biochemistry. U.S. Department of Justice, National Institute of Justice. Sections 10.14.

Baechtel, S. F. 1988. The identification and individualization of semen stains. In: Forensic Science Handbook, Volume 2. Richard Saferstein, ed. Prentice-hall, Inc., Englewood Cliffs, New jersey. Chapter 7, p. 364-366.

Abacus Diagnostics. 2011. OneStep ABAcard® p30 test for the forensic identification of semen. Product insert.

Benton, K. A., J. A. Donahue, and M. Valadez, Jr. 1998. Analysis of the ABAcard OneStep PSA test for use in the forensic laboratory. Texas Department of Public Safety Crime Laboratory Service. Unpublished.

Carradine. Cassie C., 1998. Evaluation of ABAcard® p30 Test for the Forensic Identification of Semen. Texas Department of Public Safety. Unpublished

1.6 Alternate Light Source

The Luma-Lite and Crime-Lite lamps are specially designed for detection of forensic stains, fibers, and fingerprints. Commonly called Alternate Light Sources (ALS), these lamps provide intense light of specific wavelengths usually through a hand-held wand or flashlight type device.

Many dried semen stains on cloth are detectable visually because their color, off-white or yellow, is different from that of the material on which the semen has been deposited. On many substrates, however, semen stains are not readily visible. Under ultraviolet (UV, <400 nm) or intense blue (420-470 nm) light semen stains fluoresce.

Related Documents
Serology worksheets, Laboratory Information Sheet, Laboratory Information Sheet-Worksheet, and/or Sexual Assault Examination Worksheet

Equipment / Materials / Reagents
Alternate light source (ALS) - e.g., Luma-Lite or Crime-Lites
Goggles or Amber glasses

Standards, Controls and Calibration
None

Procedure

1. Turn on and warming up the lamp, if not already on.
2. Put on the amber goggles or amber glasses.
3. Direct the ALS at the evidence.
4. Document observations. Abbreviations exist for the Crime-Lites (L, LL). If the luma-lite is used, document as such.

Interpretation
Semen stains typically appear fluorescent under ALS.

Literature / Supporting Documentation
Payton Scientific website: http://home.att.net/~paytonscientific/page6.html.

Baechtel, S. F. 1988. The identification and individualization of semen stains. In: Forensic Science Handbook, Volume 2. Richard Saferstein, ed. Prentice-hall, Inc., Englewood Cliffs, New Jersey. Chapter 7, p. 349.

Foster and Freeman website: http://www.fosterfreeman.com/

CHAPTER 2 DNA EXTRACTION

At the end of each extraction, the analyst will document in the case file the volume of extract obtained from the extraction. This volume should be measured with a pipette rather than based on what was expected as an elution volume.

2.1 QIAamp® Investigator Kit-Manual

This procedure uses the QIAamp® DNA Investigator Kit to produce a single extract of all nucleic acids from a dried body fluid stain or swab. The QIAamp® Kit has been researched for forensic application. The QIAamp silica-gel membrane has an affinity for nucleic acids under certain buffer and temperature conditions. The membrane is supported in a microcentrifuge tube, which simplifies handling. A body fluid extract is centrifuged through the membrane. The adsorbed nucleic acids are washed and then eluted.

Related Documents
DNA Extraction worksheet

Equipment / Materials / Reagents
Microcentrifuge
Vortex
Water baths (56°C and 70°C) – or 70°C oven
QIAamp DNA Investigator Kit: Expires 2 years from date of receipt
- QIAamp MinElute columns and 2-ml collection tubes
- Buffer AL
- Buffer ATL
- Buffer AW1 concentrate
- Buffer AW2 concentrate
- Buffer ATE
- Proteinase K
- RNA

Ethanol (200 proof, molecular biology grade)
Qiagen lyse and spin baskets

Standards, Controls, and Calibration
A minimum of 1 reagent blank must be processed for each extraction batch as a negative control. The extraction method must be recorded on the DNA Extraction worksheet.

Procedure

1. Do not wet the rim of the spin column when transferring liquid.
2. Do not touch the membrane with the pipette tip.
3. Prepare reagents and samples
4. Equilibrate Buffer ATE to room temperature if necessary (15–25°C).

5. Dissolve any precipitate in Buffer ATL by incubating at 70°C.
6. From the evidentiary stain or swab, remove approximately (NOTE: These are typical amounts but may vary from sample to sample):
    a. 0.1–0.50 cm2 dried bloodstain or suspected saliva
    b. 0.25–1 swab
    c. 1 cm strip of paper from outside of cigarette butt
7. Lyse cells and suspend
    a. Combine the sample with 300µl Buffer ATL and 20µl Proteinase K into a labeled microcentrifuge tube or Qiagen lyse and spin baskets.
    b. Vortex for ~10 seconds
    c. Incubate for at least one hour to overnight at **56°C**. Periodic vortexing may improve lysis.
8. Adsorb nucleic acids to membrane

    NOTE: Dissolved RNA should be aliquoted and stored frozen. Do not freeze-thaw the aliquots more than three times.

    a. Briefly spin tubes (if using microcentrifuge tubes). Remove and discard the swab or cutting while retaining as much liquid as possible in the tube. A spin basket may be used (recommended spin setting of 12,700 rpm for 5 minutes).
        i. If using a Qiagen lyse and spin basket, centrifuge at 12,700 rpm for 1 minute and remove the basket and discard.
    b. Prior to first use, RNA needs to be dissolved in 310µl ATE.
    c. Gently mix Buffer AL by inverting. Add 300µl Buffer AL and 1µl diluted carrier RNA per sample. Vortex for 15 seconds. Incubate at **70°C** for 10 minutes. Periodic vortexing may improve lysis. Briefly spin tubes.
    d. Add 150µl ethanol. Vortex for 15 seconds. Briefly spin tubes.
9. Transfer tube contents (including any precipitate) to a labeled QIAamp MinElute column in a collection tube.
10. Centrifuge at 8000 rpm for 1 minute or until all solution has passed through the membrane. Place the column in a clean 2ml collection tube and discard the collection tube containing the flow-through.
11. Wash membrane (NOTE:Before first use, add ethanol to Buffers AW1 and AW2 according to the directions on the buffer bottle.)
    a. Add 500µl Buffer AW1 to the column.
    b. Centrifuge at 8,000 rpm for 1 minute or until all solution has passed through the membrane. Place the column in a clean 2 ml collection tube and discard the collection tube containing the flow-through.
    c. Add 700µl Buffer AW2 to the column and centrifuge at 8,000 rpm for 1 minute. Transfer the column to a new collection tube.
    d. Add 700µl ethanol to the column and centrifuge at 8,000 rpm for 1 minute. Transfer the column to a new collection tube.
    e. Centrifuge at 14,000 rpm for an additional 3 minutes or until all solution has passed through the membrane.
12. Elute nucleic acids
    a. Transfer the column to a final, new, labeled collection/storage tube.

b. Add 20-100µl Buffer ATE to the column depending on the expected quantity of DNA. Incubate at room temperature for 5 minutes.
c. Centrifuge at 14,000 rpm for 1 minute.
d. Place the appropriate cap on the tube.
e. Store samples at 2–8°C or at –20°C until ready to perform PCR.

Interpretation
None

Literature/Supporting Documentation
Scherczinger, C. A., M. T. Bourke, C. Ladd, and H. C. Lee. 1997. DNA extraction from liquid blood using QIAamp. J. Forensic Sci. 42(5): 893–896.

Greenspoon, S., M. A. Scarpetta, M. L. Drayton, and S. A. Turek. 1998. QIAamp spin columns a method of DNA isolation for forensic casework. J Forensic Sci 43(5): 1024–1030.
Frégeau, C. J., K. L. Bowen, and R. M. Fourney. 1999. Validation of highly polymorphic fluorescent multiplex short tandem repeat systems using two generations of DNA sequencers. J Forensic Sci 44(1): 133–166.

Sinclair, K. and V. M. McKechnie. 2000. DNA extraction from stamps and envelope flaps using QIAamp and QIAshredder. J Forensic Sci 45(1): 229–230.

2.2 QIAamp® Investigator Kit- QIAcube

This procedure uses the QIAamp® DNA Investigator Kit to produce a single extract of all nucleic acids from a dried body fluid stain or swab. The QIAamp® Kit has been researched for forensic application. The QIAamp silica-gel membrane has an affinity for nucleic acids under certain buffer and temperature conditions. The membrane is supported in a microcentrifuge tube, which simplifies handling. A body fluid extract is centrifuged through the membrane. The adsorbed nucleic acids are washed and then eluted.

Related Documents
DNA Extraction worksheet

Equipment / Materials / Reagents
QIAcube extraction robot
Vortex
Water bath (56°C)-optional
QIAamp DNA Investigator Kit: (Expires 2 years from date of receipt)
- QIAamp MinElute column
- Buffer AL
- Buffer ATL
- Buffer AW1 concentrate
- Buffer AW2 concentrate
- Buffer ATE

Proteinase K
RNA
Ethanol (200 proof, molecular biology grade)
Rotor adapters
Qiagen lyse and spin baskets
1.5ml and 2ml microcentrifuge tubes

Standards, Controls, and Calibration
A minimum of one reagent blank must be processed for each extraction batch (robot run) as a negative control.

Procedure

1. Prepare reagents and samples
    a. Equilibrate samples and elution solution to room temperature (20-25°C).
    b. Dissolve any precipitate in Buffer ATL by incubating at 56°C.
2. From the evidentiary stain or swab, remove approximately (These are typical amounts but may vary from sample to sample):
    a. 0.1–0.50 cm^2 dried bloodstain or suspected saliva stain
    b. 0.25–1 swab
    c. 1 cm strip of paper from outside of cigarette butt
3. Lyse cells and suspend
   NOTE: Load according to the Qiagen Lysis Protocol Sheet.
    a. Robot Digestion (Use shaker adapter “2”)
        i. Place the sample into a labeled 2.0 ml microcentrifuge tube.
        ii. Place tubes in the appropriate space in the shaker.
        iii. Place the reagent bottle with Buffer ATL into Position 1
        iv. Place the appropriate amount of Pro K into a 1.5ml microcentrifuge tube and put in position A.
        v. Select from the last protocol or DNA QiaAmp DNA Investigator Forensic Casework Samples.
4. Purification
    a. If using microcentrifuge tubes, remove and discard the swab or cutting while retaining as much liquid as possible in the tube. A spin basket may be used (recommended spin setting-12,700 rpm for 5 minutes).
        i. If using a Qiagen lyse and spin basket, centrifuge at 12,700 rpm for 1 minute and remove the basket and discard.
    b. Transfer the liquid to a new labeled 2.0 ml microcentrifuge tube if the tube lid has been closed for centrifuging.
    c. Place tubes in the appropriate space in the shaker. Load the Qiacube according to the purification protocol sheet.
    d. Place the following reagents in the designated positions: Buffer AL-2, Ethanol-3; Buffer AW1-4, Buffer AW2-5, Buffer ATE-6.
    e. Prior to first use, RNA needs to be dissolved in 310µl ATE.
        i. Add 1ul of diluted carrier RNA to each sample tube.
    f. Place a labeled 1.5 ml collection tube into position 3 of the rotor adapter and a QIAamp MinElute spin column in position 1 of the rotor adapter. Load the rotor adapters into the QIAcube centrifuge per the loading chart.

g. Start the purification protocol. Elution volumes of 20µl-100µl are recommended. Select from the last or DNA QiaAmp DNA Investigator Forensic casework samples purification and follow prompts.

5. Store samples at 2–8°C or at –20°C until ready to perform PCR.

Interpretation
None

Literature/Supporting Documentation
Scherczinger, C. A., M. T. Bourke, C. Ladd, and H. C. Lee. 1997. DNA extraction from liquid blood using QIAamp. J. Forensic Sci. 42(5): 893–896.

Greenspoon, S., M. A. Scarpetta, M. L. Drayton, and S. A. Turek. 1998. QIAamp spin columns a method of DNA isolation for forensic casework. J Forensic Sci 43(5): 1024–1030.

Frégeau, C. J., K. L. Bowen, and R. M. Fourney. 1999. Validation of highly polymorphic fluorescent multiplex short tandem repeat systems using two generations of DNA sequencers. J Forensic Sci 44(1): 133–166.

Sinclair, K. and V. M. McKechnie. 2000. DNA extraction from stamps and envelope flaps using QIAamp and QIAshredder. J Forensic Sci 45(1): 229–230.

2.3 QIAamp®Investigator Kit-Manual- Hair and Nail Clippings

This procedure uses the QIAamp® DNA Investigator Kit to produce a single extract of nucleic acids from a hair root or nail clippings. The QIAamp® Kit has been researched for forensic application. The QIAamp silica-gel membrane has an affinity for nucleic acids under certain buffer and temperature conditions. The membrane is supported in a microcentrifuge tube, which simplifies handling. A hair root extract or nail clipping extract is centrifuged through the membrane. The adsorbed nucleic acids are washed and then eluted.

Related Documents
DNA Extraction worksheet

Equipment / Materials / Reagents
Microcentrifuge
Vortex
Water baths (56°C and 70°C) – or 70°C oven
QIAamp DNA Investigator Kit: (Expires 2 years from date of receipt)
- QIAamp spin columns and 2-ml collection tubes
- Buffer AL
- Buffer ATL
- Buffer AW1 concentrate
- Buffer AW2 concentrate

Buffer ATE
Proteinase K
RNA
Ethanol (200 proof, molecular biology grade)
DTT (.39M)

Standards, Controls, and Calibration
A minimum of one reagent blank must be processed for each extraction batch as a negative control. The extraction method must be recorded on the DNA Extraction worksheet.

Procedure

Do not wet the rim of the spin column when transferring liquid. Do not touch the membrane with the pipette tip.

1. Prepare reagents and samples
    a. Equilibrate Buffer ATE to room temperature (15–25°C).
    b. Dissolve any precipitate in Buffer ATL by incubating at 56°C.
2. From the evidentiary hair, remove up to:
    a. 0.5-1 cm root end of hair and add to a microcentrifuge tube (Xylene bath or freezing may be used to remove the mounted hair from a slide). Rinse hair in sterile dIH2O, if necessary, prior to removing root end.
        i. Optional: 1 cm of adjacent hair shaft may also be extracted as a control.
    b. For nail clippings, add the clippings to a microcentrifuge tube
3. Lyse cells and suspend
    a. Place the sample into a labeled microcentrifuge tube.
    b. Add 300µl Buffer ATL, 50µl .39 M DTT, and 20µl Proteinase K. Vortex for 10 seconds.
    c. Incubate for 1 hour to overnight at 56°C. Hair should dissolve. Periodic vortexing may improve lysis.
4. Adsorb nucleic acids to membrane
    a. Briefly spin tubes.
    b. Prior to first use, RNA needs to be dissolved in 310µl ATE.
    c. Gently mix Buffer AL by inverting. Add 300µl Buffer AL and 1µl diluted RNA to each sample. Vortex for 10 seconds. Incubate at 70°C for 10 minutes. Briefly centrifuge tubes.
    d. Add 150µl ethanol, cap, and vortex for 15 seconds. Briefly centrifuge tubes.
    e. Transfer the supernatant to a QIAamp MinElute column in a 2ml collection tube.
    f. Centrifuge at 8,000 rpm for 1 minute or until all solution has passed through the membrane.
5. Wash membrane
    a. Before first use, add ethanol to Buffers AW1 and AW2 according to the directions on the buffer bottle.

   b. Transfer the column to a new collection tube. Add 500µl Buffer AW1 to the column.
   c. Centrifuge at 8,000 rpm for 1 minute or until all solution has passed through the membrane. Transfer the column to a new collection tube.
   d. Add 700µl Buffer AW2 to the column and centrifuge at 8,000 rpm for 1 minute.
   e. Transfer to a new collection tube.
   f. Add 700µl of ethanol. Centrifuge at 8000 rpm for 1 minute. Transfer to a clean collection tube.
   g. Centrifuge at 14,000 rpm for an additional 3 minutes or until all solution has passed through the membrane. No Buffer AW2 should remain in or on the spin column.
6. Elute nucleic acids
   a. Transfer the column to a new, labeled collection tube.
   b. Add 20-100µl Buffer ATE to the column depending on the expected quantity of DNA. Incubate at room temperature for 5 minutes.
   c. Centrifuge at 14,000 rpm for 1 minute.
   d. Cap the tube for storage.
7. Store samples at 2–8°C or at –20°C until ready to perform PCR.

Interpretation
None

Literature/Supporting Documentation
Scherczinger, C. A., M. T. Bourke, C. Ladd, and H. C. Lee. 1997. DNA extraction from liquid blood using QIAamp. J. Forensic Sci. 42(5): 893–896.

Greenspoon, S., M. A. Scarpetta, M. L. Drayton, and S. A. Turek. 1998. QIAamp spin columns a method of DNA isolation for forensic casework. J Forensic Sci 43(5): 1024–1030.

Frégeau, C. J., K. L. Bowen, and R. M. Fourney. 1999. Validation of highly polymorphic fluorescent multiplex short tandem repeat systems using two generations of DNA sequencers. J Forensic Sci 44(1): 133–166.

Sinclair, K. and V. M. McKechnie. 2000. DNA extraction from stamps and envelope flaps using QIAamp and QIAshredder. J Forensic Sci 45(1): 229–230.

2.4 Differential Extraction of Semen Stains- QIAamp® Purification

This method of DNA extraction is appropriate for use on stains or swabs mixed with or originating from seminal fluid. The differential lysis procedure attempts to separate the stain components into a sperm fraction and a non-sperm (epithelial cell) fraction. Following the differential lysis of the cellular material, the DNA fractions are further purified using QIAamp® extraction. The resultant DNA fractions are then individually typed.

Related Documents
DNA Extraction worksheet

Equipment / Materials / Reagents
Scissors, scalpel, tweezers
Tubes –microcentrifuge
Microcentrifuge
Vortex
Water baths (56°C and 70°C) – or 70°C oven
0.39 M DTT – see Reagents section
Ethanol (200 proof, molecular biology grade)
QIAamp DNA Investigator Kit: (Expires 2 years from date of receipt)
- QIAamp spin columns and 2-ml collection tubes
- Buffer AL
- Buffer ATL
- Buffer AW1 concentrate
- Buffer AW2 concentrate
- Buffer ATE
- RNA
- Proteinase K

Qiagen lyse and spin baskets

Standards, Controls, and Calibration
A minimum of one reagent blank must be processed for each fraction type per extraction batch as a negative control. The extraction method must be recorded on the DNA Extraction worksheet.

Procedure

All extraction steps must be performed in the Extraction Work Area. Extract known samples at a different time than questioned samples. Use reagents and pipettes dedicated to this area.

Swabs should be dissected into pieces of appropriate size. Use a clean cutting surface for each sample. Sections which are not to be analyzed immediately should be stored frozen.

1. From the evidentiary semen stain remove approximately (These are typical amounts but may vary from sample to sample):
    a. 0.25 - 1 swab
    b. 0.5 cm^2 dried stain
2. Suspend the swab or stain in 500µl Buffer ATL in a microcentrifuge tube or Qiagen lyse and spin basket. Add 20µl proteinase K. Vortex for 10 seconds.
3. Incubate at 56 °C for at least 1 hour. Vortexing for 10 seconds every 10 minutes will help improve lysis.
        i. If using a Qiagen lyse and spin basket, centrifuge at 12,700 rpm for 1 minute and remove the basket and discard.

4. Without disturbing the pellet, remove 300µl of the supernatant and place in a clean labeled tube. This is the epithelial cell fraction. If any additional volume is present, either remove it at this step and discard, or it will be removed in the following steps.
5. Add 330µl Buffer ATL. Vortex for 10 seconds. Centrifuge at 12,700 for 5 minutes. Remove and discard the supernatant without disturbing the pellet.
6. Repeat step 6 at least three times.
7. Add 290µl Buffer ATL.
8. If no sperm cells were detected during the screening process, vortex for ~10 seconds and remove 10µl of the re-suspended sample and spot on a glass microscope slide for examination using NFR-PIC (Christmas tree) stain. Record the number of sperm on the DNA extraction worksheet.
9. Add 10µl Pro K and 25µl DTT to the pellet. Vortex for 10 seconds.
10. Incubate at 56°C for at least 1 hour to overnight. Vortexing for 10 seconds every 10 minutes may improve lysis.
11. Briefly spin to remove drops from the inside of the cap.
12. IMPORTANT: If using the Qiacube, follow the steps to start the forensic purification protocol. If performing Manual Purification, continue with these steps:
    a. Add 300µl Buffer AL and vortex for 10 seconds. Add 1µl dissolved carrier RNA to each sample, if applicable.
    b. Place the tube in a 70°C bath or oven and incubate for 10 minutes. Vortexing every 3 minutes may improve lysis.
    c. Add 150µl Ethanol and vortex for 15 seconds. Spin to remove drops from cap.
    d. Transfer the lysate to a QIAamp MinElute column with collection tube without wetting the rim.
    e. Centrifuge at 8000 rpm for 1 minute. Place the column in a new collection tube.
    f. Add 500µl Buffer AW1. Centrifuge at 8000 rpm for 1 minute. Place the column in a new collection tube.
    g. Add 700µl Buffer AW2 and centrifuge at 8000rpm for 1 minute. Place the column in a new collection tube.
    h. Add 700µl ethanol and centrifuge at 8000 rpm for 1 minute. Transfer to a new collection tube.
    i. Centrifuge at 14,000 rpm for 3 minutes.
    j. Place the column in a new clean 1.5ml labeled, final collection tube.
    k. Elute nucleic acids.
    l. Add 20–100µl Buffer ATE to the column depending on the expected quantity of DNA. Incubate at room temperature for 5 minutes.
    m. Centrifuge at 14,000 rpm for 1 minute.
13. Store sample at 2–8°C or at –20°C or until ready to perform PCR.

Interpretation
None

Literature / Supporting Documentation

von Beroldingen CH, Blake ET, Higuchi R, Sensabaugh GF, Erlich HA. Applications of PCR to the Analysis of Biological Evidence. In: PCR Technology: Principles and Applications for DNA Amplification, Erlich H. editor, New York: Stockton Press, 1989: 209-223.

Greenspoon SA, Scarpetta MA, Drayton ML, Turek SA. 1998. QIAamp Spin Columns as a Method of DNA Isolation for Forensic Casework. J Forensic Sci 43(5): 1024-1030.

2.5 Purification of Extracted DNA Samples Using QIAamp®

This procedure uses the QIAamp® spin columns to remove PCR inhibitors that might be present in the DNA extract of a sample.

Related Documents
None

Equipment / Materials / Reagents
Microcentrifuge
Vortex
Water baths (56°C and 70°C) – or 70°C oven
QIAamp® DNA Investigator Kit, contains the following: (Expires 2 years from date of receipt)
- QIAamp spin columns and 2-ml collection tubes
- Buffer AL
- Buffer ATL
- Buffer AW1 concentrate
- Buffer AW2 concentrate
- Buffer ATE
- Proteinase K

Ethanol (200 proof, molecular biology grade)

Standards, Controls, and Calibration
A new or original reagent blank will be purified as a negative control.

Procedure

1. Add 300µl Buffer ATL to extracted DNA.
2. Incubate at 56°C for at least one hour. Centrifuge briefly.
3. Continue with manual procedure below or use QIAcube purification procedure.
4. Gently mix Buffer AL by inverting. Add 300µl Buffer AL and 1µl diluted carrier RNA per sample. Vortex for 15 seconds.
5. Incubate at 70°C for 10 minutes. Periodic vortexing may improve lysis. Briefly spin tubes.
6. Add 150µl ethanol. Vortex for 15 s. Briefly spin tubes.
7. Transfer tube contents (including any precipitate) to a labeled QIAamp MinElute column in a collection tube.
8. Centrifuge at 8000 rpm for 1 minute or until all solution has passed through the membrane. Place the column in a clean 2ml collection tube and discard the collection tube containing the flow-through.
9. Add 500µl Buffer AW1 to the column.
10. Centrifuge at 8,000 rpm for 1 minute or until all solution has passed through the membrane. Place the column in a clean 2ml collection tube and discard the collection tube containing the flow-through.
11. Add 700µl Buffer AW2 to the column and centrifuge at 8,000 rpm for 1 minute. Transfer the column to a new collection tube.

12. Add 700µl ethanol to the column and centrifuge at 8,000 rpm for 1 minute. Transfer the column to a new collection tube.
13. Centrifuge at 14,000 rpm for an additional 3 minutes or until all solution has passed through the membrane.
14. Transfer the column to a final, new, labeled collection/storage tube.
15. Add 20-100µl Buffer ATE to the column depending on the expected quantity of DNA. Incubate at room temperature for 5 minutes.
16. Centrifuge at 14,000 rpm for 1 minute
17. Place the appropriate cap on the tube. Store samples at 2–8°C or at –20°C until ready to perform PCR.

Interpretation
None

Literature / Supporting Documentation
None

2.6 DNA Extraction-Maxwell 16

This procedure uses the DNA IQ Casework Sample Kit on the Maxwell 16 to produce an extract of nucleic acids from samples. The DNA IQ Casework Sample Kit has been researched for forensic application. The DNA IQ Casework Sample Kit uses the DNA IQ Resin to purify samples, maximizing DNA concentration and reliable STR analysis.

Related Documents
DNA Extraction worksheet

Equipment / Materials / Reagents
Maxwell 16 extraction robot
Vortex
DNA IQ Casework Sample Kit
Oven or heat block-70°C
DTT
Proteinase K

Standards, Controls, and Calibration
A minimum of one reagent blank must be processed for each extraction batch (robot run) as a negative control.

Procedure

1. Lyse cells and suspend
    a. Combine cutting or swab with 500µl Lysis Buffer, 10µl Pro K and 12µl DTT.
    b. Vortex and heat at 70°C for 30 minutes.
    c. Remove the liquid via spin basket or pipet to use in purification.

2. Purification
    a. Place the number of cartridges to be used into the Maxwell 16 Cartridge Rack in order (by rack position). Remove the seals. Ensure the cartridges snap into place.
    b. Place a plunger into well #8 of each cartridge. (Well #8 is the well closest to the tube holder).
    c. Transfer samples into well #1. (Well #1 is the well closest to the cartridge label and farthest from the user).
    d. Place 0.5ml elution tubes into the elution tube holder at the front of each of the cartridges. Add 50µl of elution buffer to each elution tube.
    e. Turn the Maxwell 16 Instrument on.
    f. Scroll to "Run" on the Menu screen and press the "Run/Stop" button to start the method prior to loading the Maxwell 16 Cartridge Rack.
    g. Open the door when prompted and press the "Run/Stop" button to extend the platform. Transfer the Cartridge Rack onto the Maxwell 16 platform. (Tube holders must be closest to the door).
    h. Press the "Run/Stop" button. The platform will retract. Close the door.

Interpretation
None

Literature/Supporting Documentation
DNA IQ Casework Sample Kit for Maxwell 16 Protocol.

2.7 Differential Extraction of Semen Stains- QIacube

This method of DNA extraction is appropriate for use on stains or swabs mixed with or originating from seminal fluid. The differential lysis procedure separates the stain components into a sperm fraction and a non-sperm (epithelial cell) fraction. Following the differential lysis of the cellular material, the DNA fractions are further purified using QIAamp® extraction. The resultant DNA fractions are then individually typed and compared to reference standards from the victim(s) and/or suspect(s).

Related Documents
DNA Extraction worksheet
QIAcube loading chart

Equipment / Materials / Reagents
Scissors, scalpel, tweezers
Microcentrifuge tubes
Microcentrifuge
Centrifuge
Vortex
.39M DTT – see Reagents section
Ethanol (200 proof, molecular biology grade)
56º water bath
QIAamp DNA Investigator Kit: (Expires 2 years from date of receipt)

QIAamp spin columns and 2-ml collection tubes
Buffer AL
Buffer ATL
Buffer AW1 concentrate
Buffer AW2 concentrate
Buffer ATE
RNA
Buffer G2
In House Pro K
Rotor adapters

Procedure

All extraction steps must be performed in the Extraction Work Area. Extract known samples at a different time than questioned samples. Use reagents and pipettes dedicated to this area.

Swabs should be dissected into pieces of appropriate size. Use a clean cutting surface for each sample. Sections which are not to be analyzed immediately should be stored frozen.

The following chart may be used to calculate the appropriate volumes needed. The use of either 1 or 11 tubes is prohibited:

G2/PK/DTT Volumes (75% G2, 5% PK, 20% DTT)

# of samples	G2 ul	PRO K (PK) ul	DTT ul
2	2925	195	780
3	3075	205	820
4	3225	215	860
5	3375	225	900
6	3525	235	940
7	3675	245	980
8	3825	255	1020
9	3975	265	1060
10	4125	275	1100
12	4425	295	1240

1. From the evidentiary semen stain remove approximately (These are typical amounts but may vary from sample to sample):
    a. 0.25 - 1 swab or 0.5 cm^2 dried stain
2. Suspend the swab or stain in 500µl Buffer G2 in a 1.5 ml microcentrifuge tube. Add 20µl proteinase K. Vortex for 10 seconds.
3. Incubate at 56 °C for at least 1.5 hours. Vortexing for 10 seconds every 10 minutes will help improve lysis
13. If using a microcentrifuge tube, briefly centrifuge the tube to remove drops from the inside of the lid. Place the cutting in a spin basket and return the spin basket to the tube. Centrifuge at 12,700 for 5 minutes. Discard the spin basket and cutting.
    a. If using a Qiagen lyse and spin basket, centrifuge at 12,700 rpm for 1 minute and remove the basket and discard.
4. Load per the separation and lysis 12A protocol sheet and Qiagen loading sheet.
5. Place sperm/epithelial mixture tube in position 3 of rotor adapter and load into centrifuge. Place labeled 2ml tubes in shaker tray for collection of epithelial cell fractions.
6. Place vial of Buffer G2 in position 1 according to the 12A Protocol Sheet
7. Load QIAcube with 1000µl wide bore tips
8. Start “Separation and Lysis 12A” procedure (DNA>pipetting> epithelial and sperm cell separation and lysis 12A). Follow prompts.
9. After completion of Lysis 12A, remove epithelial cell fractions from shaker and store for purification
10. Place vial with appropriate amount of Buffer G2/PK/DTT in position 2 and refill G2 vial in position 1 if needed
11. If no sperm were detected during the screening process, mix 1.5µl of the sperm solution sample with 3.5µl ATL and spot on a glass microscope slide for examination using NFR-PIC (Christmas tree) stain. Record the number of sperm on the DNA extraction worksheet.
12. Refill tips if necessary
13. Start “Separation and Lysis 12B” procedure. (DNA>pipetting> epithelial and sperm cell separation and lysis 12B). Follow prompts.
14. After completion of Lysis 12B, transfer sperm fractions to 2 ml tubes.
    a. Add 1µl RNA to sperm and/or epithelial cell fractions
15. Proceed to purification process

Interpretation
None

Literature / Supporting Documentation
von Beroldingen CH, Blake ET, Higuchi R, Sensabaugh GF, Erlich HA. Applications of PCR to the Analysis of Biological Evidence. In: PCR Technology: Principles and Applications for DNA Amplification, Erlich H. editor, New York: Stockton Press, 1989: 209-223.

Greenspoon SA, Scarpetta MA, Drayton ML, Turek SA. 1998. QIAamp Spin Columns as a Method of DNA Isolation for Forensic Casework. J Forensic Sci 43(5): 1024-1030.

2.8 SwabSolution™ Kit for Extraction of Reference Blood (FTA) Swabs and Buccal Swabs-Direct Amplification

The SwabSolution™ Kit is used for rapid processing of reference samples prior to amplification for human STR genotyping.

Related Documents
DNA Extraction worksheet
COC extraction worksheet
Amplification worksheets

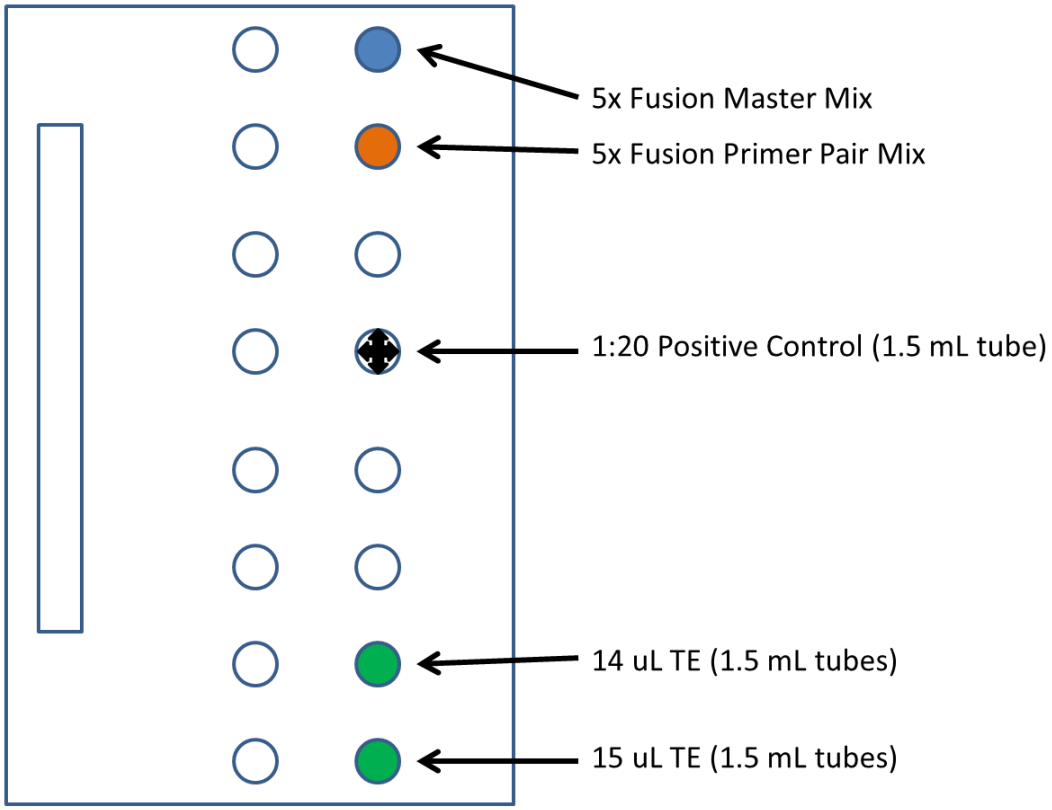
Equipment / Materials / Reagents
SwabSolution™ Kit
  SwabSolution™ Reagent
1.5ml Microtubes with locking caps
Heat block set to 70°C
Amplification reagents
Amplification tubes
TE

Standards, Controls, and Calibration
A minimum of one reagent blank must be processed for each extraction batch as a negative control.

Procedure
Storage of Kit components: Upon arrival, the SwabSolution™ Reagent should be thawed completely by placing in a 37°C water bath or oven and mixed by gentle inversion. After thawing the kit should be stored at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate and can compromise stability.

1. Add the following to a 1.5ml tube (in any order):
    a. **Swab** (or portion of swab) : Blood FTA card, see note. (FTA part or paper backing), buccal, blood
    b. 1ml of SwabSolution™ Reagent
    c. Note: For FTA bloodstain cards: use a single sterile swab and sterile water to swab the FTA card. It is necessary to transfer a good amount of blood to the swab. Extract the entire swab head.
    d. The following is recommended for FTA card processing with SwabSolution:
        i. Collect 2 circles during screening
        ii. Swab one circle and retain the other unswabbed
        iii. The swab does not need a separate LIMS number and the stick does not need to be retained after extraction.
2. Place tubes in heat block, and incubate sample at 70°C for 1 hour.
3. Briefly spin to remove condensation from caps. Ensure swab is below top liquid area within the tube. There is no need to vortex after addition of SwabSolution™ Reagent, prior to incubation or after the incubation is complete.

4. Extracts may be stored at 4°C. Promega has informed us that DNA can be amplified from extracts that have been stored for 6 months at 4°C.
5. Amplification (manual or QIAgility)-Casework samples
    a. Add 10µl PowerPlex Fusion master mix to the amplification tubes
    b. To equal a total of 15µl:
        i. Add appropriate amount of TE to each sample tube
        ii. Add appropriate amount of sample extract to the appropriate tube
            1. Typical extract amounts: saliva swabs 1µl, Blood swabs 2-4µl
6. Amplification (manual or QIAgility)-Controls
    a. Positive Control: 10µl PowerPlex Fusion master mix + 14µl TE + 1µl control
    b. Negative Control: 10µl PowerPlex Fusion master mix + 15µl TE
7. If a profile has a "degradation" like appearance, the extract can be reheated at 70°C for up to 1 hour and the process repeated. The RB would also be reheated.
8. Store original extract tube with swab frozen.
9. If using the Qiagility to set up the amplification, follow this protocol:
    a. Select the option for Fusion PCR Setup Direct Amplification
    b. Make a bank for the number of samples in your extraction batch
    c. Add your bank in the reaction list and click on the master mix, 14 uL TE from the reagent list, and enter 1 uL for the amount of sample
    d. Add a new entry to the reaction list for the positive control by clicking on the master mix, 14 uL TE from the reagent list, and the 1:20 amp positive control in the reagent list.
    e. Add a new entry to the reaction list for the negative control by clicking on the master mix, 15 uL amp negative control TE from the reagent list.
    f. Label strip tubes and place in the reaction plate
    g. Load the reagent block as follows:



h. Add extracted samples to sample block
i. Place the 1.5 mL tube in the mix plate (B1) for the master mix
    i. There is no need to remove the cap from the water vial
j. Press Go.
k. When the run it complete, the samples are ready to transfer to the thermal cycler.

Interpretation
None

Literature/Supporting Documentation
Promega SwabSolution™ Kit manual

2.9 Speedvac DNA Concentration

It may become necessary to concentrate the quantity of DNA in low yield samples. Thermo Scientific Savant® DNA SpeedVac ® Model DNA 120, is a dedicated centrifugal vaccum concentration system for drying DNA samples.

Related Documents
Speedvac User Manual

Equipment / Materials / Reagents
Thermo Scientific Savant® DNA SpeedVac ® Model DNA 120

Standards, Controls, and Calibration
None

NOTE: Samples that have a total yield of <.5ng, may be concentrated down to a final volume of ~15ul, at analyst discretion. In some cases concentrating down to different volumes may be necessary and the volume will be documented. If evidence samples are concentrated, it is also necessary to concentrate down the reagent blank at the same time.

1. Carefully spin down the microcentrifuge tubes to remove liquid from the lid
2. Open the microcentrifuge tube and place in the DNA SpeedVac ®. Balance the centrifuge as necessary.
3. Close the lid of the centrifuge and press the switch to manual. The heater should be off, the drying time switch should be in the middle position, and 2.00 on the display screen.
4. The DNA SpeedVac ® will then begin a DNA concentrator run that you can start and stop manually as necessary.
5. Allow the DNA SpeedVac ® to run for ~5-8 minutes before checking the volume (samples should be concentrated down to a volume of ~15ul)
6. It may be necessary to run the DNA SpeedVac ® longer than 8 minutes in order to obtain the correct volume
7. If the total volume is lower than 15ul, TE can be used to bring the volume up to this level. An alternative is to concentrate the samples to no volume and add 15ul of TE.
8. Once concentration is complete close the lids on the microcentrifuge tubes and proceed to amplification
9. The DNA SpeedVac should be wiped clean using a soft cloth and disinfectant after use

CHAPTER 3 QUANTITATION

3.1 Quantifiler

The Quantifiler Human DNA Quantification Kit is used to provide reliable, reproducible, and highly sensitive real-time PCR quantification of human identity samples. The kit delivers specific, robust quantification of samples with DNA concentrations of 0.023ng/ul to > 50ng/ul. The quantification assay combines two 5' nuclease assays: a target-specific (human DNA) assay, and an internal PCR (IPC) assay to allow quick identification of samples that may be inhibited or that do not contain human DNA.

The target-specific assay consists of two primers for amplifying human DNA and one Taqman ® MGB probe labeled with FAM ™ dye for detecting the amplified sequence.

Targets of Quantifiler kits:

Kit	Gene Target	Location	Amplicon Length	Region Amplified	Ploidy
Quantifiler Human Kit	Human telomerase reverse transcriptase gene (hTERT)	5p15.33	62 bases	Nontranslated region (intron)	Diploid (Single-copy target)

The IPC assay consists of IPC template DNA (a synthetic sequence not found in nature), two primers for amplifying the IPC template DNA, and one TaqMan® MGB probe labeled with VIC ® dye for detecting the amplified IPC DNA.

All forensic questioned samples must be quantitated. Reference samples extracted with Swab Solution and reagent blanks do not require quantitation.

Related Documents
Quantifiler DNA Quantitation worksheet

Equipment / Materials / Reagents
Quantifiler Kit
- Quantifiler Human Primer Mix
- Quantifiler Human DNA Standard
- Quantifiler PCR Reaction Mix

ABI Prism ® 7500 Sequence detection System
Optical Tubes (8 tubes/strip)
MicroAmp ® 96-Well Tray/retainer Set
Optical Caps (8 caps/strip)
Corbett CAS-1200 (optional)

Qiagility

Standards, Controls, and Calibration
To prepare the DNA Quantification Standard, follow the procedure listed below or use the Corbett CAS-1200 or the Qiagility(quant template option.)

1. Standard Dilution Series

Standard	Concentration (ng/ul)	Minimum Amounts	Dilution Factor
Std. 1	50.000	10ul {200ng/ul stock} + 30 ul TE Buffer	4X
Std. 2	16.700	10ul {Std. 1} + 20ul TE Buffer	3X
Std. 3	5.560	10ul {Std. 2} + 20ul TE Buffer	3X
Std. 4	1.850	10ul {Std. 3} + 20ul TE Buffer	3X
Std. 5	0.620	10ul {Std. 4} + 20ul TE Buffer	3X
Std. 6	0.210	10ul {Std. 5} + 20ul TE Buffer	3X
Std. 7	0.068	10ul {Std. 6} + 20ul TE Buffer	3X
Std. 8	0.023	10ul {Std. 7} + 20ul TE Buffer	3X

2. Label eight microcentrifuge tubes: Std. 1, Std. 2, Std. 3, etc.
3. Dispense the required amount of diluent (TE Buffer) to each tube
4. Prepare Std. 1
    a. Vortex the Quantifiler Human DNA Standard for 3-5 seconds.
    b. Using a new pipette tip, add the calculated amount of Quantifiler Human DNA Standard to the tube for Std. 1.
    c. Mix the dilution thoroughly.
5. Prepare tubes for Std. 2 through 8
6. Using a new pipette tip, add the calculated amount of the prepared standard to the tube for the next standard.
7. Mix the standard thoroughly.
8. Repeat until you complete the dilution series.

Procedure

Manual Procedure

1. Preparing the Reactions.
    a. Thaw the primer mix completely, then vortex 3 to 5 seconds.
    b. Swirl the Quantifiler PCR Reaction Mix gently before using. Do not vortex.
    c. The Qiagility or Corbett CAS-1200 may be used instead of the following manual option.

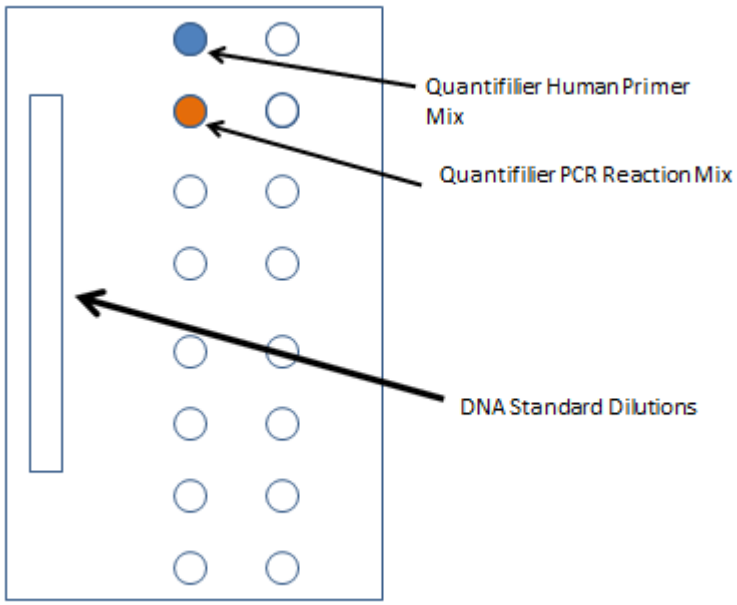
2. Calculate the volume of each component needed to prepare the reactions, using the table below.

Component	Volume Per Reaction (µl)
Quantifiler Human Primer Mix	10.5
Quantifiler PCR Reaction Mix	12.5

3. Pipette the required volumes of components into an appropriately sized tube.
4. Vortex the PCR mix 3 to 5 seconds.
5. Dispense 23µl of the PCR mix into each reaction well.
6. Add 2µl of sample, standard, or control to the appropriate wells. Reagent blanks do not need to be quantitated since they will automatically be amplified according to the largest volume of the extraction batch. The standards are run in duplicate.
7. Seal the tube strips with the Optical Caps.
8. Running the Reactions
    a. Position the tubes in the instrument thermal block.
    b. Secure the samples inside the instrument
    c. In the SDS software, Open the APD template and add the number of samples, temperature control, and select the IPC and standard.
    d. Select the Instrument tab, then click Start

CAS-1200 or Qiagility Procedure

1. Select the appropriate procedure (Quant or Quant no Standard (if standards have been previously prepared))
2. Make a bank for the number of samples in your extraction batch and place your samples on the deck
3. Add your bank in the reaction list and click on the master mix and 2ul of sample
4. Add the appropriate amount of Quantifilier Human Primer Mix to a 2 ml tube and place on the instrument deck
5. Add the appropriate amount of Quantifilier PCR Reaction Mix to a 2 ml tube and place on the instrument deck
6. Add the appropriate number of strip tubes to the deck
7. Remove the cap from the water vial
8. Place an empty 1.5ml tube on the deck next to the water vial
9. Place 8 labeled .2 ml tubes for the standards in the correct position on the deck
    a. If standards need to be made add 7.5ul of DNA standard and 23ul of TE to the first standard tube and vortex.
    b. If standards have previously been made vortex and lightly spin down before placing on the deck
10. Save the plate setup and select run following the prompts
11. Once the run is complete seal the strip tubes with the optical caps and clear off the deck
12. Run the UV light on the instrument
13. Run the reactions as described above



Interpretation

Interpretation will be according to the Quantifiler manual. If the template control shows a volume less than 0.023ng/ul, another qualified analyst will initial the Quantifiler worksheet verifying the reading. If the template control shows a reading over 0.023ng/ul the run will need to be repeated.

The R2 value should be ≥ 0.98 and the slope should be within -2.9 to -3.35. Variations may be signed by the Technical Leader or technical reviewer. Typically, the slope can be rounded to the nearest tenth. Rounding to the nearest tenth is not allowed in order to meet the minimum preferred value. These values would be expressed to the nearest hundredth.

Samples with results less than 0.023ng/ul still may be amplified.

One value of each standard amount may be omitted to better define the standard curve.

Data from each Qiagility run and Corbett CAS-1200 run (when applicable) will be stored on the DNA group drive. If exceptions occur, a summary post run report should be included in the case file with a brief explanation.

Literature / Supporting Documentation
None

CHAPTER 4 AMPLIFICATION

4.1 DNA Amplification – PowerPlex Fusion

This protocol uses the Promega PowerPlex Fusion™ PCR Amplification and Typing Kit

Related Documents
PCR Setup Worksheet
Amplification worksheet for 9700

Equipment / Materials / Reagents
Microcentrifuge
Microcentrifuge tubes
Vortex
DNA Thermal Cycler – 9700
Reaction tubes
PowerPlex Fusion™ PCR Amplification and Typing Kit
QIAgility (optional)

Standards, Controls, and Calibration
2800M Control DNA must be included with each amplification set. This amplification positive control is supplied with the kit.

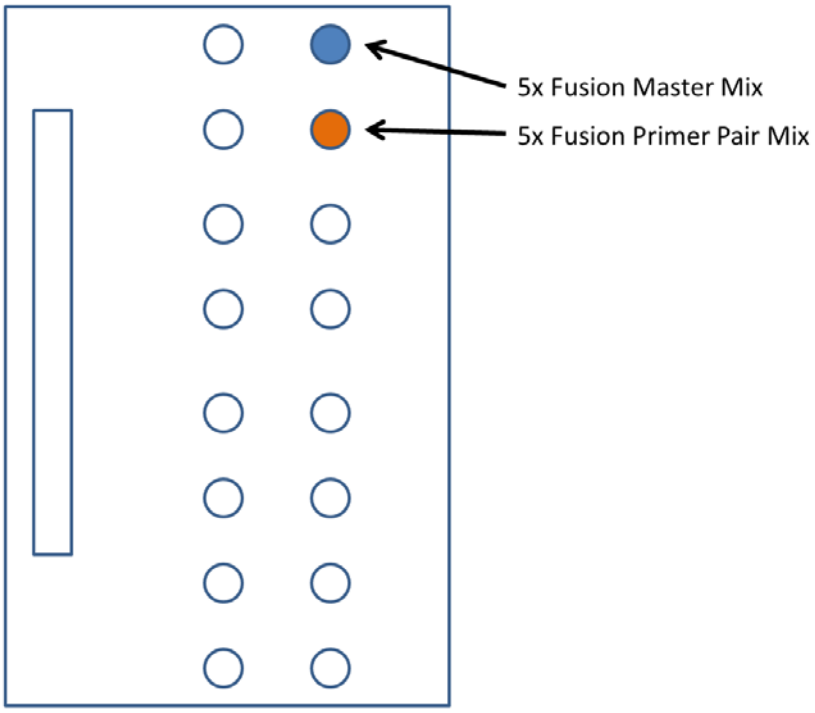
An amplification negative control must also be included with each amplification set. This negative control will consist of all amplification reagents with TE Buffer added in place of sample DNA.

Procedure

PCR Instrument	Times and Temperatures for PowerPlex Fusion Kits					
	Initial Incubation Step	30 cycles each			Final Extension	Final Step
		Melt	Anneal	Extend		
9700	96°C 1 min.	94°C 10 sec.	59°C 1 min.	72°C 30 sec.	60°C 20 min. hold	4°C hold (forever)

1. Use thermal cycler protocol according to the chart above.
2. In the PCR setup hoods, label reaction tubes with appropriate information.
3. Vortex PCR reagents and spin tubes briefly in a microcentrifuge to remove any liquid from the caps.
    a. Optional: Use the QIAgility to make the master mix and set up the reaction tubes using the QIAgility protocol. After completion, proceed to step 5.
    b. If using the Qiagility to set up the amplification, follow this protocol:
        i. Select the option for Fusion PCR Setup
        ii. There is no bank needed for this setup

iii. Only use plate C2
iv. Put empty amp tubes in plate C2 in desired wells
v. Schedule robot to add masterm ix to corresponding wells (Leave sample volume at 0)
vi. Place vials (5x master mix and 5x primer pair mix) from the kit into the reagent block
vii. Place empty 1.5 mL tube next to the TE vial for Master Mix preparation (this procedure does not actually use TE so you done need to remove the cap)
viii. Load the reagent block as follows:



ix. Press Go.
x. When the run it complete, the samples are ready to transfer to the thermal cycler.

4. Prepare a Master Mix by adding the following volumes to a tube:
   a. 25µl Reactions
      i. 5.0µl of PowerPlex Fusion 5X Primer Pair Mix X # of samples
      ii. 5.0µl PowerPlex Fusion 5X Master Mix X # of samples
   b. Mix by vortexing for approximately 5-10 seconds. Failure to vortex the PCR amplification mix sufficiently can result in poor amplification or locus-to-locus imbalance.
   c. Spin briefly in microcentrifuge to remove liquid from cap
   d. Dispense 10µl of Master Mix into each reaction tube.

5. For QIAgen and Maxwell extracts, add approximately 0.5ng of sample DNA (with any necessary TE) to the appropriate reaction tubes, not to exceed 15µl in total sample volume added.
    a. For samples with high concentrations of DNA, dilute these samples with TE buffer to attain the appropriate concentration. The same vial of TE that will be used for the negative control will be used for dilutions to ensure the TE is free of DNA. Samples with low quantitation results may be concentrated with the speed vac prior to setup. The RB would also be concentrated.
    b. For Swab Solution extracts, add ~1µl extract for saliva swabs and ~2-4µl for blood swabs plus the appropriate amount of TE to equal 15µl. These volumes may be adjusted based on the samples.
6. If not already prepared, make a 1:20 dilution of the positive control 2800M (10ng) that can be stored frozen.
    a. For each amplification set, set up a **positive control** by adding 1µl of the diluted 2800M Control DNA to a reaction tube containing 10µl of master mix and 14µl TE. If more than one positive control is set up and one does not pass due to low peak height, but the other control does pass, this can be considered as a satisfactory result.
7. For each amplification set, set up a **negative control** by adding 15µl of TE to an reaction tube containing Master Mix.
8. Place the reaction tubes into the Thermal Cycler and start the appropriate program. The UV light in the setup hoods should be run for approximately 1 hour to decontaminate the area.
9. After amplification, remove the reaction tubes from the Thermal Cycler and store at 2-6° C. Once analysis is complete, the amplicons can be disposed of.

Interpretation
None

Literature / Supporting Documentation

PowerPlex Fusion Technical Manual

CHAPTER 5 GENETIC ANALYZER SAMPLE PREPARATION

Amplified samples can be analyzed by injection into a capillary on the ABI Genetic Analyzer. An internal lane size standard is loaded with each sample to allow for automatic sizing of the PCR products and to normalize differences in electrophoretic mobility between injections.

Related Documents
CE Setup QIAgility Form (optional)
3130 Plate Record

Equipment / Materials / Reagents

ABI Prism® 3130 Genetic Analyzer
Capillaries
Plates and septa
Freezer block
Heat block
CC5 ILS 500
Buffer
Deionized formamide
Performance Optimized Polymer (POP-4 polymer)
Vortex
QIAgility (optional)
Allelic Ladder

Standards, Controls, and Calibration
Appropriate ladders and positive and negative amplification controls will be included with each run. An internal size standard will be added to each sample (QAS 9.5.4).

Procedure

1. Perform 3130 maintenance if necessary
2. Prepare a master mix of deionized formamide and CC5 ILS 500 in a microcentrifuge tube as follows and mix well
    a. 10µl deionized formamide X # of samples (include controls and ladders)
    b. 1.0µl CC5 ILS 500 X # of samples (include controls and ladders)
        i. **Do not heat to thaw CC5 ILS 500**
        ii. Optional: Use the QIAgility to make the master mix and set up the plate using the QIAgility program. Select CE setup protocol and load per instructions.. After completion, proceed to step 5.
3. Aliquot 11µl of the formamide/ CC5 ILS 500 master mix into each well.
4. Add 1.0µl of PCR product, allelic ladder, positive control, or negative control to each well
5. Seal the plate with a septum. Centrifuge briefly as needed.
6. Denature all of the sample tubes at 95° C for approximately 3 minutes in a heat block.
7. Immediately chill the sample tubes for approximately 3 minutes in a PCR cooler tray
    a. Do not reheat samples, this may cause an increase in artifacts
8. Set up plate records on the Genetic Analyzer. Inject the samples for 2-15 seconds.
    a. Any reagent blanks will be run at the injection time of the highest corresponding sample and on the same instrument model. Each run will include a positive control, a negative control, and a ladder.
9. Analyze collected data using Genemapper ID and refer to Interpretation Section for additional information.
10. Each case folder will contain a printout of the complete sample list in Genemapper ID for each run, and the electropherograms for the positive control, negative control, and reagent blanks for that case.

a. Only those electropherograms used in the final interpretation (and the final electropherogram for a sample that will be re-extracted) are required to be in the case folder. However, documentation should be made as to why an injection was not used.
b. If the interpreted data is in the next run then that should also be stated. If the interpreted data is in a later run, then that date should be stated. Lot numbers of CE reagents and instrument maintenance documents will be stored in the instrument log books. Since a complete ILS is required for the typing of a sample, it is optional for the ILS to be printed on the electropherogram.

Interpretation
None

Literature/Supporting Documentation
PowerPlex Fusion Technical Manual

CHAPTER 6 INTERPRETATION

General Guidelines

Several results are possible when conducting forensic casework analysis. These guidelines are in place to ensure that conclusions are scientifically supported by the analytical data with appropriate standards and controls. Interpretations are made as objectively as possible and as consistently from analyst to analyst as possible. Interpretation guidelines are based upon validation studies, literature, instrumentation and casework experience.

Three conclusions are generally possible:
1. inclusion (individual could have contributed to or been a source of the questioned profile)
2. exclusion (individual could not have contributed to/been a source of the questioned profile),
3. uninterpretable/inconclusive (the profile is not suitable for comparison for technical reasons)

Conclusions are determined by objective qualitative and quantitative evaluation of the entire DNA profile produced by the various loci tested.

DYS391 will be considered an information only locus in our laboratory and will not be used for interpretation and or statistical calculations. Lack of signal at DYS391 will not result in the profile being called “partial” and will not negate identity if all other loci meet the criteria. A result for DYS391 in the positive control is still required to ensure appropriate amplification.

At initial analysis of the data, all data above the analytical threshold should be assessed to determine if the sample is a mixture, if degradation/inhibition patterns exist, etc. At this

time, inconclusive results for loci or the determination that the profile is uninterpretable should be documented to indicate exclusion of that locus or profile for interpretation. Inconclusive loci may result from, but are not limited to, the following causes:

- Insufficient amounts of template DNA which can result in observation of stochastic effects
- Degradation due to environmental or chemical influences
- Preferential amplification due to the presence of inhibitors or other factors that limit the amplification of larger fragments
- Differences in the amounts of DNA present in a sample from multiple donors
- Mixtures of an excessive number of donors

It should be noted, however, that it is acceptable for an inclusion or exclusion to be determined when one or more of the loci yield inconclusive results. A consistency statement will be based only on loci that yield interpretable results. In most single source samples, exclusion will be determined if only one locus produces exclusionary results, however there may be rare exceptions to this rule.

Considerations for comparison to known profiles include the possible loss of an allele due to preferential amplification, stochastic effects, mutation, presence of a very minor contributor, or other factors are believed to have likely occurred. In these cases, a locus or sample may be determined to be inconclusive for statistics for a particular individual.

Preliminary Evaluation of Data

The first step in data evaluation is to determine whether the results are of sufficient intensity/quality for interpretation purposes. The profile should be interpreted before comparison to reference samples.

Analytical thresholds

An analytical threshold defines the minimum height requirement at and above which detected peaks can be reliably distinguished from background noise. Data below the 75 RFU threshold is considered uninterpretable or inconclusive. For any given locus, the minimum analytical threshold for evaluating profiles is 75 RFU. Reagent blanks will be analyzed at the lowest (or lower than) threshold of its interpretable corresponding samples. For any given locus, except Amelogenin, off scale data cannot be used. Off scale data in other dye colors in the Amelogenin basepair region should be interpreted with caution and consideration given to the presence of pull up. All inclusions and exclusions must be supported by above threshold data.

If the minimum analytical threshold provides unsuitable data, the analyst, at his/her discretion may choose the following:

- re-amplify the sample with more template
- re-inject that sample for a longer approved injection time
- call the sample uninterpretable/inconclusive

If the maximum threshold (sample is deemed off-scale) is exceeded at any locus (excluding Amelogenin), the analyst may choose the following:

- inject the sample for not less than 2 seconds
- dilute the amplified product in TE buffer and add the diluted amplified product to the formamide/ CC5 ILS 500 mixture (example: 2ul amplicon in 6ul TE)
- call that locus inconclusive/uninterpretable. Data in other dye colors may be interpreted with caution and evaluated for possible pull up.
- re-amplify the sample with less template.

If the maximum threshold is exceeded for the size standard, the analyst may still use the data as long as pull-up, if present, does not interfere with data interpretation. When samples are re-amplified or re-injected, typically the amplification or injection providing the most interpretable information will be used for comparisons and interpretations. If both provide the same information, either may be used. If the number of alleles obtained is the same but the alleles are not concordant, the analyst will determine which profile to use prior to comparisons of reference samples.

Internal size standard and ladder evaluation

Internal size standards are critical in STR analysis and are run with every sample. The internal lane size standard is used to normalize injection-to-injection migration differences, thereby providing sizing precision within a set of capillary injections. The minimum threshold for analysis of the ILS is 50 RFU. To ensure alleles are assigned appropriately, confirm all 21 peaks are present (additional peaks may be present as long as they do not interfere in the sample calls):
60,65,80,100,120,140,160,180,200,225,250,275,300,325,350,375,400,425,450,475,500.

If a SQ value of <1.0 is observed for a sample, the size standard should be examined to ensure that all size standard peaks are being properly called.

When interpreting results, genotypes are assigned to sample alleles by comparing their sizes to those obtained for the known alleles in the allelic ladders. Thus, a ladder must be present within each run. Each ladder used for analysis must have the appropriate alleles present for each locus (additional peaks may be present as long as they do not interfere in the sample calls) when analyzed (minimum of 75 RFU may be used). See PowerPlex Fusion Technical Manual for appropriate alleles in ladder. The ladders do not need to be printed for the paper file but the electronic data will be stored on the group drive in the run file.

Spectral

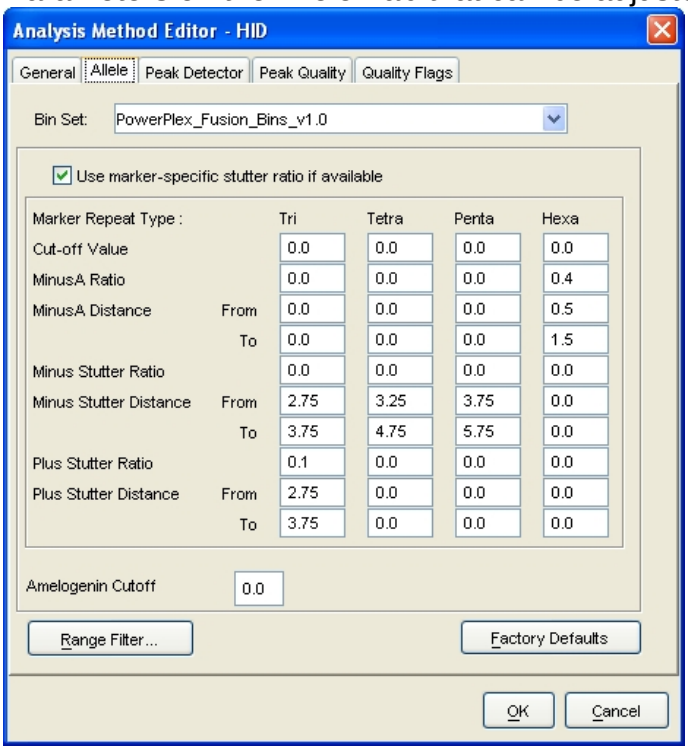
Multicomponent analysis is the process that separates the different fluorescent dye colors into distinctive spectral components. The five dyes used in the PowerPlex Fusion amplification kits are fluorescein, JOE, TMR-ET, CXR-ET, and CC5 ILS 500. Although each dye emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra. The precise spectral overlap is measured by analyzing DNA fragments labeled with each of the dyes. These dye-labeled fragments are spectral standards. A new spectral should be run on an instrument following a planned maintenance when necessary, after parts (i.e., laser, CCD camera, etc.) are replaced or realigned, or as needed.

It is critical to select the correct G5 spectral for the PowerPlex 5-dye chemistry.

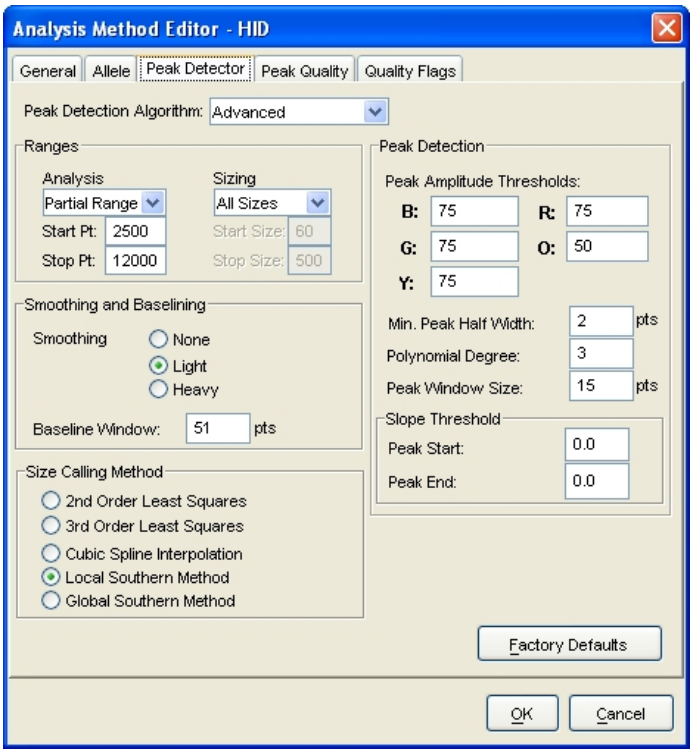
Analysis

Samples from each run will be analyzed using GeneMapper ID v3.2.1. The following settings will be required for analysis. The parameters in the screenshots below should be used for all analysis. Exceptions to this rule are noted above the screenshot:

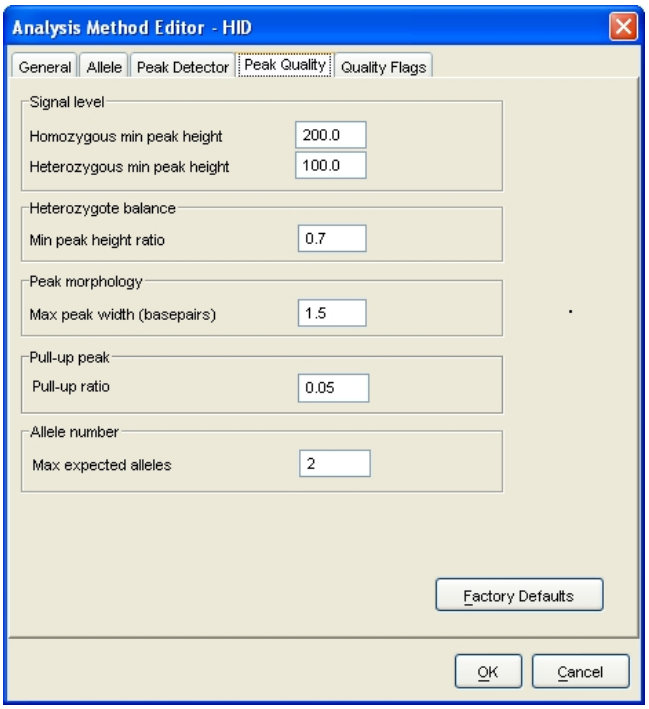
Parameters on the Allele Tab that can be adjusted at analyst discretion: None



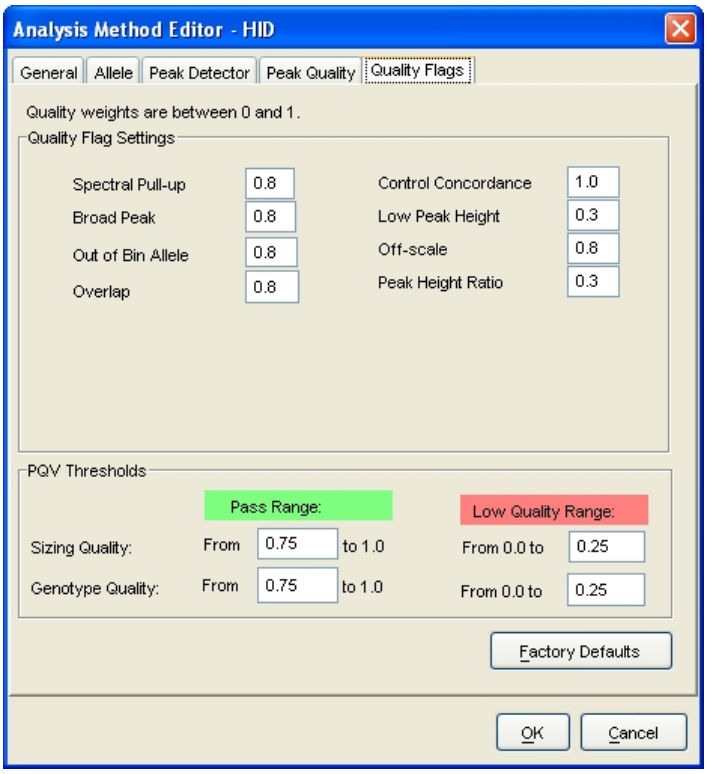
Parameters on the Peak Detector Tab that can be adjusted at analyst discretion: Ranges, Analysis or Sizing



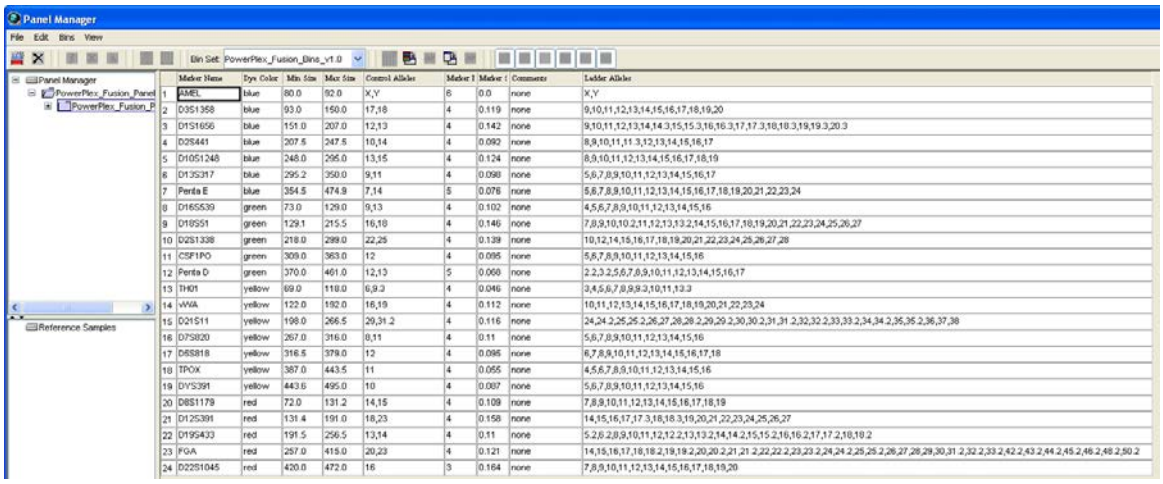
Parameters on the Peak Quality Tab that can be adjusted at analyst discretion: All. These are flag settings and generally flags are not used in our analysis in a formalized way. If individual analysts would like to use flags for their purpose, they are free to do so.



Parameters on the Quality Flags Tab that can be adjusted at analyst discretion: All. These are flag settings and generally flags are not used in our analysis in a formalized way. If individual analysts would like to use flags for their purpose, they are free to do so.



Parameters in the Panel Manager that can be adjusted at analyst discretion: None



Second readings by technical reviewer

A second qualified analyst will perform second readings of the electropherograms/runs by reanalyzing sample data with GeneMapper ID v3.2.1.

Controls

Controls are required to assess the effectiveness, accuracy and precision of the analytical procedures. Appropriate controls must be analyzed with each sample batch. Appropriate controls may include, but are not limited to, reagent blanks, negative controls, and positive controls.

Reagent Blank

The reagent blank is a test for contamination of the extraction reagents. See section on investigating contaminations for more information.

Negative Amplification Control

The negative amplification control is a test for contamination during amplification set-up. See section on investigating contaminations for more information.

Positive Amplification Control

The positive amplification control tests for proper amplification of samples, as well as ensuring that GeneMapper ID™ v3.2.1 is working properly. A positive amplification control is included in the amplification kit. This control must exhibit the following typing results:

NOTE: Amelogenin may have OL allele due to minus A or pull up

Amel	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539
X,Y	17,18	12,13	10,14	13,15	9,11	7,14	9,13

D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818
16,18	22,25	12,12	12,13	6,9.3	16,19	29,31.2	8,11	12,12

TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
11,11	10	14,15	18,23	13,14	20,23	16,16

If the correct alleles are not achievable, re-amplification of the positive control and all samples in the batch will be necessary. If the positive control is off-scale, it will be re-injected for 2 seconds. If it is still off-scale, it may be used with Technical Leader approval.

Allele Identification

True alleles are defined as peaks that are clearly visible above baseline noise, cannot be determined to be caused by an artifact or extra peak, and are of a size and shape indicative of an allele. The following describes other types of information that may be detected.

Artifacts and Extra Peaks

Peaks other than target alleles may be detected on an electropherogram. Some common artifacts are described in this manual. These artifacts or anomalies occur routinely during STR analysis. It is important to attempt to identify the cause of extra peaks. The analyst should use their training, knowledge, and skills to determine the likely cause of the artifact, and the analyst interpretation should be documented in the case record. Additional print outs of base pair sizing or raw data may be useful to help demonstrate the probable cause of an artifact.

An attempt should be made to rule out common causes of extra peaks such as spikes, pull up, excessive stutter, or other commonly encountered anomalies. All interpretations of artifacts must be made prior to comparison of the unknown profile to any reference profiles. Some signal that can be observed in the Powerplex Fusion chemistry are indicated in the manufacturer's user manual.

Spikes

Spikes are generally present in at least two colors and have the same data points. Confirmation of spikes may be confirmed in the raw data view of the software and removed from the interpretable profile.

Stutter

A stutter peak is a reproducible minor product peak shorter or longer than the corresponding main allele peak that is produced during amplification of STR loci. Stutter products may be caused by slippage of the DNA polymerase during amplification, probably due to out-of-alignment re-annealing of complementary target sequences during extension or out-of-alignment re-annealing of incomplete PCR products prior to extension. If a stutter peak exceeds the percent stutter associated with a locus, the analyst may choose to interpret that peak as excessive stutter and remove it as an allele designation. Like other data interpretations, this must occur prior to comparison to reference DNA samples. The stutter filter percentages for PowerPlex Fusion represent the manufacturer published values and are as follows:

Amel	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539
0%	11.9%	14.2% / (N-2) 3.6%	9.2%	12.4%	9.8%	7.6%	10.2%

D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818
14.6%	13.9%	9.5%	6.8%	4.6%	11.2%	11.6%	11.0%	9.5%

TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
5.5%	8.7%	10.9%	15.8%	11.0%	12.1%	(-)16.4%/(+) 8.6%

Minus A

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 that is one base pair longer than the actual target DNA

sequence. STR/PCR amplifications have been optimized to favor the "A" nucleotide addition. Incomplete "A" nucleotide addition may occur when too much amplification product is generated due to over addition of template DNA. Clear documentation will exist in the case record regarding the analysts interpretation of –A and agreement should exist between the analyst and the technical reviewer.

Pull-up

Pull-up is the result of the instrument's inability to separate colors (spectral overlap) used to fluorescently label STR products. Pull-up is observed as a peak beneath a peak or as an elevation of the baselines for any color. Pull-up is identified as a minor peak of similar base pair size as the true allele but in a different color, and interpretation is often assisted by utilizing the raw data view in the data analysis software. Pull-up can occur when too much template DNA has been added to the amplification mix or when a new spectral is needed. Samples with pull-up caused by over-amplification may be re-injected for a shorter period of time, diluted and re-run, or re-amplified with less DNA. Alternatively, in single source samples or mixtures when the pull-up is called "OL", the analyst may document the peak as pull-up and use the injection. Clear documentation will exist in the case record regarding the analysts interpretation of pull up and agreement should exist between the analyst and the technical reviewer.

Microvariants

Microvariants are defined as alleles that contain an incomplete repeat unit. The designation of alleles containing an incomplete repeat unit should include the number of complete repeats, and separated by a decimal point, the number of base pairs in the incomplete repeat. If an analyst is unable to determine the size of a microvariant, it will be documented as undetermined. If an allele falls outside (shorter or longer) the ladder alleles at a locus, it will be designated as greater than or less than the appropriate ladder allele (i.e., >16 for CSF1PO) for CODIS entry.

Off ladder alleles must be verified by re-injection of the sample unless the off ladder allele is called consistently in two or more samples (reference or questioned samples). Off ladder alleles often require the use of the minimum allele frequency for statistical calculations.

Full profiles (Single Source)

An evidentiary sample may be considered to be from a single person if the number of observed alleles at each locus is no more than two (except in the rare event of a tri-allele) and the peak heights are balanced (≥ 60%) for heterozygous alleles. All loci must be evaluated in total when making determination of single source or mixed profile. If a sample has unbalanced peak heights (< 60%) with no other indication of a mixture, the sample can still be considered a single source for statistical purposes with the approval of the technical reviewer.

Partial profiles

Partial profiles are profiles (insufficient data at one or more loci) can result from degraded/inhibited template DNA or low concentrations of template DNA. The entirety of the profile should be considered when determining whether a profile is a partial profile or not, and all individual locus interpretations must occur prior to comparing to the known reference samples in the case. Some profiles may contain too many contributors, or be of poor quality, to allow the profile to be used for interpretation. The profile should be designated as inconclusive and the analyst's reason for doing so shall be documented in the case record. This determination shall be agreed to by the technical reviewer and, if necessary in the case of dispute, agreed to by the technical leader. See below for more guidance on interpreting and reporting partial profiles.

Stochastic effects

Decreasing levels of template DNA may lead to stochastic effects which may under-represent one of the alleles in a locus. Using a minimum analytical threshold of 75 RFU, the following guidelines will be followed for interpreting data from low concentration samples:

Concentration	Single Source	Mixture with Major Component	Mixture with no Major Component
>0.3 ng	X	X	X
Between 0.0625 ng and 0.3 ng	X	Interpret loci from the major profile that contain heterozygous loci. The minor profile will be deemed uninterpretable.	The entire profile is uninterpretable
<0.0625	May interpret heterozygous loci (>75 RFU) or designate entire profile as uninterpretable	The entire profile is uninterpretable	The entire profile is uninterpretable

NOTE: X indicates that this combination of criteria does not meet the minimum criteria for stochastic amplification and the special guidelines for stochastic amplification are not applicable. Interpret according to the standard interpretation guidelines.

The table above represents commonly encountered general guidelines. If a departure from the above guidelines is determined to be necessary after discussion between the analyst and technical reviewer, approval from the technical leader is necessary prior to issuance of a test report.

Mixtures

Samples from crime scene evidence may contain DNA from more than one individual. The entire profile should be used to determine if there is sufficient information to

conclude that the sample contains DNA from more than one person. The analyst should be aware that mixtures can consist of full and/or partial profiles from multiple individuals, and a full profile from each contributor is not assumed due to potential dropout, especially in low template samples. Since quantitation does not allow for the individual quantitation of each person in a mixture, the exact concentration of each contributor cannot be known.

Some common indicators of potential signs of mixed profiles are:

- The presence of greater than two alleles at a locus

- The presence of a peak at a stutter position that is significantly greater in percentage than what is typically observed in validation or single source samples

- Significantly unbalanced alleles for a heterozygous genotype (i.e. <60% PHR). The peak height ratio, or PHR, is defined as the height of the lower peak (in RFU) divided by the height of the higher peak in (RFU), expressed as a percentage.

  Factors causing peak height imbalances include, but are not limited to, the following:
  - degraded DNA
  - inhibitors
  - very low amounts of input DNA
  - SNPs, mutation, and other genetic anomalies (i.e., Down's syndrome, Klinefelters, XYY)
  - multiple contributors or mixed profile

Minimum number of contributors: If a mixture is present, prior to including an individual, the data should be examined carefully to determine the minimum number of contributors (2, 3, 4, etc.) to a mixture. The minimum number of contributors is determined by the analyst considering the allele pattern of the entire profile, requires agreement from the technical reviewer and, if necessary, disputes can be arbitrated by the technical leader.

Some considerations when the analyst is estimating the minimum number of contributors are:

1. Only alleles that are present above the analytical threshold (75 RFU) may be used for interpretation. Signal below the analytical threshold should not be used for interpretation either for inclusion, exclusion, or an estimation of the number of contributors.
2. Examples of some signal patterns that may be removed at the analyst discretion are pull up, elevated stutter, spikes, free dye signal, and PCR artifacts, among others. This signal, if removed as uninterpretable signal, should be documented as such in the case file. The analyst may use data sources such as the raw data

to assist in arriving at an opinion that a signal is likely not a true allele that should be attributed to the profile.

3. After removing artifacts and signal that is not interpreted by the analyst as alleles, divide the number of alleles per locus and divide by two and round up, if necessary.
4. The analyst will document their interpretation of the minimum number of contributors in the case record and determine this prior to examining any reference samples in the case.

NOTE: if, after the above analysis is performed, the profile contains too many contributors (minimum number of contributors determined to be 4 or greater and a major component cannot be deconvoluted), this profile will be deemed inconclusive and not used for comparison to known reference samples or statistical analysis.

Mixture with Major/Minor Components

Specific genotype combinations and input DNA ratios of the samples contained in a mixture contribute to the complexity of resolving the genotypes of contributors to a mixed profile.

Whether a major component is discernable from a mixture is determined by the analyst considering the allele pattern of the entire profile, requires agreement from the technical reviewer and, if necessary, disputes can be arbitrated by the technical leader. Some factors that assist the analyst in determining a major component are:

- The analyst can determine which alleles are sourced from the major component at each interpretable locus
- Definition of Major Component: Where no minor allele is greater than 40% of the height of the shortest major peak in the same locus.
    - For example, if the allele that is determined to be from the major component is 1,000 RFU, no minor alleles should be greater than 400 RFU. This is not to be confused with a CODIS Eligible Profile (CEP) which is discussed in the context of CODIS uploads. (See section on CODIS for more information).

Prior to comparisons to reference samples, analysts will determine if a major component exists. If so, the major component profile will be documented in the case record.

Mixtures with indistinguishable contributors: An evidentiary sample should be considered a mixture with indistinguishable contributors when the major or minor contributors cannot be distinguished because of signal intensities or shared or masked alleles. Individuals may still be included or excluded as possible contributors assuming more than the minimum number of contributors does not exceed 3. If, after the analyst has corrected for artifacts and extra signal, the analyst interpretation is that the minimum number of contributors is 4 or more and a major contributor cannot be deduced, the profile will be identified as uninterpretable and not comparisons to knowns will be

performed. This determination should be made by the analyst, agreed to by the technical reviewer and, if necessary, disputes can be arbitrated by the technical leader. All loci in the profile should be considered when making this determination.

Comparisons to Known Reference Samples: Mixtures should be carefully evaluated to determine whether or not the reference profile of the individual being compared is consistent with being a component of the mixture. The totality of the profile including, but not limited to peak height ratios, homozygosity or heterozygosity of the individual, evidence of preferential amplification/ degradation/inhibition, should be considered.

Interpretation Guidelines for Very Low Level Profiles

An interpretation that includes an individual as a possible contributor to a questioned sample may be made even if evidence of locus or allele dropout exists. Some indicators, among others, that may lead the analyst to render a profile too weak for comparison are low RFU values, preponderance of homozygous alleles, and entire loci with no alleles above threshold. However, the following rules should be followed:

1. The analyst must determine which loci are interpretable and uninterpretable, or inconclusive, prior to comparing the profile to known reference samples.
2. Loci marked as inconclusive are not to be used in the comparison, for inclusionary or exclusionary purposes, to known reference samples. Once the locus is marked as inconclusive, it is to be ignored for comparison purposes.
3. A minimum number of 4 autosomal loci (in the Powerplex Fusion chemistry) should be determined to be interpretable in order to be suitable for comparison purposes. If only 3 loci or less are determined to be interpretable, the profile will be marked inconclusive. The relative weight or significance of the inclusion will be reflected in the statistical weight assigned to the profile.
4. Depending on the totality of the circumstances surrounding a profile, the analyst has the discretion of marking any profile inconclusive even if more than 3 loci are deemed interpretable. This determination shall be agreed to by the technical reviewer and, in the case of dispute, by the technical leader.
5. Any deviation from these guidelines regarding low level samples should be clearly stated by the analyst in the case record, agreed to by the technical reviewer or, in the case of dispute, agreed to by the technical leader.

CHAPTER 7 STATISTICS (QAS 9.6.4c)

Once an individual cannot be excluded as a possible component of a sample, the weight of the significance of the inclusion is determined statistically. Entire profiles or individual loci in profiles that are determined to be inconclusive are not included in the statistical analysis. Because it is the significance of the match that is important, only the interpretable loci where the person is included can be taken into account in estimating significance. DYS391 and amelogenin will not be used for statistical purposes since they are not autosomal loci.

Related Documents

None

Equipment/Materials/Reagents
None

Standards, Controls, and Calibration
None

Procedure

Source Attribution (QAS 9.6.2)

When reporting a source attribution statement, the following will be considered:

The analyst may choose to assign source attribution when a certain statistical threshold is met for **unrelated individuals**. Consideration will not be considered for the possibility of related individuals. (For more on source attribution, see Forensic Science Communications, July 2000, Volume 2, Number 3, Source Attribution of a Forensic DNA Profile). Source attribution of evidence does not require that the profile be unique, but instead that there is reasonable scientific certainty regarding the source of the evidence.

The base calculation is provided in the article as $p \leq 1 - (1-\alpha)1/N$ where:

- p=the adjusted probability
  - F x 10 where F is the most conservative estimate of genotype frequency across all the populations examined (i.e. African American, Caucasians, Hispanics, etc).
- $\alpha = 0.01$
- N = 314 million (the total suspect population which is taken to be the approximate population of the US).

In addition, the frequency is then increased by a factor of 10 to produce a more conservative estimate and encapsulate the expected range of uncertainty in the estimate.

1. *Basic Calculation:* $P \leq 1 - (1 - \alpha)^{1/N}$
2. *The factor of 10, correction for subpopulations, and current US population is applied:* $F \times 10 \leq 1 - (1 - 0.01)^{1/314,000,000}$
3. *Equation is reduced:* $F \leq (1 - (0.99)^{3.185E-9}) \div 10$
4. *Equation is reduced:* $F \leq 3.201^{E-12}$
   Exponential is converted to a probability: $1/F \geq$ 312,000,000,000 or the inverse probability of inclusion is 1 in at least 312 billion

The reporting statement will only include the use of a source attribution statement when:

1. the estimate for each calculated population group indicates a 1 in at least 312 billion probability of a random match
2. All autosomal loci tested must have interpretable results in order to use the source attribution statement.
3. there are no known identical siblings or close relatives in the case in question.

3.4 Statistical Significance Calculations

The single source significance calculation can be applied if the evidentiary profile compared fits the criteria for either a single source or a major component of a mixed source. If the profile fits the criteria for either a single source or a major component of a mixture, the analyst is encouraged to apply single source significance calculations and use single source or major component reporting statements.

- Determination of a major component (see above for previous definition of a major component) is determined by the analyst prior to examination of the known reference samples in the case and must be agreed upon by the technical reviewer or, in the case of dispute, the technical leader. The technical reviewer, when reviewing the case, should also examine the unknowns and make this assessment before examining the known reference samples in the case.
- Possible contribution of stutter is not subtracted prior to application of the 40% rule, assuming the allele in the stutter position has not been removed for interpretation by the analyst. The sample may still be considered single source with unbalanced alleles if agreed to by the technical reviewer and, in the case of dispute, the technical leader.

Single Source or Major Component Calculations

For evidence profiles that meet the criteria for either single source or major component of a mixture, calculations will use NRC II Recommendation formulae at a given locus:

For homozygotes, $f = p^2 + p(1-p)\theta$ where $\theta = 0.01$.
For heterozygotes, $f = 2pq$

For all loci used to identify the match, $F = (f_1 \times f_2 \times f_3 \times f_4 \times \ldots \times f_k)$, where k is the number of loci.

Inverse probability = 1/F.

Mixture calculations

Evidence profiles that do not fit the criteria for a single source are considered mixtures of two or more sources of biological material.

Major component profiles (2 or 3 person mixtures)

For evidence profiles that meet major component criteria and are considered to be mixtures of 2 or 3 people, the major component profile can be calculated using the single source profile calculation. If the minor component is probative, the mixture calculation described below may be used for all above threshold alleles at the included loci. Single source statistics should never be applied to a minor component of a mixture.

Mixtures with indistinguishable contributors
For probative mixtures with indistinguishable contributors (do not meet major component criteria), the following steps will be used to determine the mixed source significance:

For each individual locus, $f = (p_1 + p_2 + p_3 + p_4 + \ldots + p_k)^2$

- where p is the estimated frequency of the allele detected for each allele 1 through k, and k is the number of alleles detected at the locus.

- For all loci that are not determined to be inconclusive for comparison purposes, $F = (f_1 \times f_2 \times f_3 \times f_4 \times \ldots \times f_k)$, where k is the number of loci.

Minimum allele frequencies

Minimum allele frequencies are calculated using 5/2N where N is the number of individuals in the population database.

Off-ladder alleles

Reasonable attempts should be made to determine the reason for the off-ladder allele, whether PCR, sequence, or capillary electrophoresis based. Methods to deduce this include reinjection, analyzing on a different instrument, reamplification, or presence of the same off-ladder allele in more than one sample believed to be of a similar source. If the interpretation of the off-ladder is that it is not just due to poor migration that can't be further resolved on the instrument, then the allele frequency of the off-ladder allele may be the calculated using minimum allele frequency for the or the locus designated as inconclusive.

Software

The latest available and installed version of the FBI's Popstats software will be configured to use the above formulae and used to calculate significance estimates. Appendix 7A describes the source data and the data was provided to the CODIS Program in electronic format by John Butler at NIST. The Popstats files were reviewed for correctness by the FBI CODIS unit in Quantico, VA.

Population Databases

The significance estimate calculations above use empirically determined allele frequencies for each of the represented population groups and are described in detail in Appendix 7A.

Population groups

Analysts will routinely calculate and report significance estimates using Caucasian, African American, and Hispanic databases. Statistical calculations using other databases available in the Popstats program may be applied as required by the courts or deemed necessary by the analyst, and this require technical leader approval.

Interpretation
None

Literature/Supporting Documentation

Committee on DNA Technology in Forensic Science: an Update. II. National Research Council (U.S.). Commission on DNA Technology in Forensic Science: an Update. 1996. The Evaluation of Forensic DNA Evidence. National Academy Press, Washington, D.C. p. 122.

Committee on DNA Technology in Forensic Science: an Update. II. National Research Council (U.S.). Commission on DNA Technology in Forensic Science: an Update. 1996. The Evaluation of Forensic DNA Evidence. National Academy Press, Washington, D.C. p. 148.

Evett, I. W., Gill, P. D., Scranage, J. K., and B. S. Weir. 1996. Establishing the robustness of short-tandem-repeat statistics for forensic applications. Am J Hum Genet 58: 398-407.

Federal Bureau of Investigation. CODIS Users Guide.

Borys, S., R. Iwamoto, J. Miyakoshi, G. Carmody, and R. Fourney. 1999. Allele frequency distributions for nine STR loci in the Japanese population. J Forensic Sci 44(6):1319.

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Budowle, B., Moretti, T. R., Baumstark, A. L., Defenbaugh, D. A., and K. M. Keys. 1999. Population data on the thirteen CODIS core short tandem repeat loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians. J Forensic Sci 44(6):1277-1286.

Forensic Science International: Genetics Journal [7 (2013) e82-83]. The data is also referenced in the following article: Butler, J.M., Hill, C.R. and Coble, M.D. Variability of New STR Loci and Kits in US Population Groups. [Internet] 2012. Available from: http://www.promega.com/resources/articles/profiles-in-dna/2012/variability-of-new-str-loci-and-kits-in-us-population-groups/

APPENDIX 7A DATABASE INFORMATION

The following allele frequencies were determined by NIST. They are described in the Letter to the Editor entitled "U.S. Population data for 29 Autosomal STR Loci" published in the Forensic Science International: Genetics Journal [7 (2013) e82-83]. The data is also referenced in the following article: Butler, J.M., Hill, C.R. and Coble, M.D. Variability of New STR Loci and Kits in US Population Groups. [Internet] 2012. Available from: http://www.promega.com/resources/articles/profiles-in-dna/2012/variability-of-new-str-loci-and-kits-in-us-population-groups/

Any values less than the minimum allele frequency have been replaced with the minimum allele frequency in this table.

MARKER D3S1358

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<12	0.0069	0.0106	0.0073
12	0.0069	0.0106	0.0073
13	0.0069	0.0106	0.0073
14	0.1066	0.0784	0.0906
15	0.2729	0.3220	0.3085
15.2	0.0069	0.0106	0.0073
16	0.2382	0.2797	0.3187
17	0.2105	0.1843	0.2120
18	0.1510	0.1229	0.0570
19	0.0166	0.0106	0.0073
20	0.0069	0.0106	0.0073
>20	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER D1S1656

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN

<10	0.0069	0.0106	0.0073
10	0.0069	0.0106	0.0146
11	0.0776	0.0275	0.0453
12	0.1163	0.0890	0.0643
13	0.0665	0.1144	0.1009
13.3	0.0069	0.0106	0.0073
14	0.0789	0.1165	0.2573
14.3	0.0069	0.0106	0.0073
15	0.1496	0.1377	0.1579
15.3	0.0582	0.0508	0.0292
16	0.1357	0.1758	0.1096
16.3	0.0609	0.0508	0.1023
17	0.0471	0.0424	0.0278
17.3	0.1330	0.1483	0.0497
18	0.0069	0.0106	0.0073
18.3	0.0499	0.0254	0.0234
19	0.0069	0.0106	0.0073
19.3	0.0152	0.0106	0.0073
> 19.3	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER D2S441

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<9	0.0069	0.0106	0.0073
9	0.0069	0.0106	0.0073
9.1	0.0069	0.0106	0.0073
10	0.2105	0.3369	0.0848
11	0.3435	0.2987	0.3626
11.3	0.0609	0.0445	0.0439
12	0.0471	0.0360	0.1652
12.3	0.0069	0.0106	0.0073
13	0.0291	0.0233	0.0439
13.3	0.0069	0.0106	0.0073
14	0.2410	0.2055	0.2675
14.3	0.0069	0.0106	0.0073
15	0.0596	0.0487	0.0190
16	0.0069	0.0106	0.0073
17	0.0069	0.0106	0.0073
>17	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER D10S1248

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN

<8	0.0069	0.0106	0.0073
8	0.0069	0.0106	0.0073
9	0.0069	0.0106	0.0073
10	0.0069	0.0106	0.0073
11	0.0069	0.0106	0.0351
12	0.0319	0.0424	0.1301
13	0.3075	0.2733	0.2339
14	0.2978	0.3390	0.2763
15	0.1967	0.2119	0.1974
16	0.1330	0.0996	0.0877
17	0.0277	0.0254	0.0249
18	0.0069	0.0106	0.0073
19	0.0069	0.0106	0.0073
>19	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER D13S317

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<8	0.0069	0.0106	0.0073
8	0.1205	0.1102	0.0278
9	0.0776	0.1653	0.0336
10	0.0471	0.0996	0.0307
11	0.3255	0.2182	0.3099
12	0.2687	0.2352	0.4181
13	0.1163	0.1059	0.1404
14	0.0429	0.0614	0.0395
15	0.0069	0.0106	0.0073
>15	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER PENTA E

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<5	0.0069	0.0106	0.0073

5	0.0762	0.0360	0.0950
6	0.0069	0.0106	0.0073
7	0.1690	0.1186	0.1038
8	0.0139	0.0254	0.1667
9	0.0125	0.0169	0.0512
10	0.0859	0.0847	0.0468
11	0.0873	0.0742	0.0643
12	0.1994	0.1737	0.1287
13	0.0859	0.0932	0.1038
14	0.0623	0.0720	0.0687
15	0.0429	0.0911	0.0556
15.4	0.0069	0.0106	0.0073
16	0.0512	0.0614	0.0409
17	0.0485	0.0551	0.0439
18	0.0332	0.0339	0.0161
19	0.0152	0.0212	0.0073
20	0.0097	0.0212	0.0073
21	0.0069	0.0106	0.0073
22	0.0069	0.0106	0.0073
23	0.0069	0.0106	0.0073
24	0.0069	0.0106	0.0073
25	0.0069	0.0106	0.0073
>25	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.007

MARKER D16S539

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<5	0.0069	0.0106	0.0073
5	0.0069	0.0106	0.0073
8	0.0180	0.0191	0.0322
9	0.1066	0.1398	0.1827
10	0.0568	0.1504	0.1170
11	0.3144	0.2648	0.3143
12	0.3144	0.2775	0.2047
13	0.1634	0.1335	0.1228
14	0.0263	0.0127	0.0249
15	0.0069	0.0106	0.0073
>15	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER D18S51

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<9	0.0069	0.0106	0.0073

9	0.0069	0.0106	0.0073
10	0.0083	0.0106	0.0073
11	0.0097	0.0148	0.0073
12	0.1136	0.1144	0.0760
13	0.1233	0.1229	0.0409
13.2	0.0069	0.0106	0.0073
14	0.1343	0.1610	0.0716
14.2	0.0069	0.0106	0.0073
15	0.1704	0.1589	0.1652
15.2	0.0069	0.0106	0.0073
16	0.1468	0.1250	0.1711
16.2	0.0069	0.0106	0.0073
17	0.1385	0.1250	0.1520
18	0.0776	0.0784	0.1213
19	0.0402	0.0466	0.0994
20	0.0180	0.0275	0.0629
21	0.0097	0.0106	0.0102
21.2	0.0069	0.0106	0.0073
22	0.0069	0.0106	0.0073
23	0.0069	0.0106	0.0073
24	0.0069	0.0106	0.0073
>24	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER D2S1338

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<15	0.0069	0.0106	0.0073
15	0.0069	0.0106	0.0073
16	0.0374	0.0297	0.0556
17	0.1856	0.1695	0.1009
18	0.0734	0.0805	0.0424
19	0.1205	0.1928	0.1389
20	0.1565	0.1271	0.1038
21	0.0374	0.0318	0.1360
22	0.0346	0.0572	0.1374
23	0.1053	0.1398	0.1038
24	0.1150	0.0763	0.0833
25	0.1025	0.0784	0.0775
26	0.0305	0.0169	0.0146
27	0.0069	0.0106	0.0073
>27	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER CSF1PO

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<7	0.0069	0.0106	0.0073

7	0.0069	0.0127	0.0556
8	0.0069	0.0106	0.0556
9	0.0139	0.0233	0.0395
10	0.2202	0.2373	0.2500
11	0.3089	0.2797	0.2485
12	0.3601	0.3750	0.2953
13	0.0817	0.0593	0.0468
14	0.0097	0.0106	0.0088
15	0.0069	0.0106	0.0073
>15	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER PENTA D

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
2.2	0.0069	0.0169	0.1140
3.2	0.0069	0.0106	0.0088
5	0.0069	0.0106	0.0439
6	0.0069	0.0106	0.0102
7	0.0069	0.0106	0.0439
8	0.0208	0.0191	0.1082
9	0.2216	0.2415	0.1681
10	0.1150	0.1568	0.0994
11	0.1260	0.1568	0.1798
12	0.2327	0.1631	0.1082
13	0.1967	0.1441	0.0833
13.4	0.0069	0.0106	0.0073
14	0.0609	0.0720	0.0249
15	0.0097	0.0106	0.0073
16	0.0069	0.0106	0.0073
17	0.0069	0.0106	0.0073
>17	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER TH01

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<5	0.0069	0.0106	0.0073
5	0.0069	0.0106	0.0073
6	0.2355	0.2394	0.1316
7	0.1939	0.2966	0.4079
8	0.0956	0.0911	0.1959
9	0.1191	0.1462	0.1594
9.3	0.3449	0.2182	0.0965
10	0.0083	0.0106	0.0073
11	0.0069	0.0106	0.0073
>11	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER vWA

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<11	0.0069	0.0106	0.0073
11	0.0069	0.0106	0.0073

12	0.0069	0.0106	0.0073
13	0.0069	0.0106	0.0088
14	0.0928	0.0805	0.0804
15	0.1053	0.1441	0.1915
16	0.2008	0.2839	0.2500
17	0.2839	0.2458	0.2354
18	0.2022	0.1801	0.1491
19	0.1039	0.0508	0.0629
20	0.0069	0.0106	0.0161
21	0.0069	0.0106	0.0073
>21	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER D21S11

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<24.2	0.0069	0.0106	0.0073
24.2	0.0069	0.0106	0.0073
25.2	0.0069	0.0106	0.0073
26	0.0069	0.0106	0.0073
26.2	0.0069	0.0106	0.0073
27	0.0222	0.0275	0.0746
28	0.1593	0.0996	0.2456
29	0.2022	0.2076	0.2047
29.2	0.0069	0.0021	0.0073
29.3	0.0069	0.0106	0.0073
30	0.2825	0.2733	0.1696
30.2	0.0291	0.0233	0.0175
31	0.0720	0.0763	0.0789
31.2	0.0983	0.0996	0.0512
32	0.0069	0.0169	0.0088
32.2	0.0900	0.1271	0.0614
33	0.0069	0.0106	0.0073
33.1	0.0069	0.0106	0.0073
33.2	0.0263	0.0339	0.0351
34	0.0069	0.0106	0.0058
34.2	0.0069	0.0106	0.0073
35	0.0069	0.0106	0.0219
36	0.0069	0.0106	0.0088
37	0.0069	0.0106	0.0073
38	0.0069	0.0106	0.0073
39	0.0069	0.0106	0.0073
>39	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER D7S820

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
6	0.0069	0.0106	0.0073

7	0.0277	0.0106	0.0117
8	0.1440	0.1208	0.2281
8.1	0.0069	0.0106	0.0073
9	0.1676	0.0911	0.1155
10	0.2562	0.3072	0.3363
10.3	0.0069	0.0021	0.0073
11	0.2050	0.2775	0.2032
12	0.1593	0.1547	0.0877
13	0.0346	0.0360	0.0146
14	0.0069	0.0106	0.0073
>14	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER D5S818

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<7	0.0069	0.0106	0.0073
7	0.0069	0.0339	0.0073
8	0.0069	0.0106	0.0468
9	0.0416	0.0530	0.0322
10	0.0554	0.0572	0.0731
11	0.3560	0.3898	0.2339
12	0.3878	0.3390	0.3699
13	0.1427	0.1081	0.2237
14	0.0069	0.0106	0.0161
15	0.0069	0.0106	0.0073
>15	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER TPOX

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<5	0.0069	0.0106	0.0073
5	0.0069	0.0106	0.0073
6	0.0069	0.0106	0.0892
7	0.0069	0.0106	0.0175
8	0.5249	0.4852	0.3670
9	0.1274	0.0932	0.1959
10	0.0499	0.0487	0.0863
11	0.2521	0.2542	0.2164
12	0.0416	0.1038	0.0263
13	0.0069	0.0106	0.0073
>13	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER D8S1179

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<8	0.0069	0.0106	0.0073

8	0.0139	0.0148	0.0073
9	0.0069	0.0106	0.0073
10	0.1025	0.0932	0.0307
11	0.0762	0.0530	0.0526
12	0.1676	0.1292	0.1301
13	0.3296	0.2733	0.2193
14	0.1662	0.2627	0.2939
15	0.1039	0.1292	0.1901
16	0.0332	0.0318	0.0643
17	0.0069	0.0106	0.0073
18	0.0069	0.0106	0.0073
>18	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER D12S391

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<14	0.0069	0.0106	0.0073
14	0.0069	0.0106	0.0073
15	0.0319	0.0445	0.0775
16	0.0222	0.0424	0.0673
17	0.1274	0.0763	0.1667
17.1	0.0069	0.0106	0.0073
17.3	0.0208	0.0169	0.0073
18	0.1717	0.1780	0.2529
18.1	0.0069	0.0106	0.0073
18.3	0.0249	0.0127	0.0073
19	0.1247	0.1886	0.1477
19.1	0.0069	0.0106	0.0088
19.3	0.0069	0.0106	0.0073
20	0.1108	0.1547	0.1038
20.1	0.0069	0.0106	0.0073
20.3	0.0069	0.0106	0.0073
21	0.1288	0.1123	0.0643
22	0.0956	0.0678	0.0365
22.2	0.0069	0.0106	0.0073
23	0.0693	0.0572	0.0292
24	0.0471	0.0169	0.0132
24.3	0.0069	0.0106	0.0073
25	0.0166	0.0106	0.0088
26	0.0069	0.0106	0.0073
27	0.0069	0.0106	0.0073
>27	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER D19S433

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<9	0.0069	0.0106	0.0073
9	0.0069	0.0106	0.0073

10	0.0069	0.0106	0.0102
11	0.0069	0.0148	0.0629
12	0.0706	0.0657	0.1228
12.2	0.0069	0.0127	0.0365
13	0.2548	0.2225	0.2456
13.2	0.0069	0.0445	0.0526
14	0.3615	0.3538	0.2105
14.2	0.0235	0.0381	0.0746
15	0.1565	0.1356	0.0804
15.2	0.0360	0.0551	0.0614
16	0.0568	0.0254	0.0073
16.2	0.0152	0.0275	0.0263
17	0.0069	0.0106	0.0073
17.2	0.0069	0.0106	0.0088
18	0.0069	0.0106	0.0073
18.2	0.0069	0.0106	0.0073
>18.2	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

MARKER FGA

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<16.2	0.0069	0.0106	0.0073
16.2	0.0069	0.0106	0.0073
17.2	0.0069	0.0106	0.0073
18	0.0249	0.0127	0.0073
18.2	0.0069	0.0106	0.0175
19	0.0499	0.0805	0.0512
19.2	0.0069	0.0106	0.0073
20	0.1233	0.0847	0.0541
21	0.1787	0.1525	0.1228
21.2	0.0069	0.0106	0.0073
22	0.2050	0.1653	0.1988
22.2	0.0125	0.0106	0.0073
22.3	0.0069	0.0106	0.0073
23	0.1524	0.1208	0.1696
23.2	0.0069	0.0106	0.0073
24	0.1343	0.1419	0.1330
24.2	0.0069	0.0106	0.0073
25	0.0789	0.1186	0.1184
25.2	0.0069	0.0106	0.0073
26	0.0263	0.0614	0.0702
27	0.0069	0.0445	0.0234
28	0.0069	0.0106	0.0146
29	0.0069	0.0106	0.0073
30	0.0069	0.0106	0.0073
30.2	0.0069	0.0106	0.0073
31.2	0.0069	0.0106	0.0073
>31.2	0.0069	0.0106	0.0073

N	361	236	342
Min. allele frequency	0.0069	0.0021	0.0073

MARKER D22S1045

	US CAUCASIAN	US HISPANIC-AMERICAN	AFRICAN AMERICAN
<8	0.0069	0.0106	0.0073
8	0.0069	0.0106	0.0073
9	0.0069	0.0106	0.0073
10	0.0069	0.0148	0.0409
11	0.1399	0.0636	0.1447
12	0.0125	0.0127	0.0541
13	0.0069	0.0106	0.0073
14	0.0568	0.0275	0.0775
15	0.3213	0.4258	0.2515
16	0.3823	0.3496	0.1915
17	0.0748	0.0911	0.2091
18	0.0069	0.0106	0.0146
19	0.0069	0.0106	0.0073
>19	0.0069	0.0106	0.0073
N	361	236	342
Min. allele frequency	0.0069	0.0106	0.0073

CHAPTER 8 CODIS (Combined DNA Index System)

1 Scope

The 1994 Crime Act included provisions establishing the FBI's Combined DNA Index System, a national DNA database program. As of June 1998, all 50 States require the collection of DNA samples.

In June 1998, the FBI announced the establishment of a set of 13 core STR loci for use in the National DNA Index System (NDIS). The set of core loci required for participation in NDIS is as follows: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, TH01, TPOX, and CSF1PO. The APD DNA section will use only NDIS approved PCR Amplification kits to satisfy this core loci requirement for CODIS entry. All specimen categories, loci requirements, and match estimator requirements apply to CODIS core loci only.

2 Related Documents

The CODIS software is the property of the FBI. It is a requirement to follow NDIS guidelines in order to obtain Federal financial support for the CODIS system and to maintain participation. Refer to the NDIS Procedures Manual for additional information. (available on the CJIS WAN) The system is secured by the FBI and is restricted to users approved by NDIS.

3 Case Evaluation

All cases and samples containing biological evidence should be evaluated for possible entry into CODIS. These samples must be evaluated for CODIS eligibility prior to upload or search in CODIS. A good faith effort shall be made to obtain results at each locus for inclusion into the appropriate index for CODIS. Only interpretable profiles which are above the stochastic threshold are eligible for CODIS entry. All forensic unknowns, forensic mixtures, and forensic partial profiles that are submitted for entry into CODIS shall originate from or be associated with a crime scene and the source should be associated with the putative perpetrator. Alleles that are unambiguously attributed to a victim or individuals other than the perpetrator(s) shall not be submitted to CODIS.

3 Index Systems

1. LDIS
    a. LDIS (Local DNA Index System) Does not require a minimum number of loci for searching.
2. SDIS

a. SDIS (State DNA Index System) requires a minimum of 7 loci (not including Amelogenin) for searching. LEGAL index PROFILES require all 13 core loci.

3. NDIS
   a. NDIS (National DNA Index System) requires that all 13 core loci be attempted and will accept specimens with data for 10 loci (not including Amelogenin) for searching. Other loci may be uploaded, but they are not required to report a match.

Any evidence profile that does not meet the NDIS or SDIS requirements for upload can be maintained at LDIS with the approval of the technical reviewer and Local CODIS Administrator.

5 Specimen Identification/ Information

Specimen naming policies are up to the laboratory entering the profile. There is no statewide requirement for standardization of naming between laboratories as long as each laboratory can identify their own samples. The only requirement for CODIS is that each DNA profile has a unique specimen ID. The ID for cases should include the case number, item number, and item description (i.e. vs for vaginal swab). All DNA casework extracts produced in the laboratory, including those which result in a profile uploaded to CODIS, will be stored and maintained as evidence according to laboratory procedures.

CODIS Eligible Profiles (CEP) are defined as:

- Profiles deduced from mixtures in compliance with the CODIS rules published by the FBI. CEPs may or may not correlate to an interpretation of a major component as defined by this laboratory.
- Determination of a CEP is determined by the analyst and must be agreed upon by the technical reviewer or, in the case of dispute, the CODIS Administrator. The DNA technical leader should be involved in the discussions.

6 Forensic Unknown Specimen Category

If a profile can be reliably deduced from a mixture profile, ONLY that portion of the mixture will be entered into CODIS.

1. The CEP shall have no more than 3 alleles at one locus and all remaining loci can have up to 2 alleles.
2. Any profile that has more than 50% homozygous alleles must meet a statistical threshold for rarity using the Match estimator tool in the CODIS software. This match rarity should not be more than 1 in the size of the NDIS database and 20 in the size of the SDIS database. The match estimator tool only needs to be used against the index it will be searched/ stored against (i.e SDIS or NDIS). The technical reviewer should initial the match estimation

report. If a profile falls outside of this match rarity it can still be uploaded with approval of the CODIS Administrator.

7 Forensic Mixture Specimen Category (FM)

1. No more than four alleles at any locus
2. Profiles submitted for inclusion in the Forensic Mixture Index must meet a statistical threshold for rarity using the Match estimator tool in the CODIS software. This match rarity should not be more than 1 in the size of the NDIS database and 20 in the size of the SDIS database. The match estimator tool only needs to be used against the index it will be searched/ stored against (i.e SDIS or NDIS). The technical reviewer should initial the match estimation report. If a profile falls outside of this match rarity it can still be uploaded with approval of the CODIS Administrator.
3. Individual loci that are believed to exhibit allelic dropout or have other conditions where additional information may aide in match resolution should be designated with a p on the CODIS entry form (i.e. 18, p).
4. State level only profiles should be clearly identified on the CODIS sheet and put into the state partial forensic mixture category.

8 Forensic Partial Profile Specimen Category (FP)

1. An evidence profile originating from a single source sample or a deduced profile originating from a mixture that has either locus or allelic dropout at the core loci.
2. Profiles submitted for inclusion in the Forensic Partial Profile Index must meet a statistical threshold for rarity using the Match estimator tool in the CODIS software. This match rarity should not be more than 1 in the size of the NDIS database and 20 in the size of the SDIS database. The match estimator tool only needs to be used against the index it will be searched/ stored against (i.e SDIS or NDIS). The technical reviewer should initial the match estimation report. If a profile falls outside of this match rarity it can still be uploaded with approval of the CODIS Administrator.
3. The profile shall have no more than 3 alleles at one locus and all remaining loci can have up to 2 alleles.
4. Individual loci that are believed to exhibit allelic dropout or have other conditions where additional information may aide in match resolution should be designated with a p on the CODIS entry form (i.e. 18, p).
5. Profiles in this category do not necessarily require a designation as a partial profile. (A 10 locus profile that is completely heterozygous and does not exhibit any allelic dropout; therefore does not have any additional information to aide in match resolution is not considered a partial profile).

9 Suspect (S)/ Legal Specimen Category (L)

Texas CODIS law allows for the inclusion of any sample legally obtained in the investigation of a crime, regardless of origin. The law further allows for the inclusion of

voluntarily submitted samples. These samples are collected under applicable legal authorities.

Profiles will be entered in the "Suspect Known" index or the "Legal" Index if the sample was:

- obtained legally
- originally collected for the investigation of a crime
- a biological sample from a suspect in a criminal investigation
- DNA typed for comparison to evidence in a submitted case
- not typed solely for the purpose of eliminating a non-suspect (e.g., husband / boyfriend)

These Suspect knowns will be regularly searched against the forensic database at the local level and will be regularly uploaded to SDIS (Suspect Index) or NDIS (Legal Index). Suspect profiles will be identified in the database by the case number and sample designation and not include the name of the individual. Inclusion into either of these categories is at analyst discretion. Match estimator is not required.

Suspect Specimen Category

- Any suspect profile that is incomplete for the Core CODIS Loci
- At least 7 Core CODIS Loci
- Alternate reference samples
- These profiles are not uploaded to NDIS

Legal Specimen Category

- Any suspect profile that is complete for the 13 Core CODIS Loci
- These profiles are uploaded to NDIS

The records in the CODIS database are confidential and not subject to open records disclosure. A record includes both the profile and the identity of the individual whose profile is in the database. Neither the profile nor the identity of the individual whose profile is in the database will be released except (QAS 11.3.3):

- to a criminal justice agency for law enforcement investigative purposes
- for a judicial proceeding, if otherwise admissible under law
- for criminal defense purposes to a defendant, if related to the case in which the defendant is charged

- to criminal justice agencies, if personally identifiable information is removed, for a population statistical database, for forensic identification, forensic protocol development purposes, forensic research, or for quality control purposes

10 Expunctions

1. Federal law requires that a laboratory, as a participant in NDIS, expunge the DNA records of persons (whose qualifying offense has been overturned, they have been acquitted, no charges have been filed and for other reasons as described in the Federal DNA Identification Act at *42 U.S.C.A. §14132 (d)* and the DNA Fingerprint Act of 2005 *(P.L. 109-162)* when court ordered to do so.
2. Any suspect known profile will be expunged from the database if court ordered to do so.
    - When a court order is received the CODIS Administrator will remove the sample from all levels of CODIS
    - The reference sample will be destroyed
    - A delete report is generated through CODIS and shall serve as documentation of the removal
    - The APD DNA lab is not required to destroy an item of physical evidence obtained from a sample if evidence relating to another person would thereby be destroyed
    - Any identification, warrant, probable cause to arrest or arrest based upon a database match is not invalidated due to a failure to expunge or delay in expunging records

11 Additional Considerations

1. Prior to upload or search all profiles must have undergone a documented technical review (this review can be separate from the technical review of the case file).
2. By signing the CODIS entry form the analyst and technical reviewer are verifying sample name, specimen category, allele calls, CEP, and eligibility.
3. Source ID, partial profile designation, and Offense code are considered optional on the CODIS entry form for Legal and Suspect profiles.
4. When necessary; below threshold alleles may be used for CODIS upload with the appropriate designation () if above the stochastic threshold and can be used for exclusionary purposes.

12 Searching

The APD DNA Laboratory will routinely search at moderate stringency with 7 loci needed to report a match and allow for one mismatch locus. If profiles are entered or searched with less than 7 loci these parameters must be adjusted and these profiles can be seared periodically.

Autosearcher should be run after new profiles are entered into CODIS or a minimum of weekly if new profiles are entered frequently. Match messages should be checked and dispositioned by the Local CODIS Administrator weekly.

Staff profiles are not maintained in the CODIS software but are maintained as a batch target file. These profiles should be searched weekly prior to upload or as needed.

13 CODIS Reporting

After appropriate ”Forensic Unknowns”, “Forensic Mixtures”, “Forensic Partials”, “Legal” or “Suspect” knowns have been entered into the CODIS system, a report needs to be generated to the investigative official reporting the CODIS entry. This can be accomplished by incorporating the CODIS information into the report containing DNA results.

Letters that pertain to CODIS (matches, hits, dispositions, requests, etc) will be generated in the LIMS system under the CODIS Section. Cases without lab numbers (non LIMS cases) letters will be generated manually and scanned into the appropriate system. All CODIS letters are for information purposes only and require an administrative review. This administrative review is documented in the LIMS system or on the letter itself in non LIMS cases.

14 Matches/ Hits/ Dispositions

All matches will be dispositioned per current NDIS guidelines available on the CJIS WAN. A CODIS hit follow up form should be used to help track the notification process to ensure investigating officers have been informed of the matches. All the duties performed by the CODIS Administrator can also be performed by the Backup CODIS Administrator or designee. Documentation will be maintained in the case record.

Forensic Hits (Case to case Hits)

A forensic match occurs when DNA profiles from two or more forensic samples match one another. These matches will be reviewed by the CODIS Administrator and case files will be examined as needed.

If the case is unsolved a letter, or email, will be generated from the local CODIS Administrator to the other laboratory exchanging information. The letter will include specific case information along with contact information for the investigating officers. No personally identifiable information will be released.

If our case is solved and we have confirmed the information through a direct comparison a letter or email will be generated and it may include personally identifying information.

When these matches occur outside the state of Texas a confirm interstate match form may be used and letters only should be generated.

Offender Matches

An offender match occurs when a DNA profile developed from crime scene evidence matches an offender, suspect, or legal DNA profile.

When a solved case matches with an offender, legal or suspect known the identity of that individual must be confirmed. This is usually done by comparing names between the two laboratories. If names can't be confirmed refer to NDIS procedures.

When an unsolved case matches with an offender, legal or suspect profile a letter will be generated providing information that the hit occurred. In many cases an Offender Request form in addition to the letter can be used to contact the Offender laboratory. In some cases Offender laboratories have their own forms and/or require additional information. The CODIS Administrator is responsible for ensuring the other agencies have the information necessary to confirm the match.

Case to Suspect/ Legal Index Hits

Matches between suspect profiles and previously unlinked forensic profiles provide an investigative lead and may be sufficient probable cause for suspicion. Such a match is not to be used as the basis of a final comparison in any case. In order to make a comparison for a final case report, the suspect profile must be verified with a newly collected suspect sample. In some cases the court may request comparison to a previously collected sample.

When a forensic sample matches to a sample in our Suspect or Legal index it will be treated the same way as Offender matches. If the case is solved the identity of the individual is confirmed. If the case is unsolved letters or emails will be generated providing specific case information along with contact information for the investigating officers. No personally identifiable information will be released. The CODIS Administrator is responsible for ensuring the other agencies have the information necessary.

A letter will be generated from the Local CODIS Administrator for other matches when necessary.

15 Partial Matches

A "partial match" is a moderate stringency match between two single source DNA profiles that have at least one allele in common at each locus. This is a fortuitous event. The APD DNA lab will not routinely follow up on partial matches. If contacted by an outside agency requesting partial match information the CODIS Administrator and DNA

technical Leader will review the request and make a determination based on case information, DNA profiles, and NDIS procedures.

16 Uploads

Uploads to SDIS should be performed each Friday unless no new data has been entered. Uploads can be performed at other times when necessary. Full uploads should be performed a minimum of twice yearly.

17 Backups

Backups should be performed weekly. Backups should be generated with one copy stored off-site at a location with at least equal security to the crime lab. A check of the backup system should be performed and documented once annually.

17 Contingency Plan

The following items are to be stored off site in case of an emergency.

- Install CD's
- Backup
- An emergency Contact list
- Information for starting up the system

18 Search Requests

If the laboratory receives a search request from an NDIS approved laboratory and the profile has been developed according to the Quality Assurance Standards, then that profile may be searched through the Local DNA Index System (LDIS). Any matches originating from that search will be dispositioned and reports issued per section SOP.

With sufficient cause and with approval from the DNA technical leader and the CODIS Administrator, a search request can be made for a profile that does not meet the SDIS or NDIS minimum requirements for upload.

19 CODIS Assessments

An internal CODIS Assessment should be performed and documented yearly.

APPENDIX 8A CODIS Manager and local CODIS Administrator Duties / Qualifications

State CODIS Manager

The Texas Department of Public Safety Crime Laboratory Service will have an individual in the position of CODIS Manager. The Manager has the authority to terminate a laboratory's participation in CODIS in the event of a problem until the reliability of the data can be assured. This termination authority applies to all of the Local CODIS sites that are under the jurisdiction of the CODIS Manager.

The CODIS Manager is responsible for adding or removing laboratories from NDIS. The Manager is responsible for adding or removing CODIS users from NDIS. The Manager is responsible for uploading the state's DNA data to NDIS. The duties of the CODIS Manager are to be performed according to the NDIS Procedures Manual. The CODIS manager is responsible for coordinating with the local CODIS administrator for uploads to SDIS and for performing SDIS searches.

Local CODIS Manager/Administrator

The Austin Police Department DNA section will have an individual in the position of local CODIS Administrator who meets NDIS requirements. This individual will be part of the DNA staff of the laboratory and will meet the educational and experience qualifications specified in the DNA Audit document. The CODIS Administrator shall have successfully completed the QAS auditor training within one year of assuming these duties and shall also successfully complete the CODIS software training within six months of assuming these duties. The local CODIS Administrator will serve as a point of contact for the NDIS custodian, CODIS Unit, CODIS software provider, the CODIS manager or an outside agency in the event of a match in the CODIS system. All duties of the local CODIS Administrator are to be performed according to NDIS procedures and the CODIS Administrators handbook, available on the CJIS WAN. The Local CODIS Administrator is responsible for the oversight of CODIS computer training and the quality assurance of data. The Local CODIS Administrator is also responsible for completing and maintaining FBI security access and successfully completing the annual training. The local CODIS Administrator is also responsible for adding or removing CODIS users from the system and has the authority to terminate an analyst's participation in CODIS **(QAS 5.3.5)**. They should also attend the annual Conference sponsored by the FBI. If they are unavailable to do so the Backup Administrator/ or designee shall attend.

Other responsibilities include:

- Completion of the Annual Audit certification Backup
- Completion of the annual CODIS survey
- Compilation of monthly reports to the State CODIS Manager
- Updating antivirus software
- Ensuring all CODIS users (except IT users) successfully complete annual training

- Ensure backups of CODIS data are being done and perform a check of these backups at least once a year
- Keeping all CODIS Users informed as to changes/ updates to NDIS procedures
- Coordinating the entering, searching, and uploading of DNA profiles
- Resolving matches and compliance with the CODIS Memorandum of Understanding and NDIS procedures.
- Serve as the gatekeeper of the CODIS database and assist in determining eligibility of samples when necessary

Backup Local CODIS Administrator

There should also be someone in the position of backup Administrator who meets the NDIS requirements. This individual will be part of the DNA staff of the laboratory. The Backup Administrator is responsible for assisting in the entering, searching, and uploading of DNA profiles. They are also responsible for assisting in the resolution of matches and being another point of contact for the laboratory. The Backup Administrator shall successfully complete QAS auditor training within one year and CODIS software training within six months of assuming these duties. The Backup Administrator must complete and maintain FBI security access and successfully complete the annual training. The Backup CODIS Administrator is responsible for all duties of the CODIS Administrator in their absence or when unavailable.

APPENDIX 8B: CODIS Specimen Abbreviations

Please use the following specimen designation format and abbreviations when naming a specimen for CODIS entry:

Forensic Specimens:
Lab # / Item # / Abbreviation / Fraction (if applicable)

Suspect/Legal Samples:
Lab # / Item # / Initials / SUS

Item	Abbreviation	Item	Abbreviation
Suspect	SUS	Bra	BR
Vaginal swab	VS	Skirt	SK
Anal Swab (rectal)	RS	Glove	GLV
Oral Swab	OS	Envelope	ENV
Penile Swab	PS	Cigarette Butt	CB
Face Swab	FS	Towel	TW

Breast Swab	BRS	Beer/ Soda Can	CAN
Neck Swab	NS	Bed/ Mattress/ Pad	BD
Misc. Swab/ Stain	SWB	Bed Sheet	BDS
Fingernails	FGN	Blanket	BK
Panties/ Underwear	PN	Pillow	PW
Pantyhose	PH	Bag	BAG
Condom	CDM	Toilet Paper	TP
Shorts	SH	Airbag	AB
Shirt	ST	Ligature	LG
Dress	DR	Gun	GUN
Pants	PT	Knife	KN
Jeans	JN	Clitoral Swab	CS
Socks	SO	Hair	HR
Jacket	JK	Finger Swab	FS
Sweater	SW	Body Swab	BS
Tampon/ Pad	FH	Hat	HT

CHAPTER 9 REAGENTS

9.1 AP Test Reagent

Acid phosphatase (AP) test reagent undergoes a color change in the presence of acid phosphatase, which is found in highest concentration in semen. Instructions for use and interpretation are in the AP Test protocol.

Specification
AP

Quality Control
Test a positive and negative control before use (once per day) by testing a known semen sample (positive control) and an unstained swab or substrate (negative control). A 4+ result is required for the positive control to pass and no color change at 1 minute is required for the negative control to pass. Is either control fails the reagent will not be used on casework.

Special Equipment and Supplies
Beaker
Graduated cylinder
Bottle
Reagents
Deionized water (dIH2O) 10ml
AP test reagent 0.26g

Procedure

1. Measure 10ml of deionized water (dIH2O) into a beaker.
2. Add 0.26g AP test reagent
3. Mix

Testing, storage, expiration, and disposal
Store in a darkened container. AP Test Reagent can be stored at 2-8°C until a 4+ rating is no longer achieved with the control. A positive and negative control must be tested on day of use.

Literature/Supporting Documentation
Kind, Stuart S. 1957. The use of acid phosphatase in searching for seminal stains. Journal of Criminal Law, Criminology, and Police Science. 47(5):597-600.

Smith, Charles. 1979. An acid phosphatase reagent for use in searching large areas for seminal stains. Unpublished.

9.2 DTT, 0.39 M

DTT (dithiothreitol) is a reducing agent used in DNA extraction buffers to allow lysis of cells with thiol-rich membrane proteins, such as spermatozoa. Instructions for use and interpretation are in the test procedures for extraction.

Specification
DTT, 0.39M

Quality Control
Each new lot of prepared DTT will undergo a QC check. Place a semen sample containing sperm in a microcentrifuge tube with 290ul Buffer ATL. Allow to extract for ~1 hour. Place ~10ul of the extract on a slide. Stain and view per the spermatozoa examination chapter. Confirm the presence of in-tact sperm heads. Add 25ul DTT to the microcentrifuge tube and incubate at 56°C for at least 1 hour to overnight. Prepare and stain a slide per the spermatozoa examination chapter. Look for intact sperm heads with a microscope.

If no sperm heads are detected, the lot of DTT passes and is available for use on casework samples. If sperm heads are detected the reagent will not be used.

Document results on form DNA 043.

Special Equipment and Supplies
Sterile glassware

Reagents

Dithiothreitol (DTT), molecular biology grade	601.2 mg
Sterile deionized water (diH_2O)	10 ml

Procedure

1. Dissolve 601.2 mg DTT and bring to 10 ml with sterile diH_2O.
2. Aliquot single-use volumes into sterile tubes.

Testing, storage, expiration, and disposal
Store in microcentrifuge tubes and place tubes in a storage container in the freezer. Minimum labeling includes reagent name, lot #, initials, and date prepared. Store aliquots frozen at -15°C to -25°C for up to two years. Thaw tubes as needed for appropriate number of extractions and discard unused portion.

Literature/Supporting Documentation
Budowle, B., J. Smith, T. Moretti, and J. DiZinno. 2000. DNA typing protocols: molecular biology and forensic analysis. Eaton Publishing, Natick, MA.

9.3 Proteinase K Solution

Proteinases are protein and peptide splitting enzymes. Proteinase K in combination with other reagents is used in DNA extraction procedures. The source of Proteinase K is from Tritirachium album.

Specification
Pro K

Quality Control
Each new prepared lot of proteinase K will be subjected to a QC check prior to use on casework. A known blood or saliva sample will be extracted with the QIAgen procedure except that the in house pro K will be used in lieu of the pro K present in the investigator kits. The extract will then be quantitated. If a quant value of 0.023ng/ul is achieved the QC passes. If the quant value is less than 0.023ng/ul the QC fails and the reagent will not be used. The quant can be repeated or a new lot can be prepared.

Document results on form DNA 044.

Special Equipment and Supplies
Microcentrifugetubes

Reagents

Proteinase K, molecular biology grade	100mg
Sterile diH_2O	10 ml

Procedure

1. Dissolve 100 mg Proteinase K in 10 ml sterile diH_2O.
2. Aliquot single-use volumes into tubes.

Testing, storage, expiration, and disposal
Store in microcentrifuge tubes and place tubes in a storage container in the freezer. Minimum labeling includes reagent name, lot #, initials, and date prepared. Store aliquots frozen at -15°C to -25°C for up to two years. Thaw tubes as needed for appropriate number of extractions and discard unused portion.

Discard in appropriate container.

Literature/Supporting Documentation
None

9.4 Tetramethylbenzidine (tmb) solution

Tetramethylbenzidine (TMB) solution is used for presumptive blood identification because it oxidizes to a blue-green form in the presence of heme and hydrogen peroxide.

Specification
TMB

Quality Control
Positive and negative controls must be tested each day of use by testing on a known blood source (positive control) and testing an unstained swab or substrate (negative control). If either control fails the reagent will not be used.

Special Equipment and Supplies
Hood

Reagents

3, 3', 5, 5' - tetramethylbenzidine	0.2	g
Ethanol	10	ml
Glacial acetic acid	5	drops

Procedure

1. Dissolve tetramethylbenzidine (0.2g) in 10 ml ethanol.
2. Add 5 drops glacial acetic acid and swirl to mix.

Testing, storage, expiration, and disposal
Minimum labeling includes specification above, initials, and date prepared. TMB solution can be stored at 2-8°C for one year.

Discard in appropriate container according to the MSDS.

Literature/Supporting Documentation

Lee, Henry C. 1982. Identification and grouping of bloodstains. In: Forensic Science Handbook, Volume 1. Richard Saferstein, ed. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. Chapter 7, p. 273.