

Forensic Biology Casework Procedures – Part 1

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Section 1 Documentation Generated During Screening and DNA Analysis

All paperwork generated during the course of analysis will be maintained either in the case record, or in a central log record in the LIMS or on the laboratory network share drive.

All bench notes pages in the case record contain the case number, analyst's initials, item # (if appropriate, i.e. digital images) and page number (page X). The total number of pages is indicated either by "page X of Y" numbering or by marking the last page as "last page".

When one analyst performs an analysis on behalf of another analyst, the resulting data pages should contain the handwritten initials (or secure electronic equivalent) of each analyst.

1.1 Biological Screening Bench Notes

Biological screening bench notes consist of any documentation generated during the analysis of a case and are specific to that case. The biological screening bench notes are placed in the case images for the request in the LIMS. All bench notes must be in LIMS and the report marked draft complete (by the analyst) prior to submitting the case for technical review. Upon completion of the technical and administrative reviews, the review checklist is also added to the case images.

The bench notes for each case may contain the following worksheets:

- Victim Assault Kit Worksheet
- Suspect Assault Kit Worksheet
- Evidence Examination Worksheet
- Microscopic Worksheet
- PSA/Sealant Identification Worksheet
- HemaTrace® Species Identification Worksheet
- Case Images Worksheet

The worksheets contain details of all of the items processed and include the item packaging, contents and description, images of the evidence processed (when applicable), documentation of all presumptive tests performed and the test results, the location of all testing, the location of all isolated stains/samples, trace evidence collected (if applicable), the reagents used, and the date the evidence was opened and sealed (where applicable) in accordance with laboratory policies and procedures outlined in the Laboratory QA Manual.

Note: For Proficiency Tests, the results pages of the paperwork from the test provider are completed and become the first pages of the bench notes.

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1.2 DNA Central Log Records

Each batch of cases assigned to a DNA analyst will be named with the batch date (typically, this is the day you take custody of the evidence) and the analyst's initials (i.e. YY-MMDDinitials 06-0102MLC). This batch name is recorded for each case in the LIMS. Upon completion of the technical review of a batch, the central log documentation is retained in LIMS, in the annual DNA record.

The templates for the worksheets that comprise the central log are controlled documents. The templates may be modified as required for the batch. The central log for each batch of cases will contain at least the following information in an appropriate format:

- A cover sheet containing:
 - batch name
 - the name of the analyst(s)
 - a list of cases worked in the batch
 - the raw data folders and GeneMapper™ (current version) projects for all data used to generate results/conclusions for cases in the batch.
- A Casework Standards, Controls and Reagents worksheet detailing:
 - the extraction methods used
 - specific instrument used
 - elution volumes
 - the extraction controls
 - date of reagent blank (should be on or after the date of extraction)
 - extraction and quantification reagent lot numbers and reagent expiration dates. (reagents not used can be deleted from worksheet)
- The quantification Experiment Results Report, to include the following:
 - Experiment summary
 - Plate layout
 - human standard curve
 - male standard curve
 - Results table
- The amplification/electrophoresis worksheet(s) detailing:
 - the samples/controls amplified (identify clearly the Positive Control sample provided by the PCR kit vendor).
 - the well plate locations
 - the amount of sample amplified
 - the amount of sample (amplified product) injected for electrophoresis,
 - the electrophoresis injection module,
 - lot numbers and expiration dates of all reagents used in amplification and electrophoresis.

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- If electrophoresis results indicate that a sample should be re-extracted, re-amplified or re-injected, the reason should be documented in the comments/notes field of the amp/3500 worksheet (Section 4 of Part 2 of this manual contains a list of defined abbreviations)
 - Instrument failure
 - ILS failure
 - Noisy baseline
 - Data below reporting threshold
 - Drop-out
 - Saturation
 - Off-ladders
 - Artifacts (ie pull-up, dye blobs, spike, hump, les, shoulder)
 - Potential Tri-allele
 - Data above reporting threshold in a blank/negative control
- Electropherograms for all control samples generated during analysis of the batch.
 - ILS - The printouts must show the correct labeling of the 80-550 peaks of the internal lane standard and the tops of every peak.
 - Negative/blank controls - primer peaks must be visible by selecting 3500 Blank-Casework as the Analysis Method.
 - ICS – print and label ICS and add to central log as a control. The analyst should include the necessary information for the reviewer to verify that the obtained profile matches the expected profile. This additional documentation is not retained once the technical review is completed.
 - Root/shaft hair extraction internal controls
- Electropherograms of all allelic ladders (with the ILS) used for genotyping (best viewed by printing in landscape view).
- It is recognized that not all cases may be submitted to technical review simultaneously. Therefore, page numbering of the central log does not need to be completed until all laboratory work is completed for all cases in the batch.

1.3 DNA Bench Notes

DNA bench notes consist of any documentation generated during the analysis of a case that is specific to that case. Upon completion of a case, the DNA bench notes and the DNA laboratory report are maintained in the LIMS. The templates for the DNA worksheets that comprise the case record are controlled documents. The templates may be modified as required for the case. The bench notes for each case will contain at least the following:

- The STR Results table (an administrative document) comprises the first page(s) of the bench notes and contains genetic profiles from questioned and known samples suitable for comparison.

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- It is not necessary to include in the STR Results table the profiles obtained from samples that serve as internal controls (i.e. the epithelial fraction of a vaginal swab) or intimate samples (body or underwear from a SART kit), when the profile is consistent with the owner or in the case of an epithelial fraction, the owner and the sperm fraction.
- If results are not suitable for comparison, do not include in the STR Results Table the Questioned sample profile (ie. majority of alleles are not reproducible, minor profile not suitable for comparison, etc).
- If interpretable genetic profiles are not obtained from any of the questioned samples, the known sample profiles need not be included in an STR Results Table.
- Add ^{DBRT} after the item description if there is data – i.e. alleles - below reporting threshold in a sample.
- Add “epithelial” or “sperm fraction” to sample description when necessary.
- Minor alleles go in () below the major alleles
- Add “major component” in the item description when only reporting the major component of a mixture.
- Deduced profiles are reported in a separate column, in addition to reporting the entire mixture.
- If an allele is determined to be an A/TA, this notation goes after the allele call in superscript (i.e. 10^{A/TA}).
- Loci with no reportable alleles are left blank and the boxes grayed out.
- All notes at the bottom of the STR Results Table are retained.

Note: For Proficiency Tests, it is not necessary to create a separate a STR Results table. The DNA results and conclusions pages of the paperwork from the test provider are completed and become the first pages of the DNA bench notes.

- The DNA extraction worksheet(s) contains:
 - All of the questioned and known items processed,
 - Sample code (within the batch)
 - Documentation of item packaging
 - Presumptive testing performed and the results, if applicable
 - Amount of sample used (sample size)
 - Date sampled (this is the date that you cut your evidence)
 - Extraction methods and specific instrument used
 - Elution volumes
 - Disposition of the DNA extract
- Digital image printouts of questioned samples, when appropriate (ie cigarette butts, fingernail clippings, hairs).

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- Electropherograms for all of the items amplified, showing the correct labeling of the 80-550 peaks of the internal lane standard. All electropherograms must contain the lab # and item # in the Sample Name column.
 - Zoomed in views (that include the peak heights and base sizes) are also required for samples that contain artifacts. Only loci with artifacts need to be included.
 - If the sample has no labeled peaks, the electropherogram must also include the primer peaks [Analysis method: 3500 Blank-Casework]
 - Special case: When an OL is a true allele or a potential tri-allele exists, a casework artifact view is also required that includes the relevant locus for an allelic ladder and the sample with the potential tri-allele.
- The following handwritten notations may be included on the electropherogram and/or the Mixture Interpretation Worksheet:
 - “DBRT” in the upper right hand corner
 - Artifacts (i.e. pull-up, dye blobs, etc.) are struck and initialed (or electronic equivalent) on the full view electropherogram
 - All non-reproducible alleles are struck and initialed (or electronic equivalent) on full view electropherogram.
 - For samples that were double amplified and did not yield data suitable for comparison, add “Reproducible alleles noted” and the reason that the data is not suitable for comparison to the bottom of the electropherogram
 - For peaks in stutter positions (that appear close to the expected stutter percentages) indicate the % of the peak relative to the main peak and the maximum expected stutter percentage (for example, 12%>5% or 5.2%~5%). When the peak can be reasonably interpreted as elevated stutter, add a notation to this effect. When it is not possible to discern whether the peak is a stutter peak or a true allele, add “A/TA”.
 - If data is not suitable for comparison, add a note at bottom of electropherogram i.e. “data not included in STR Results table due to insufficient DNA”, “minor component not suitable for comparison therefore not included in STR Results table”, “minor component consistent with owner and therefore not included in STR Results table” etc.
 - If able to determine a major vs. minor profile add () around the minor alleles.
 - ‘NS’ at loci determined not to be suitable for inclusion in a statistical analysis

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- Popstats printouts for all samples for which a statistical analysis was performed will include:
 - Specimen ID: lab # and Item#
 - Comments section: add any additional info (ie sperm fraction, minor profile)
 - Print for all reported populations
- The casework review checklist, completed and initialed by the analyst and reviewers.
 - Analyst will complete the first column of boxes before handing in for technical review

Note: All pages in file must be page numbered before handing in for technical review.

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Section 2 Screening of Biological Evidence

The laboratory does not typically examine every piece of evidence submitted for analysis. The only items routinely screened in a sexual assault case will be the assault kit(s). Underwear, clothing or other items, such as condoms and bedding, may require screening when no probative biological material is observed/detected in the assault kit, no kit was collected, or other under special circumstances.

The probative value of an item and the amount of time between the alleged assault and the collection of the evidence should be considered when deciding if the analysis of these samples/items is appropriate. Samples present in the assault kit(s) may not be examined due to the length of time between the offense and collection. Information pertaining to the date of offense and date of examination may be found on the Forensic History Form, the Victim Information/Medical History Form or on the outside of the kit box/envelope.

The following abbreviations are commonly used in bench notes and on other documents generated during biological screening.

Biological Screening Abbreviations and Definitions	
Phenolphthalein/Kastle-Meyer	PH, Pheno, or KM
Fluorescence/Alternated Light Source	F or ALS
Acid Phosphatase activity	AP, BCIP, FBB
Nucleated Epithelial Cells	NECs
Prostate-Specific Antigen	PSA or p30
Human Hemoglobin	hHb, Species
Positive test result	Pos or (+)
Negative test result	Neg or (=)
CIDI	Case,item,date & initials

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2.1 Hair and Fiber Evidence

Depending on individual case circumstances, it may be appropriate to collect/evaluate trace evidence. Collection and examination of trace evidence need not occur in cases where the trace is not likely to have probative value. Hair and fiber evidence may be recovered from evidentiary items by the following methods:

Tape Lifting

Clear plastic latent fingerprint tape (or other suitable adhesive tape) can be applied adhesive side down to the surface of the evidence item. Press the tape down and then pull away. Hairs and fibers will adhere to the adhesive on the tape. Place the tape adhesive side down on the shiny-side of freezer paper (or on another appropriate surface).

Scraping

The item to be examined is suspended above the examination surface and gently scraped with a clean metal spatula. Scraping in a downward direction allows surface debris to fall onto the examination surface for collection. The debris is transferred to an appropriate storage container.

Hand-picking

Clean forceps are used to collect trace evidence from evidence items. The isolated material is then placed in an appropriate container. Isolated trace evidence should be sealed in a suitable container and clearly marked with identifying information.

Isolated trace evidence may be placed within the originating item or it may be repackaged as an entirely new item of evidence.

The collection and any subsequent examination of trace evidence is documented in the laboratory bench notes.

The laboratory does not currently provide microscopic comparisons for hair and fiber evidence submitted or recovered in casework. If required, trace evidence may be sent to another laboratory for such analysis.

2.1.1 Screening Hairs

An intact hair will have a root portion and a shaft portion. It is important to start with visual examination followed by macroscopic examination of the morphology of individual hairs. An initial examination at a magnification of 5-10X, followed by an examination at 30X or higher, will enable the analyst to record the overall shape of the hair, whether or not a root is present and, where present, its shape and appearance, the basic features of the shaft, and medullary structure.

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Hairs recovered in case work will be examined visually and macroscopically, using a stereoscope, to determine the following:

- Is the hair Animal or Human in origin
- If Human, is the hair suitable for nuclear DNA analysis

The analyst will also document the observed characteristics of the hair that were used to make the above described determinations. The analyst should also document characteristics indicative of a specific somatic region, although this determination is not being conclusively made. These characteristics may include the following:

Animal vs. Human

- color(s) of the hair
- medullary structure
- root shape
- hair shape or form

Head Hairs

- length of hair
- moderate shaft diameter and diameter variation.
- medulla absent to continuous and relatively narrow when compared with its structure in hairs from other body areas.
- often with cut or split tips
- may show artificial treatment (solar bleaching, dying, or mechanical damage)
- Soft texture (pliable)
- little or no taper

Pubic Hairs

- length of hair
- shaft diameter coarse with wide variations and buckling
- medulla relatively broad and usually continuous when present
- follicular tag often present on the root
- tips usually rounded or abraded
- stiff texture (wiry)

Suitable for Autosomal STR Analysis

- anagen or early catagen growth phase
- visible tissue/sheath material
- follicular tag

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DNA analysis of a hair consumes a portion of the hair, preventing subsequent examinations. If the analyst concludes that the hair(s) examined may be suitable for nuclear DNA analysis, a digital image of the hair's root will be included in the bench notes.

2.2 Vaginal/Cervical, Rectal and Oral Swabs and Smears

Samples collected within the following time frame should be examined for the presence of spermatozoa:

- Vaginal/Cervical - 7 days
- Rectal - 2 days
- Oral - 1 day

These guidelines, based on published literature, apply only to living adult female victims. Different considerations may apply to samples collected from children, adult males, and homicide victims.

2.2.1 Microscopic Examination of Samples

This procedure is used for the examination of swab samples as well as for stains that test positive for the presence of acid phosphatase. The following procedure will provide an extract of the soluble substances and a pellet of the particulate material for analysis. This procedure is also used for extraction of swabs.

Microscope slides are packaged with the item after examination. Screening extracts are discarded after all analyses are completed.

Sample Extraction

- Label a sterile 2.0 mL tube for each sample to be extracted.
- Add 200 μ L of sterile, deionized water and a small cutting of the stain/swab(s) into each tube. Stains/swabs collected from the same area may be grouped.
- Soak for at least 30 minutes at 37°C. The extraction may also be accomplished overnight at 37°C or in the refrigerator at 4°C.
- Label a microscope slide for each sample.
- Agitate the material in each tube with a clean toothpick or a sterile pipette tip and then spot 3 μ L of the extract onto a labeled microscope slide.

Optional: To improve recovery of the spermatozoa, the following procedure may be substituted:

- Agitate the cutting/sample using a clean toothpick for approximately one minute.

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- Use a clean toothpick to transfer the cutting into a spin basket and insert into the 2.0mL tube containing the extract.
- Centrifuge for 1 minute (at speed sufficient to pellet the cellular material) and then discard the basket with the cutting.
- Re-suspend the pellet and then spot 3 μ L of the extract onto a labeled microscope slide. The 3 μ L may be withdrawn from the pellet, but this should be clearly stated in the bench notes.
- The extract is retained in the refrigerator, until completion of the case, for possible P30/PSA testing.

Staining of Slides

This is a differential staining process to aide in the conclusive identification of spermatozoa.

- Heat fix cells to a microscope slide by gently flaming or by placing the slide in a 37°C oven for 15 minutes.
- Slides may be examined microscopically prior to staining. Intact spermatozoa can often be detected. If intact spermatozoa are observed, staining is not required.
- Cover the slide/debris with Nuclear Fast Red stain and let stand for at least 10 minutes.
- Gently wash away the Nuclear Fast Red with deionized water.
- Immediately cover the slide/debris with Picroindigocarmine stain (PICS) and let stand for 15-30 seconds.
- Gently wash the slide with reagent grade ethanol. Allow to air dry.
- Add Permount and a cover slip.
- Examine the slide microscopically (200-400x) and record the results based on the interpretations given below.

Interpretation of Results

Nuclear material is stained red by the Nuclear Fast Red dye. Sperm heads are usually well differentiated with the acrosome staining significantly less densely than the distal region of the head. Picroindigocarmine stains the epithelial membranes green. Nuclei inside the epithelial cells may appear purple. Yeast cells also stain red; however, the stain is uniform throughout the cells and extends into polyp-like structures, which are occasionally observed with yeast cells.

If spermatozoa are detected, note if they are intact and score the number observed according to the scale below. Record the presence of nucleated epithelial cells (NECs) and non-nucleated cellular debris. NECs may also be graded using the scale given below.

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- <10 Few
- 1 + Hard to find
- 2 + Some in fields, easy to find
- 3 + Many or some in most fields
- 4 + Many in every field

If no spermatozoa are observed, one of the following should be performed to confirm the negative result:

- P30 analysis on the aqueous portion of the extract (supernatant)
- Preparation of a second slide (may occur after a 2nd epithelial digestion during DNA extraction)
- Verification of the negative slide by a second qualified analyst

2.2.2 Detection of PSA by the Abacus ABACard[®]

This is a rapid and sensitive detection method for PSA (P-30 or Prostate-specific antigen), a component of seminal fluid.

This test is normally performed after obtaining a positive presumptive test for seminal fluid (acid phosphatase) with negative sperm search results.

Procedure

- This procedure is done following extraction and a microscopic examination as previously described.
- Retrieve the extract prepared in the previous section.
 - Option: place the substrate into a spin basket and centrifuge the sample for 3-5 minutes.
- Remove the device and dropper from the sealed pouch. Label the PSA card with the case number, item number, your initials and the date.
- If refrigerated, allow the sample(s) to come to room temperature prior to testing.
- Add 4-5 drops of the extract, using the dropper provided, into the sample region (S) on the card.

Allow the sample to stand for 10 minutes. Positive results can be seen as early as 1 minute depending on the P-30 concentration.

- The results of the test are documented using digital imaging and included in the analyst's bench notes.

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Interpretation of Results

Positive: The formation of two pink lines, one in the test area (T) and in the control area (I) is a positive result, indicating the concentration of P-30 is at least 4ng/ml. Another analyst should confirm weak results. This verification will be documented in the bench notes.

Negative: The formation of only one pink line in the control area (I) indicates a negative test result. This may indicate that (a) No PSA is present above 4ng/ml or (b) presence of “high dose hook effect”. Presence of “high dose hook effect” may give a false negative result due to the presence of high concentrations of PSA in the sample. If this is suspected, the sample should be retested using a 1:10 to a 1:1,000 fold dilution of the sample in question using the remaining 100µl of sample.

Inconclusive: There is no formation of a pink line in either the test area (T) or the control area (I) of the card. Repeat the test and reexamine the test procedure carefully.

2.3 Miscellaneous Evidence Swabs

Suspected blood or semen samples may be examined using the screening methods described in this manual.

The laboratory does not conduct presumptive testing for the presence of saliva, urine or feces. Currently there are no confirmatory tests for the presence of these body fluids.

Miscellaneous swabs suspected to contain foreign saliva or skin cells do not require biological screening before proceeding to DNA analysis.

2.4 External Genitalia Swabs

Swabs collected from the external genitalia area may be examined for the presence of semen/spermatozoa using the screening methods described in this manual. Penile swabs obtained from the victim/suspect within 24 hours of the offense may be examined for the presence of blood, epithelial cells or spermatozoa (useful in cases involving multiple suspects or to help establish recent contact).

2.5 Fingernail Scrapings / Finger and Hand Swabs

Fingernail scrapings and finger/hand swabs do not routinely require biological screening. Screening may be appropriate if the presence of blood or semen would be pertinent to the investigation.

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2.6 Condoms

Condoms may contain evidence that can be isolated and compared to the DNA profiles of suspects or victims in sexual assault cases.

- Document the condition of the condom. Imaging may be used as needed.
- Label one pair of sterile cotton swabs “outside”. Moisten the swabs using sterile deionized water. Swab the “outside” surface of the condom, as received.
- Prepare a smear from the swabs collected.
- Repeat for the “inside” surface of the condom.
- Stain and grade the prepared smears as previously described in this manual.
- The swabs and prepared slides are retained as a new item of evidence.

Note: Acid phosphatase/BCIP/FBB testing should not be conducted when screening condoms. The laboratory has encountered condoms containing seminal fluid/spermatozoa that tested negative for acid phosphatase.

2.7 Contact/Wearer Sources of DNA

Items not examined for the presence of biological evidence may be sampled by swabbing the item for contact/touch sources of DNA. Standard screening protocols apply.

- Document the item by digital imaging.
- Moisten a sterile cotton-tipped swab using sterile water.
- Swab the area(s) of interest (those likely to have the most contact with bare skin).
- The isolated sample(s) are packaged and retained as a separate item of evidence.

2.8 Semen Stain Analysis

The detection and identification of semen is achieved by visual examination, presumptive chemical testing for the presence of acid phosphatase, confirmation by microscopic identification of spermatozoa, and occasionally by testing for human seminal protein p30.

Suspected semen stains can be located by a number of methods, including careful visual examination, the use of tactile senses, fluorescence under alternate light sources, and chemical presumptive testing.

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2.8.1 Visual and Tactile Examination

Most dried semen stains on cloth are detectable visually because their color is different from that of the material on which the semen has been deposited. Semen stains that have been exposed to warm, moist conditions may assume a yellow coloration due to the growth of bacteria. Seminal fluid frequently has a stiffening effect on fabric, forming a crusty stain.

2.8.1.1 Alternate Light Source Examination using the Omniprint™ 1000B

Reference: Omniprint™ 1000B-110 Operating Instructions

Semen stains frequently fluoresce when examined with alternative wavelengths of light. They can appear as a bright fluorescence on a dark background or as a dark area when the background itself fluoresces. Mark the suspect stains with a pen and proceed with chemical presumptive tests and microscopic confirmatory tests.

General Safety Guidelines

- It is essential that proper eye protection be provided for and worn by anyone operating an intense light source such as the Omniprint™ 1000B. Permanent eye damage can occur from specular (direct illumination to the eye) or reflected or refractive light hitting the eye. Label goggles for the specific wavelength. Do not allow use of inappropriate or incorrect goggles. Some goggles offer only a narrow band of protection.
- Remove all unnecessary reflective surfaces from the area or exam room. Avoid looking at reflections in shiny spherical objects such as door knobs, watch crystals, tools, jewelry, window panes, mirrors or any other surface that may reflect light.
- Exposing the skin to the beam of light (directly from the unit) can cause burns and other skin damage. There is no hazard with skin exposures to the beam emitting from the liquid light guide or fiber optic cables as temperatures are decreased, but the direct emission of the light from the discrete setting is very warm.

Proper Operation of the System

- Check to see that both switches are in the “off” position.
- Plug the unit into a three-prong grounded outlet. If an extension cord is used, it must be a heavy duty grounded cord.
- Turn on the power rocker switch (marked “Line”). The switch will light, and the fan will begin to operate. You may now turn the lamp switch on (marked “Lamp”). The lamp should light within a few seconds.

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- You may hear a ticking noise and see brief flashes of light while the lamp is attempting to ignite. This is normal.
- If the lamp fails to ignite within two minutes, and you hear a ticking noise, turn the unit off. The lamp may have failed. Lamps are guaranteed for 500 hours of use, provided it has been used in the prescribed manner (periods of at least 15 minutes). Replace the lamp (see Lamp Changing Instructions provided with the manual). The lamp should be left running for periods of at least 15 minutes. The lamp must cool after it has been turned off and should not be restarted until it has fully cooled.
- Although the minimum suggested operating time is 15 minutes, it is important to note it is better to operate the lamp for continuous periods, rather than turning the lamp on and off. This procedure will increase lamp life.
- If you do not hear a ticking noise and the lamp does not light, this is an indication that the power supply is not functioning properly. Please contact Omnichrome for further instructions.
- Operation in high ambient temperatures: the Omniprint™ 1000B is equipped with over temperature protection. If the instrument is being operated at ambient temperatures exceeding 100° F, the over temperature protector may shut off the lamp. When the unit cools sufficiently, the lamp will come back on automatically.

Filter Selections

The wavelengths are selected by simply turning the knob marked “Wavelength Selector” in either direction. A green LED light will appear next to the selected wavelength.

- 450nm band when used with yellow goggles or filters provides near UV excitation. Generally the optimum setting for the detection of physiological stains such as semen, saliva, urine and blood. Forensic odontologists and pathologists prefer this setting for bite mark and bruising detection and photographic documentation.
- Prior to the examination of casework evidence, a known semen stain control is examined with the wavelength set at 450nm. This ensures that the system is functioning properly. The stain must exhibit the expected fluorescence for the unit to be used in casework. This check is performed each day that the unit is in use and is documented in the bench notes.

Shutdown Procedure

- Push the “Lamp” rocker switch off. You must now wait for the unit to cool down. The system includes dual fans for cooling.
- After feeling that the body of the unit and the exhaust are cool, the “Power” rocker switch may be turned off. The cool-down period is approximately 5 minutes.
- Never turn the lamp back on until the unit has completely cooled.

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- If the unit is to be moved, remove the light guide(s) by gently pulling the cable out of the aperture. Gently wind the cable into a loose coil and place cable in a safe place or back into the case.
- The liquid light guide must not be wrapped or coiled too tightly as this can permanently damage the cable. The liquid light guide is very fragile and will become damaged if it is kinked, stepped on, bent, or frozen. Damage may not be evident, but there will be a decrease in output power (brightness).

2.8.2 Chemical Examination

Semen stains can be located by detecting the presence of acid phosphatase activity. Visible stains can be lightly swabbed with a water-moistened cotton swab and tested by Fast Blue B (or BCIP for challenging substrates).

2.8.2.1 Detection of Acid Phosphatase using Fast Blue B

This is a presumptive test for the detection of seminal fluid. Samples with positive results are further analyzed by performing a microscopic examination and/or a PSA test as previously described.

Procedure

- Positive & Negative Controls: A human semen standard control and sterile water blank must be tested each day that the reagents are used in casework.
- Moisten a sterile swab with a minimal amount of sterile deionized water. Rub the questioned stain with moistened swab.
- Add one drop of Solution #1 (α -Naphthyl Phosphate).
- Add one drop of Solution #2 (Fast Blue B).

Note: this test may also be used to 'map' the location of semen stains by pressing a large section of moistened filter paper on the test item. After the paper is removed from the item, add the reagents to the paper.

Interpretation of Results

The development of a purple color within one minute is a preliminary indication of the presence of acid phosphatase, a component of semen. For stains older than 1 year, it may be necessary to wait up to 2 minutes for a color change. The positive control should portray a purple color and the negative control should not exhibit a color change. The controls must function as expected for the test results on questioned stains to be valid.

Additional Notes

- The FBB procedure is not specific for semen.

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- Possible sources of acid phosphatase other than semen, i.e. vaginal fluids or feces, generally produce slow, weak reactions.

2.8.2.2 Detection of Acid Phosphatase using BCIP

This is a presumptive test for the detection of seminal fluid. Samples with a positive result are further analyzed by performing a microscopic exam and/or PSA test as previously described.

Procedure

- Place 200µL of BCIP substrate solution in labeled glass test tubes, one test tube for each sample. (Prepare a test tube for a positive control, a negative control, and for each Q sample).
- Negative Control: Moisten a sterile swab with a minimal amount of sterile deionized water. Place the swab in labeled test tube with BCIP solution. (A Negative Control is required for each run and should be the first sample prepared).
- Q Samples: Moisten sterile swab with a minimal amount of sterile deionized water. Lightly swab the questioned stain with the swab. Place the swab in a labeled test tube with BCIP solution.
- Positive Control: Moisten a sterile swab with a minimal amount of sterile deionized water. Swab the human semen sample with the swab for a positive control. Place the swab in labeled test tube with BCIP solution. (A Positive Control is required for each run and should be the last sample prepared).
- Put the test tubes in a rack. Place the rack in a 37°C water bath and incubate for 15 minutes. Document the water bath temperature in the log book. Record the following in case notes: lot # and expiration dates for the BCIP solution, lot # for the sterile water, and the sample # of the human semen stain used.

Interpretation of Results

A positive test result is an aqua (blue-green) color. The appearance of an aqua color indicates the presence of acid phosphatase activity. The positive control should give an aqua color and the negative control should not exhibit a color change. The controls must function as expected for the test results on Q samples to be valid.

Additional Notes

- The BCIP procedure is not specific for semen.
- The BCIP procedure is 99% accurate in predicting a true negative stain, i.e. one out of 100 negative BCIP tests may be false.
- The BCIP procedure has not been found to be useful in screening latex condoms. The laboratory has encountered condoms containing numerous spermatozoa that yielded negative BCIP results.

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- This test should not be performed on body cavity swabs since spermatozoa may be found on vaginal swabs which do not test positive to the BCIP reagent.

2.9 Blood Stain Analysis

Blood may be present in the form of pools, spatters, or stains. The detection and characterization of blood is achieved by visual examination and subsequent presumptive testing for peroxidase-like activity of hemoglobin. Stains testing positive to a presumptive test for blood may be further characterized by determining if the stain is animal or human in origin, or by DNA profiling to provide information as to the blood's source.

Suspected bloodstains can be located by a number of methods, including visual and stereoscopic examination, the use of alternate light sources and chemical presumptive testing.

2.9.1 Visual Examination

Most dried bloodstains on cloth can be detected visually because their color is different from that of the material on which the blood has been deposited. Bloodstains range in color from a reddish-brown to black.

Blood deposited on dark colored items may be difficult to locate visually. The use of an infrared hand held scope, video imaging system using an infrared filter, or Omniprint™ 1000B (as previously described) may assist in the location of possible bloodstains. Use of these sources will provide the contrast needed to search for stains that are not visible otherwise.

Bloodstains on clothing and other porous substrates tend to be wicked into the substrate unlike dirt and debris, which will be found on and around the fibers. This can easily be seen microscopically.

Bloodstains deposited on non-porous substrates will dry on the surface as a film or a crust.

2.9.2 Chemical Examination

Suspected blood stains can be tested for peroxidase-like activity with Phenolphthalein reagent and hydrogen peroxide. Stains can be sampled with a dry or moistened swab or piece of filter paper, or by teasing a few stained fibers from the substrate.

Stains testing positive to this presumptive test for blood may be further characterized as to their origin (human or higher primate) using Human Hemoglobin analysis by immunoassay.

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2.9.2.1 Detection of Heme with Phenolphthalein (Kastle-Meyer Test)

This is an oxidative test for the presumptive identification of blood based on the catalytic activity of the heme group of hemoglobin. If the sample is of a limited nature and the presumptive test is likely to consume sufficient sample to prevent successful DNA typing, then the presumptive test should not be performed. The limited nature of the sample will be documented in the analyst's bench notes.

Procedure

- Record all lot numbers and expiration dates.
- Positive & Negative Controls: A human blood standard control is swabbed and tested each day that the presumptive test reagents are used. A swab moistened with the same lot of sterile water used for the questioned samples is also tested to function as a reagent control.
- Questioned samples: Moisten a sterile cotton-tipped swab with sterile water. Rub the swab over the suspected bloodstain. Stains may also be sampled with a dry swab or a folded piece of filter paper, or by teasing a few stained fibers from the substrate.
- Add 1-2 drops of phenolphthalein working solution to the swab. Wait a few seconds and observe any color changes.
- Add 1-2 drops of a 3% hydrogen peroxide solution.

Interpretation of Results

The appearance of a rapidly developing pink color after the addition of 3% hydrogen peroxide (H_2O_2) is a presumptive positive test for the presence of blood. A pink color forming after one minute should not be considered as a positive result, as auto-oxidation can occur in air and light.

The positive control must yield a positive result (i.e. rapid pink color change) and the negative control give no color change, for the test results on evidentiary samples to be valid.

Color catalytic tests are very sensitive, but not specific. The positive color test alone should not be interpreted as positive proof of blood. The major sources of "false positive" reactions are chemical oxidants and vegetable peroxidases. Color development before the addition of H_2O_2 may be due to the presence of chemical oxidant. Fruit and vegetable peroxidases react similar to blood but slower and more weakly. Additionally, the presumptive test for blood is not species specific. Blood and other tissues from animals besides humans will also give a positive reaction with this test.

A negative result is indicative of the absence of detectable quantities of heme or its derivatives.

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2.9.2.2 Detection of Human Hemoglobin (hHb) using OneStep ABACard HemaTrace

This test is a simple, rapid and a sensitive confirmatory test for human (and other higher primates) blood.

Procedure

- Place a portion of the suspected bloodstain or swab into an entire labeled tube of the extraction buffer provided.
- Allow the sample(s) to extract for 1 – 5 minutes.
- If refrigerated, allow the sample(s) to warm to room temperature.
- Remove the device/card and dropper from the sealed pouch and label the HemaTrace card with the lab number, item number, your initials and the date.
- Add 4-5 drops of the extract, using the dropper provided, into the sample region (S) on the card.
- Allow the sample to stand for 10 minutes. Positive results can be seen as early as 2 minutes depending on the hHb concentration.
- Document the test results by digital imaging and include in the bench notes.

Interpretation of Results

The control line in the control area (I) is an internal procedural control. A distinct pinkish line will always appear if the test has been performed correctly and the reagents are working properly. If the control line does not appear, the test is invalid and a new test must be performed.

Positive: The formation of two pink lines, one in the test area (T) and in the control area (I) is a positive result, indicating that the human hemoglobin concentration is at least 0.05µg/ml. Another analyst should confirm weak results. This verification will be documented in the bench notes.

Negative: The formation of only one pink line in the control area I indicates a negative test result. This may indicate that:

- No human hemoglobin is present above 0.05µg/mL, or
- Presence of “high dose hook effect”. Presence of “high dose hook effect” may give a false negative result due to the presence of high concentrations of human hemoglobin in the sample. If suspected, the sample should be retested using a 1:100 or 1:1,000 fold dilution of the sample.

Inconclusive: There is no formation of a pink line in either the test area (T) or the control area (I) of the card. Repeat the test and re-examine the test procedure carefully.

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2.10 Processing, Documentation and Isolation of Biological Stains

General Instructions

- Do not allow one evidence stain to come into contact with other biological samples and do not collect or package two separate stains together.
- Change paper between items or between pertinent groups of items (i.e., suspect and victim or items from different crime scenes).
- When possible, isolate and manipulate stains with sterile, disposable implements. When non-disposable tools are used, they can be cleaned by thoroughly rinsing with a stream of distilled water and drying with paper tissue, or by rinsing with ethanol and flaming. This process should be repeated twice before using the tool to manipulate another sample.
- Small biological stains (i.e., 2mm size bloodstain) and items suspected of containing minimal stains should be handled with clean gloves and before items containing larger stains.
- It is important to save as much sample as possible to permit re-analysis at a later date, if needed. Presumptive and/or confirmatory testing methods should not be performed if the testing would consume the entire sample or would prevent re-analysis by the crime lab or any outside laboratory/agency.
- Received items are labeled with the case and item numbers, and the analyst's initials and date of examination.
- Items of evidence are examined one at a time, on clean laboratory paper. Take care, when opening items, to minimize unnecessary destruction of the packaging or previous seals.
- Where possible, the item itself should be labeled away from stained areas. Take care not to obscure possible latent print evidence when labeling articles that will be submitted for latent print examinations. A latent fingerprint analyst should be consulted prior to conducting any testing on these items.
- If any previous examination/testing is apparent or known, record the nature of the test and the results obtained. Identify field-tested stains as "possible" blood or semen stains.
- Take detailed notes describing each item and its packaging. When practical, digital images of items should be included in the bench notes.
- Collect other trace evidence that may be present and of possible forensic significance.
- Isolate stains that have tested positive to a presumptive test for blood, the presence of spermatozoa, or PSA activity for further analysis. If the stain is large, only a generous portion needs to be isolated. The portion of the stain sampled should be documented in the bench notes.

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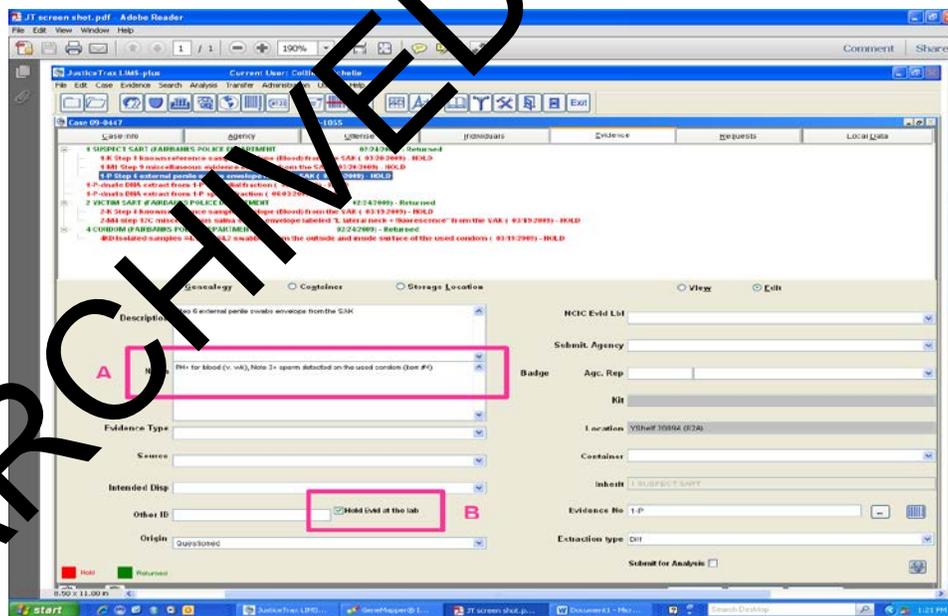
2.11 Packaging of Isolated Stains

Secure dried swabs back in their original package or a swab carton. Secure isolated cuttings into a glassine envelope. Individual glassine sleeves containing separate samples from the same item can be placed together into one larger envelope; as can individual swab packages from a single item (i.e. swabs from inside/outside of condom). Stains/samples from separate items are packaged in separate envelopes. Evidence tape is only required when sealing the final external package.

Each individual swab package, swab carton, or glassine envelope is labeled with the lab case number, unique identifier (item # / analyst initials), a brief description / isolated stain number, and the analyst's initials along the edge of the label. The required information may be on a printed label or may be handwritten on the package.

The external packaging must contain the previously described labeling as well as a LIMS barcode.

The “Notes” section (A) in the evidence tab in LIMS can be used to document any relevant case/sample information that should be brought to the attention of the DNA analyst.



Mark all items to be retained for DNA analysis by checking the box labeled “Hold Evid at the lab” (B) in the LIMS. Typically, entire assault kits will be retained by the laboratory and itemization of contents in LIMS is not necessary.

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Section 3 Biological Screening Report Writing and Review

Proficiency test reports will be written in a manner similar to casework reports.

3.1 Guidelines for Biological Screening Examinations / Reports

All biological screening reports will contain the following:

- Date report was issued (automatically populates)
- Laboratory and agency case numbers (automatically populates)
- Name of submitting agency and case officer (automatically populates)
- List of all items analyzed
 - include the agency item # in () if the item was re-numbered at the laboratory
 - this is not necessary if the laboratory number contains the original agency item #
- Results of presumptive/confirmatory tests (in body of report)
- Results, conclusions and opinions for all tested items (guidelines for reporting are provided in the tables below and may be modified, as necessary, on a case by case basis)
- Explanation of why testing was stopped, if appropriate
- The disposition of all retained items
- Any known samples that are required for DNA analysis, when applicable
- Statement regarding Y-STR testing, when applicable
- Signatures of the reporting analyst and the technical reviewer (the analyst should electronically sign the report by setting the milestone to Draft Complete prior to submitting for technical review; only the analyst is required to scan their barcode and enter a pin when signing the report in LIMS)

3.2 Results and Conclusions for Biological Screening Examinations

Sperm Findings	Report
Microscopic examination for spermatozoa	Spermatozoa/few spermatozoa/no spermatozoa were observed/detected (microscopically) in/on ...
Blood Findings	Report
Phenolphthalein (Kastle-Meyer)	<p>A stain(s) testing positive/negative with a presumptive test for blood was/were located/detected on (item).</p> <p>No blood was detected on (item) with a presumptive test for blood.</p> <p>This stain/sample tested positive/negative to a presumptive test for blood.</p>

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Seminal Fluid	Report
FBB/BCIP negative	No semen was detected chemically (FBB/BCIP) on (item)
FBB/BCIP positive	Positive results are not reported
P-30/ABA card positive (no spermatozoa observed)	No spermatozoa were observed microscopically, but the presence of the human prostate specific antigen (PSA/p30), found in seminal fluid, was detected by immunoassay on (item) and the item may be suitable for Y-STR analysis. For more information please contact the laboratory's DNA Technical Manager, XXXXX XXXXX (269-XXXX) or (xxxxx.xxxxx@alaska.gov)
P-30/ABA card negative (no spermatozoa observed)	No spermatozoa were observed microscopically and no semen/PSA was detected by immunoassay on (item).

Speciation	Report
ABA card positive	Stain(s) present on (item) tested positive using an immunoassay test for the presence of human hemoglobin. This test is specific to human, higher primate and ferret blood.
ABA card negative	No human hemoglobin was detected by immunoassay.

Hair/Fiber Evidence	Report
Hairs/debris recovered/not recovered	(No) (Animal) Hairs/debris were observed/recovered from (item).
If human hairs...	A human hair(s)/hair fragment(s) was/were recovered from (item) and is not/may be suitable for nuclear DNA analysis.

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3.2 Review of Biological Screening Reports

All case reports issued by the Forensic Biology discipline, and all supporting documentation that is part of the case record, will be subjected to a technical review and an administrative review.

Technical and administrative reviews of biological screening casework will be conducted by a second qualified analyst, in accordance with the laboratory Quality Assurance Manual (current version).

The analyst and the reviewer may consult a third qualified analyst, if necessary, when there is a disagreement on how to report a result. If the analysts are unable to come to an agreement, the DNA Technical Manager will be consulted to make the final decision.

A discipline checklist is used to document completion of the individual components of the technical and administrative reviews. The completion of the final laboratory report, technical review and administrative review are tracked electronically in LIMS.

When a case has been through technical and administrative review, the report will be issued in accordance with laboratory policies and procedures.

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Section 4 DNA Extraction

General instructions

- Analysts will use a clean cutting surface and sterile consumables for sampling items of evidence. Non-disposable items used for evidence handling, such as hole punches, will be cleaned before and after use by repeated punching of clean paper.
- Questioned samples and reference samples will be extracted separately in time and/or location.
- Extraction order is planned so that samples with potentially high levels of DNA are processed after samples with potentially lower levels of DNA.
- Sample tubes must be centrifuged before opening and only one sample tube is to be open at a time.
- DNA extraction information is documented on the designated worksheet, provided at the back of this document. This worksheet must include the batch name, a description of the item, extraction procedure used (e.g. EZ1 Trace or Large Volume (LV) protocol for casework samples), extraction instrument, sample size, elution volume (for casework extractions), and the date extraction is started (e.g. the date the evidence is cut/sampled). If the questioned samples and reference samples are extracted on the same day, the time when each set of extractions was begun is documented.
- Each batch of extractions must include appropriate reagent blanks for each type of extraction performed. All reagents (lot # and expiration date) and reagent blanks in a batch of samples are documented on the designated worksheet, provided at the back of this document.
- The volumes specified in the procedure are suitable for most forensic stains and reference samples. These volumes may be adjusted appropriate to the size and nature of the sample(s).
- Digital imaging of evidence
 - It may be appropriate for a DNA analyst to image an item of evidence that has not previously been examined and documented by another discipline in the laboratory (for example, a cigarette butt). These images should include a scale, are labeled with the case #, item #, date and analyst's initials, and will be included in the bench notes.
- Presumptive blood tests and microscopic examinations performed and reported in Biological Screening do not need to be repeated during DNA analysis.
 - Slides created during the differential extraction process only need to be retained if results were not previously reported by the biological screener. Otherwise, slides may be discarded upon completion of the case technical review.

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- For items not routinely screened prior to DNA analysis (e.g. penile swabs, fingernail scrapings), the DNA analyst should check the evidence item record in LIMS for pertinent notes recorded by the biological screener.

4.1 Evidence Sampling

4.1.1 Bloodstains

- Typical bloodstains on fabric are sampled by cutting approximately 0.5sq cm of the stain using a sterile, disposable scalpel. The size of the cutting may vary depending on the size and condition of the stain. The entire stain may be sampled for very small or potentially degraded stains.
- Bloodstains on thick fabric or filter paper may be cut, or sampled using a 3mm punch.
- Bloodstains may also be sampled by swabbing with a damp, sterile swab if the substrate is difficult to cut, or potentially contains PCR inhibitors (e.g. cigars or denim).
- Bloodstained swabs are usually sampled by cutting a portion of the stained area of the swab.

4.1.2 Swabs

- Swabs without visible staining are generally sampled by cutting approximately half of the swab lengthwise.
- Any swab(s) that is sampled should be tagged with a label containing the case number and item number.
- Typically, contact DNA swabs (not thought to contain biological fluids) will be sampled in their entirety, maximizing the chance of obtaining an interpretable DNA profile. When an entire sample is extracted, at least half of the extract must be retained for future use.
- Typically, known buccal swabs require only 1/3 of one swab or a small portion of two swabs.
- When the sampled swab material is too large for a single tube, it should be split into multiple tubes and the extracts recombined at the end of the EZ1 run. The elution volume should be adjusted so that the final volume allows for at least half to be retained after analysis.

4.1.3 Fingernail Swabs/Scrapings

- Samples are received either as swabs or debris that is scraped into a paper bindle.

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- Swabs from under the nails are generally sampled by cutting the swab tips into the sample tube. However, as in the case of contact swabs, the entire swab may be used with at least half of the extract being retained for future use.
- When sampling debris in a paper bindle, a moistened swab is used to swab the scraper, visible debris and the inside of the paper bindle. Typically one swab is used per hand. The entire swab is then cut into the sample tube. When the bindle contains a large amount of debris, a representative amount may be sampled.
- Any extract remaining after analysis shall be retained since these samples may not be homogeneous.

4.1.4 Fingernails or Fingernail Clippings

- Fingernails or fingernail clippings should be imaged prior to sampling.
- Swabs from the nail(s) may be collected for profiling of tissue and cellular debris external to the nail itself. The entire swab tips are cut into the sample tube.
- If surface material, i.e. blood or dirt, is observed on the nail, the nail may be washed by immersing in 200 μ L of dilute G2 buffer (1:1 solution of H₂O:G2 buffer) in a microcentrifuge tube with gentle agitation. This may be appropriate when attempting to obtain a DNA profile of the surface material separately from the nail itself.
- The washing is then transferred to a new microcentrifuge tube and digested by adding 10 μ L Proteinase K.
- Any clippings that remain after digestion are re-packaged with the evidence.

4.1.5 Hairs

Note: Prior to DNA analysis, hair evidence shall be examined by a qualified Biological Screening analyst to determine suitability for DNA analysis.

For mounted hairs, score the edge around the cover slip with a sterile scalpel. Remove the cover slip by prying it off or by soaking the slide in xylene substitute. Use a pipette to wash the mounting medium away with xylene substitute. Pick up the hair with clean forceps and wash in a beaker, on a watch glass, or on a microscope slide with absolute ethanol, then wash the hair with sterile, de-ionized water in a clean container.

Note: A reference (known) hair sample is processed in the batch alongside the questioned hair sample(s) as an internal control. Processing of the control hair should include shaft and root samples.

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- Examine the hair, microscopically if necessary, for the presence of surface materials (i.e. blood or dirt) and document the findings. The hair may be placed on a clean piece of white paper or on a microscope slide for examination.
- If surface material is observed on the hair, the hair should be washed by immersing in 200 μ L of dilute G2 buffer in a microcentrifuge tube with gentle agitation. This may be appropriate when attempting to obtain a DNA profile of the surface material separately from the hair itself.
- The washing is then transferred to a new microcentrifuge tube and may be extracted by adding 10 μ L Proteinase K.
- Cut approximately 1cm of the proximal (root) end of the hair for digestion. Using clean forceps, place the hair root into a sterile 1.5mL tube.
- Cut approximately 1cm of the shaft adjacent to the root for separate analysis as a substrate/shaft control. Add the shaft portion of the hair to a sterile 1.5mL tube.
- The remaining portion of the hair is re-packaged with the evidence.
- DNA extracts from hairs will always be retained.

4.1.6 Other tissue samples

Refer to the EZ1 DNA Investigator handbook for instructions on the appropriate sample size based on tissue type. The extraction procedure is as for other sample types.

4.2 Qiagen BioRobot EZ1 Advanced-XL DNA Extraction

Reference: *EZ1 Advanced XL User Manual*

General Instructions:

- Use the Trace or Large Volume protocols for all questioned and known forensic casework samples.
- The Large Volume protocol requires an additional reagent blank because of the addition of Buffer MTL after digestion. When using the Large Volume protocol, be sure to aliquot enough dilute G2 to your reagent blank tube ($\geq 700\mu$ l of dilute G2 buffer) before digestion to have enough post-digestion solution to extract two separate reagent blanks to accompany the Trace and Large Volume protocols, respectively. Alternatively, a second reagent blank tube can be set up prior to digestion and used exclusively as the reagent blank for Large Volume protocol sample(s).
- Questioned samples shall be eluted in TE buffer.
- Known samples may be eluted in water or TE buffer.
- Elution volumes are selected based on sample type and/or quantity:

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- Larger elution volumes (200 μ l) may be selected for most reference samples, large blood stains, 4+ sperm samples, 4+ epithelial fractions and reference samples
- Smaller elution volumes (no less than 40 μ L) are recommended for reference blood samples from decedents, previously extracted data bank samples, contact DNA samples and samples with few sperm or epithelial cells.
- If the entire sample was consumed, the total elution volume shall be no less than 40 μ L, and at least half of the extract volume shall be retained.
- If more than one elution volume is used in a set of extractions, the corresponding reagent blank(s) should use the most stringent elution volume used in that set.

4.2.1 Direct (non-differential) Extraction

1. Prepare the pre-digest solution:
 - (Number of samples + 3) x 230 μ l EZ buffer
 - (Number of samples + 3) x 230 μ l sterile de-ionized water
 - (Number of samples + 3) x 10 μ l Proteinase K
2. Add enough pre-digest solution (no less than 450 μ l) to each sample cutting to allow for at least 200 μ l of free liquid in the tube after absorption by the substrate.

Note: for hair samples and nail clippings*, also add 10 μ l DTT.
(* when the source or owner of the nail needs to be determined)

3. Incubate at 56 $^{\circ}$ C. Known samples shall be incubated for at least one hour. Question samples will typically be incubated overnight. Exceptions will be noted in the bench notes.
 - If the reagent cartridges are being stored between 2 $^{\circ}$ C and 8 $^{\circ}$ C, place them at room temperature overnight. Alternatively, they may be warmed in a 37 $^{\circ}$ C incubator for approximately 1 hour prior to placing on the EZ1.
4. Following incubation, transfer 200 μ l of the digest buffer into a sterile 1.5mL screw-cap tube. If absorption by the swab is unusually great, transfer as much of the digest as possible, up to 200 μ l. For all casework questioned samples, add 1 μ l carrier RNA solution to the transferred digest solution. It is not necessary to add carrier RNA to reference sample extracts.

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Option 1 – Large-Volume Protocol:

- *For samples that typically yield less DNA or if the sample required additional digest buffer for thorough cell lysis, the analyst may transfer up to 500 μ l of the digest solution to an EZ1 sample tube, add 1 μ l carrier RNA solution, and add 400 μ l of Buffer MTL.*
- *Load the sample(s) onto the EZ1, select the Large-Volume Protocol, and elute in no less than 40 μ l TE buffer. An additional reagent blank with Buffer MTL added must be extracted with all Large-Volume Protocol samples.*

Option 2 – Large-Volume Protocol for Consumed Samples:

- *If the entire evidence sample has been consumed and digested, the analyst shall transfer the entire volume of digest solution to an EZ1 sample tube (or tubes – with no more than 500 μ l in each tube), add 1 μ l of carrier RNA solution and add 400 μ l of Buffer MTL.*
- *Run the Large-Volume protocol and elute in no less than 40 μ l TE buffer.*
- *On occasion, use of more than one tube may be required to digest and extract the entire sample efficiently. In such situations, the EZ1 extracts obtained from all such digests may be combined in one tube and proceed to the quantitation step.*
NOTE: *If any sample is extracted using the Large-Volume protocol and eluted in a small volume (such as 40 or 50 μ l), then the reagent blank accompanying that sample will suffice as the reagent blank for a consumed sample extracted using the Large-Volume protocol and eluted in any larger volume (such as 100 or 200 μ l). If no other Large-Volume protocol samples are extracted, ensure that a reagent blank is extracted using the Large-Volume protocol and eluted in the lowest elution volume used.*

5. Insert the DNA Investigator card into the slot located on the front of the BioRobot EZ1 Advance -XL.
6. Turn on the power switch on the back of the instrument.
7. Directions are displayed on the screen on the front of the instrument. Press the START button to select a protocol (Trace or Large-Volume), elution buffer and elution volume.
8. Follow the instructions displayed on the screen to set up the reagent cartridges, digest tubes, tip holders, and elution tubes.

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Note: Ensure that the reagent cartridges snap into place in the cartridge rack and that the caps are removed from the sample tubes and elution tubes prior to loading on the deck. The caps from the sample tubes may be discarded. The caps from the elution tubes are retained and replaced upon completion of the protocol.

If fewer than 14 samples are being extracted, reagent cartridges and consumables need to be used only for the occupied channels.

9. Upon completion of the protocol, remove the elution tubes containing the purified DNA and cap the tubes.
10. The extracted DNA is now ready for quantification (optional for known samples) and/or amplification. DNA extracts can be stored for several weeks at 2°C to 8°C. Extracts should be dried down with DNASTable LD for long-term storage.
11. Clean the instrument by wiping down with ethanol, followed by distilled water, after each use. **Do NOT use bleach**, as it may react with the extraction reagents.
12. Clean the piercing unit after each use by selecting option 2 (“Man”) on the main menu, then option 3 (“Clean”). Then wipe each piercing unit down with ethanol.

4.2.2 Differential Extraction

1. Prepare the 1:1 diluted G2 buffer:
 - (Number of samples + 3) x 1040µl G2 buffer
 - (Number of samples + 3) x 1040µl distilled water
2. Cut the sample and place in a 2.0ml tube.
3. Add at least 600µl of sterile de-ionized water to each of the tubes. The volume of water added should allow for full submersion of the swabs.
4. Vortex briefly. Incubate overnight at 37°C. Pre-soaks/water soaks with less incubation time shall be performed only with documented pre-approval of the Forensic Biology Supervisor or DNA Technical Manager after evaluation of the sample.
5. Agitate the samples by vortexing or using a sterile pipette tip or a sterile toothpick to remove the cells from the substrate.
6. For each sample, transfer the substrate to a spin basket and place the basket back in the sample tube. Centrifuge the sample tubes for 5 minutes at approximately 14,500 RPM.

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7. Transfer approximately 550 μ l of the supernatant to a new, labeled 2.0mL tube. Also transfer the spin basket containing the substrate. Retain until the case has been completed.
8. Re-suspend the cell pellet and spot 3 μ l on a microscope slide. Stain and grade the slide (as described in Section 2).
9. If sperm and epithelial cells are observed proceed to step 10.

OPTIONS:

- If few/no sperm/epithelial cells are observed, a second extraction may be performed by adding more of the original evidence item to the sample tube and repeating steps 2-8.
 - Alternatively, the substrate may be added back to the sample tube for digestion or it may be digested separately.
 - If adding the substrate back to the sample tube, place substrate back in the tube and proceed to step 10.
 - If digesting separately, place substrate into a new 1.5mL tube and label it as "Q#sub"; analysis of the substrate resumes at step 17.
 - When no sperm are observed, it is not necessary to divide the sample into separate epithelial and sperm fractions (document on the DNA worksheet). If not separating the sample, add up to 390 μ l of 1:1 diluted G2 buffer + 10 μ l of Proteinase K solution and proceed to step 18.
 - When no NECs are observed, it is not necessary to divide the sample into separate fractions (document on the DNA worksheet). Add 190 μ l of 1:1 diluted G2 buffer + 10 μ l Proteinase K solution + 10 μ l DTT and proceed to step 18.
10. To the re-suspended cell pellet (may include substrate if added back), add up to 390 μ l of 1:1 diluted G2 buffer + 10 μ l of Proteinase K solution.
 11. Vortex and spin briefly to force the material into the extraction fluid.
 12. Incubate for at least two hours at 56°C. During the incubation step label a new sterile tube for each sample, including the reagent blank. Label these as the epithelial fractions.

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13. Centrifuge the sample tubes for 5 minutes at approximately 14,500 RPM. If the substrate was added back to the tube (step 9 option), using a sterile toothpick to place the substrate into a spin basket and centrifuge for 5 minutes at approximately 14,500 RPM. Remove all but 50 μ l of the supernatant and transfer to the epithelial fraction tube. Analysis of the epithelial fraction resumes at step 19.

Optional: At this stage, the analyst may perform a second epithelial cell digest (repeat steps 10-13) on samples with a large number of nucleated epithelial cells. This is called a “double digest” and should be documented on the DNA worksheet and on the Standards and controls worksheet for the accompanying reagent blank. This may be based on the initial microscopic examination or on a second examination.

14. Wash the sperm pellet by adding approximately 50 μ l of dilute G2 buffer. Vortex briefly and centrifuge at approximately 14,500 RPM for 5 minutes.
15. Remove and discard the supernatant.
16. Repeat the wash two more times for a total of three washes. If few sperm were observed, the number of washes for the sperm fraction may be decreased.
17. Add 190 μ l of 1:1 diluted G2 buffer + 10 μ l Proteinase K solution + 10 μ l DTT.

Note: If the substrate was digested separately, the volume of diluted G2 buffer may be increased to account for absorption by the substrate.

18. Vortex and spin briefly to force the material into the extraction fluid.
19. Incubate overnight at 56°C.
 - If the reagent cartridges are being stored between 2°C and 8°C, place them at room temperature overnight. Alternatively, they may be warmed in a 37°C incubator for approximately 1 hour prior to placing on the EZ1.

20. Insert the DNA Investigator card into the slot located on the front of the BioRobot EZ1 Advanced-XL.

21. Turn on the EZ1 power switch on the back of the instrument.

22. Directions are displayed on the screen on the front of the instrument. Press the START button to select a protocol (Trace or Large-Volume), elution buffer and elution volume.

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23. Follow the instructions displayed on the screen to set up the reagent cartridges, digest tubes, tip holders, and elution tubes.

Note: Ensure that the reagent cartridges snap into place in the cartridge rack and that the caps are removed from the sample tubes and elution tubes prior to loading on the deck. The caps from the sample tubes may be discarded. The caps from the elution tubes are retained and replaced upon completion of the protocol.

If fewer than 14 samples are being extracted, reagent cartridges and consumables need to be used only for the occupied channels.

24. For sperm fractions:

- Transfer 200 μ l of the digest to a 1.5mL screw cap tube, add 1 μ l carrier RNA solution, run Trace protocol and elute in 40 μ l to 200 μ l TE buffer.

For epithelial fractions:

- Transfer 200 μ l of the digest to a 1.5mL screw cap tube, add 1 μ l carrier RNA solution, run Trace protocol and elute in 40 μ l to 200 μ l TE buffer.

Options for fractions with none/few/1+ cells and samples not separated:

Option 1 – Large-Volume Protocol

- *For samples that typically yield less DNA or if the sample required additional digest buffer for thorough cell lysis, the analyst may transfer up to 500 μ l of the digest solution to an EZ1 sample tube, add 1 μ l carrier RNA solution, and add 400 μ l of Buffer MTL to each tube(s).*
- *Load the sample(s) onto the EZ1, select the Large-Volume Protocol, and elute in no less than 40 μ l TE buffer. An additional reagent blank with Buffer MTL added must be extracted with all Large-Volume Protocol samples.*

Option 2 – Large-Volume Protocol for Consumed Samples:

If the entire evidence sample has been consumed and digested, the analyst shall transfer the entire volume of digest solution to an EZ1 sample tube (or tubes – with no more than 500 μ l in each tube), add 1 μ l of carrier RNA solution and add 400 μ l of Buffer MTL to the tube(s).

- *Run the Large-Volume protocol and elute in no less than 40 μ l TE buffer.*

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NOTE: *If any sample is extracted using the Large-Volume protocol and eluted in a small volume (such as 40 or 50 μ l), then the reagent blank accompanying that sample will suffice as the reagent blank for a consumed sample extracted using the Large-Volume protocol and eluted in a larger volume (such as 100 or 200 μ l). If no other Large-Volume protocol samples are extracted, ensure that a reagent blank is extracted using the Large-Volume protocol and eluted in the largest elution volume used.*

25. Upon completion of the EZ1 protocol, remove and cap the microtubes containing the purified DNA.
26. The extracted DNA is now ready for quantification and amplification. DNA extracts can be stored for several weeks at 2°C to 8°C. Extracts should be dried down with DNASTable LD for long-term storage.
27. Clean the instrument by wiping down with ethanol, followed by distilled water, after each use. Do NOT use bleach, as it may react with the extraction reagents.
28. Clean the piercing unit after each use by selecting option 2 (“Man”) on the main menu, then option 3 (“Clean”). Then wipe each piercing unit down with ethanol.

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Section 5 DNA Quantification

Questioned samples are routinely quantified in duplicate. However, no more than 10% of the non-retained portion of a questioned extract from a consumed sample should be used in quantification. When consumed samples are eluted in 40 or 50uL, only one replicate is to be quantified. Quantification is not mandatory for database samples or forensic known samples.

References: ABI Prism 7000 Sequence Detection and Applied Biosystems 7500 Real Time PCR System User Bulletin and Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance Guide

5.1 Preparing the 7500 Real-Time PCR Instrument for a Run

- Open the HID Real-Time PCR Analysis software:
 - Log in under analyst name or initials
- Select the Quantifier Duo icon on the left
- On the left is a menu with three parts: Experimental Properties, Plate Set Up and Run Method
 - Run method is correct as default – do not alter these settings.
 - Under Experimental Properties, enter a name for the project that begins with the batch name. An additional designator such as Q or K can be added to distinguish between multiple quants in a single batch (ex. 13-1230MTC-Q). Default settings below are correct and should not be changed (Instrument: 7500 (96 wells); Experiment Type: Quantitation – HID Standard Curve; Reagents: TaqMan Reagents; Ramp Speed: Standard (~2 hours to complete a run).
 - Under Plate Set Up, the default settings include Standards and NTC.
 - Under the Define Samples tab, add samples. These can be left as Sample 1, Sample 2, and so on, with Q or K codes manually written on printouts later; or they can be entered with Q or K codes.
 - Under the Assign Targets and Samples tab, highlight a box (or boxes) on the map, then check the box from the list on the left (Heading: Assign samples to the selected wells) for the corresponding reaction well(s).
 - Standard Curve wells are already correctly assigned. The template default includes one NTC reaction. Be sure that the final plate includes two NTC reactions.
 - Selected samples are automatically assigned three targets: Duo Human, Duo IPC, and Duo Male.

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- Save the experiment in the experiments folder (Applied Biosystems > 7500 > experiments)

5.2 Preparing the DNA Quantitation Standards

Note: The volumes listed below are minimum volumes to optimize accuracy in pipetting. Larger quantities of standard curve may be prepared by scaling all volumes as needed. Prepared standard curves can be stored for up to two weeks at 2 to 8 °C with documentation of the date made, the expiration date, and the reagent lot numbers.

- Bring the reagents to room temperature before pipetting.
- Label eight sterile microcentrifuge tubes for standards 1 through 8.
- Dispense 30 uL of Quantifiler Duo DNA Quantitation Buffer into Std 1 and 20 uL into each of the remaining tubes
- Prepare Standard 1:
 - Vortex the Quantifiler Duo DNA Standard for 3 – 5 seconds.
 - Using a new pipette tip, add 10 uL of Quantifiler Duo DNA Standard to the tube for standard 1.
 - Mix the dilution thoroughly.
- Prepare Standards 2 through 8:
 - Using a new pipette tip, add 10 uL of the prepared standard 1 to the tube for the next standard.
 - Mix the standard thoroughly.
 - Repeat until you complete the dilution series.

The approximate quantities of DNA in prepared Standards 1 through 8 are as follows:

- Standard 1 ≈ 50ng/μL
- Standard 2 ≈ 16.7ng/μL
- Standard 3 ≈ 5.56ng/μL
- Standard 4 ≈ 1.85ng/μL
- Standard 5 ≈ 0.62ng/μL
- Standard 6 ≈ 0.21ng/μL
- Standard 7 ≈ 0.068ng/μL
- Standard 8 ≈ 0.023ng/μL

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5.3 Preparing the Reactions

- Bring the reagents to room temperature before pipetting.
- It is recommended that preparation of the reactions take place in a designated hood in order to minimize the introduction of contamination as well as dust or particulate matter.
- While preparing the reactions, keep the 96-well reaction plate in a base (not directly on the bench top) to protect the plate from scratches and particulate matter.
- Calculate the volume of each component needed to prepare a master mix, including a few additional reactions. This calculation is made automatically on the designated 96-well plate QPCR worksheet that is used for well mapping.
 - Quantifiler™ Duo Primer Mix at 10.5µL per reaction
 - Quantifiler™ Duo PCR Reaction Mix at 12.5µL per reaction.
- Vortex the Quantifiler Duo Primer Mix for 3-5 seconds and centrifuge briefly before opening the tube.
- Swirl the Quantifiler Duo PCR Reaction Mix gently before using. Do not vortex it.
- Pipette the required volumes of the components into a sterile tube. Vortex and centrifuge briefly.
- Dispense 23µL of reaction mix into each reaction well.
- Add 2µL of sample, standard or control to the applicable wells. 2µL of dilution buffer is added to each NTC well.
- Seal the plate with the Optical Adhesive Cover. Use an applicator to create a firm seal between each of the wells.
- Centrifuge the plate at 3000 rpm for at least 20 seconds to remove any bubbles.

5.4 Sample Loading

- Push the tray door on the 7500 instrument to open it.
- Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder, with the notched A12 position in the top right of the tray.
- Close the tray door. Apply pressure to the right side of the tray door at an angle.

5.5 Starting the Run

- Click the green box labeled START RUN in the upper left hand corner.

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5.6 Analyzing the Data

- When the run is complete and the quality checks described below in 5.6.1 through 5.6.4 have been completed, print the Experiment Results Report for documentation in the central log file.

5.6.1 Checking the Standard Curve

Both the human and male DNA Standard curves need to be assessed for their R^2 values, slopes and Y-intercept values. No more than two data points may be removed from a standard curve in order to achieve passing quality metrics.

R^2 is a measure of the closeness of fit between the standard curve regression line and the individual data points of the quantification standard reactions. A value of 1.0 indicates a perfect fit between the regression line and the data points. Passing value for R^2 is ≥ 0.98 . It is acceptable to remove one or both of the Standard 8 data points in order to achieve a passing R^2 value. If, after removing up to two data points, the R^2 value is < 0.98 , the plate must be re-run.

Slope is an indicator of amplification efficiency. A slope value of -3.3 corresponds to true doubling of the amplicon at each cycle. Acceptable slope values fall in the range of -3.0 to -3.6. Slope values outside this range require approval by the DNA Technical Manager.

Y-intercept is the theoretical expected quantity for 1 ng/uL of DNA. Y-intercept is used to compare performance among different QD kit lot numbers. If an average Y-intercept value drops by one C_T value, the standard curve will shift to the left and samples that previously quantified at 2 ng/uL will now quantify at 1 ng/uL. This will cause an underestimation of DNA quantity. Conversely, if the average Y-intercept value increases by one C_T value, the standard curve will shift to the right and samples that previously quantified at 1 ng/uL will now quantify at 2 ng/uL. This will cause an overestimation of DNA quantity, which will lead to lower peak heights in the analysis of amplified samples. Typical accepted ranges for Y-intercept values are 28.8 – 29.2 for the human standard curve and 29.8 – 30.2 for the male standard curve. During QD kit verification, if either Y-intercept value falls outside the accepted range, the plate must be re-run. If the Y-intercept value(s) is reproducible, note the average Y-intercept values (human and male) both on the verification paperwork and the reagent storage container. Subsequent Y-intercept values for that QD kit lot number must fall within ± 0.5 of the verified Y-intercept values. Non-reproducible Y-intercept values, or values falling outside the accepted range, should be brought to the DNA Technical Manager to determine a course of action.

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5.6.2 Checking the Internal PCR Control (IPC)

IPC values are documented in the Experiment Results Report for each reaction. The IPC C_T value for each reaction is expected to fall in the range of 28 to 31. When a C_T for an IPC is greater than 31, the sample should be assessed for possible amplification inhibition. Note that excessive quantities of DNA do not exhibit excessive concentration inhibition.

5.6.3 Checking the Passive Reference (ROX)

The passive reference signal flag (on the QC summary page of the Experimental Results Report) indicates if any samples exhibited a bad passive reference signal. The results of this check should be documented on the 1st page of the report and included in the central log. The Technical Manager is to be consulted when any samples fail this check.

5.6.4 Checking the No Template Controls (NTC)

In the Results Table of the Experiment Results Report, confirm that the quantity of DNA for both Duo Human and Duo Male is negative (C_T = undetermined). A detected quantity of DNA could indicate contamination of the master mix. If the quantity of either Duo Human or Duo Male is greater than zero, consult the DNA Technical Manager for a course of action.

5.6.5 Checking the Initial Template Quantity

Quantities of DNA, including both total human and male DNA, are listed for each reaction in the Results Table. The Quantity (Mean) column provides the average of any wells assigned to the same sample identifier in 5.1.1. When STR analysis will not be performed based on the quantification results (as described below), this shall be reflected on the DNA Case Extraction Worksheet.

5.6.5.1 Using female:male ratio to assess suitability for STR analysis

When female DNA is in excess, an estimate of the ratio of female to male DNA can be made by subtracting the male quantification value from the total human concentration to obtain the female contribution. This ratio is documented on the relevant page of the Experiment Results Report.

- When male DNA quantity is <0.050 ng/ μ L, stochastic effects can limit the accuracy of assessment of the true female to male ratio.
- The calculation of minor female DNA in the presence of excess male DNA is not accurate and should **not** be used to assess the suitability for analysis.

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Because of the low success rate of obtaining data suitable for probative comparisons for the minor contributor(s) in mixtures where the ratio of contributors is greater than 10:1, this ratio may be used to determine course of future use of the extract. Extracts for which the male DNA is the probative component, but for which the female:male ratio is greater than 10:1, should be recommended for Y-STR testing and retained with no STR analysis. Exceptions might include non-suspect cases or event-specific scenarios; these extracts may proceed to STR testing with documented approval by the discipline supervisor or the DNA Technical Manager.

5.6.5.2 Absence of male DNA where male DNA is probative

In cases such as sexual assaults with female victims and alleged male assailants, the absence of detectable levels of male DNA (as demonstrated by an undetermined Quant Duo Male result) in the presence of any female DNA means that no STR analysis will be performed on that sample.

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Biological Screening Review Checklist

LAB NUMBER:		Technical Review	Administrative Review
ANALYST:			
Date Submitted for Review:			
Technical Review Started:	Reviewer:		
Administrative Review Started:	Reviewer:		
Pages are numbered correctly, lab case #, item # and analyst initials are on each page			
Requesting agency, agency case #, lab case #, and officer's name are correct			
Item numbers / packaging / descriptions on report / notes are consistent with RLS/LIMS			
The type of examination (visual, stereoscopic, ALS) and testing performed is documented in notes			
Item descriptions are consistent with clothing/evidence images present (if applicable)			
The location of all chemical testing performed is documented in the notes (if applicable)			
All isolated stains/samples are documented and numbered correctly (if applicable)			
Verification reviews conducted are documented in the notes (if applicable)			
The location and disposition of all trace evidence is documented			
Worksheets contain all lot #s and expiration dates for all reagents used			
The date evidence was opened and/or sealed is documented			
Retained items created in LIMS; all retained and unexamined items HELD in LIMS			
The "FUTURE TECH" flag has been flagged for the case, if applicable (samples suitable for Y-STR)			
Check grammar/spelling/punctuation in report			
Results/conclusions/opinions are given for each item tested			
Conclusions/opinions drawn from results comply with laboratory guidelines			
Conclusions/opinions drawn from results are supported by documentation in the notes			
Known samples requested, if appropriate			
Report signed in LIMS			
All case related notes and attached/scanned documents are present			
Technical reviewer is in review history for each page of the bench notes			
SCPs are added to request in LIMS			
Chain of Custody for all tested items can be tracked through RLS and LIMS			
Assign DNA holding to DNA Supervisor			
VERIFICATION REVIEW	Analyst:	Date:	
Semen/ABACard® p30 Test			
Species/ABACard® HemaTrace®			
Hairs - Stereoscopic			

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Appendix A Revision History

FBCP1 2014 R1 Page	FBCP1 2014 R0 Page	Location	Revision made
2	2	Section 1	Revised the sentence beginning “All pages in the case record and central log record contain the case number...” to read “All bench notes pages in the case record contain the case number...”
2	2	Section 1	Revised the page numbering requirement to allow for “page X of Y” also
3	3	Section 1.2	Removed “both the case record and” from the sentence beginning “Upon completion of the technical review.....”
8	8	Section 2	Added BCIP to table
9	9	Section 2.1.1	Changed “7X” to “5-10X” in the sentence beginning “An initial examination.....”
11	11	Section 2.2.1	Moved the addition of water from the 3 rd bullet to the 2 nd bullet in Sample Extraction.
12	12	Section 2.2.1	Revised the stain time for Nuclear Fast Red from “10-15 minutes” to “at least 10 minutes”.
13	13	Section 2.2.2	Revised the 2 nd bulleted statement to make use of a spin basket optional
15	15	Section 2.6	Added BCIP to the Note:
18	18	Section 2.7.2	Added “(or BCIP for challenging substrates)”
18	18	Section 2.8.2	Replaced “Lightly stroke” with “Rub” in the procedure.
18	18	Section 2.8.2	Added the sentence beginning “For stains older than 1 year....” to the interpretation section.
19	n/a	Section 2.8.2	Added procedure for BCIP testing (taken from archived FBCP1 2013 R1)
26	25	Section 3.2	Added BCIP to the Seminal Fluid results and conclusions table
39	38	Section 5	Revised the 1 st sentence and added details regarding quantification of consumed extracts in 40 or 50uL.
40	39	Section 5.2	Replaced Standard A, Standard B, etc. with Standard 1, Standard 2, etc.
41	40	Section 5.3	Added “2uL of dilution buffer is added to each NTC well.” to the bulleted statement beginning with “Add 2uL of sample.....”
45	44	Forms	Added a line for “Known samples requested, if appropriate” to the review checklist

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