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Department of Forensic Science

VIRGINIA
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FORENSIC BIOLOGY SECTION PROCEDURES MANUAL

SECTION II

PRESUMPTIVE AND CONFIRMATORY TESTS FOR BIOLOGICAL SUBSTANCES

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1 DETECTION OF BLOOD

1.1 Combined Phenolphthalein-Tetramethylbenzidine (PTMB) Test (References 1, 2, 3, Appendix A)

1.1.1 Safety Considerations

- Phenolphthalin - Caution! Avoid contact and inhalation!
- Potassium hydroxide - Caution! Corrosive! Poisonous!
- Tetramethylbenzidine - Caution! Harmful if swallowed, inhaled or absorbed through skin! Emits toxic fumes under fire conditions!
- Glacial acetic acid - Caution! Corrosive! Flammable!
- Ethanol - Caution! Flammable! Poisonous!
- Oxidized zinc - Caution! Danger of spontaneous combustion if allowed to dry!

1.1.2 Materials and Equipment

- Dropper bottles
- Cotton swabs
- Test tubes, microtiter plates, or filter paper
- 100 ml graduated cylinder
- Weigh boats or weigh paper
- Balance
- Spatula
- Scissors
- Tweezers
- Magnetic stir plate

1.1.3 Stock Solutions

1.1.3.1 Phenolphthalin Stock Solution

- 1 g Phenolphthalin
- 25 g Potassium Hydroxide (KOH)
- 100 ml Distilled water
- The above ingredients are mixed until thoroughly dissolved.

1.1.3.1.1 Storage

This colorless solution is stored under refrigeration over fresh granular zinc to keep it in the reduced form. The oxidized zinc in the bottle should not be allowed to dry (see 1.1.1 Safety Considerations and 1.1.3.1.3 Disposal).

1.1.3.1.2 Labeling

1.1.3.1.2.1 Label the bottle as Phenolphthalin Stock Solution with a lot number (the date of preparation followed by the initials of the person preparing the stock solution).

Example: Phenolphthalin Stock Solution Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

1.1.3.1.2.2 There is no expiration date (see 1.1.5.1 Minimum Standards and Controls).

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1.1.3.1.3 Disposal

1.1.3.1.3.1 When the reduced phenolphthalin stock solution is depleted, cover the zinc in the bottom of the bottle completely with a solution of potassium hydroxide in distilled water (25 g KOH/100 ml dH_20).

1.1.3.1.3.2 Label the bottle with the contents and "For Disposal" and refrigerate. Notify the Safety Officer that the zinc is ready to be disposed of in accordance with Department procedures.

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1.1.3.2 Tetramethylbenzidine (TMB) Stock Solution

- 10 mg Tetramethylbenzidine (TMB)
- 30 ml Glacial acetic acid
- Mix the above ingredients until thoroughly dissolved.

1.1.3.2.1 Storage

The TMB stock solution should be stored at room temperature.

1.1.3.2.2 Labeling

1.1.3.2.2.1 Label the bottle as TMB Stock Solution with a lot number (the date of preparation followed by the initials of the person preparing the stock solution).

Example: TMB Stock Solution Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

1.1.3.2.2.2 There is no expiration date (see 1.1.5.1 Minimum Standards and Controls).

1.1.3.2.3 Disposal

Dispose of the TMB stock solution and other materials contaminated with this solution as hazardous waste in accordance with Department procedures.

1.1.4 Working Solutions

- Distilled water
- Ethanol
- 3% Hydrogen peroxide
- 1:5 dilution of phenolphthalin stock solution in distilled water (1 part of the phenolphthalin stock solution diluted with 4 parts of distilled water)
- TMB stock solution

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1.1.4.1 Storage

All bottles of working solutions are stable at room temperature.

1.1.4.2 Labeling

1.1.4.2.1 Bottles containing working solutions of ethanol and 3% hydrogen peroxide will be labeled with the contents and the lot number.

1.1.4.2.2 The bottle containing the 1:5 dilution of phenolphthalin stock solution must be labeled appropriately. If the lot number of the diluted stock solution is recorded in the reagent log book, then the bottle must be labeled with this lot number. If only the neat stock solution is recorded in the reagent log book, then the bottle must be labeled with the lot number of the neat stock solution, the date of the dilution, and the initials of the person making the dilution.

1.1.4.2.3 There is no expiration date for the working solutions (see 1.1.5.1 Minimum Standards and Controls).

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1.1.5 Minimum Standards and Controls

1.1.5.1 On the day of use a positive reagent control (known bloodstain) and a negative reagent control (distilled water) must be tested to ensure that the reagents are working properly. The results of this testing must be documented in the case file.

1.1.5.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.

NOTE: Caution should be used when testing leather and suede items.

1.1.6 Combined Phenolphthalein-Tetramethylbenzidine (PTMB) Test Procedure

If it is possible that the stain will be consumed, do not perform the PTMB testing and go directly to DNA testing.

1.1.6.1 Gently rub a suspected stain with a cotton swab which has been moistened with distilled water or place a small cutting of the stain in a small test tube or microtiter plate, or on filter paper and moisten with distilled water if desired.

1.1.6.2 Add one drop of ethanol.

1.1.6.3 Add one drop of 1:5 dilution of phenolphthalin (i.e., the working solution of phenolphthalin).

1.1.6.4 Add one drop of 3% hydrogen peroxide.

1.1.6.5 Note any color change. An immediate pink color is expected if blood is present.

1.1.6.6 Add one drop of tetramethylbenzidine stock solution.

1.1.6.7 Note any color change. An immediate blue-green color is expected if blood is present.

1.1.6.8 Interpretation

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1.1.6.8.1 Positive Reaction = Immediate pink color at 1.1.6.5, followed by immediate blue-green color at 1.1.6.7

1.1.6.8.2 Negative Reaction = No color change at 1.1.6.5, followed by no color change at 1.1.6.7

1.1.6.8.3 Inconclusive Reaction = Development of color combinations other than those specified for a positive reaction, including one test positive and the other test negative

1.1.6.9 Reporting Results

1.1.6.9.1 Report positive test results as “blood was indicated…”

1.1.6.9.2 Report negative test results as “no blood was indicated...”

1.1.6.9.3 Report inconclusive test results as “the possible presence or absence of blood could not be determined 1) due to insufficient information obtained from the chemical analysis or 2) since the controls used in this testing did not meet the quality control requirements.”

1.1.6.9.4 Report a negative observation as “no blood was observed on ….”

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1.1.6.9.5 Report the absence of PTMB testing as “in order to conserve the sample for DNA analysis, no blood detection testing was conducted.”

1.2 Luminol Test{tc \l2 "LUMINOL TEST} (Reference 4, Appendix A)

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1.2.1 Safety Considerations

- Sodium perborate - Caution! Harmful if swallowed, inhaled or absorbed through skin!
- Aminophthalhydrazide (luminol) - Caution! Irritant! Emits toxic fumes under fire conditions!

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1.2.2 Materials and Equipment

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- Spray bottle (must contain no metal parts as the luminol reacts with some metals)
- 50 ml graduated cylinder
- Balance
- Weigh boats or weigh paper
- Spatula
- Ziploc bags, conical tubes, or other appropriate containers (optional)
- Magnetic stir plate

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1.2.3 Stock Solutions

1.2.3.1 Solution A

- 0.7 g Sodium perborate
- 50.0 ml Distilled water
- Mix above ingredients until thoroughly dissolved.
- Mixed solution should be stored in the dark at room temperature for up to 6 months.

1.2.3.2 Solution B

- 0.1 g Aminophthalhydrazide (luminol)
- 5.0 g Sodium carbonate
- 50.0 ml Distilled water
- Mix the above ingredients until thoroughly dissolved.
- Mixed solution should be stored in the dark at room temperature for up to 6 months.

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NOTE: The dry chemicals in Solutions A and B can be weighed out and placed in appropriately labeled containers and stored in the dark at room temperature. Each container must be labeled with the date prepared, the initials of the person who prepared each package, and the amount of distilled water to be added. Water can be added when needed.

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1.2.4 Minimum Standards and Controls

1.2.4.1 Test a positive reagent control (known bloodstain) and a negative reagent control (distilled water) to ensure that the reagents are working properly. The results of this testing must be documented in the case file.

1.2.4.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.

1.2.4.3 If the results of the test are positive, as a general rule a substrate control will not be tested.

1.2.5 Luminol Procedure

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1.2.5.1 **When the test is ready to be conducted,** mix equal parts of solutions A and B and place in a sprayer bottle.

1.2.5.2 **Under darkened conditions**, immediately after mixing equal parts of solutions A and B, spray the positive and negative controls to ensure that the reagents are working properly. If both controls give the expected results, proceed with spraying the area of interest. Document results in the case file.

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1.2.5.3 Areas containing blood will luminesce immediately.

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1.2.5.4 Mark luminescent areas for subsequent testing with the Combined Phenolphthalein-Tetramethylbenzidine Test.

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1.2.5.5 Interpretation

1.2.5.5.1 Positive Reaction = Immediate luminescence

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1.2.5.5.2 Negative Reaction = No luminescence

1.2.5.5.3 Inconclusive Reaction = Slow and/or weak luminescence

1.2.5.6 Reporting results

1.2.5.6.1 A negative reaction will be reported as “no blood was observed.”

1.2.5.6.2 A positive reaction will always be confirmed with PTMB testing.

1.3 BLUESTAR® Forensic Test{tc \l2 "LUMINOL TEST}

BlueStar® Forensic Test**{**tc \l2 "LUMINOL TEST**}** Kit information available at http://www.bluestar-forensic.com

1.3.1 Safety Considerations

- Beige Tablet - Caution! Avoid contact with skin and eyes, gloves must be worn
- White Tablet - Caution! Avoid contact with skin and eyes, gloves must be worn

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1.3.2 Materials and Equipment

- Spray bottle (must contain no metal parts, chemicals may reacts with some metals)
- 100 ml graduated cylinder

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1.3.3 Stock Solutions

BlueStar® Forensic Working Solution

- White and beige BlueStar® Forensic tablets
- 125 ml Distilled water

- Dissolve white and beige tablets in water
- USE IMMEDIATELY! DO NOT STORE.

1.3.4 Minimum Standards and Controls

1.3.4.1 Test a positive reagent control (known bloodstain) and a negative reagent control (distilled water) to ensure that the reagents are working properly. The results of this testing must be documented in the case file.

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1.3.4.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.

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1.3.5 BLUESTAR® Forensic Procedure

1.3.5.1 **Under darkened conditions**, immediately after dissolving the tablets in water, spray the positive and negative controls to ensure that the reagents are working properly. If both controls give the expected results, proceed with spraying the area of interest. Document results in the case file.

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1.3.5.2 Areas containing blood will luminesce immediately.

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1.3.5.3 Mark luminescent areas for subsequent testing with the Combined Phenolphthalein-Tetramethylbenzidine Test.

1.3.5.4 Interpretation

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1.3.5.4.1 Positive Reaction = Immediate luminescence

1.3.5.4.2 Negative Reaction = No luminescence

1.3.5.4.3 Inconclusive Reaction = Slow and/or weak luminescence

1.3.5.4.4 Reporting Results See 1.2.5.6

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3 SEMEN IDENTIFICATION

3.1 Acid Phosphatase Test {tc \l2 "ACID PHOSPHATASE TEST}(Reference 5, pp. 162-163, Appendix A)

3.1.1 Safety Considerations

- Glacial acetic acid - Caution! Corrosive! Flammable!
- Sodium acetate - Caution! Irritant!
- Sodium α-naphthyl acid phosphate - Caution! Irritant! Emits toxic fumes under fire conditions!
- o-Dianisidine (Naphthanil diazo blue B) - Caution! Highly toxic! Emits toxic fumes under fire conditions!
- Naphthanil diazo red - Caution! Avoid contact and inhalation! Emits toxic fumes under fire conditions!

3.1.2 Equipment

- 5 ml and 500 ml Graduated cylinders
- Balance
- Spatula
- Scissors
- Tweezers

3.1.3 Materials

- Filter paper or microtiter plate (optional)
- Weigh boats or weigh paper
- Cotton swabs
- Test tubes or bottles
- Disposable transfer pipets or droppers

3.1.4 Working Solutions

3.1.4.1 Acid Phosphatase (AP) Buffer

- 2.5 ml Glacial acetic acid
- 10.0 g Sodium acetate (anhydrous)
- 450.0 ml Distilled water
- Mix the above ingredients until thoroughly dissolved.

3.1.4.1.1 Storage

The AP Buffer is stable at room temperature.

3.1.4.1.2 Labeling

3.1.4.1.2.1 Label the bottle as AP Buffer with a lot number (the date of preparation followed by the initials of the person preparing the stock solution).

Example: AP Buffer Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

3.1.4.1.2.2 There is no expiration date (see 3.1.5 Minimum Standards and Controls).

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3.1.4.2 Sodium α-Naphthyl Acid Phosphate Solution

3.1.4.2.1 Add a small amount (approximately 4 mg) of sodium α-naphthyl acid phosphate to approximately 3 ml of Acid Phosphatase buffer in an appropriately labeled 10 X 75 mm test tube or bottle.

3.1.4.2.2 Discard the solution at the end of the day.

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3.1.4.3 Dye Solution

3.1.4.3.1 Add a small amount (approximately 4 mg) of o-dianisidine or naphthanil diazo red to approximately 3 ml of buffer in an appropriately labeled 10 X 75 mm test tube or bottle.

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NOTE: Protect from light by covering tube with foil or use a brown bottle.

3.1.4.3.2 Discard the solution at the end of the day.

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3.1.4.4 Distilled water

3.1.5 Minimum Standards and Controls

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3.1.5.1 On the day of use a positive reagent control (known semen stain) and a negative reagent control (distilled water) must be tested to ensure that the reagents are working properly. The results of this testing must be documented in the case file.

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3.1.5.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.

3.1.6 Acid Phosphatase (AP) Test Procedure (Sodium α-Napthyl Acid Phosphate)

If it is possible that the stain will be consumed, do not perform the AP testing and go directly to DNA testing.

3.1.6.1 Moisten filter paper/swab with distilled water. (Do not use buffer solution, as this will contaminate the stained area.) Press the filter paper against the suspected stain or gently rub the stained area with the moistened swab. Alternatively, a small piece of the stain/swab can be placed on filter paper, in a small test tube, or in a microtiter plate.

3.1.6.1.1 If filter paper is used for AP mapping, the appropriate sized piece of filter paper should be moistened with distilled water, taking care not to soak the paper as this can dilute the stain and/or transfer the stain to another layer or portion of the item.

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3.1.6.1.2 Apply the filter paper to the questionable stain or stained area by pressing the filter paper down onto the item carefully with moderate pressure long enough to transfer just a small amount of stain for testing (typically ~15 to 30 seconds).

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3.1.6.1.3 Remember the orientation of the filter paper or mark a corner.

3.1.6.1.4 Apply the solutions in the order described below (see 3.1.6.2); however, in order to ensure each solution covers the entire piece of filter paper, disposable pipettes can be used to apply each solution over the paper while holding at an angle; place the paper down on bench paper or paper towel and read the reaction after 10-15 seconds as described below.

3.1.6.1.5 Mark the item or document in a diagram using the filter paper as reference where any positive areas were detected and note strength of reaction.

3.1.6.1.6 Be sure to change gloves between each application of filter paper to additional stains/stained areas for testing.

3.1.6.1.7 If necessary air dry the evidence item before repackaging.

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3.1.6.2 Add 1-2 drops of sodium α-naphthyl acid phosphate solution.

3.1.6.3 Add 1-2 drops of dye solution.

3.1.6.4 The development of a blue/purple color with o-dianisidine or an orange/red color with naphthanil diazo red within 10 to 15 seconds is indicative of acid phosphatase levels in semen.

NOTE: Slow color development or development of a color not defined above may be attributed to increased acid phosphatase levels from non-seminal fluid sources (e.g., increased acid phospatase levels in vaginal fluid due to the victim's age, bacteria and/or white blood cells)

3.1.6.5 The presence of semen in all samples exhibiting an inconclusive result (not attributable to an increase in phosphatase levels from non-seminal fluid sources) or a positive result must be either confirmed by identifying spermatozoa or, in the absence of spermatozoa indicated with p30.

3.1.6.6 Interpretation

3.1.6.6.1 Positive Reaction = Blue/purple color with o-dianisidine within 10 to 15 seconds OR Orange/red color with naphthanil diazo red within 10 to 15 seconds

3.1.6.6.2 Negative Reaction = No color development, slight/slow color development

3.1.6.6.3 Inconclusive Reaction = Slow moderate to strong color development

3.1.6.7 Refer to Section 3.8 for reporting results.

3.2 Extraction of Spermatozoa from a Substrate

3.2.1 Equipment

- Rotator, vortex, sonicator, or centrifuge (depending on extraction method used)
- Scissors
- Tweezers
- Dissecting needle (optional)

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3.2.2 Materials

- Microscope slides
- Test tubes

3.2.3 Reagents

Distilled water

3.2.4 Extraction Methods

3.2.4.1 Cut a small portion of a stain and soak in a test tube overnight in distilled water.

3.2.4.2 Soak a small portion of a stain in distilled water and rotate overnight.

3.2.4.3 Soak a small portion of a stain in distilled water and sonicate for 10 seconds, followed by a 30 second sonication.

3.2.4.4 Tease fibers apart and soak in a small amount of distilled water.

3.2.4.5 Soak a small portion of a stain in distilled water and vortex.

3.2.4.6 Soak a small portion of a stain in distilled water on a microscope slide, stain side down (may be followed by maceration).

3.2.4.7 Cut the stain into small pieces, place the pieces on a microscope slide, and soak in a small amount of distilled water (may be followed by maceration).

3.2.4.8 Cut a small portion of the stain and perform the DNA isolation method in accordance with the Forensic Biology Section Procedures Manual.

NOTE: This approach may be appropriate when it is believed that the sample may be consumed prior to the DNA analysis step.

3.2.4.9 For the ABAcard® p30 Test extraction method, refer to 3.7.9.1 through 3.7.9.7.

NOTES:

- Always soak the material first; prolong the soaking for difficult stains.
- Use the sonicator on low (high setting will disintegrate spermatozoa).
- To concentrate an extract, after soaking a small portion of a stain or swab, centrifuge and make a smear of the sediment.
- DNA extracts can also be used to search for spermatozoa.

3.3 Kernechtrot-Picroindigocarmine Stain{tc \l2 "KERNECHTROT-PICROINDIGOCARMINE STAIN} (Christmas Tree Stain) (Reference 7, p. 141, Appendix A)

3.3.1 Kernechtrot-Picroindigocarmine (KPIC) differential biological stain is used to assist in the microscopic identification of spermatozoa. The solutions for this procedure can either be purchased (SERI) or prepared in-house.

3.3.2 Safety Considerations

- Aluminum sulfate - Caution! Harmful if inhaled, in contact with skin, and if swallowed! Emits toxic fumes under fire conditions!
- Nuclear fast red - Caution! Irritant! Emits toxic fumes under fire conditions!
- Saturated picric acid solution - Caution! Toxic! Explosive when dry! Emits toxic fumes under fire conditions!
- Indigocarmine dye - Caution! Harmful if swallowed! Emits toxic fumes under fire conditions!

3.3.3 Equipment

Flame or heat block

3.3.4 Materials

Fixative (optional)

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3.3.5 Reagents

- Kernechtrot staining solution (KS)
- Picroindigocarmine staining solution (PICS)
- Distilled water
- 95% ethanol or methanol

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3.3.6 Stock Solutions (In-house Preparation)

3.3.6.1 Equipment

- Filtration apparatus
- 500 ml glass beakers
- Balance
- Spatula
- Plastic bottles
- Glass rod

3.3.6.2 Materials

- Filter paper
- Weigh boats or weigh paper

3.3.6.3 Reagents

- Aluminum sulfate
- Nuclear Fast Red
- Distilled water
- Picroindigocarmine dye
- Saturated picric acid solution **(Purchase saturated solution. DO NOT PURCHASE DRY PRODUCT! See 3.3.2.3 Safety Considerations.)**

3.3.6.4 Kernechtrot Solution (KS)

- In a beaker dissolve 5 g of aluminum sulfate in 100 ml of hot distilled water.
- Immediately add 0.1 g of Nuclear Fast Red and stir with a glass rod.
- Allow to cool and filter through filter paper.

3.3.6.4.1 Storage

The Kernechtrot Solution is stable at room temperature for up to 6 months, but may need to be re-filtered after standing.

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3.3.6.4.2 Labeling

Label the bottle as KS with the expiration date and a lot number (the date of preparation followed by the initials of person preparing the solution).

Example: KS Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

3.3.6.5 Picroindigocarmine Solution (PICS)

- Dissolve 1 g of Indigocarmine dye in 300 ml of a commercially purchased saturated solution of picric acid.
- Filter and store.

3.3.6.5.1 Storage

The Picroindigocarmine Solution is stable at room temperature for up to 6 months, but may need to be re-filtered after standing.

3.3.6.5.2 Labeling

Label the bottle as PICS with an expiration date and a lot number (the date of preparation followed by the initials of person preparing the solution).

Example: PICS Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

3.3.7 SERI Christmas Tree Stain (R540) Kit

3.3.7.1 Contents

- Solution A (Kernechtrot Solution - KS) - 30 ml
- Solution B (Picroindigocarmine Solution - PICS) - 30 ml
- Directions for use.

3.3.7.2 Store under refrigeration in bottles provided.

3.3.7.3 Shelf life: 6 months

3.3.8 KPICS/Christmas Tree Staining Procedure

3.3.8.1 Prepare a thin smear of an extract of a suspected semen stain and allow to dry, or use a smear from the Physical Evidence Recovery Kit (PERK). Fix the smear with a quick flame or fixative, or by placing it on a heat block overnight.

3.3.8.2 Add a sufficient amount (2-5 drops) of KS (red reagent) to cover the stained portion of the microscope slide.

3.3.8.3 Let the slide stand at room temperature for at least 15 minutes.

3.3.8.4 Wash KS off of the slide with a gentle stream of distilled water and drain the slide.

3.3.8.5 Add a sufficient amount (2-5 drops) of PICS (green reagent) to cover the stained portion of the slide.

3.3.8.6 Allow PICS to stain the smear for 5-15 seconds.

3.3.8.7 Wash PICS off of the slide with 95% ethanol, methanol or with water.

3.3.8.8 Dry the slide at room temperature.

3.4 Procedure for Köehler Illumination (Reference 8, Appendix A)

3.4.1 Determine that the lamp is centered according to the instructions for the microscope in use.

3.4.2 Using a medium to low power objective (approximately 10X), place a specimen in position and focus.

3.4.3 Close the field diaphragm.

3.4.4 Focus the image of the field diaphragm by adjusting the sub-stage condenser.

3.4.5 Center the field diaphragm using the centering screws on the condenser.

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3.4.6 Open the field diaphragm so that the rim just disappears beyond the field of view.

3.4.7 Adjust the condenser diaphragm (aperture diaphragm) to about ½ of the full aperture.

NOTE: Resolution, contrast, and depth of field can be regulated with the condenser diaphragm. It should not be used to regulate the brightness. For this purpose, either the regulating transformer or neutral density filters should be used.

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3.5 Microscopic Examination of Stained Slides for Spermatozoa

3.5.1 Equipment

Microscope (with approximately 200X – 400X total magnification, with or without phase capability)

3.5.2 Materials

- Distilled water, xylene substitute, or other appropriate mounting medium.
- Coverslips

3.5.3 Procedure

3.5.3.1 Quickly scan at approximately 200X total magnification. Confirm at approximately 400X total magnification.

3.5.3.1.1 With phase microscopy: Spermatozoa heads are neon-like pink/red with darker pink/purple acrosomal caps and green tails. Epithelial cells and most bacteria stain green with some of the nuclei pink/red; however, these are shaped differently than spermatozoa. Yeast cells take on the same color as spermatozoa, but are shaped differently.

3.5.3.1.2 Without phase microscopy: Spermatozoa heads are neon-like pink/red with pale pink (almost colorless) acrosomal caps, blue-green necks/midpieces, and green tails. Epithelial cells appear bright blue with red to purple nuclei.

3.5.3.2 Document the approximate number of spermatozoa and spermatozoa heads on the smear per hpf (approximately 400X total magnification), per lpf (approximately 200X total magnification), per length of slide, or per slide, as appropriate. **If only 1 spermatozoon or spermatozoon head is observed, there must be documented confirmation of its presence by a second qualified examiner.**

3.5.3.3 Place all smears submitted in the PERK back into the PERK. Properly label and return all other spermatozoa positive smears, including slides prepared by the laboratory, with the evidence.

NOTE: If a stain is consumed during the DNA isolation step and subsequent preparation of a smear, properly label and return the smear even when no spermatozoa are identified.

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3.5.4 Refer to Section 3.8 for reporting results.

3.6 Microscopic Examination of Unstained Slides for Spermatozoa

3.6.1 Unstained smears may be examined using phase contrast microscopy.

3.6.2 Equipment

Microscope (approximately 200X – 400X total magnification) with phase capability

3.6.3 Materials

- Microscope slides
- Coverslips
- Applicator sticks

3.6.4 Reagents

Distilled water

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3.6.5 Procedure

3.6.5.1 Place a small amount of an extract of a suspected semen stain on a microscope slide and cover with a coverslip, or add a drop of distilled water to a smear from the PERK, use an applicator stick to mix the water and the material on the smear, and cover with a coverslip.

3.6.5.2 Scan quickly with phase at approximately 200X total magnification. Confirm with phase at approximately 400X total magnification.

3.6.5.3 When the coverslip is touched gently, the spermatozoa and/or spermatozoa heads will roll, exhibiting their characteristic 3-dimensional shape. Use the distinctive size and morphology to identify the spermatozoa/spermatozoa heads.

3.6.5.4 Document the approximate number of spermatozoa and spermatozoa heads on the smear per hpf (approximately 400X total magnification), per lpf (approximately 200X total magnification), per length of slide, or per slide, as appropriate. **If only 1 spermatozoon or spermatozoon head is observed, there must be documented confirmation of its presence by a second qualified examiner.**

3.6.5.5 Place all smears submitted in the PERK back into the PERK. Properly label and return all other spermatozoa positive smears, including slides prepared by the laboratory.

NOTE: If a stain is consumed during the DNA isolation step and subsequent preparation of a smear, properly label and return the smear even when no spermatozoa are identified.

3.6.5.6 Refer to Section 3.8 for reporting results.

3.7 P30 ABACARD® (References 9, 10, 11, Appendix A)

3.7.1 Quality Control

3.7.1.1 Before using a new lot number of the ABAcard® p30 Test, its specificity must be tested and appropriately documented in the laboratory's quality control records.

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3.7.1.2 The ABAcards® ("test devices") must be tested against human blood, vaginal fluid, saliva, feces, urine, a positive control (semen), and a negative control (buffer) to ensure that the test is working properly.

3.7.1.2.1 Samples of human blood, vaginal fluid, saliva, feces, urine, and semen will be prepared in-house. Samples should be air dried onto a swab or similar material prior to testing.

3.7.1.2.2 Label the known samples with the name of the substance (i.e., human semen, etc.) and the lot number (the date of preparation followed by the initials of the person preparing the sample).

Example: human semen Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

3.7.1.2.3 Store known samples in the freezer.

3.7.1.2.4 It is also desirable to test dilutions of semen to determine the sensitivity of the test.

3.7.1.3 The quality control documentation will include (Refer to Document 210-F615):

- The lot number, receipt date, expiration date, and manufacturer of the ABAcard® p30 Test.
- The date of testing.
- Initials of the person conducting the testing.
- Results of the testing.

3.7.1.4 Once the appropriate tests have been performed on a lot number of the ABAcard® p30 Test, they need not be repeated for each case. If another shipment of the same lot number is received on a different date, they are considered QC'd.

3.7.2 "High Dose Hook Effect"

The "High Dose Hook Effect" is a false negative result that is obtained in the presence of high concentrations of p30 (usually undiluted semen). This effect results from large amounts of human p30 binding to the antibody to form an antigen-antibody complex and free p30 migrating toward the test area "T". The antibody in the test area "T" is blocked by this free p30. Therefore, the mobile antigen-antibody complex cannot bind to the antibody. As a result no pink line will form in the test area "T". To confirm the presence of "High Dose Hook Effect", repeat the test using a 10-10,000 fold dilution of the sample.

3.7.3 Stability, Storage and Shelf Life

3.7.3.1 The ABAcard® p30 Test should be stored below 82° F (28° C).

3.7.3.2 The test can be stored in the sealed pouch below 82° F (28° C) until the expiration date as printed on the sealed test pouch.

3.7.3.3 DO NOT FREEZE.

3.7.3.4 Do not use the test after the expiration date.

3.7.4 Reagents and Materials Provided

- Test Device (25 pieces, each individually sealed in a test pouch) - one device needed per sample tested
- A dropper and a desiccant sealed inside each of the test pouches
- Test Instructions
- Buffer

3.7.5 Equipment Required But Not Provided

- Microcentrifuge
- Timer
- Scissors
- Tweezers
- Microcentrifuge tube rack
- Pipettors (1000 µl and/or 200 µl)
- Dissecting needle

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3.7.6 Materials Required But Not Provided

- Microcentrifuge tubes
- Microcentrifuge tube lids
- Pipette tips

3.7.7 Reagents Required But Not Provided

Known semen sample

3.7.8 Minimum Standards and Controls

3.7.8.1 On the day of use a positive reagent control (known seminal fluid) and a negative reagent control (buffer) must be tested to ensure that the reagents and test device are working properly. The results of this testing must be documented in the case file.

3.7.8.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.

3.7.8.3 As a general rule a substrate control will not be tested nor is not necessary to test submitted control swabs.

3.7.9 P30 ABACARD® Procedure

3.7.9.1 Cut a portion of the dried stain into small pieces (size based upon the substrate and the intensity of the acid phosphatase test) and place into a labeled microcentrifuge tube.

3.7.9.2 Add 350 µl of the buffer provided within the kit and cap the tube.

3.7.9.3 Allow the sample to extract at room temperature for a minimum of 2 hours. Extraction can be done overnight if desired.

3.7.9.4 Punch holes in the lid of the tube.

3.7.9.5 Place the cuttings into the lid.

Option: Alternatively the cutting may be placed into a Spin-Ease basket

3.7.9.6 Centrifuge for 5 minutes at approximately 10,000 rpm to recover the liquid.

3.7.9.7 If a microscopic sperm search is to be conducted, remove approximately 320 µl of the extract and place into a new labeled microcentrifuge tube. This aliquot will be used for the test procedure and may be stored between 2-8°C or frozen if not used immediately. The remaining extract and pellet can be used for the sperm search.

3.7.9.8 **Allow the sample to warm to room temperature** if it has been refrigerated or frozen.

3.7.9.9 Remove the device and dropper from the sealed pouch.

3.7.9.10 Add approximately 200 µl (or 8 drops with the dropper) of the sample to the sample well “S” on a labeled test device.

3.7.9.11 Record result at 10 minutes. A positive result can be seen as early as 1 minute. For negative results, one must wait for the full 10 minutes. Do not record the results after 10 minutes. All control samples must give the expected results before the result on an unknown sample can be

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called, i.e., the reagent blank is negative and the known semen sample is positive. A diagrammatic representation of the results is located at the end of this chapter.

3.7.9.12 Interpretation

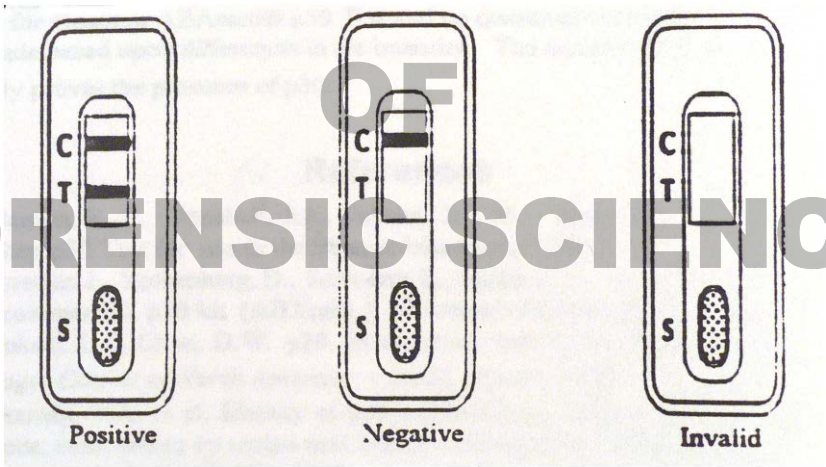
3.7.9.12.1 Positive Result = 2 pink lines, one in the test area "T" and one in the control area "C". p 30 level is at or above 4 ng/ml.

3.7.9.12.2 Negative Result = 1 pink line in the control area "C". No p30 is present above 4 ng/ml OR presence of "High Dose Hook Effect".

3.7.9.12.3 Invalid Result = No pink line in the control area "C" or incomplete line in the test area "T". The test is inconclusive. Repeat the test if sufficient sample remains.

3.7.9.13 Refer to Section 3.8 for reporting results.

ABAcard® p30 TEST RESULTS DIAGRAMMATIC REPRESENTATION



NOTE: The diagrammatic representation of the ABAcard® p30 Test results is taken from Abacus Diagnostics, ABAcard® p30 Test For The Forensic Identification of Semen, Technical Information Sheet (Revised 05/2011).

3.8 Reporting Results

Report the results of semen testing using the statements which follow:

3.8.1 Positive findings

3.8.1.1 "Spermatozoa were identified …"

3.8.1.2 "A spermatozoon was identified …"

3.8.1.3 "Chemical and immunological testing indicated the presence of seminal fluid; however, no spermatozoa were identified… " (i.e., Positive AP, Positive p30 and Negative Sperm)

3.8.2 Negative findings

3.8.2.1 "No spermatozoa were identified and no seminal fluid was indicated…" (i.e., Negative AP and Negative Sperm)

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3.8.2.2 "No seminal fluid was indicated…" (i.e., Negative AP)

3.8.2.3 "Immunological testing did not indicate the presence of seminal fluid and no spermatozoa were identified…" (i.e., Positive AP, Negative p30 and Negative Sperm)

3.8.3 Inconclusive findings

3.8.3.1 "The possible presence or absence of seminal fluid could not be determined 1) due to insufficient information obtained from the chemical analysis or 2) since the controls used in this testing did not meet the quality control requirements."

3.8.3.2 "The possible presence or absence of seminal fluid could not be determined due to insufficient information obtained from the chemical analysis and the stain was insufficient for further body fluid identification testing…" (i.e., Positive AP, Negative Sperm, QNS P30).

3.8.4 Testing not conducted

Report the absence of seminal fluid or semen testing as "in order to conserve the sample for DNA analysis, no seminal fluid detection testing was conducted."

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4 DETECTION OF URINE

4.1 Urease Test ({tc \l2 "UREASE TEST}Reference 5, pp. 191-193, Appendix A)

4.1.1 Equipment

- Scissors
- Tweezers
- Scalpel or other sharp instrument (to cut cork)
- Heat block (37° C)

4.1.2 Materials

- Test tubes (10 X 75 mm)
- Corks
- Disposable pipettes

4.1.3 Reagents

- Distilled water
- Urease reagent
  - Store at 2-8° C.
  - According to the manufacturer (Sigma), reagents stored at 2-8° C will have a shelf life of 2 years from the manufacturer's quality control date.
- Red litmus paper
- Positive control (known urine)

4.1.4 Minimum Standards and Controls

A positive reagent control (known urine stain) and a negative control distilled water) must be tested and results documented in the case file.

4.1.5 Urease Test Procedure

If it is possible that the stain will be consumed, do not perform the Urease testing and go directly to DNA testing.

4.1.5.1 Cut an approximate 2 cm² piece of a suspected urine stain and the appropriate controls into small pieces. Place the cuttings into appropriately labeled 10 X 75 mm test tubes.

4.1.5.2 Add 3-4 drops of distilled water and 6-7 drops of urease reagent to each tube.

4.1.5.3 Cut a slit into the small end of a cork and insert a strip of red litmus paper into this slit.

4.1.5.4 Place the cork (with red litmus paper) into each test tube. Do not allow the litmus paper to touch the liquid.

4.1.5.5 Incubate the samples in a 37° C heat block for 30 minutes.

4.1.5.6 Observe any change in the color of the litmus paper. Document results in the case file.

4.1.5.7 All controls must give the expected results before a conclusion can be reached on an unknown sample. When all controls work properly and a positive reaction is observed for the unknown sample, urine is <u>indicated</u> to be present.

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4.1.5.8 Interpretation

4.1.5.8.1 Positive Reaction = Red litmus paper turns blue

4.1.5.8.2 Negative Reaction = No color change to red litmus paper

4.1.5.8.3 Inconclusive Reaction = No color change of the positive control to the red litmus paper

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4.1.5.9 Reporting Results

4.1.5.9.1 Report positive test results as "Urine was indicated…"

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4.1.5.9.2 Report negative test results as "No urine was indicated…"

4.1.5.9.3 Report inconclusive test results as "the possible presence or absence of urine could not be determined 1) due to insufficient information obtained from the chemical analysis or 2) since the controls used in this testing did not meet the quality control requirements."

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4.1.5.9.4 Report the absence of urease testing as "in order to conserve the sample for DNA analysis, no urine detection testing was conducted."

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5 DETECTION OF FECAL MATTER

5.1 Edelman's Test ({tc \l2 "EDELMAN'S TEST}Reference 12, pp. 4-7, Appendix A)

5.1.1 Safety Considerations

- Mercuric chloride - Caution! Very toxic if inhaled or swallowed, or if in contact with skin! Poisonous! Dangerous! May be fatal!
- Zinc chloride - Caution! Corrosive!
- Amyl alcohol (isopentyl alcohol) - Caution! Harmful if swallowed or inhaled! Irritant! Combustible!

5.1.2 Equipment

- Scissors
- Tweezers
- Centrifuge
- Long wavelength ultraviolet light source
- Vortex

5.1.3 Materials

- Disposable pipettes
- Test tubes and/or microcentrifuge tubes

5.1.4 Reagents

- 10% Saturated mercuric chloride solution (1 g in 10 ml of 95% ethanol)
- 10% Saturated zinc chloride solution (1 g in 10 ml of 95% ethanol)
- Amyl alcohol (isopentyl alcohol)
- Distilled water
- Positive control (known feces)

5.1.5 Storage

The 10% saturated solutions of mercuric chloride and zinc chloride are stable at room temperature.

5.1.6 Labeling

5.1.6.1 Label each bottle with the contents and lot number (date of preparation followed by the initials of person preparing the solution).

Example: 10% saturated zinc chloride solution Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

5.1.6.2 There is no expiration date (see 5.1.7 Minimum Standards and Controls).

5.1.7 Minimum Standards and Controls

A positive reagent control (known fecal stain), and a negative control (distilled water) must be tested and results documented in the case file. As a general rule a substrate control will not be tested nor is not necessary to test submitted control swabs.

5.1.8 Edelman's Test Procedure

If it is possible that the stain will be consumed, do not perform the Edelman's testing and go directly to DNA testing.

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5.1.8.1 To prepare an extract of the stain, place an approximate ½ cm² piece of suspected fecal stain and controls in appropriately labeled test tubes or microcentrifuge tubes, add a minimum of 3 drops of distilled water to each tube (use only the amount of distilled water necessary to saturate the stain) and leave at room temperature for at least 15 minutes.

5.1.8.2 Remove the material and add a minimum of 3 drops of 10% saturated zinc chloride solution to the extract.

5.1.8.3 Add 5 drops of amyl alcohol (isopentyl alcohol) to the extract and vortex.

5.1.8.4 Centrifuge for 5 minutes. Pipet the supernatant layer into an appropriately labeled test tube.

5.1.8.5 Add 3 drops of 10% saturated mercuric chloride solution.

5.1.8.6 Observe color changes in both white and ultraviolet light. Document results. If urobilin is present the solution may become rose-pink, but will show a crab apple green fluorescence under long wave ultraviolet light.

5.1.8.7 All controls must give the expected results before a conclusion can be reached on an unknown sample. When all controls work properly and a positive reaction is observed for the unknown sample, presence of feces is indicated.

5.1.8.8 Interpretation

5.1.8.8.1 Positive Reaction = Crab apple green fluorescence under long wave ultraviolet light

5.1.8.8.2 Negative Reaction = No green fluorescence under long wave ultraviolet light

5.1.8.8.3 Inconclusive Reaction = No green fluorescence of the positive control under long wave ultraviolet light

5.1.8.9 Reporting Results

5.1.8.9.1 Report positive test results as “Fecal material was indicated…”

5.1.8.9.2 Report negative test results as “No fecal material was indicated”

5.1.8.9.3 Report inconclusive test results as “the possible presence or absence of fecal material could not be determined 1) due to insufficient information obtained from the chemical analysis or 2) since the controls used in this testing did not meet the quality control requirements.”

5.1.8.9.4 Report the absence of fecal testing as “in order to conserve the sample for DNA analysis, no fecal detection testing was conducted.”

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6 GENERAL REPORT WRITING

6.1 Basic Report Terminology

6.1.1 Examination performed using an alternate light source (ALS) is considered a screening test.

6.1.1.1 If no stains are observed with the use of the ALS, the negative observation will be reported as "No [body fluid] was observed."

6.1.1.2 If a stain fluoresces during the use of the ALS and upon subsequent chemical testing produces a negative result, the test result will be reported as "No [body fluid] was indicated." Positive test results will be tested further if applicable and reported according to those test results.

6.1.2 The determination of the possible existence of a body fluid performed through visualization (bright light and/or ALS) without chemical testing will be reported as an observation. A negative observation will be reported as "No [body fluid] was observed." Positive observations will be tested further if applicable and reported according to those results.

6.1.3 For presumptive testing, demonstrated through the act of testing, the term "indicated" will be used when a positive result is obtained. If no result/negative result is obtained this result will be reported as "no [body fluid] was indicated".

6.1.4 For confirmatory testing, the terms "identified" and "not identified" will be used.

6.2 Reporting Results of Analysis

6.2.1 When body fluid testing is conducted (seminal fluid, spermatozoa, blood, urine or feces), the following will be included in the METHODS section: the methods section can include all body fluids as listed below or can be edited to reflect the body fluid that was tested for in each case (for example blood only).

BODY FLUID DETECTION METHODS:

- The indication of blood, seminal fluid, urine and fecal material is based on physical characteristics innate, but not unique, to the body fluid tested. Therefore, the testing for these body fluids is not confirmatory.
  - The indication of blood, fecal material and urine is conducted through visual and chemical examinations.
  - The indication of seminal fluid is conducted through visual, chemical and immunological testing.
- The identification of spermatozoa is conducted through microscopic examinations and is confirmatory.

6.2.2 When body fluid presumptive/identification tests (including visual examination for blood) are conducted, the conclusions (based on the results) will be reported as "Results".

6.2.2.1 The observation of the absence of blood on trauma-related evidence (evidence in homicides, assaults, rapes, etc.) will be reported as this may provide investigative information.

6.2.3 In the report, it is not necessary to distinguish whether an item was swabbed or cut by the examiner during analysis. This information is maintained in the case file notes.

6.2.3.1 Example: Where the blade was swabbed and the swab tested - "Blood was indicated on the blade of the knife."

6.2.3.2 Example: Where a cutting of the shirt was tested - "Blood was indicated on the collar of the shirt."

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6.3 Reporting Locations of Biological Stains on Items

6.3.1 Examiners will report the location of biological stains or sample recovery on an item in the report. This may be helpful to the investigator and/or attorneys as they sort through the facts of the case.

6.3.1.1 Example: "Blood was indicated on the front left thigh of the jeans and on the center back of the shirt."

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6.3.1.2 Example: "Blood was indicated in stains on the left front thigh, lower left cuff and right front knee of the jeans, designated A-C respectively."

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6.3.1.3 It is recommended that when reporting results on bottles and cans, the use of the term "mouth area" will be used to differentiate this area from a "touch" area on the body of the bottle or can.

6.3.1.4 As appropriate, it may be important to specify the surface of stain deposition or sample recovery on the item.

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6.3.1.4.1 If it is not possible to determine the inner and outer surface of an item (i.e., a condom), the results may be reported with respect to how the item was submitted.

6.3.1.4.1.1 Example: "Spermatozoa were identified on the inside of the condom (as submitted)."

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6.3.2 When multiple stains are located in the same general vicinity, the results may be reported with respect to an "area", "multiple stains", or "selected stains", as necessary. However, the report should be clear if individual stains were tested or if stains were grouped for testing.

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6.3.2.1 Example: "Blood was indicated in stains on the front right thigh area of the jeans. A DNA profile was developed from one of these stains. "

6.3.2.2 Example: "Blood was indicated in multiple stains on the front right thigh area of the jeans. A DNA profile was developed from a representative sample of these stains (or from combined stains from this area)"

6.3.3 When an RFLE or other communication from an investigator/detective requests that specific stains on an item be examined, the results will be reported with respect to those and any other stains tested.

6.3.3.1 Example: RFLE states "Please test the stain on the left sleeve of the T-shirt for blood." The results would be reported as "No blood was indicated in the stain on the left sleeve or elsewhere on the T-shirt".

6.4 Reporting Results from a Physical Evidence Recovery Kit (PERK)

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6.4.1 The swabs and smears submitted from the same body area in the Physical Evidence Recovery Kit will be combined to be reported as a "sample" (i.e., vaginal/cervical smears and swabs will be reported as vaginal/cervical sample). Notes will address what was done with the smear and swabs separately; however, the report will only address the results for the body area sample.

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6.4.1.1 Example: "Chemical and immunological testing indicated the presence of seminal fluid; however, no spermatozoa were identified on the vaginal/cervical sample."

6.4.1.2 Example: "Spermatozoa were identified and blood was indicated on the vaginal sample."

6.4.2 Only the final interpretation based on all presumptive and/or confirmatory tests for body fluids will be reported for each sample.

6.4.3 All contents (including known samples and buccal swabs) included in the Physical Evidence Recovery Kit which were not examined will be specified in the report.

6.4.3.1 Example: "The oral rinse, lips/lip area, perianal/buttocks and anal samples, as well as the pubic combings and debris collection, were not examined."

6.5 Reporting Results from "Saliva" Evidence

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Cigarette butts, bottles, cans, etc. will not routinely be tested for blood. If the observation is made that no blood was seen, this will be documented in the notes, but does not need to be reported. If testing for blood is conducted, the results must be reported.

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6.5.1 Cigarette butts submitted in conjunction with homicides and assaults must be evaluated on a case-by-case basis with regard to blood testing and reporting since the presence or absence of blood may be probative in these situations, depending on the scenario.

6.6 Reporting Results from Buccal Swabs Submitted as a Known Sample

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6.6.1 It is not necessary to report the presence or absence of blood on buccal swabs submitted as a known sample.

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6.6.2 If blood is observed on buccal swabs, document this in the case file.

6.7 Reporting Results from "Touch" Evidence

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Swabs from light switches, steering wheels, door handles, etc. submitted for analysis of possible touch evidence (perspiration, skin cells, etc.) do not routinely need to be tested for blood. If the observation is made that no blood was seen, this will be documented in the notes, but does not need to be reported. If blood detection is performed, the results must be reported.

6.8 "Touch" evidence submitted in conjunction with homicides and assaults must be evaluated on a case-by-case basis with regard to blood testing and reporting since the presence or absence of blood may be probative in these situations, depending on the scenario.

6.9 Reporting Results of Samples Requiring Preservation

If no analysis will be conducted on an evidence sample that required preservation, the case notes will indicate that sample has been preserved; the results will be reported as "this sample was not analyzed/examined".

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APPENDIX A – REFERENCES FOR PROCEDURES

1. Forensic Science Handbook, edited by Richard Saferstein, Prentice-Hall, Inc., Englewood Cliffs, NJ (1982).

2. Garner, D. D., Cano, K. M., Peimer, R. S., and Yeshion, T. E., "An Evaluation of Tetramethylbenzidine as a Presumptive Test for Blood," JFS, Vol. 21: 816-821 (1976).

3. Culliford, Bryan J., Examination and Typing of Bloodstains in the Crime Laboratory, U. S. Government Printing Office, Washington, DC (1971).

4. Kirk, Paul L., Crime Investigation, John Wiley and Sons, New York, NY (1974), pp. 650-651.

5. Gaensslen, R. E., Sourcebook in Forensic Serology, Immunology, and Biochemistry, U. S. Government Printing Office, Washington, DC (1983).

6. Wraxall, Brian G. D., Bloodstain Analysis System Procedures Manual (October 1978), prepared for Forensic Serology Workshops at the Serological Research Institute 1977-1978.

7. Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence, Laboratory Division of the Federal Bureau of Investigation, U. S. Department of Justice, Washington, DC (July 6-8, 1983).

8. McCrone, Walter C. And Delly, John Gustav, The Particle Atlas, Volume I, 2nd ed., Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan (1973), page 23.

9. Abacus Diagnostics, ABAcard® p30 Test For The Forensic Identification of Semen, Technical Information Sheet, Revised 05/2011.

10. Hochmeister, M. N., Budowle, B., Rudin, O., Gehrig, C., Borer, U., Thali, M., and Dirnhofer, R., "Evaluation of Prostate-Specific Antigen (PSA) Membrane Test Assays for the Forensic Identification of Seminal Fluid", J. Forensic Sci., Vol. 44: 1057-1060.

11. Spear, T. F. and Khoskebari, N., "The Evaluation of ABAcard® p30 Test for the Identification of Semen", The CAC News, 1st Quarter 2001, pages 22-24.

12. Metropolitan Police Forensic Science Laboratory, Biological Methods Manual, London, England (1978).

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