Forensic Chemistry Section

Identification of Semen Method

1. Scope

This document outlines the methods for examining various items for the presence of semen.

2. Safety

2.1 Disposable laboratory coats and disposable gloves will be worn when handling evidence and when preparing reagents.

2.2 Disposable face masks will be worn when necessary.

2.3 Reagents will be prepared in the total exhaust hood.

2.4 Protective goggles will be worn whenever the alternative light source is in use.

3. Alternate Light Source Examination

3.1 Clothing, bedding, and other larger items will first be screened using an alternate light source.

3.2 For general semen, saliva, perspiration, etc. screening, the alternate light source will be set at 530 nm. Alternatively, a BattleLite with a blue 455 nm light head may be used as the light source.

3.3 The light source will be tested against a known semen standard. If a known semen standard does not fluoresce, the alternate light source will not be used until the problem is resolved.

3.4 The room must be as dark as possible to analyze the sample.

3.5 A marking pen or chalk will be used to mark all areas of fluorescence.

4. Presumptive Chemical Testing

4.1 Presumptive chemical testing may be conducted on stains identified using the ALS and swabs found in sex crimes kits, etc. If case information does not suggest semen stain location and the alternate light source fails to reveal latent stains, the analyst should test relevant surfaces of the item for the presence of acid phosphatase.

4.2 The reagents will be prepared as follows:

4.2.1 Fast Blue B

- A 1% acetic acid solution will be prepared by adding 5ml glacial acetic acid to 495ml reagent water.

- A 2% sodium acetate solution will be prepared by dissolving 10.0 g anhydrous sodium acetate in 500 ml 1% acetic acid solution.
- 4 g of fast blue salt B will be added to 200 ml of the sodium acetate solution and stirred.
- The solution may need to be filtered through Whatman No. 1 filter paper.

4.2.2 α-naphthyl phosphate

- Acetic acid and sodium acetate solutions as described above will be used.
- 0.4 g of α-naphthyl phosphate will be added to 200 ml of the sodium acetate solution and stirred.

4.2.3 The solutions will be tested with positive and negative controls. If the positive and negative controls do not respond appropriately, the reagent will not be used until the problem can be rectified. If the problem cannot be rectified, the reagent(s) will be discarded and new reagents prepared.

4.2.4 The solutions then will be divided into 4ml cryo-tubes, capped, and placed in a rack.

4.2.5 Each tube will be labeled with chemical name, lot number with initials (ex. 070202BLC), and expiration date, which will be 6 months from the date of preparation.

4.2.6 The tubes will be stored frozen and thawed before use.

4.3 Prior to examination, all trace evidence should be removed if necessary.

4.4 Positive and negative controls will be tested with each set of reagents prior to evidence examination. If the positive or negative controls do not respond appropriately, the reagent will not be used until the problem can be rectified.

4.5 Suspected semen stains on clothing, bedding, or other fabric or hard substrate should be tested as follows:

4.5.1 A swab or piece of filter paper will be moistened with a minimal amount of reagent water.

4.5.2 The dampened swab or filter paper will be pressed against the suspected stain to transfer a portion of the stain to the swab or the filter paper.

4.5.3 Fast Blue B will be added to the swab or the filter paper.

4.5.4 After approximately 20 seconds, the α-naphthyl phosphate solution will be added to the swab or filter paper.

4.6 The examiner may choose one of two methods to process swabs, direct or indirect testing.

4.7 To test the swab directly:

4.7.1 A small portion of the swab head will be cut and placed in a spot plate.

4.7.2 Fast Blue B will be added to the well of the spot plate.

4.7.3 After approximately 20 seconds, the α-naphthyl phosphate solution will be added to the spot plate.

4.8 To test the swab indirectly:

4.8.1 A piece of filter paper will be dampened.

4.8.2 The swab will be pressed onto the dampened filter paper.

4.8.3 Fast Blue B will be added to the area where the swab was pressed.

4.8.4 After approximately 20 seconds, the α-naphthyl phosphate solution will be added to the same area where the swab was pressed.

4.9 A positive reaction results in a deep purple color within approximately 20 – 40 seconds after addition of the second solution.

4.10 A negative reaction will result in no color change.

4.11 A weak reaction will result in a slow color change, a diminished or minimal color change, or both.

4.12 If a piece of evidence has numerous stains, the examiner will determine which stains will be tested. Those stains tested will be recorded in the notes.

4.13 If a stain reacts positively with the presumptive chemical test, or the examiner decides that a stain should otherwise be tested, a sample may be prepared and further tested for semen / seminal fluid confirmation by sperm cell search and/or PSA/p30 examination.

5. Sample Preparation for Confirmatory Testing

5.1 Samples for PSA/p30 extraction and semen / seminal fluid confirmation will be placed in microcentrifuge tubes.

5.2 Microcentrifuge tubes will be poured individually out of the container.

5.3 Generally, the sample size will be one swab head or an approximately $1cm^2$ cutting if enough sample exists.

5.4 If the entire sample will be extracted for sperm cell identification and/or PSA/p30 identification, the tube will be marked with a **blue** marker and a notation made on the Assignment Notification.

5.5 Generally, the Forensic Chemist Technician will conduct sperm cell extraction, slide preparation, sperm cell screening, and testing for the presence of PSA/p30. However, the examiner may choose to perform these analyses.

6. Sperm Cell Extraction Procedure

6.1 The work area and pipets will be cleaned with a 10% bleach solution.

6.2 Disposable lab coats, disposable gloves, and aerosol pipette tips will be used to avoid contamination.

6.3 Pipette tips will be changed with each transfer or addition of reagent or sample.

6.4 Gloves will be changed between cases.

6.5 Gloves will be cleaned with a 10% bleach solution between each sample within a case. Gloves will be changed between each sample if any solution splashes on gloves.

6.6 The tubes will be allowed to come to room temperature.

6.7 800 µl of TE buffer will be added to the tubes and sample.

6.8 The sample should be gently agitated with a pipette tip.

6.9 The tubes will be vortexed for at least 10 seconds.

6.10 The tubes will typically be incubated at 4 degrees Celsius overnight or 16 hours. However, the tubes may be incubated at room temperature for a minimum of 30 minutes or for a minimum of 4 hours at 4 degrees Celsius.

6.11 After incubation, the tubes will be vortexed for 10 seconds (or agitated with a sterile pipette tip) to aid in releasing cells from the substrate.

6.12 The evidence will be transferred to the 800µl filter insert of the SPIN-EASE extraction tube. The filter insert will be placed in the 2ml receiver tube and the snap top closed.

6.13 The tubes will be placed in the centrifuge for 3 minutes at maximum speed (14,000 rpm).

6.14 If the entire sample is being extracted, the filter insert with the sample will be retained. Otherwise, the filter insert containing the sample swab or cutting may be discarded.

6.15 All but approximately 50µl of the supernatant (or twice the volume of the cell pellet) will be removed to a 1.7 ml microcentrifuge tube, being careful not to disturb the cell pellet. The tube with the supernatant will be refrigerated and held for PSA analysis. For long-term storage, the supernatant should be frozen.

6.16 The cell pellet will be re-suspended in the remaining supernatant.

6.17 The cell pellet will be examined for the presence of sperm cells and epithelial cells using a 1/10 aliquot.

6.18 The remaining cell pellet will be frozen upright prior to packaging in a heat-sealed bag for long term storage in a freezer.

6.19 The sample will be re-labeled in LIMS and is either “consumed in analysis” or retained.

7. Microscopic Examination of Cellular Debris

7.1 The 1/10 aliquot will be transferred to a glass microscope slide.

7.2 The cells will be fixed to a microscope slide on a hot plate for approximately 2 minutes or by air-drying.

7.3 An outline of the mounted specimen will be etched on the bottom of the slide with a carbide-tipped scribe.

7.4 The slide may be gently rinsed with reagent water if the slide is particularly viscous.

7.5 The area of the slide with the cells will be flooded with Xmas Stain A and allowed to incubate for at least 5 minutes.

7.6 The slide will be gently rinsed with reagent water.

7.7 The area of the slide with the cells will be flooded with Xmas Stain B and allowed to incubate for 1-3 minutes.

7.8 The slide will be gently rinsed with ethanol and allowed to completely air dry.

7.9 Mounting medium will be added to the slide and a cover slip attached for permanent storage.

7.10 The slides will be stored at room temperature in a slide mailer until microscopic examination/interpretation.

7.11 Interpretation of the sperm search slides will be conducted as follows:

7.11.1 The slides will be observed at 400X.

7.11.2 Human sperm heads have a distinctive size and morphology and stain differentially in a very characteristic fashion, with slight variation from head to tail. Sperm heads appear clear or light pink at the tip of the head, intensely red stained at the base of the head, and green at the tail (tail may not be present).

7.11.3 Epithelial cells have a large green cytoplasm surrounding a faintly red nucleus.

7.11.4 Leukocytes (White blood cells) appear pink with a single, large nuclei or multiple, small nuclei.

7.11.5 Yeast cells stain a uniform red throughout the cell and extend into polyp-like structures, which may be observed occasionally.

7.12 If sperm cells are identified on the microscope slide, the examiner will determine which, if any, samples will be submitted to the Forensic Biology Section of the Crime Laboratory for further analysis.

7.13 If the microscope slide is particularly dense with epithelial cells or other debris, the examiner may decide to further dilute the re-suspended cell pellet in a separate microcentrifuge tube with an additional volume of TE Buffer. A second slide will be prepared with the dilute sample, stained, and searched for sperm cells as described above.

7.14 If no sperm cells are identified on the microscope slide, the supernatant of the cell debris extract will be tested for the presence of PSA/p30 which is present in seminal fluid and in some male urine.

8. PSA/p30 Testing

8.1 PSA/p30 testing is conducted using the Abacus Diagnostics ABAcard p30 Test.

8.2 The sample and test devices will be brought to room temperature.

8.3 A positive control will consist of a known semen stain extracted in 800µl of TE buffer for a minimum of 30 minutes.

8.4 A negative control will consist of the reagent blank supernatant.

8.5 Each test device should be labeled in such a way any examiner could identify the samples.

8.6 80µl of the specimen extract will be added to the sample well marked “S” of the test device.

8.7 The results will be determined after a reaction time of 10 minutes. Positive results can be apparent as early as 1 minute. Negative results must be allowed to react for 10 minutes.

8.8 The presence of a pink line in the ‘C’ and ‘T’ areas of the test device indicate positive results. This indicates that p30 (“seminal fluid”) is present at the level of 4.0 ng/ml or more.

8.9 Negative results are indicated by the presence of a pink line in the ‘C’ area of the test and no reaction in the ‘T’ area of the test device. A negative result indicates one of the following:

- no PSA/p30 is present
- PSA/p30 is present at less than 4.0 ng/ml

8.10 The product insert indicates that a High Dose Hook Effect could cause a false negative. However, a study by this laboratory has found no instance of a high dose hook effect causing false negatives in laboratory samples. If an examiner suspects a sample may be producing a false negative due to High Dose Hook Effect, the examiner will perform a 1:10 dilution of the supernatant. The 1:10 dilution would then be tested for PSA/p30.

8.11 When no pink line develops in the 'C' area of the test device, the test is invalid and must be repeated. If the results from the positive control or the negative control are erroneous, the controls and the associated samples must be re-tested.

8.12 A Forensic Chemist will be called upon to witness the final test device result when this analysis is conducted by the Forensic Chemist Technician. The written result will be initialed and dated by the Chemist.

8.13 After final interpretation, the sperm search slide(s) will be placed into the sex crimes kit or packaged with the parent item for return to the submitting agency.

8.14 If a sample is also to be tested for human blood, the remaining supernatant can be used for the Abacus Diagnostics HemaTrace test.