Procedure for Semen and Sperm Analysis

1.0 Purpose - This procedure specifies the method for conducting analysis for semen and sperm in non-Sexual Assault Evidence Collection Kit (SAECK) related evidence in forensic casework.

2.0 Scope - This procedure applies to those Forensic Scientists who have been released to conduct semen and/or sperm analysis in forensic casework.

3.0 Definition - N/A

4.0 Equipment, Materials and Reagents

- Working solution (see Forensic Biology Section QC procedure)
- Disposable scissors or disposable scalpel blade
- Glass culture tube (10 x 75 mm)
- Whatman 55 mm filter papers
- Disposable transfer pipettes
- Known seminal stain
- Kernechtrot and Picroindigocarmine stain (see Forensic Biology Section QC Procedure)
- Microscope slides
- 22 x 50 cover slips
- Olympus BX41 microscope
- Hot plate
- Deionized water
- Methanol
- Permount
- Wooden applicator sticks
- RSID kits which contain the test cards and universal buffer
- 1.5 mL centrifuge tube

5.0 Procedure

5.1 Sample Screening

5.1.1 The general work flow for items being examined for the presence of semen/sperm that are not a part of a SAECK submission will be as follows:

- Examine item with alternate light source and identify areas of interest
- Test areas of interest with Acid Phosphatase test
- Send samples for DNA testing based on case Type I or II and case information. Forensic scientists will use all information in addition to location of potential semen stains in decision making on what is the best sample to send for DNA testing.

5.1.2 When examining items other than those contained in a SAECK submission, a visual/alternate light source examination shall be conducted for semen-like stains (refer to the Forensic Biology Section Procedure for Use of an Alternate Light Source). These stains shall be marked then photographed (refer to Forensic Biology Section Procedure for Photographing Evidence)..

5.1.3 When multiple articles of clothing are contained together as one item, not all of the articles need to be examined if information is received that accounts for only one possible semen donor.

5.1.4 The initial pair of underwear submitted in the case (whether located inside or outside the kit) shall be analyzed per the direct to DNA procedure. Additional pairs shall be treated as clothing and be examined according to **5.1.2** and **5.1.3**.

5.1.5 Confirmatory testing (microscopic and/or RSID-Semen testing) shall be performed only upon specific request made by the District Attorney following the completion of DNA testing. This testing shall be performed on the slides prepared during the QIAcube extraction process or from the cuttings prepared for the DNA processing.

5.2 Acid Phosphatase Test

5.2.1 After a visual examination (using an alternate light source), the Forensic Scientist will test stains of interest based upon their training and experience in combination with the information provided in the case.

5.2.2 Using a disposable pair of scissors or a disposable scalpel blade, remove a cutting of the suspected stained area or the tip of each swab and place the sample in a separately labeled clean 10 x 75 mm glass tube or on a piece of filter paper.

5.2.3 Add enough working solution to cover each sample and agitate for one minute. The results shall be read immediately following this incubation.

5.2.4 Results

5.2.4.1 A positive result occurs when a purple color develops quickly on the material or bleeds into the solution/test paper. This positive result is indicative for the presence of semen.

5.2.4.2 A negative result occurs if no purple color change is observed. This negative result fails to indicate the presence of semen.

5.2.4.3 If the substrate has a color that could affect the ability to see a potential color change when the reagents are applied and the test is recorded as inconclusive, the reason shall be documented in the notes.

5.2.5 If the Forensic Scientist performs further testing due to the nature of the sample, the reason shall be documented in the worksheet.

5.2.6 If the sample is limited in quantity so that performing the presumptive test could limit the ability to obtain results from DNA testing, the analyst can bypass the presumptive testing and go directly to DNA testing.

5.3 Confirmatory Testing

5.3.1 Sperm Identification

5.3.1.1 Slide Preparation

5.3.1.1.1 Using a sterile disposable utensil, cut a sample from the item of evidence which contains the suspected stain or the tip of each swab and place the sample on a clean

microscope slide.

5.3.1.1.2 Add 1-2 drops of deionized water to the sample and tease the sample apart with wooden applicator sticks.

5.3.1.1.3 Heat fix the sample onto the slide by placing the slide on a hot plate until the sample is dry.

5.3.1.2 Place the slide(s) on a rack and apply the Kernechtrot stain to the slides for a minimum of 15 minutes. Note: The stain should not be allowed to dry onto the slide.

5.3.1.3 Remove the Kernechtrot stain by pouring it into a biological waste container and immediately apply the Picroindigocarmine stain to each slide. Leave this stain on for no more than 15 seconds. Pour the stain into a biological waste container.

5.3.1.4 Wash off the stain with methanol. Let the slides dry.

5.3.1.5 Once dry, apply 1-2 drops of Permount onto the slide (enough to cover the sample portion of the slide) and add a cover slip over the slide.

5.3.1.6 Observe the slide under the microscope at 200X or 400X magnifications and confirm the microscopic characteristics of the sperm head at 400X. Spermatozoa have a clear acrosomal cap, a red head and a green tail. Spermatozoa may be identified without the presence of a tail, but the clear acrosomal cap must be present and clearly visible.

5.3.1.7 Sperm shall be quantitated in a microscopic field at 200X. The following ranges shall be noted for the quantitation of spermatozoa:

- Rare – one sperm per slide up to 1 sperm per field of view (FOV)
- Greater than Rare (GTR) – any sperm quantity greater than 1 sperm per field of view

5.3.1.8 If multiple slides are made from an item (with the exception of slides prepared from condoms) and some of the slides are positive for sperm and some are negative for sperm, RSID shall be run on those areas which were AP positive and the slides failed to reveal sperm. These results shall be documented in the case notes and Laboratory Report for both the positive sperm and positive or negative semen areas.

5.3.1.9 If a single spermatozoon is observed on a slide, a verification of that sperm and verification review shall be performed by another qualified Forensic Scientist.

5.3.2 RSID-Semen Test

5.3.2.1 Cut a small sample, approximately 0.5 cm^2 (depending on the concentration of the stain), from the evidence sample using sterile disposable scissors or a sterile scalpel blade and place the cutting into a 1.5 mL centrifuge tube.

5.3.2.2 Add a minimum of 150 µL, up to 1 mL, of RSID universal buffer to each sample and mix well. (The amount of buffer added will depend on the sample size; buffer should cover the sample completely.)

5.3.2.3 Allow the sample to extract for a minimum of 2 hours. For weak or older samples,

Forensic Scientists should use a larger quantity of material and/or an extended extraction time to include overnight (not to exceed 24 hours).

5.3.2.4 After completing the extraction process, pipette 100 µL of the extracted sample into the sample well on the RSID card.

5.3.2.5 Read and record the results of the card after 10 minutes. A second qualified analyst Perform a second read of the results.

5.3.2.6 A verification review shall be done in FA by the second analyst to record the results of the second read of the test card.

5.3.2.7 Results

5.3.2.7.1 A positive reaction will have two lines appear in the test window. One line will appear in the area marked "C" for control and one line will appear in the area marked "T" for test. A positive result can be recorded as soon as both of these lines appear, but no later than 10 minutes. The lines must be reddish in color.

5.3.2.7.2 If a line does not appear in the "T" area within 10 minutes, for both neat and diluted samples (if applicable) then the test is considered negative. A line must appear at the area marked "C" to ensure that the test is working properly.

5.3.2.7.3 If no line appears at the area marked "C," the test shall be repeated. If no line is seen in the "C" window in the repeated test, the Body Fluid Technical Leader shall be notified as soon as possible. Refer to Forensic Biology Section Administrative Policy and Procedure.

5.3.2.7.4 If the analyst, through training and experience, believes a sample that was indicative for semen and RSID-Semen test negative was a result of high-dose hook effect then a 1:10 dilution of the sample shall be made using the RSID buffer and an additional test performed and results recorded.

5.4 Reporting Guidelines - The results statements shall reflect only the work that is performed. Portions of the statements may be omitted to address what testing is actually performed. This interpretation may include or build upon one (1) or more of the following responses depending on the circumstances of the case and the nature of the examination.

5.4.1 This phrase shall be used if the Acid Phosphatase Test is negative:

Examination of a sample(s) taken from_____ (Item(s) ___), using the Acid Phosphatase Test, failed to indicate the presence of semen.

5.4.2 This phrase shall be used if the Acid Phosphatase Test is positive:

Examination of a sample(s) taken from_____ (Item(s) ___), using the Acid Phosphatase Test indicates, but is not specific for, the presence of semen.

5.4.3 This phrase shall be used when an inconclusive test is indicated and there is possible interference of the substrate:

Examination of a sample(s) taken from _____ (Item(s) ___), using the Acid Phosphatase Test, failed to reveal conclusive results to indicate the presence of semen because of possible interference of the substrate.

5.4.4 This phrase shall be used if no confirmatory semen testing was done.

No confirmatory semen testing was performed.

5.4.5 This phrase shall be used if, due to limited sample, no confirmatory semen testing was done.

Due to the limited quantity of the sample, no confirmatory semen testing was done.

5.4.6 This phrase shall be used if spermatozoa/spermatazoon are seen microscopically:

Microscopic examination of the slide prepared from _____ (Item(s) ___) revealed the presence of spermatozoa/spermatazoon.

5.4.7 This phrase shall be used if spermatozoa were not seen microscopically:

Microscopic examination of the slide prepared from _____ (Item(s) ___) failed to reveal the presence of spermatozoa.

5.4.8 This phrase shall be used if the cellular material contained on the slides is not microscopically human in origin:

Microscopic examination of the slide prepared from _____ (Item(s) ___) was conducted. The morphology of the cellular material is not consistent with human spermatozoa.

5.4.9 This phrase will be used if the RSID Semen test is positive:

Further examination of sample(s) taken from ____ (Item(s) ____), using the RSID Semen Test, revealed the presence of human semen.

5.4.10 This phrase shall be used if the RSID Semen test is negative:

Further examination of sample(s) taken from ____ (Item(s) ____), using the RSID Semen Test failed to reveal the presence of human semen.

5.4.11 This phrase shall be used if the RSID Semen test reads invalid:

Examination of a sample(s) taken from ____(Item ___), using the RSID Semen Test failed to give conclusive results for the presence of human semen.

5.5 Controls

5.5.1 Acid Phosphatase Controls: A known seminal stain is used as a positive control and the working solution is used as a reagent control. A substrate control, if available, is set up using a control cutting from an apparently "unstained" area of the same material from which the suspected stain has

been cut. A positive and negative control shall be tested prior to analysis once each day the Acid Phosphatase Test is performed per each lot used and the results shall be recorded in the case notes as positive or negative for each case that was worked that day. The controls must react appropriately.

5.5.2 RSID-Semen Controls: A positive control (known semen standard), and a negative control (100 µL of universal buffer) shall be run with every case or every batch of cases and the results will be recorded in the case notes. If a reddish line is seen in the negative control "T" area, the test shall be rerun. If a reddish line appears again in the negative control "T" area, the test shall be considered inconclusive. If this occurs, the Technical Leader shall be notified immediately.

6.0 Limitations – Limitations include, but are not limited to, the following: The Acid Phosphatase Test is a presumptive test for semen. It detects the enzyme acid phosphatase which is present in semen. If the enzymatic activity is low, it is possible for a seminal stain to give a negative Acid Phosphatase reaction. Enzyme activity used to screen for semen (Acid Phosphatase) is more easily degraded than sperm cells, can be affected by various disease states, and is extremely water soluble. For these reasons, it is not unexpected that with older samples one may find a sample which yields a negative Acid Phosphatase result, but is positive for sperm cells. If an older case (samples are 15+ years after collection) is being examined, a determination will be made by the Forensic Scientist and the Technical Leader on the best course of action for analysis of the case. This determination will be made in writing and retained in the case file.

RSID-Semen- High dose hook effect can occur.

7.0 Safety – Refer to Appendix 1 for chemical hygiene and safety precautions.

8.0 References

Forensic Biology Section Procedure for Use of an Alternate Light Source

Forensic Biology Section Procedure for Photographing Evidence

Forensic Biology Section Procedure for Aseptic Technique and Contamination Control

Forensic Biology Section Body Fluid training documents

Forensic Biology Section Procedure for Calibration and Maintenance

Forensic Biology Section Administrative Policy and Procedure

9.0 Records - N/A

10.0 Attachments - N/A

Revision History		
Effective Date	Version Number	Reason
03/09/2020	3	5.1.4 – updated wording; removed revision history; added Appendix 1.

Appendix 1

Methanol **DANGER: HIGH RISK SUBSTANCE**	
	**HEALTH 2** **FLAMMABILITY 3** **REACTIVITY 0**
Detection of Release	Colorless liquid with a sweet, pungent odor.
Signs/Symptoms of Exposure	Headache, Nausea, Dizziness, Eye damage. May cause intoxication that includes central nervous system depression, headache, dizziness, nausea, lack of coordination, and confusion.
PEL	OSHA (TWA) 200 ppm
Associated Hazards	Flammable. Acute oral, dermal, and inhalation toxin. Toxic if swallowed, comes in contact with skin, or inhaled. Specific target organ toxicity of eyes.
Controls	Use under fume hood. Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product. Use eye protection. Handle with gloves. Wear lab coat. Gloves: nitrile (break through time less than 1minute), butyl-rubber (break through time greater than 8 hours)
Safe handling, storage, disposal	Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Use explosion-proof equipment. Keep away from sources of ignition. Take measures to prevent the build-up of electrostatic charge. Dispose in Hazardous Chemical Waste. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Emergency Procedures	**Eye Contact:** Flush eyes with water as a precaution. **Inhalation Exposure:** If inhaled, move person into fresh air. If not breathing, give artificial respiration. Consult a physician. **Ingestion:** After swallowing: fresh air. Make victim drink ethanol (e.g. 1 drinking glass of a 40% alcoholic beverage). Call a doctor immediately (mention methanol ingestion). Only in exceptional cases, if no medical care is available within one hour, induce vomiting (only in fully conscious persons) and make victim drink ethanol again (approx. 0.3 ml of a 40% alcoholic beverage/kg body weight/hour). **Skin Contact:** Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician. **Spills:** Avoid breathing vapors, mist, or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapors accumulating to form explosive concentrations. Vapors can accumulate in low areas. Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Small spills: Contain spillage, and then collect with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and place in container for disposal. Large spills: Turn off sources of heat if possible; evacuate area and call 911 (Haz Mat).