

Procedure for Sperm Identification

1.0 Purpose - This procedure specifies the method for conducting sperm identification in forensic casework.

2.0 Scope - This procedure applies to those Forensic Scientists who have been released to conduct sperm identification in forensic casework.

3.0 Definitions

- Spermatozoa (sperm) - Male reproductive cell found in semen.

4.0 Equipment, Materials and Reagents

- Kernechtrot and Picroindigocarmine stain (see Forensic Biology Section Procedure for Body Fluid Quality Control)
- Microscope slides
- 22 x 50 cover slips
- Olympus BX41 microscope
- Hot plate
- Sterile disposable scissors or sterile scalpel blade
- Deionized water
- Methanol
- Permunt
- Wooden applicator sticks

5.0 Procedure

5.1 A microscopic examination is conducted to confirm the presence of spermatozoa. This shall be done on samples meeting any of the following criteria:

5.1.1 All vaginal and rectal swabs in the sexual assault kit. If oral assault is indicated from the paperwork, a slide shall be made from the oral swabs.

5.1.2 If limited spermatozoa (less than 1 sperm/3 fields of vision) or no spermatozoa are seen on the slide prepared from the vaginal swabs and swabbings are received from the external labia area, a slide shall be made from the external labia.

5.1.3 Any area that gives a positive AP test (refer to Forensic Biology Section Procedure for Acid Phosphatase Test (Walker Test)). If multiple areas tested from one item give a positive AP result, a slide needs to be prepared only from the area that gives the strongest color change unless there is reason to believe more than one semen donor may be present on the item.

5.1.4 Condoms: Swab both the inside and the outside of a condom (if possible) and make a slide directly from each of the swabbings. An AP Test on these swabbings is not required prior to doing a sperm search.

5.1.5 On blood and saliva evidence if sperm may exist on that evidence due to location (e.g., crotch area of the suspect's pants).

- 5.1.6** If sperm is suspected in a liquid urine sample, spin down the sample to pellet any cellular material. Return liquid to submitted container. Re-suspend the pellet in TE (approximately 25-50 μ L). Pipette 10 μ L onto slide and continue with **5.5**. If the re-suspended sample will be transferred to another analyst for DNA examination, the remaining sample shall be placed on sterile swabs and air dried before it is transferred to the analyst for DNA testing.
- 5.2** If a slide has been prepared previously, proceed to **5.5**. If a slide is to be prepared by the Forensic Scientist, continue with **5.3**.
- 5.3** Using a sterile disposable utensil, cut approximately $\frac{1}{2}$ cm² 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.
- 5.4** Add 1-2 drops of deionized water to the sample and tease the sample apart with wooden applicator sticks.
- 5.5** Heat fix the sample onto the slide by placing the slide on a hot plate.
- 5.6** Place the slide(s) on a rack and apply the Kernechtrot stain to the slides. Leave the stain on for a minimum of 15 minutes. The stain can be left on the slide longer; however, the stain should not be allowed to dry onto the slide.
- 5.7** Remove the Kernechtrot stain by pouring it into a biological waste container (see biohazard safety procedure) and immediately apply the Picroindigocarmine stain to each slide. Leave this stain on for no more than 15 seconds. Then pour the stain into a biological waste container.
- 5.8** Wash off the stain with methanol. Let the slides air dry.
- 5.9** Once dry, apply a small amount (a couple of drops) of Permunt onto the slide and add a 22 x 50 mm cover slip over the slide.
- 5.10** 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.
- 5.11** Sperm shall be quantitated in a microscopic field at 200X and the approximate amount documented for quantitative purposes.
- 5.11.1** Forensic Scientist who is doing body fluid identification only - If the sample has a low sperm quantity (less than one sperm/3 fields of vision) on an intimate sample, an attempt should be made to locate an additional sample of evidence with a higher quantity of sperm for DNA testing.
- 5.12** If multiple slides are made from an item and some of the slides are positive for sperm and some are negative for sperm, RSID shall be run on those slides that failed to reveal sperm. The results shall be documented in the case notes and Laboratory Report for both the positive sperm and positive or negative semen areas.
- 5.13** If only one spermatozoon is observed on a slide, that spermatozoon shall be verified by another Forensic Scientist and a verification review shall be scheduled in FA.
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5.14 If more than one vaginal, rectal or oral smear is collected and spermatozoa is identified on one of the smears, the other smear does not need to be examined.

5.15 Reporting Guidelines

5.15.1 Slides which are prepared by the Forensic Scientist from vaginal, rectal or oral swabs which were present in the Sexual Assault Kit shall be labeled and placed in a labeled slide mailer. The slide mailers shall be taped together and given a sub-item number if the swabs are to be removed for DNA analysis by a different Forensic Scientist. The report shall reflect the following statement:

Item# _____: Slides prepared from Items _____.

5.15.2 If the Acid Phosphatase Test is positive and no spermatozoa are seen microscopically and an RSID Semen test is performed refer to the Procedure for RSID Test.

5.15.3 This phrase shall be used if the Acid Phosphatase Test is positive and spermatozoa are seen microscopically:

Examination of a sample(s) taken from _____ (Item(s) _____), using the Acid Phosphatase test, revealed elevated levels of acid phosphatase, which is an indicator of, but not specific for, semen. Microscopic examination of a slide prepared from _____ (Item____) revealed the presence of spermatozoa.

5.15.4 This phrase shall be used if the Acid Phosphatase Test is negative but, due to the nature of the sample, a slide is prepared and spermatozoa are seen microscopically:

Examination of a sample(s) taken from _____ (Item(s) _____), using the Acid Phosphatase test, failed to reveal chemical indications for the presence of semen, Due to the nature of the sample, further microscopic examination of a slide prepared from _____ (Item____) was performed and revealed the presence of spermatozoa.

5.15.4 This phrase shall be used if only one sperm cell is seen microscopically:

Microscopic examination of a slide prepared from _____ (Item____) revealed the presence of a spermatozoon.

5.15.5 This phrase shall be used if the slides have been prepared previously and submitted in a Sexual Assault Kit and spermatozoa were not seen microscopically:

Microscopic examination of the _____ smears (Item____) failed to reveal the presence of spermatozoa.

5.15.6 This phrase shall be used if the slides have been prepared previously and submitted in a Sexual Assault Kit and spermatozoa were seen microscopically:

Microscopic examination of the _____ smears (Item____) revealed the presence of spermatozoa.

5.15.7 This phrase shall be used if the slides have been prepared previously and submitted in a Sexual Assault Kit and only one sperm cell was seen microscopically:

Microscopic examination of the _____ smears (Item____) revealed the presence of _____ a spermatozoon.

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

Microscopic examination of _____ (Item_____) was conducted. The morphology of the cellular material is not consistent with human spermatozoa.

6.0 Limitations - N/A

7.0 Safety - Picric Acid is an explosive if allowed to dry; therefore, ensure that the picric acid remains covered by the liquid in which it is packaged.

8.0 References

Forensic Biology Section Body Fluid training documents

Forensic Biology Section Procedure for the Acid Phosphatase Test (Walker test)

Forensic Biology Section Procedure for Aseptic Technique and Contamination Control

Forensic Biology Section Procedure for Calibration and Maintenance

9.0 Records - N/A

10.0 Attachments - N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document