

FBS06 – P30 Antigen Test for the Presence of Semen

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1. Scope

- 1.1. This procedure is used to confirm the presence of semen.

2. Background

- 2.1. To establish the practices for documenting the examination of evidence to conform to the requirements of the Department of Forensic Sciences (DFS) Forensic Science Laboratory (FSL) *Quality Assurance Manual*, the accreditation standards under ISO/IEC 17025:2005, and any supplemental standards.
- 2.2. P30, also known as Prostate Specific Antigen (PSA), is a glycoprotein produced in the prostate gland and is secreted in seminal fluid independently of the production of spermatozoa. The presence of p30 is used as another means of seminal fluid identification, particularly in samples with little or no spermatozoa. While p30 is not restricted to seminal fluid, it's extremely high concentration in seminal fluid makes it an effective marker to confirm the presence of semen on evidence stains.
- 2.3. P30 can be detected using a chromatographic immunoassay method. A stain extract is placed on a porous membrane in the presence of a monoclonal PSA antibody that is linked to a dye. If PSA is present in the extract, a PSA antigen-monoclonal PSA antibody complex will form. This complex will then migrate along the membrane where it will interact with monoclonal PSA antibody imbedded in the membrane at the test region. The antibody-antigen-antibody

“sandwich” that is formed will result in a colored line confirming the presence of p30.

3. Safety

- 3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures.
- 3.2. Read Material Safety Data Sheets to determine the safety hazards for chemicals and reagents used in the standard operating procedures.

4. Materials Required

4.1. P30 Cards

4.1.1. Note: Each lot of p30 Cards must be evaluated prior to use and periodically before expiration date. See QSOP 20 for the information regarding the procedure for evaluation.

4.2. TE Buffer (FBR14)

4.2.1. NOTE: Never use solutions directly from the stock bottles. Use Reagent SOPs for preparation and labeling instructions.

5. Standards and Controls

5.1. The Positive and Negative Controls are cut after all the questioned stains. Control extracts should be the last samples added to the cards in a sample set. One set of controls may be tested with each sample set. Record the results in the casework documentation.

5.1.1. A portion of a known semen swab (FBR03) is cut and extracted in the proper volume and labeled as a Positive Control. This control should exhibit a solid pink line at the “T” (test) region, the “C” (control) region, and the Internal Standard 4 ng region. A positive result may be recorded at any time within the 10 minute development period.

5.1.2. A portion of a sterile swab is cut and extracted in the proper volume and labeled as a Negative Control. This control should exhibit a solid pink line at the “C” (control) region and the Internal Standard 4 ng region. A

negative result is valid if a card remains negative at the "T" (test) region for the full 10 minute development period.

6. Calibration

6.1. Not applicable

7. Procedures

7.1. Take a cutting from a suspected semen stain (see chart below for cutting sizes) and place it in an appropriately labeled 1.5 ml or 2.0 ml microcentrifuge tube.

7.2. Following the chart below, add an appropriate volume of TE to samples followed by the controls.

7.3. Dimensions of Cutting (cm)	7.4. Extraction Volume (µl)
7.5. 0.5 x 0.5	7.6. 250
7.7. 0.7 x 0.7	7.8. 500
7.9. 1.0 x 1.0	7.10. 1000
7.11. ¼ of swab	7.12. 1000

7.13. Allow the samples to incubate at room temperature for two hours or overnight at 4°C. Samples may be placed on an orbital shaker during the incubation time. If refrigerated, be sure to allow samples to come to room temperature prior to proceeding to the next step.

7.14. Vortex briefly and centrifuge for 3 minutes at maximum angular velocity. If a sperm search is to be performed, the sample may be piggybacked using a centrifugal filter device such as a spin basket. The supernatant is removed for p30 testing and the remaining pellet may be used for slide preparation (FBS07) or in DNA Differential Extraction (FBS11). The cutting may be retained for future testing or may be disposed of in the biohazard waste.

7.15. Unwrap the Seratec card from packaging. Take care to confirm that the lot number being used has previously been approved for casework and has not expired.

7.16. Label the card with the appropriate sample identification number or control name.

- 7.17. Add 200 µl of each sample's extract to the sample well of card. Controls should be added last. If using the remainder of the extract for sperm search, take care not to disturb the cell pellet.
- 7.18. Allow the card to remain at room temperature for 10 minutes.
- 7.19. Read and record the results on the designated p30 worksheet. A positive result may be recorded at any time within the 10 minute period, however an inconclusive or negative result is not confirmed until the full 10 minutes has elapsed. Inconclusive results should be retested if possible.

7.20. Seratec Results

7.21. Card Result	7.22. Recorded Result
7.23. Pink lines at C, 4 ng and T	7.24. Positive
7.25. Pink lines at C, 4 ng	7.26. Negative
7.27. Pink line at C	7.28. Inconclusive
7.29. Pink line at T	7.30. Inconclusive



- 7.31. The results may be confirmed and initialed by another qualified analyst. A photograph of the cards is taken and included in case documentation.

8. Sampling

- 8.1. Not applicable

9. Calculations

- 9.1. Not applicable

10. Uncertainty of Measurement

- 10.1. When quantitative results are obtained, and the significance of the value may impact the report, the uncertainty of measurement must be determined. The method used to determine the estimation of uncertainty can be found in the *FSL Quality Assurance Manual – Estimation of Uncertainty of Measurement (Section 5.4.6)*.

11. Limitations

- 11.1. No test result should be recorded after the 10 minute development period has elapsed. An unlimited detection time could lead to a false positive reaction.
- 11.2. Insufficient sample quality and/or quantity could limit the development of a positive reaction.
- 11.3. Samples that are weak positive may require a second reader (qualified analyst) whose initials are recorded on the p30 worksheet.
- 11.4. The samples must be properly diluted in order to avoid the High Dose Hook Effect. If there is an excess amount of p30 in the sample, p30 will not completely bind to the gold-labeled antibody. Free p30 will reach the test result zone and bind to the p30 antibody fixed in this zone. The binding sites of the antibody become blocked so that the p30 bound to the gold-labeled antibody can no longer bind. The formation of the sandwich complex is repressed and no pink test result line is formed, resulting in a false negative. If High Dose Hook Effect is suspected, the sample should be further diluted and retested.

12. Documentation

- 12.1. FBU Serology Examination Worksheets
- 12.2. P30 Worksheet
- 12.3. Diagram / Photo Worksheet
- 12.4. FBU Report of Results

13. References

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- 13.30. FBS05 - Acid Phosphatase Presumptive Chemical Test for the Presence of Semen (Current Version)
- 13.31. FBS07 - Microscopic Examination for the Presence of Spermatozoa by Christmas Tree Stain (Current Version)
- 13.32. FBS09 - Differential Organic DNA Extraction (Current Version)
- 13.33. FBQ20 - Quality Control of P30 Antigen Cards (Current Version)