

FBS06 – P30 Antigen Test for the Presence of Seminal Fluid

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1. Scope

- 1.1. This procedure is used to confirm the presence of seminal fluid on evidentiary material.

2. Background

- 2.1. 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, its extremely high concentration in seminal fluid makes it an effective marker to confirm the presence of seminal fluid on evidence stains.
- 2.2. P30 can be detected using a chromatographic immunoassay method. A stain or sample 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 pink 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. Seratec p30 Cards

4.1.1. Note: Each lot of p30 Cards must be evaluated prior to use and periodically before expiration date. See FBQ20 for the information regarding the procedure for evaluation.

4.1.2. Store cards at room temperature.

4.1.3. Do not use past the expiration date.

4.2. TE Buffer

4.3. 1.5 ml microcentrifuge tubes

4.4. 2.0 ml microcentrifuge tubes

4.5. Microcentrifuge

4.6. Spin baskets

5. Standards and Controls

5.1. The Positive and Negative Controls are cut after all the questioned stains. Control extracts will 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 appropriate p30 examination worksheet (Document Control Number: 1576; 2154).

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 will 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 will exhibit a solid pink line at

the “C” (control) region and at 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 or sample (see chart below for cutting sizes) and place it in a 1.5 ml or 2.0 ml microcentrifuge tube labeled with case/sample identifying information.

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

Dimensions of Cutting (cm)	Extraction Volume (µl)
0.5 x 0.5	250
0.7 x 0.7	500
1.0 x 1.0	1000
¼ of swab	1000

7.3. Allow the samples to incubate at room temperature for two hours or in the refrigerator 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.

7.4. Vortex briefly and microcentrifuge for 3 minutes at maximum speed. If a sperm search is to be performed (FBS07 – Microscopic Examination of Spermatozoa by Christmas Tree Stain (Document Control Number: 1577)), the sample may be piggybacked using a centrifugal filter device such as a spin basket as referenced in step 7.2 of FBS07. The cutting may be retained for future testing or may be disposed of in the biohazard waste.

7.5. 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.

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

7.7. Add 200 µl of each sample’s extract to the appropriate sample well of card. Controls will be added last. If using the remainder of the extract for sperm search

(FBS07 – Microscopic Examination of Spermatozoa by Christmas Tree Stain (Document Control Number: 1577)), take care not to disturb the cell pellet when removing the p30 sample extract.

- 7.8. Allow the card to remain at room temperature for 10 minutes.
- 7.9. Read and record the results on the appropriate p30 examination worksheet (Document Control Number: 1576; 2154). 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. Retest inconclusive results using the original prepared extract if possible.
- 7.10. Seratec Results

Card Result	Recorded Result
Pink lines at C, 4 ng and T	Positive
Pink lines at C, 4 ng	Negative
Pink line at C	Inconclusive
Pink line at T	Inconclusive



8. Sampling

- 8.1. Not applicable

9. Calculations

- 9.1. Not applicable

10. Uncertainty of Measurement

10.1. Not applicable

11. Limitations

- 11.1. No test result will 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 will be noted as such 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 will be further diluted and retested.

12. Documentation

- 12.1. FBU Serology Examination Worksheets:
 - 12.1.1. P30 Worksheet – Document Control Number: 1576
 - 12.1.2. PERK Worksheet – Document Control Number: 2154
- 12.2. Diagram / Photo Worksheet – Document Control Number: 1606; 1607
- 12.3. FBU Report of Examination

13. References

- 13.1. *Abacus Diagnostics, Inc. (1999) OneStep ABACard p30 Test for the Forensic Identification of Semen-Technical Information Sheet/Product Insert.*
- 13.2. *Baechtel, F.S., Jung, J.C., and Terrell, L.D. Use of an enzyme-linked immunosorbent assay for the detection of p30 in questioned semen stains. In: Proceedings of the International Symposium on Forensic Immunology, FBI Laboratory, Washington, DC, 1986.*

- 13.3. *Benton, K.A., Donahue, J.A., Valadez, Jr., M, "Analysis of the ABACard p30 test for use in the forensic laboratory" 1998.*
- 13.4. *Caradine, C.C. "Evaluation of the OneStep ABACard p30 test for the forensic identification of Semen," 1998.*
- 13.5. *Hochmeister, et.al. (1999) "Evaluation of Prostate-Specific Antigen Membrane Tests for the Forensic Identification of Semen," Journal of Forensic Sciences, 44: 1057-1060.*
- 13.6. *Kamenev, L., Leclercq, M. and C. Francois-Gerard (1990) "Detection of p30 antigen in sexual assault case material," Journal of Forensic Science Society, 30(4), p 193-200.*
- 13.7. *Kearsy, J., Louie, H., Poon, H. (2001) "Validation of the 'OneStep ABACard PSA Test' kit for RCMP Casework," Canadian Society Forensic Science Journal v34, No2 (2001) p. 63-72.*
- 13.8. *Kristaly, A., Smith, D.A.S. "Validation of the OneStep ABACard PSA test for the rapid forensic identification of semen," 1999.*
- 13.9. *Kuester, J., Rothenburg, D., Schwartz, E., Eustace, M, Adamo, R. "Validation of a commercial p30 kit (ABA card) for forensic identification of semen," 1998.*
- 13.10. *Sensabaugh, G.F. (1976) "Isolation and Characterization of a Semen Specific Protein from Human Seminal Plasma: A Potential Marker for Semen Identification," Journal of Forensic Sciences, 23, 106-115.*
- 13.11. *Seratec, PSA Semiquant Cards for the Identification of Semen, Pamphlet Insert.*
- 13.12. *Spears, F.T., Khoskebari, N. "The Evaluation of ABACard p30 Test for the Identification of Semen." Crime Scene. 2000.*
- 13.13. *Wraxall, B. and L. DeHaan (1983) "The Use of p30 Antiserum in Sexual Assault Cases," presented at the Joint Canadian Society of Forensic Science/Northwest Association of Forensic Science Meeting, Vancouver, B.C.*
- 13.14. *Quality Control of p30 Antigen Cards (FBQ20)*
- 13.15. *Microscopic Examination for the Presence of Spermatozoa by Christmas Tree Stain (FBS07)*
- 13.16. *Forensic Science Laboratory Quality Assurance Manual (Current Version)*
- 13.17. *DFS Departmental Operations Manuals (Current Versions)*

13.18. FSL Laboratory Operations Manuals (Current Versions)