RAPID IMMUNOASSAY FOR SEMEN

15.1 PURPOSE

15.1.1: To identify p30 (ABAcard®) in Forensic samples.

A. Theory

1. The ABAcard® p30 test uses an immunochromatographic lateral flow assay to detect the presence of the p30 protein to indicate the presence of semen in questioned stains.

2. The chromatographic strip within the test device contains an excess of mobile pink-dye tagged anti-p30 antibodies located on the area of the strip underneath the sample well. Embedded anti-p30 antibodies (immobile) are located on the "Test line" of the test membrane. Embedded anti-IgG antibodies (immobile) are located on the "Control line" of the membrane and are used as an internal control. The test and control lines can be viewed through the test result window of the device.

3. When an extract of the questioned sample is deposited in the sample well, the extract comes in contact with the mobile tagged antibodies. If p30 (antigen) is present in the questioned sample, p30/tagged antibody complexes will form and be transported by bulk fluid flow through the length of the test membrane.

4. The embedded anti-p30 antibodies on the Test line will capture the p30/tagged antibody complexes, creating antibody/p30/tagged antibody "sandwiches". When the p30 concentration sample exceeds 4ng/ml, a pink colored line will develop in the Test position, indicating a positive result.

5. If p30 is not present in the questioned sample, the p30/tagged antibody complexes do not form in the sample well area and will not be captured at the Test position to form the antibody/p30 antigen/tagged antibody "sandwiches". Therefore, the p30 concentration remains below the level of detection (4ng/ml) and no pink line will develop at this position.

6. The anti-IgG antibody on the Control line captures the tagged antibodies flowing past the Test line position, producing a pink line at the Control position. This demonstrates that the sample fluid was transported through the length of the test, and that the components of the strip test are working correctly.

B. Limitations

1. P30 may be detected in adult male urine.

2. A positive result may be obtained from undiluted urine (male or female) applied directly to the test device.

3. Heavy fecal-type material and breast milk may give false positive results. Therefore, a sample heavily stained with fecal-type material or if the presence of breast milk is suspected (based on case information), the sample is considered unsuitable for p30 testing.

4. High Dose Hook Effect may occur when the concentration of p30 is too high which can cause a false negative result.

15.1.2: To quality control new p30 rapid immunoassay kits.

15.2 RESPONSIBILITY

15.2.1: Forensic Science Examiners (however titled) from the Division of Scientific Services who have been trained in the discipline of rapid immunoassay tests for semen according to FB SOP-26 (Training Manual and Checklist).

15.2.2: Forensic Science Examiners in the Forensic Biology Unit. Ordering information is maintained in a log book and/or electronically in the Forensic Biology Unit. New kits are purchased according to GL-6 (Purchasing). For additional information, refer to the Biological Inventory located in Appendix 3.

15.3 SAFETY

Use appropriate measures for the proper handling of biohazardous material according to GL-2 (Safety Manual) and the Safety Data Sheets.

15.4 DEFINITIONS

A. ABAcard®: Rapid Immunoassay
B. RSID™: Rapid Stain Identification
C. QRW(s): Quality Record Worksheet(s) (Appendix 1)

15.5 TEST PROCEDURE

A. The p30 ABAcard® test will be performed at the discretion of the examiner, with input from the Unit Lead(s) when applicable, based on the submitting agency requests, case information and the condition of the evidence.

B. A sample heavily stained with fecal-type material or if the presence of breast milk is suspected (based on case information), the sample is considered unsuitable. Therefore, the p30 test should not be conducted but the sample may be forwarded for DNA analysis, at the discretion of the examiner, with input from the Unit Lead(s).

C. Undiluted urine should not be tested directly utilizing this test.

For additional information, refer to FB SOP-13 (Extraction of Samples for Semen, section13.5.2.B) and FB SOP-17 (Rapid Immunoassay Test for Urine, section 17.5.2.B).

15.5.1: Materials

A. Extracted samples
B. ABAcard® test device and enclosed dropper
D. Spot plates
E. Micropipette and tips

15.5.2: Procedure

A. Record the test device lot # used on the General Reagent Sheet (FBQR-09).

B. The sample should be at room temperature before testing.

C. Label the test device with the case and item number.

D. Using a micropipette, add approximately 200µl (~6-7 drops with the enclosed dropper) of supernatant from the extracted sample to well "S" of the test device. Note the time immediately after adding the sample.

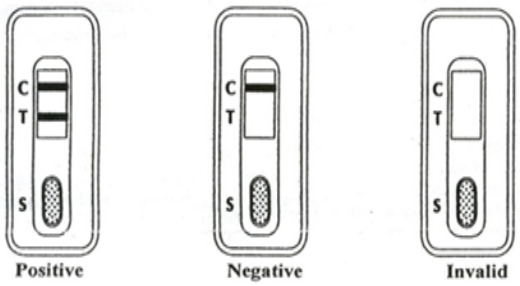
Note: If the sample is not migrating, additional RSID™-Universal buffer may be added to the 'S' well of the device to facilitate migration. Record the additional volume added on the appropriate QRW.

E. Monitor the progress of the test result for up to 10 minutes.
1. Record the final result no later than 10 minutes.
2. Record the time elapsed when the positive result was first observed.
3. DO NOT record any changes which occur after 10 minutes. Any change in test results after 10 minutes is invalid.
4. A second qualified examiner will observe and confirm the final results and initial the appropriate QWR. Note: The time elapsed when the analyst first observes a positive result will not be confirmed.

F. If the internal control fails (see Invalid Test under results below), review the test procedure and, if the quantity of sample allows retesting, repeat the test with a new device. If the test does not yield the appropriate results again, then inform the Unit Lead to try to determine the root cause.

G. Record the results of the control(s) and sample(s) on the appropriate QRW.

15.5.3: Results and Conclusions



A. Positive

1. If there are two pink lines, one each in the test area 'T' and in the control area 'C', the test result is positive and indicates that the p30 level is present at or above 4ng/ml.

2. Suggested Report Wording:

a.

Testing Performed	Result	Conclusion
Immunological - p30 Protein	Positive	Semen indicated

Appendix:
p30 is a component of semen.

b. *This/these extract(s) gave a positive result(s) with an immunological test for the presence of p30, a component of semen.*

B. Negative

1. If there is only one pink line in the control area 'C', the test result is negative. This may indicate that no p30 is present, the p30 level is below 4ng/ml or the presence of the "High Dose Hook Effect".

a. Presence of "High Dose Hook Effect" may give false negative results due to the presence of a high concentration of p30 in the sample.

b. "High Dose Hook Effect" may be suspected when a strong positive Acid Phosphatase reaction was observed and a negative p30 result was obtained. The extract may be diluted 1:20 and rerun with the ABAcard® Procedure.

2. Suggested Report Wording:

a.

Testing Performed	**Result**	**Conclusion**
Immunological - p30 Protein	Negative	Semen not detected

Appendix:
p30 is a component of semen.

b. *An immunological test for the presence of p30, a component of semen, was performed on this/these extract(s). Semen was not detected with this test.*

C. Inconclusive

1. If a pink line in the test area 'T' could not be determined.

2. Suggested Report Wording:

a.

Testing Performed	**Result**	**Conclusion**
Immunological - p30 Protein	Indeterminate	Inconclusive[1]
Comment: [1]Due to indeterminate results and/or substrate interference, this/these test(s) was/were determined to be inconclusive.		

Appendix:
p30 is a component of semen.

b. *An immunological test for the presence of p30, a component of semen, was performed on this/these extract(s). Due to indeterminate results and/or substrate interference, this/these test(s) was/were determined to be inconclusive.*

3. Record the reason a result is determined to be inconclusive on the appropriate QRW.

D. Invalid (Failed)

1. If there is no pink line visible in the control area ‘C’.

2. If there is not enough sample to repeat the test then no conclusion is possible.

3. Suggested Report Wording:

a.

Testing Performed	Result	Conclusion
Immunological - p30 Protein	Failed	No conclusion possible

Appendix:
p30 is a component of semen.

b. *An immunological test for the presence of p30, a component of semen, was performed on this/these extract(s). Due to the failure of this/these test(s), no conclusion(s) is/are possible.*

4. Record the reason the test failed on the appropriate QRW.

E. Unsuitable

1. See 15.5.B for additional information.

2. Suggested Report Wording:

Note: The conclusion for the result of the microscopic examination for spermatozoa will be superscripted since the sample is unsuitable for the next serological test (i.e. p30).

a.

Testing Performed	Result	Conclusion
Microscopic - Spermatozoa	Negative	Spermatozoa not identified[1]
Comments: [1]This sample was determined to be unsuitable for further serological testing due to the presence of heavy fecal-type material.		

b. *This sample was determined to be unsuitable for further serological testing due to [ ].*

[ ] = the presence of heavy fecal-type material.

[ ] = case information indicating the presence of breast milk.

F. It should be noted that any result above does not preclude the sample from being forwarded for DNA analysis.

15.6 QC PROCEDURE

A. Test the new lot before use according to the test procedure above, FB SOP-13 (Extraction of Samples for Semen) and the ABAcard® p30 Reagent Log Sheet. Record required information.

A second qualified examiner will observe and confirm the final results and initial the ABAcard® p30 Reagent Log Sheet. Note: The time elapsed when the analyst first observes a positive result will not be confirmed.

B. If the appropriate results are not obtained, review the procedure and repeat the test. If the test still does not yield the appropriate results, then inform the Unit Lead to try to determine the root cause.

C. If the lot is acceptable for use, record the date received, date opened and examiner's initials on each box and store according to the manufacturer's instructions.

The lot is acceptable for use when a positive result is obtained with the semen control, and negative results are obtained with the blank/negative control, female urine control and breast milk control.

D. Discard/replace according to the manufacturer's expiration date or according to 21.4.3.E in FB SOP-21 (General Chemical and Reagent QC).

Manufacturer's expiration dates with only month and year indicated (i.e. 04/2014) expire the last day of the month noted.

15.7 REFERENCES

A. Abacus Diagnostics' OneStep ABAcard® p30 Test For The Forensic Identification of Semen, provided Technical Information and Protocol sheet.
B. Connecticut State Forensic Science Laboratory, ABAcard® p30 Internal Validation, 1998.
C. Connecticut State Forensic Science Laboratory, RSID™-Semen Internal Validation, 2010.
D. Independent Forensics, RSID™-Universal Buffer Technical Information and Protocol sheet.
E. Connecticut State Forensic Science Laboratory, RSID™-Universal Buffer Internal Validation, 2011.
F. Connecticut State Forensic Science Laboratory, ABAcard® p30 Internal Validation, 1998.
G. GL-2 (Safety Manual)
H. GL-6 (Purchasing)
I. Safety Data Sheets