Document Title: Rapid Immunoassay Tests for Human Semen
Controlled: Yes, with red stamp present
Controlled By: Quality Manager

Prepared By: _____ Date: _____

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A. PURPOSE:

To identify Semenogelin (RSID™-Semen) or p30 (ABAcard®) in Forensic samples.

B. RESPONSIBILITY:

Forensic Science Examiners from the Connecticut State Forensic Science Laboratory who have been trained in the discipline of rapid immunoassay tests for human semen according to SOP-FB-31 (Training Manual).

C. DEFINITIONS:

1. RSID™: Rapid Stain Identification
2. ABAcard®: Rapid Immunoassay

D. PROCEDURE:

RSID™-Semen and/or p30 ABAcard® tests will be performed at the discretion of the examiner based on the submitting agency requests, case information and the condition of the evidence.

1. Materials:
   a. Extracted samples
   b. RSID™-Semen test cassettes
   c. RSID™-Semen running buffer
   d. ABAcard® test device and enclosed dropper
   e. Microcentrifuge tubes
   f. Spot plates
   g. Micropipet and tips

2. Procedures:

   RSID™-Semen Test with Universal Buffer

   a. Bring extracted samples to room temperature before use (approximately 10 minutes).

   b. Label RSID™-Semen cassettes with case and item number.

   d. Using a micropipet, add 100µl of extract to well 'S' of the cassette. Note the time immediately after adding the sample.

D. 2. e. Monitor progress of test results for a 10 minute period. Record final result at 10 minutes. DO NOT record any changes which occur after 10 minutes. Any change in test results after 10 minutes is invalid.

f. Subsequently, the ABAcard® p30 procedure can be run with the remaining extract.

RSID™-Semen Test with Extraction Buffer or PBS

a. Bring extracted samples to room temperature before use (approximately 10 minutes).

b. Label RSID™-Semen cassettes with case and item number.

c. Using a micropipet, place 20µl of the supernatant into a microcentrifuge tube or spot plate and add 80µl of RSID™-Semen running buffer for a total extract volume of 100µl.

d. Add 100µl of extract to well 'S' of the cassette. Note the time immediately after adding the sample.

e. Monitor progress of test results for a 10 minute period. Record final result at 10 minutes. DO NOT record any changes which occur after 10 minutes. Any change in test results after 10 minutes is invalid.

f. Subsequently, the ABAcard® p30 procedure can be run with the remaining extract.

ABAcard® p30 Test

a. Bring extracted samples and test devices to room temperature before use (approximately 10 minutes).

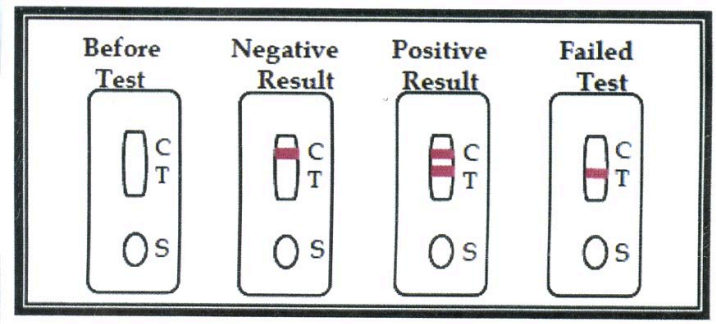
b. Label ABAcard® with case and item numbers.

c. Using the enclosed dropper, add 5-6 drops (~150-200µl) of supernatant to well "S" of the test device. Note the time immediately after adding the sample.

d. Monitor progress of test results for a 10-minute period. Record final result at 10 minutes. DO NOT record any changes which occur after 10 minutes. Any change in test results after 10 minutes is invalid.

D 3. Results and Interpretation:

RSID™-Semen Test



a. *Negative.* A visible red line at the Control 'C' position only, indicates a negative result. *No human semen detected or the sample is below the detectable level.*

aa. "High Dose Hook Effect" refers to weak a positive or false negative result seen when very high levels of human semen are present in the sample.

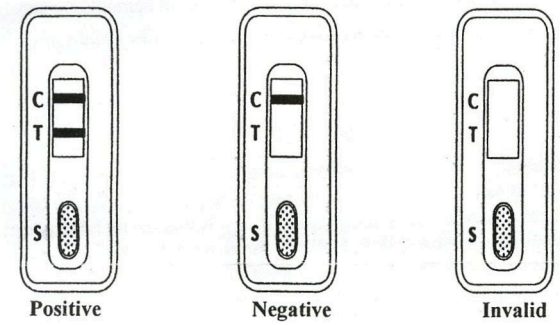
bb. In such cases or when a strong Acid Phosphatase reaction is observed, the extract may be diluted 1:20 and rerun with the RSID™-Semen Extraction Buffer or Universal Buffer procedure.

b. *Positive.* Visible red lines at both the Control 'C' and Test 'T' positions indicate a positive result. *Human semen detected.*

c. *Failed(Invalid).* No visible red line at the Control 'C' position indicates a failed test. *No conclusion possible.* Review the test procedure carefully and repeat the test with a new plate.

d. If a negative result is obtained, the ABAcard® p30 procedure below may be used.

ABAcard® p30 Test



D. 3. ABAcard® p30 Test

a. *Positive.* 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.

b. *Negative.* 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".

aa. "High Dose Hook Effect" refers to weak positive or false negative results due to the presence of a high concentration of p30 in the sample.

bb. In such cases or when a strong Acid Phosphatase reaction is observed, the extract may be diluted 1:20 and rerun with the ABAcard® Procedure.

c. *Invalid.* If there is no pink line visible in the control area 'C', the test is *inconclusive*. Review the test procedure carefully and repeat the test with a new plate.

4. Record the results of the control(s) and sample(s) on the appropriate Quality Record Worksheet.

E. REFERENCES:

1. Abacus Diagnostics' *OneStep* ABAcard® p30 Test For The Forensic Identification of Semen, provided Technical Information and Protocol sheet.

2. Connecticut State Forensic Science Laboratory, ABAcard® p30 Internal Validation, 1998.

3. Independent Forensics, Rapid Stain Identification of Human Semen (RSID™-Semen) Technical Information and Protocol sheets.

4. Old, Dr. Jennifer, Schweers, Dr. Brett A., Boonlayangoor, Dr. P. W., Reich, Dr. Karl, Developmental Validation Studies of RSID™-Semen Lateral Flow Immunochromatographic Strip test for the forensic detection of Seminal Fluid, p 1-36.

5. Connecticut State Forensic Science Laboratory, RSID™ - Semen Internal Validation, 2010.

6. Independent Forensics, RSID™ - Universal Buffer Technical Information and Protocol sheet.

7. Connecticut State Forensic Science Laboratory, RSID™ - Universal Buffer Internal Validation, 2011.