TITLE: Rapid Immunoassay for Human Blood

A. PURPOSE:

To identify glycophorin A (RSID™-Blood) or hemoglobin (ABAcard® HemaTrace®) 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 blood according to SOP-FB-31 (Training Manual).

C. DEFINITIONS:

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

D. PROCEDURE:

RSID™-Blood and/or HemaTrace® 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. RSID™ - Blood Extraction Buffer
   b. RSID™ - Blood Running Buffer
   c. RSID™ - Universal Buffer
   d. RSID™ - Blood test cassettes
   e. HemaTrace® extraction buffer
   f. 0.5% Ammonia
   g. pH paper
   h. ABAcard® test device and enclosed dropper
   i. Microcentrifuge tubes and spin baskets
   j. Spot plates
   k. Micropipet and tips
   l. Wooden sticks
   m. Shaker
   n. Ultrasonic bath
   o. Centrifuge

D. 2. Procedures:

RSID™ - Blood Test with Dual Buffers

a. Extract a portion of the questioned sample or stain in a microcentrifuge tube with 30 µl of extraction buffer and mix with a wooden stick.

b. Allow to extract on a shaker for a minimum of one (1) hour at room temperature or overnight at 4°C. A 30 minute sonication step may be included during the extraction process.

If sample was refrigerated, bring to room temperature before use (approximately10 minutes).

c. Place sample in a spin basket and centrifuge for five (5) minutes at 13,000 rpm.

d. In a microcentrifuge tube or spot plate, combine a maximum volume of 20µl of the extract with RSID™-Blood running buffer to a final volume of 100µl.

e. Label RSID™ cassettes with case and item numbers.

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

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

h. Subsequently, the HemaTrace procedure can be run either by re-extracting the same sample or by extracting a new portion of sample.

RSID™ - Blood Test with Universal Buffer

a. Extract a portion of the questioned sample in a microcentrifuge tube with 125µl of RSID Universal Buffer and mix with a wooden stick.

b. Allow to extract on a shaker for a minimum of one (1) hour at room temperature or overnight at 4°C. A 30 minute sonication step may be included during the extraction process.

If sample was refrigerated, bring to room temperature before use (approximately10 minutes).

c. Place sample in a spin basket and centrifuge for five (5) minutes at 13,000 rpm.

d. Label RSID™ cassettes with case and item numbers.

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

D. 2. RSID™ - Blood Test with Universal Buffer

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

g. Subsequently, the HemaTrace procedure can be run either by re-extracting the same sample or by extracting a new portion of sample.

ABAcard® HemaTrace®

a. Extract a portion of the questioned sample or stain in a microcentrifuge tube with approximately 5-6 drops (~150-200 µl) of HemaTrace® extraction buffer for a minimum of five (5) minutes at room temperature.

aa. Sample extract should be no darker than straw color; dilute with buffer as necessary to make a straw-colored extract.

bb. If necessary, sample can be extracted for a longer period of time on a shaker at room temperature or overnight at 4°C.

cc. Bring refrigerated extracts and test devices to room temperature before use (approximately 10 minutes).

b. 0.5% ammonia may be used to extract aged samples as follows:

aa. Place the sample in a microcentrifuge tube with enough 0.5% ammonia to cover the sample for a minimum of five (5) minutes at room temperature. Otherwise, extract the sample for a longer period of time on a shaker at room temperature or overnight at 4°C.

bb. Evaporate off excess ammonia from the extract for approximately 5-10 minutes at room temperature.

cc. Bring to a volume of approximately 150-200µl with HemaTrace® extraction buffer.

Mix thoroughly.

dd. Test the extract with pH paper. The pH of the extract must be between 1 and 9.

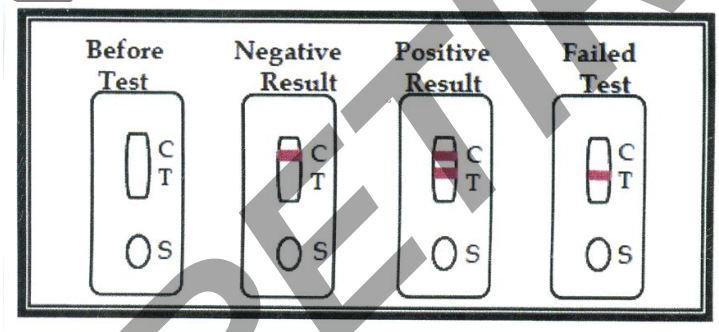
c. Label ABAcard® with case and item numbers.

d. Using the enclosed dropper, add 5-6 drops (~150 - 200µl) of extract to well "S" of the test device.

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

D. 3. Results:

RSID™ - Blood Test



a. *Negative.* A visible red line at the Control 'C' position only, indicates a *negative* result. *No human blood detected.*

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

bb. Under standard laboratory testing and relevant blood concentration ranges, the "High Dose Hook Effect" is not observed with the RSID™-Blood Test.

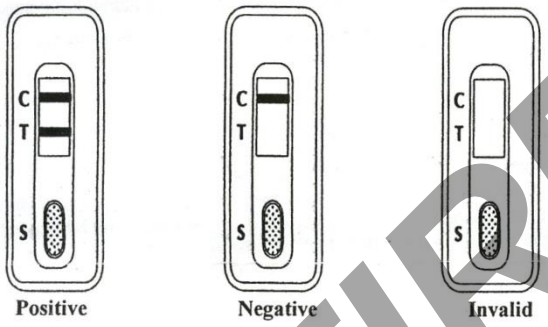
b. *Positive.* Visible red lines at both the Control 'C' and Test 'T' positions indicate a positive result. *Human blood detected.*

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® HemaTrace® procedure may be used or if an animal source is suspected, test the sample according to SOP-FB-09 (Species Double Diffusion Test).

ABAcard® HemaTrace®



D. 3. ABAcard® HemaTrace®

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 human hemoglobin level is present at or above 0.05μg/ml. Human hemoglobin has been detected.

b. *Negative.* If there is only one pink line in the control area 'C', the test result is *negative*. This indicates either that no human hemoglobin is present, the human hemoglobin level is below 0.05μg/ml or the presence of the "High Dose Hook Effect". Human hemoglobin has not been detected.

"High Dose Hook Effect" refers to weak positive or false negative results due to the presence of a high concentration of human hemoglobin in the sample. In such cases, the extract may be diluted 1:20 and re-run with the HemaTrace® 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.

d. If a negative result is obtained and an animal source is suspected, test the sample according to SOP-FB-09 (Species Double Diffusion Test).

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

E. REFERENCES:

1. Kristaly, A., Smith, D.A.S. Validation of the Onestep ABAcard® HemaTrace® for the rapid Forensic identification of human blood. 1999.

2. Connecticut State Forensic Science Laboratory, ABAcard HemaTrace Internal Validation, 2004

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

4. Schweers, Dr. Brett A., Old, Dr. Jennifer, Boonlayangoor, Dr. P. W., Reich, Dr. Karl, Developmental Validation of a Novel Lateral Flow Strip Test for Rapid Identification of Human Blood, Rapid Stain Identification - Blood, RSID™ - Blood, p1-13.

5. Connecticut State Forensic Science Laboratory, RSID™ - Blood Internal Validation, 2007.

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

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