

Document Title: Rapid Immunoassay Tests for Human Blood

Controlled: Yes, with red stamp present

Controlled By: Quality Manager

Prepared By: _____ Date: _____

Approved By: _____ Date: _____

A. PURPOSE:

To identify glycophorin A (RSID™-Blood) or hemoglobin (ABAcad® 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. ABAcad® 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™ - Blood test cassettes
 - d. HemaTrace® extraction buffer
 - e. 0.5% Ammonia
 - f. pH paper
 - g. ABAcad® test device and enclosed dropper
 - h. Microcentrifuge tubes and spin baskets
 - i. Spot plates
 - j. Micropipet and tips
 - k. Wooden sticks
 - l. Shaker
 - m. Ultrasonic bath
 - n. Centrifuge

D. 2. Procedures:

RSID™ - Blood Test

- 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 (approximately 10 minutes).
- c. Place sample in a spin basket and centrifuge for approximately five (5) minutes within the range of 10,000 – 14,000rpm (13,000 rpm is recommended).
- 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.

ABAcad® HemaTrace®

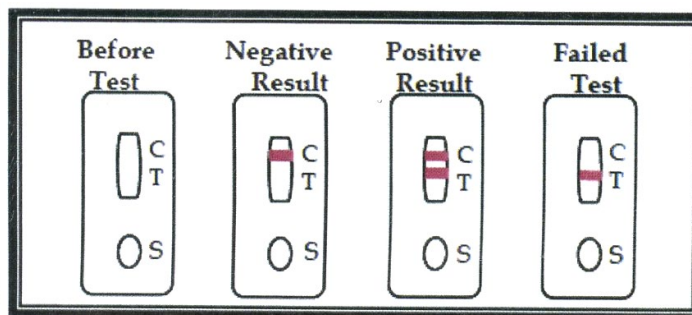
- a. Extract a portion of the questioned sample or stain in a microcentrifuge tube with approximately 5-6 drops (~150-200µl) of HemaTrace® extraction buffer for a minimum of five (5) minutes at room temperature.
 - aa. Sample extract should be no darker than straw color; dilute with buffer as necessary 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).

- D. 2. 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.
- cc. If 0.5% ammonia is used, record on the appropriate Quality Record Worksheet and the General Reagent Sheet (FBQR-09).
- 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.

3. Results:

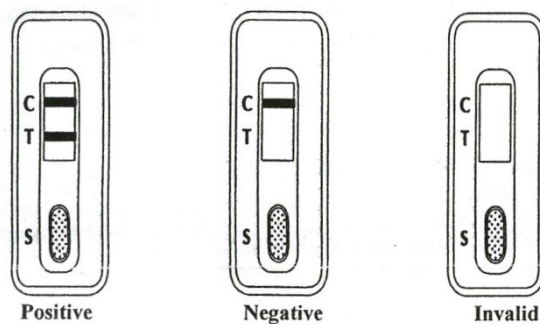
RSID™ - Blood Test



- a. *Negative.* A visible red line at the Control 'C' position only, indicates a *negative* result.
No Glycophorin A 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.
- D. 3. a. 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. *Glycophorin A detected.*
- c. *Inconclusive/Failed (Invalid).*
 - aa. 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.
 - bb. No distinguishable red line at the Test 'T' position.
- 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®



- 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.
 - 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".

"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. *Inconclusive/Invalid.*
 - aa. 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.
 - bb. If there is no distinguishable pink line in the test area 'T'.
- D. 3. c. bb. If there is no distinguishable pink line in the test area 'T'.
- 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.

Note: The reason a result is determined to be inconclusive must also be recorded.

5. Record test(s) used on the General Reagent Sheet (FBQR-09).

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.