RAPID IMMUNOASSAY FOR BLOOD

11.1 PURPOSE

11.1.1: To identify glycophorin A (RSID™-Blood) or hemoglobin (ABAcard® HemaTrace®) in Forensic samples.

11.1.2: To quality control new RSID™-Blood and ABAcard® HemaTrace® rapid immunoassay kits.

11.2 RESPONSIBILITY

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

11.2.2: Forensic Science Examiners in the Forensic Biology Unit. Ordering information is maintained in a log book in the Forensic Biology Unit. New kits are purchased according to GL-6 (Purchasing). For additional information, refer to the Biological Inventory in Appendix 2.

11.3 SAFETY

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

11.4 DEFINITIONS

A. RSID™: Rapid Stain Identification

B. ABAcard® HemaTrace®: Rapid Immunoassay

11.5 TEST PROCEDURE

RSID™-Blood and/or HemaTrace® Tests will be performed when deemed necessary and/or based on the submitting agency requests, case information and the condition of the evidence.

A. The RSID™ - Blood test is typically conducted when there is sufficient quantity of blood-like substance present to perform the test without compromising future DNA testing.

B. The ABAcard® HemaTrace® test is typically conducted when the RSID™ - Blood test is negative/inconclusive or when the sample appears limited/compromised.

C. A sample is considered limited/compromised when it appears to be of low quantity and/or in poor condition. The conditions the evidence may have been exposed to prior to submission shall be considered when assessing the sample tested and/or collected.

Extracts prepared from these types of samples should be retained according to section 1.5.4.V in FB SOP-01 (Evidence Examination).

11.5.1: Materials

A. RSID™ - Blood Extraction Buffer (RSID™ -EB)
B. RSID™ - Blood Running Buffer (RSID™ -RuB)
C. RSID™ - Blood test cassettes
D. HemaTrace® extraction buffer
E. 0.5% Ammonia
F. pH paper
G. ABAcard® test device and enclosed dropper
H. Centrifuge tubes and spin baskets
I. Spot plates
J. Micropipet and tips
K. Wooden sticks
L. Shaker
M. Ultrasonic bath
N. Centrifuge

11.5.2: RSID™ - Blood Test Procedure

A. Record the test used on the General Reagent Sheet (FBQR-09) and appropriate Quality Record Worksheet.

B. Extract a portion of the questioned sample or stain in a centrifuge tube with 30µl of extraction buffer and agitate to ensure sample is submerged.

C. Allow the sample to extract on a shaker for one (1) hour at room temperature. A 30 minute sonication step may be included during the extraction process.
If sample was refrigerated, bring to room temperature before use (approximately10 minutes).

D. Place sample in a spin basket and centrifuge for approximately five (5) minutes. The centrifuge will be set at approximately 13,000 rpm.

E. Add 80µl of RSID™-Blood running buffer to the extract.

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

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

H. Monitor the progress of the test result for a 10 minute period.
   1. Record the final result at 10 minutes.
   2. If a positive result is obtained prior to ten minutes, the test may be observed for up to but no longer than ten minutes.
   3. DO NOT record any changes that occur after 10 minutes. Any change in the test results after 10 minutes is invalid.

I. If the internal control fails (see Failed Test under RSID™-Blood results below), review the test procedure. If the quantity of sample allows retesting, repeat the test with a new cassette. If the test does not yield the appropriate results again, then inform the Unit Lead, determine the root cause and correct.

   If the quantity of sample limits retesting, the same portion of sample that was extracted for the RSID™ test may be retested according to the ABAcard® HemaTrace® procedure (section 11.5.3 below).

J. If a negative or inconclusive RSID™-Blood result is obtained and the quantity of sample is sufficient, test a new portion of sample according to the ABAcard® HemaTrace® procedure (section 11.5.3 below).

   If a negative or inconclusive RSID™-Blood result is obtained and the quantity of sample limits retesting, the same portion of sample that was extracted for the RSID™ test may be retested according to the ABAcard® HemaTrace® procedure (section 11.5.3 below).

K. If a negative result is obtained and an animal source is suspected, the sample may be tested according to FB SOP-10 (Species Double DiffusionTest).

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

11.5.3: ABAcard® HemaTrace® Procedure

A. Record the test used on the General Reagent Sheet (FBQR-09) and appropriate Quality Record Worksheet.

B. Extract a portion of the questioned sample or stain in a centrifuge tube with approximately 150-200µl (~5-7 drops with the enclosed dropper) of HemaTrace® extraction buffer for a minimum of five (5) minutes at room temperature. Agitate to ensure sample is submerged.

If the sample was previously tested with the RSID-Blood test, remove the extracted portion from the basket and place back into the centrifuge tube before adding HemaTrace® extraction buffer.

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

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

3. Bring refrigerated extracts to room temperature before use (approximately 10 minutes).

C. 0.5% ammonia may be used for samples that are difficult to extract as follows:

1. Place the sample in a centrifuge tube with enough 0.5% ammonia to cover the sample and extract for a minimum of five (5) minutes at room temperature. Agitate to ensure sample is submerged.

2. If necessary, the sample can be extracted on a shaker at room temperature or overnight at 4°C.

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

4. Bring to a volume of approximately 150-200µl with HemaTrace® extraction buffer. Mix (vortex) thoroughly.

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

6. Record the use of 0.5% if ammonia on the appropriate Quality Record Worksheet and the General Reagent Sheet (FBQR-09).

D. Label ABAcard® with case and item numbers.

E. Using a micropipet, add approximately 150-200µl (~5-7 drops with the enclosed dropper) of extract to well "S" of the test device.

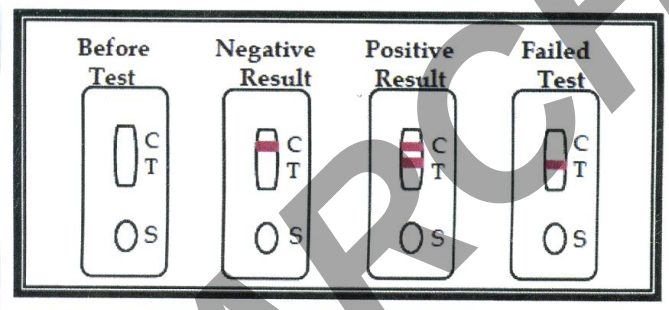
F. Monitor the progress of the test result for a 10 minute period.
   1. Record the final result at 10 minutes.
   2. If a positive result is obtained prior to 10 minutes, the test may be observed for up to but no longer than 10 minutes.
   3. DO NOT record any changes that occur after 10 minutes. Any change in test results after 10 minutes is invalid.

G. If the internal control fails (see Invalid Test under ABAcard® HemaTrace® 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, determine the root cause and correct.

H. If a negative result is obtained and an animal source is suspected, the sample may be tested according to FB SOP-10 (Species Double Diffusion Test).

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

11.5.4: RSID™ - Blood Test Results



A. Negative
A visible red line at the Control ‘C’ position only, indicates a negative result. No Glycophorin A detected.
   1. “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.

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

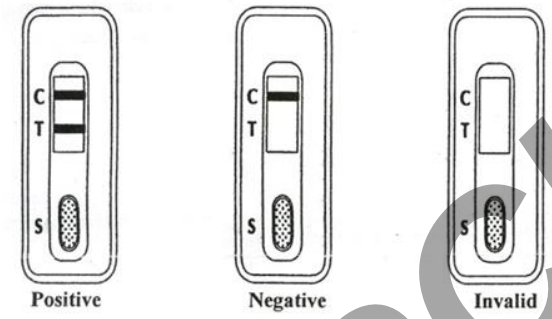
B. Positive
Visible red lines at both the Control ‘C’ and Test ‘T’ positions indicate a positive result. Glycophorin A detected.

C. Inconclusive
   1. Not able to determine a red line at the Test 'T' position.
   2. Record the reason a result is determined to be inconclusive on the appropriate Quality Record Worksheet.

D. Failed (Invalid)
   1. No visible red line at the Control 'C' position.
   2. If there is not enough sample to repeat the test then no conclusion is possible.
   3. Record the reason the test failed on the appropriate Quality Record Worksheet.

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

11.5.5: 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 0.05ug/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 0.05ug/ml or the presence of the "High Dose Hook Effect".

   1. "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.

   2. In such cases, the extract may be diluted 1:20 and re-run with the HemaTrace® Procedure.

C. Inconclusive
   1. If a pink line in the test area 'T' could not be determined.
   2. Record the reason a result is determined to be inconclusive on the appropriate Quality Record Worksheet.

D. Invalid (Failed)
   1. 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. Record the reason the test failed on the appropriate Quality Record Worksheet.

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

11.5.6: ***Suggested Report Statements***

A. Positive

[ ] gave a positive result(s) with an immunological test(s) for the presence of human glycophorin A/human hemoglobin, a component(s) of human blood.

B. Negative

An immunological test(s) for the presence of human glycophorin A/human hemoglobin, a component(s) of human blood, was/were performed on [ ]. Human blood was not detected with this/these test(s).

C. Inconclusive

An immunological test(s) for the presence of human glycophorin A/human hemoglobin, a component(s) of human blood, was/were performed on [ ]. Due to indeterminate results and/or substrate interference, this/these test(s) was/were determined to be inconclusive.

D. Failed/Invalid

An immunological test(s) for the presence of human glycophorin A/human hemoglobin, a component(s) of human blood, was/were performed on [ ]. Due to the failure of this/these test(s), no conclusion(s) is/are possible.

11.6 QC PROCEDURE

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

11.6.1 RSID™ - Blood

A. Test the new lot before use according to the test procedure and the RSID™ - Blood Reagent Log Sheet. Record the required information.

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 then inform the Unit Lead, determine the root cause and correct.

C. If the lot is suitable for use, record the date received, date opened and examiner's initials on each box and the provided buffer bottles. Store according to manufacturer's instructions.

D. Discard according to the manufacturer's expiration date.

11.6.1.1: Running a Buffer Blank

A. Buffers will be tested prior to each use with questioned samples (not more than once per day) by mixing 80µl of RSID™-Blood running buffer and 20µl of RSID™-Blood extraction buffer and adding the mixture to the RSID™-Blood cassette (see steps G and H in section 11.5.2).

B. Record the results on the Buffer Log Sheet (see section 11.5.4 above).

C. Notify the Unit Lead if the expected results are not obtained, remove from service and replace.

11.6.2: ABAcard® HemaTrace®

A. Test the new lot before use according to the test procedure and the ABAcard® HemaTrace® Reagent Log Sheet and record required information.

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 then inform the Unit Lead, determine the root cause and correct.

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

D. Discard according to the manufacturer's expiration date.

E. Please refer to FB SOP-08 for the Preparation/QC procedure for 0.5% ammonia.

11.7 REFERENCES

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

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

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

D. 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.

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

F. GL-2 (Safety Manual)

G. GL-6 (Purchasing)

H. Safety Data Sheets