RAPID IMMUNOASSAYS FOR BLOOD

11.1 PURPOSE

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

A. Theory

Both tests use an immunochromatographic lateral flow assay to detect their respective blood components. Each assay takes place on a chromatographic strip within a test cassette. This strip is comprised of three distinct areas. The conjugate pad, located beneath the sample window, is where the sample is deposited. The test membrane, viewed through the results window, is where the extract flows during testing and where results can be read. The wick, located within the cassette, is where the sample is absorbed, preventing sample backflow.

1. For Glycophorin A Identification (RSID™-Blood):

Two monoclonal antibodies are used for this test. One monoclonal antibody (mobile) is conjugated to colloidal gold and deposited onto the conjugate pad. The other monoclonal antibody (immobile) is embedded onto the "Test line" of the test membrane. An anti-IgG antibody (immobile) is used as an internal control and is embedded onto the "Control line" of the test membrane.

When an extract of the questioned sample is added to the sample window, it diffuses through the conjugate pad, dissolving the gold-conjugate antibodies. If human glycophorin A (antigen) is present in the questioned sample, antigen/antibody-colloidal gold complexes will form. The questioned sample extract and antibodies (complexed and free) are transported by bulk fluid flow to the test membrane.

The embedded anti-glycophorin A antibodies on the Test line capture the glycophorin A antigen/antibody-colloidal gold complexes. When a sufficient number of complexes have been captured, a red line at the Test position is produced.

If no human glycophorin A is present in the sample, antigen/antibody-colloidal gold complexes do not form and will not be captured at the Test position. Therefore, no red line will form at this position.

The anti-IgG antibody on the Control line captures the colloidal gold conjugated antibodies flowing past the Test line position, producing a red 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.

Note:

a. There is no known cross-reactivity with blood tested from animals (ferret, skunk, opossum, dog, cat, cow, pig, chicken, owl, horse, goat, turtle, elk, deer, tiger, alpaca), including higher primates (orangutan, gorilla, spider monkey, bonobo, and baboon).
b. There is no known cross-reactivity with human saliva, semen, breast milk, amniotic fluid, vaginal fluid or urine.
c. Under standard laboratory testing and relevant blood concentration ranges, users will not observe false negative results due to the High Dose Hook Effect.
d. It is reported that this test can detect as little as 1 microliter of human blood.

2. <u>For Hemoglobin Identification (ABAcard® HemaTrace®):</u>
The chromatographic strip within the test device contains an excess of mobile pink-dye tagged anti-hHb antibodies located on the area of the strip underneath the sample well. Embedded anti-hHb 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.

When an extract of the questioned sample is deposited in the sample well, it dissolves the tagged antibodies. If human Hb (antigen) is present in the sample, antigen/tagged antibody complexes will form and be transported by bulk fluid flow through the length of the test membrane.

The embedded anti-hHb antibodies on the Test line capture the hHb antigen/tagged antibody complexes, creating antibody/antigen/tagged antibody "sandwiches". When a sufficient quantity of sandwiches are created at the Test line, a pink line will appear.

If no hHb is present in the questioned sample, the antigen/tagged antibody complexes do not form in the sample well area and the antibody/antigen/tagged antibody "sandwiches" cannot be formed at the Test line position. Therefore, no pink line will develop at this position.

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 for Glycophorin A (RSID™-Blood):
The viscosity of an undiluted blood sample prevents proper release of the conjugate from the conjugate pad. Therefore, undiluted blood should not be used.

C. Limitations for Hemoglobin (ABAcard® HemaTrace®):
1. This test is not human specific and is known to cross react with blood from higher primates and the weasel family.
2. The High Dose Hook Effect may occur with a very high concentration of human hemoglobin, causing a false negative result.

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

11.2 RESPONSIBILITY

11.2.1: Test Procedure – Personnel qualified to perform Forensic Biology duties.
11.2.2: Preparation/QC Procedure – Personnel qualified to perform Forensic Biology duties. Ordering information is maintained in a logbook 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.

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/ABBREVIATIONS

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

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 or compromised.

C. A sample is considered limited when it appears to be of low quantity or compromised when it appears to be 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.

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. ABAcard® test device and enclosed dropper
F. Centrifuge tubes and spin baskets
G. Spot plates
H. Micropipette and tips
I. Forceps
J. Scissors/scalpel(s)
K. Wooden sticks
L. Shaker
M. Centrifuge

11.5.2: RSID™-Blood Test Procedure

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

B. The RSID™-Blood extraction buffers will be tested prior to each use. See section 11.6.1.1 below.

C. 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.

D. Allow the sample to extract on a shaker for approximately one (1) hour at room temperature.

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

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

G. Mix 20µl of extract with 80µl of RSID™-Blood Running Buffer.

H. Using a micropipette, add 100µl of the extract/running buffer mixture to well 'S' of the cassette. Note the time immediately after adding the sample.

I. Monitor the progress of the test result for up to 10 minutes.
   1. Record the final result no later than 10 minutes.
   2. DO NOT record any changes that occur after 10 minutes. Any change in the test result after 10 minutes is invalid.
   3. A second qualified examiner will observe/confirm the result and initial/date the appropriate QRW(s).

J. 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 to try to determine the root cause.

   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).

K. If a negative or inconclusive RSID™-Blood result is obtained and the quantity of sample is sufficient, a new portion of sample may be tested 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).

L. Record the sample test result and document that the test ran appropriately (i.e. controls ok) on the appropriate QRW(s).

11.5.3: ABAcard® HemaTrace® Test Procedure

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

B. Extract a portion of the questioned sample or stain in a centrifuge tube with 150µl of HemaTrace® extraction buffer for a minimum of five (5) minutes at room temperature. Agitate to ensure the 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, the 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. Label ABAcard® with case and item numbers.

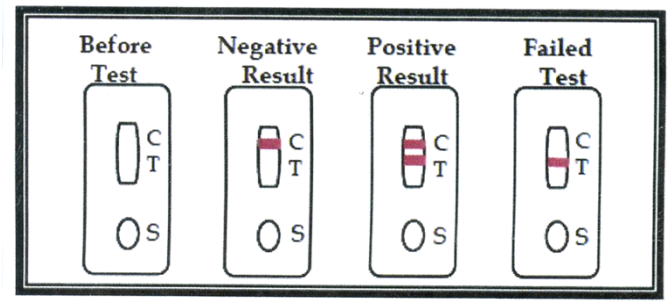
D. Using a micropipette, add 80µl of extract to well 'S' of the test device. Note the time immediately after adding the sample.

E. Monitor the progress of the test result up to 10 minutes.
1. Record the final result no later than 10 minutes.
2. DO NOT record any changes that occur after 10 minutes. Any change in the test result after 10 minutes is invalid.
3. A second qualified examiner will observe/confirm the result and initial/date the appropriate QRW(s).

F. 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 to try to determine the root cause.

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

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.

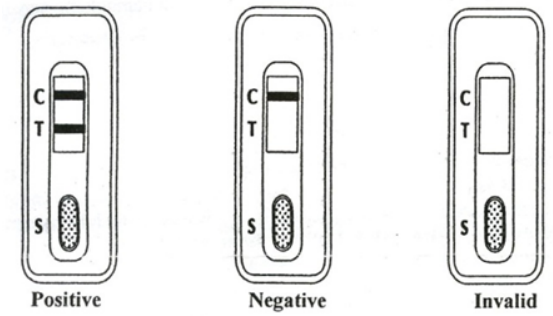
B. Positive
Visible red lines at both the Control ‘C’ and Test ‘T’ positions indicate a positive result. Glycophorin A detected.

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 QRW(s).

D. Failed (Invalid)
1. No visible red line at the Control ‘C’ position due to a migration failure or other run failure.
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 QRW(s).

E. It should be noted that any result above does not preclude the sample from being forwarded for DNA analysis, as determined on a case-by-case basis.

11.5.5: ABAcard® HemaTrace® Test Results



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 QRW(s).

D. Invalid (Failed)

1. There is no pink line visible in the control area 'C' due to a migration failure or other run failure.
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 QRW(s).

E. It should be noted that any result above does not preclude the sample from being forwarded for DNA analysis, as determined on a case-by-case basis.

11.5.6: Suggested Report Statements and Conclusions

A. Positive

1.

Testing Performed	Results	Conclusion
Immunological – Human Glycophorin A	Positive	Human blood detected
Immunological – Human Hemoglobin	Positive	Human blood indicated

Appendix:
Human Glycophorin A is a component of human blood.
Human hemoglobin is a component of human blood.

2. *[ ] 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

1.

Testing Performed	Results	Conclusion
Immunological – Human Glycophorin A	Negative	Human blood not detected
Immunological – Human Hemoglobin	Negative	Human blood not detected

Appendix:
Human Glycophorin A is a component of human blood.
Human hemoglobin is a component of human blood.

2. *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

1.

Testing Performed	Results	Conclusion
Immunological – Human Glycophorin A	Indeterminate	Inconclusive[1]
Immunological – Human Hemoglobin	Indeterminate	Inconclusive[1]

Comment:
[1]Due to an indeterminate result and/or substrate interference, this test was determined to be inconclusive.

Appendix:
Human Glycophorin A is a component of human blood.
Human hemoglobin is a component of human blood.

2. *An immunological test for the presence of human glycophorin A/human hemoglobin, a component of human blood, was performed on [ ]. Due to an indeterminate result and/or substrate interference, this test was determined to be inconclusive.*

D. Failed/Invalid

1.

Testing Performed	**Results**	**Conclusion**
Immunological – Human Glycophorin A	Failed Test	No conclusion possible
Immunological – Human Hemoglobin	Failed Test	No conclusion possible

Appendix:
Human Glycophorin A is a component of human blood.
Human hemoglobin is a component of human blood.

2. *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.*

E. See FB SOP-05 (Case Records and Reports) for additional information.

11.6 QC PROCEDURE

11.6.1 RSID™ - Blood

A. Test the new lot before use according to the test procedure and the RSID™-Blood Reagent Log Sheet (Appendix 2). Record the required information.

A second qualified examiner will observe/confirm the result and initial/date the appropriate QRW(s).

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 the provided buffer bottles. Store according to the manufacturer's instructions.

The lot is considered acceptable for use when a positive result is obtained with the human blood control and a negative result is obtained with the blank/negative control.

D. Discard 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.

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 through J in section 11.5.2).

B. Record the results on the Buffer Log Sheet (Appendix 2), see section 11.5.4 above.

A second qualified examiner will observe/confirm the results and initial/date the Buffer Log Sheet.

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 (Appendix 2). Record the required information.

A second qualified examiner will observe/confirm the result and initial/date the appropriate QRW(s).

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 considered acceptable for use when a positive result is obtained with the human blood control and a negative result is obtained with the blank/negative 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.

11.7 REFERENCES

A. Abacus Diagnostics, Inc., ABAcard® HemaTrace® for the Forensic Identification of Human Blood, Technical Information Sheet, Rev: 07/13.
B. Kristaly, A., Smith, D.A.S. Validation of the Onestep ABAcard® HemaTrace® for the rapid Forensic identification of human blood, 1999.
C. Connecticut State Forensic Science Laboratory, ABAcard HemaTrace Internal Validation, 2004.
D. Independent Forensics, Rapid Stain Identification of Human Blood (RSID™-Blood) Technical Information and Protocol sheet.
E. 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.
F. Connecticut State Forensic Science Laboratory, RSID™-Blood Internal Validation, 2007.
G. Li, Richard, Forensic Biology Second Edition, CRC Press, FL, 2015:
Chapter 11: "Serology Techniques", pp. 200 (11.1.4) - 204 (11.2.2)
Chapter 13: "Species Identification", pp. 245-250
H. GL-2 (Safety Manual)
I. GL-6 (Purchasing)
J. Safety Data Sheets