RAPID IMMUNOASSAY FOR URINE

17.1 PURPOSE

17.1.1: To identify Tamm-Horsfall Protein (RSID™-Urine) in Forensic samples.

A. Theory

The RSID™-Urine test uses an immunochromatographic lateral flow assay to detect the presence of Tamm-Horsfall glycoprotein (THP) in questioned stains to identify urine.

1. The chromatographic strip within the test cassette 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 results can be read; and the wick, located within the cassette, is where the sample is absorbed, preventing sample backflow.

2. Two polyclonal antibodies are used for this test. One polyclonal antibody (mobile) is conjugated to blue latex beads and deposited onto the conjugate pad. The other polyclonal 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.

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

4. The embedded anti-THP antibodies on the Test line capture the THP antigen/antibody-blue latex bead complexes, producing a blue line at the Test position.

5. If no THP is present in the questioned sample, THP antigen/antibody-blue latex bead complexes do not form and will not be captured at the Test position. Therefore, no blue line will form at this position.

6. The anti-IgG on the Control line captures free antibody-blue latex bead conjugates flowing past the Test line position, producing a blue 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

1. This test is not human-specific.
2. Fecal material is a known inhibitor and its presence may result in a failed test.
3. Blood may inhibit the signal which can cause a false negative result.
4. Undiluted urine should not be used with this test.
5. Minor High Dose Hook Effect may occur which can cause a slight decrease in signal.

17.1.2: To quality control new RSID™-Urine rapid immunoassay kits.

17.2 RESPONSIBILITY

17.2.1: Forensic Science Examiners (however titled) from the Division of Scientific Services who have been trained in the discipline of the rapid immunoassay test for urine according to FB SOP-26 (Training Manual and Checklist).

17.2.2: 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 (Appendix 3) in the FB folder on the shared drive.

17.3 SAFETY

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

17.4 DEFINITIONS

A. RSID™: Rapid Stain Identification
B. THP: Tamm-Horsfall Protein
C. QRW(s): Quality Record Worksheet(s) (Appendix 1)

17.5 TEST PROCEDURE

A. This test will be performed at the discretion of the examiner, with input from the Unit Lead(s), based on the submitting agency requests, case information and the condition of the evidence.

B. Do not use this test with samples containing fecal-type material, refer to FB SOP-18 (Test for Fecal Material).

17.5.1: Materials

A. RSID™-Urine Buffer
B. RSID™-Urine test cassettes
C. Centrifuge tubes and spin baskets
D. Micropipette and tips
E. Wooden sticks
F. Ultrasonic bath
G. Shaker
H. Centrifuge
I. Sterile disposable pipets
J. Filter paper or sterile swabs

17.5.2: Procedure:

A. Record the lot numbers of the test and buffer used on the General Reagent Sheet (FBQR-09).

Note: See section 17.6.2 for running a buffer blank prior to sample extraction.

B. Do not use liquid urine with this test. A stain of the questioned urine sample may be made as follows:
   1. Allow the cellular material in the sample to settle toward the bottom of the container.
   2. Using a sterile disposable pipet, draw up the bottom sediment and make a stain on filter paper or sterile swabs.
   3. Allow to air dry under a hood.

   Note: A portion of this stain may be forwarded for DNA analysis if necessary.

C. Extract a portion of the questioned sample or stain in a centrifuge tube with 150ul of RSID™-Urine Buffer and agitate to ensure sample is submerged.

D. Place the sample in an ultrasonic bath for fifteen (15) minutes and then place on the shaker for one (1) hour and forty-five (45) minutes at room temperature for a total extraction time of two (2) hours.

E. If necessary, the sample may be extracted overnight at 4°C after the sonication step.

F. If the sample was refrigerated, bring to room temperature for approximately ten (10) minutes before testing.

G. Place the sample in a spin basket, return to tube and centrifuge for approximately ten (10) minutes. The centrifuge will be set at approximately 13,000 rpm.

H. Label the RSID™ cassette with the case and item number.

I. Using a micropipette, add 100µl of extract to well 'S' of the cassette. Note the time

immediately after adding the sample.

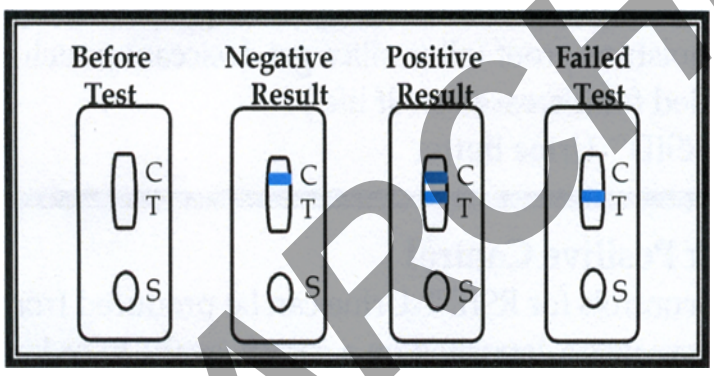
J. Monitor the progress of the test results for a fifteen (15) minute period.
   1. Record final result at fifteen (15) minutes.
   2. DO NOT record any changes that occur after fifteen (15) minutes. Any change in the test results after fifteen (15) minutes is invalid.

K. If the internal control fails (see Failed Test under results below), review the test procedure and, 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.

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

M. A second qualified examiner will observe and confirm the results and initial the appropriate QRW.

17.5.3: Results and Conclusions



A. <u>Negative</u>
   1. A visible blue line at the Control 'C' position only, indicates a negative result. No Tamm-Horsfall Protein detected.

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

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

   2. Suggested Report Wording:

a.

Testing Performed	Result	Conclusion
Tamm-Horsfall Protein	Negative	Urine not detected

Appendix:
Tamm-Horsfall protein is a component of urine.

b. *An immunological test for the presence of Tamm-Horsfall protein, a component of urine, was performed on [ ]. Urine was not detected with this test.*

B. Positive

1. Visible blue lines at both the Control 'C' and Test 'T' positions indicate a positive result. Tamm-Horsfall Protein detected.

2. Suggested Report Wording:

a.

Testing Performed	Result	Conclusion
Tamm-Horsfall Protein	Positive	Urine indicated

Appendix:
Tamm-Horsfall protein is a component of urine.

b. *[ ] gave a positive result(s) with an immunological test for the presence of Tamm-Horsfall protein, a component of urine.*

C. Inconclusive

1. Not able to determine a blue line at the Test 'T' position.

2. Suggested Report Wording:

a.

Testing Performed	Result	Conclusion
Tamm-Horsfall Protein	Indeterminate	Inconclusive[1]

Appendix:
Tamm-Horsfall protein is a component of urine.
[1]Due to indeterminate results and/or substrate interference, this/these test(s) was/were determined to be inconclusive.

b. *An immunological test for the presence of Tamm-Horsfall protein, a component of*

urine, was performed on [ ]. Due to indeterminate results and/or substrate interference, this/these test(s) was/were determined to be inconclusive.

3. Record the reason a result is determined to be inconclusive on the appropriate QRW.

D. Failed (Invalid)

1. No visible blue line at the Control 'C' position.

2. If there is not enough sample to repeat the test then no conclusion is possible.

3. Suggested Report Wording:

a.

Testing Performed	**Result**	**Conclusion**
Tamm-Horsfall Protein	Failed	No conclusion possible

Appendix:
Tamm-Horsfall protein is a component of urine.

b. *An immunological test for the presence of Tamm-Horsfall protein, a component of urine, was performed on [ ]. Due to the failure of this/these test(s), no conclusion(s) is/are possible.*

4. Record the reason the test failed on the appropriate QRW.

17.6 QC PROCEDURE

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

17.6.1: Lot QC

A. Test the new lot before use according to the test procedure and the RSID™ - Urine 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 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 manufacturer's instructions.

1. The lot is acceptable for use when a positive result is obtained with the urine control and a negative result is obtained with a blank/negative control.

2. A second qualified examiner will observe and confirm the results and initial the RSID™ - Urine Reagent Log Sheet.

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

17.6.2: Running a Buffer Blank

A. Buffer will be tested prior to use with each case but no more often than once a week, by adding 100µl directly to the RSID™ cassette (see steps I and J in section 17.5.2 above).

B. Record the results on the Buffer Log Sheet (see section 17.5.3 above).

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

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

17.7 REFERENCES

A. Independent Forensics, Rapid Stain Identification of Urine (RSID™ - Urine) Technical Information and Protocol sheet.
B. Old, Dr. Jennifer, Reich, Dr. Karl, Developmental Validation of RSID™ - Urine, p1-19.
C. Connecticut State Forensic Science Laboratory, RSID™ - Urine Internal Validation, 2012.
D. GL-2 (Safety Manual)
E. GL-6 (Purchasing)
F. Safety Data Sheets