BOCA RATON POLICE SERVICES DEPARTMENT
BIOLOGICAL PROCESSING LABORATORY

MEMORANDUM

TO: **Amy Flagler, FSM**

FROM: **Kristen Archuleta, BPL Analyst**
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DATE: **September 16, 2024**

SUBJECT: **Urine Testing Internal Validation Report**

INTRODUCTION

The indication of urine can yield important insights in forensic casework. There are several off-the-shelf products on the market for urine testing, most of which involve chemical color change tests. RSID™-Urine was evaluated for use at the Boca Raton Police Services Department (BRPD) Biological Processing Laboratory (BPL).

Independent Forensics' RSID™-Urine tests for the presence of Tamm-Horsfall protein (THP) using an immunochromatographic assay. Also known as uromodulin, THP plays a significant role in kidney function and is the most abundant protein in human urine. If THP is present in the sample, it will bind to blue latex bead-labeled rabbit polyclonal anti-THP antibody in the sample well. The antigen-antibody complex will migrate across the test membrane by capillary action. The test (T) region contains immobilized rabbit polyclonal anti-THP antibody. The control (C) region contains immobilized anti-rabbit immunoglobulin G antibody. As dye-labeled antigen-antibody complexes are captured by the immobilized anti-THP antibody in the test region and accumulate, a line will form. Free dye-labeled antibody that was unbound in the sample well will bind to the immobilized immunoglobulin G in the control area, forming a line as they accumulate.

OVERVIEW

RSID™-Urine was selected for evaluation for potential implementation as the urine test method at BPL. This internal validation study was designed and conducted to satisfy ANSI/ASB Standard 077 *Standard for the Developmental and Internal Validation of Forensic Serological Methods*. All currently employed analytical personnel at BPL were involved in sample preparation and the validation studies conducted as summarized herein.

PROCEDURE

The manufacturer's Frequently Asked Questions page provides a shortened incubation protocol of 20 minutes in comparison to the originally published method, which specified a 1-2 hour incubation. Per correspondence with the manufacturer, 1 hour incubation was recommended and selected for validation purposes:

1. Place a small cutting of the stain or swab(s) into a microcentrifuge tube with 200µL RSID™-Urine Buffer.

2. Incubate for 1 hour at room temperature, frequently vortexing.
3. Add 100µL of the eluate to the sample well of the RSID™ card.
4. Read the results at 15 minutes; alternatively, a positive result may be read within 15 minutes.
    a. Positive: 2 lines (1 in the control region, 1 in the test region).
    b. Negative: 1 line in the control region.
    c. Invalid: No line in the control region. Test must be repeated.
    d. Inconclusive: 1 line in the control region, uncertainty about the presence or absence of a line in the test region.

DEVELOPMENTAL VALIDATION

Records of the published validation study (Independent Forensics, n.d.) were obtained online and will be retained at BPL, accessible to all laboratory personnel. The developmental validation predates the publication of the ANSI/ASB Standard 077 *Standard for the Developmental and Internal Validation of Forensic Serological Methods* and may not reflect all studies specified by Standard 077. Where possible, these elements were incorporated into the internal validation study.

STUDY RESULTS

Control Study

Control studies were performed to establish the control samples needed for each procedure, the frequency with which the controls will be performed, and the performance expectations for each control. The test under evaluation is purchased and will be handled as a single "lot" from the manufacturer. The control study was repeated three weeks after the initial date of testing to ensure that there is consistency in test performance across a single manufacturer's lot. Positive controls were prepared by adding approximately 100 µL neat urine to cotton swabs. A cotton swab was used as the negative control for RSID™-Urine.

Sample	Result
Positive Control (Neat Urine)	+
Negative Control (Cotton Swab)	N
Positive Control (Neat Urine) – 3 weeks	+
Negative Control (Cotton Swab) – 3 weeks	N

RSID™-Urine showed no performance differences after three weeks when stored as recommended by the manufacturer. There was no performance difference across the lot detected during the control study.

Based on the results of the control study, positive and negative controls will be required to verify the lot's performance prior to implementation in casework. Positive and negative controls do not need to be repeated on the day of analysis once a lot has been verified.

Sensitivity Study

Sensitivity studies are performed to determine the upper and lower limits of a test to accurately detect the analyte of interest and are performed using serial dilutions. Since neat urine was tested as the positive control and concentrated urine samples are unlikely to be forensically relevant, the sensitivity

study was performed to determine the lower limit of test performance (limit of detection). Dilutions were prepared using deionized water. Due to the variability observed between donors in the developmental validation study, the sensitivity study was conducted using samples prepared from three donors, one male and two female. All samples were prepared from the donor's first urine output of the day. Approximately 100 µL was applied to each swab and allowed to dry completely prior to testing. The Donor 1 and Donor 2 samples were tested by Scientist 1 and the Donor 3 samples were tested by Scientist 2. Due to the results obtained from Donor 1, all prepared dilutions from Donors 2 and 3 were not tested to preserve resources.

Sample	Donor 1 (F)	Donor 2 (M)	Donor 3 (F)
1:2 Urine Dilution	+	+	+
1:5 Urine Dilution	N	+	+
1:10 Urine Dilution	N	N	N
1:50 Urine Dilution	N	Not performed	Not performed
1:100 Urine Dilution	N	Not performed	Not performed
1:500 Urine Dilution	N	Not performed	Not performed
1:1000 Urine Dilution	N	Not performed	Not performed

*Retested to clarify the limit of detection.

As observed in the developmental validation study, THP levels are variable for a single donor across a single day and vary between donors. The sample from Donor 1 appeared to have a lower level of THP than those collected from Donors 2 and 3. RSID™-Urine could reproducibly detect 1:2 dilutions of urine. Results were variable or negative at greater dilutions; therefore, 1:2 dilution of urine will be considered the limit of detection.

Repeatability Study

Repeatability studies were performed to verify the results of the test by the same personnel. The scientist who performed testing of the Donor 1 samples in the sensitivity study (Scientist 1) performed two additional tests of the 1:10 urine dilution to assess the repeatability of their results. Additional swabs from Donor 1 prepared concurrently with those used in the sensitivity study (same prepared dilution solutions) were used for the repeatability study.

Sample	Result
1:10 Urine Dilution (Test 1, from Sensitivity Study)	N
1:10 Urine Dilution (Test 2)	N
1:10 Urine Dilution (Test 3)	N

The repeatability study demonstrated that the test results were repeatable when a sample was retested under the same testing conditions.

Reproducibility Study

Reproducibility studies are performed to assess the ability to obtain the same test results when an experiment is repeated between different operators. A second scientist (Scientist 3) repeated a subset

of the samples from the sensitivity study and their results were compared to the results obtained by Scientist 1. Additional swabs from Donor 1 prepared concurrently with those used in the sensitivity study (same prepared dilution solutions) were used for the reproducibility study.

Sample	Result
1:10 Urine Dilution (Scientist 3)	N
1:100 Urine Dilution (Scientist 3)	N
1:1000 Urine Dilution (Scientist 3)	N

The reproducibility study indicated that two examiners testing similar samples under the same test conditions produce consistent results.

Population Study

Population studies are performed to determine the effectiveness and utility of the test with representative samples from the population. Due to the variability observed throughout the day within and between donors in the developmental validation study, three donors their first five urine samples of the day for evaluation. Approximately 100 µL was applied to each swab and allowed to dry completely prior to testing.

Sample	Donor 1 (F)*	Donor 2 (M)	Donor 3 (F)
Sample 1	+	+	+
Sample 2	+	+	+
Sample 3	N	+	+
Sample 4	+	+	+
Sample 5	+	+	+

*Donor 1 samples were originally tested with very small cuttings and all yielded negative results. The same samples were repeated using larger cuttings consistent with the cutting size used by the other two scientists.

As observed in the developmental validation study, THP levels are variable for a single donor across a single day and vary between donors. In the BPL internal validation study, Donor 1's third sample demonstrated a level significantly higher (resulting in high dose hook effect) or significantly lower (resulting in a false negative) than the remaining samples collected on the same day. It may be recommended to dilute a sample 1:2 and re-testing if a false negative result is suspected in casework. Due to cassette availability and the demonstrably lower levels of THP for this donor in the sensitivity study, dilution and re-testing of this sample was not conducted in the validation study.

Donors 2 and 3 did not demonstrate variability in their THP levels over the course of a day to the degree that test results were impacted.

Contamination Study

Contamination studies are performed to assess the risk that unintended material may be introduced into a sample from test components, instrumentation, the operator, or test procedures. No instrumentation is required for the performance of RSID™-Urine. Positive and negative control samples were completed side-by-side for the control study on the first day of testing and when repeated three

weeks later. No cross-contamination was observed from test components or the operator following the test procedures in the laboratory environment.

BPL assessed the risk of unintended material introduced into the sample prior to submission of evidence to the laboratory to see if sample contaminants or substrates may interfere with either test. Samples were prepared as follows:

- Cleaning solutions (e.g. reagent alcohol, bleach): approximately 100 µL applied to cotton swabs.
- Cleaning solutions applied to neat urine: approximately 100 µL urine applied to cotton swabs, dried, and subsequently saturated in approximately 100 µL solution.
- Urine on substrates (e.g. fabrics): approximately 100 uL urine applied to substrate.
- Consumption samples: approximately 100 µL urine applied to cotton swabs.

Sample	Result
Reagent Alcohol	N
Reagent Alcohol Applied to Neat Urine	+
Bleach	N
Bleach Applied to Neat Urine	N
Hydrogen Peroxide	N
Hydrogen Peroxide Applied to Neat Urine	+
Windex	N
Windex Applied to Neat Urine	+
Neat Urine on Denim	+
Neat Urine on Green Fabric	+
Neat Urine in Dirt/Soil	+ (non-human)
Urine Collected After Soda Consumption	N
Urine Collected After Coffee Consumption	N
Urine Collected After Alcohol Consumption*	+

*Sample was collected voluntarily on a non-working day.

Commonly encountered cleaning products and first aid supplies (alcohol, bleach, hydrogen peroxide, and Windex) did not cause false positive results. When applied to neat urine, alcohol, hydrogen peroxide, and Windex did not interfere with the ability to detect urine. Bleach applied to urine interfered with the ability to detect urine using RSID™-Urine (false negative).

Urine was detectable using both methods when deposited on various fabric substrates.

Donors consumed various beverages prior to sample donation to see if consumption impacted the ability to detect urine in their next urine output. Soda and coffee consumption samples yielded negative results; however, due to the variability observed within a single day's samples (see population study), it is possible that THP variability was not solely due to the consumption of these substances.

Specificity Study

Specificity studies are performed to assess the ability of the system to provide reliable results for targeted analytes in the presence of cross-reactive substances. Our specificity study assessed the possible cross-reactivity of forensically relevant human body fluids as well as urine from regularly encountered household pets. Samples were prepared as follows:

- Vaginal and nasal secretions: saturated (approximately 100 µL) swab.
- Menstrual blood: transferred fluid from used tampon to dry cotton swab.
- Fecal: dry swabs used to swab toilet paper after use.
- Non-human urine: swabs used to swab fresh pool of non-human urine in dirt.
- Perspiration: dry swabs used to swab sweat on skin.
- All other fluids: approximately 100 µL applied to each swab.

Sample	Result
Semen	N
Blood	N
Saliva	N
Perspiration	N
Fecal	N
Breast Milk	N
Vaginal Secretions	N
Menstrual Blood	N
Nasal Secretions	N
Dog Urine	+

Cross-reactivity was not observed with forensically relevant body fluids. Cat urine could not be obtained not commingled with litter and was therefore not tested as originally planned. Dog urine tested positive with RSID™-Urine. Consistent with the findings of the developmental validation, the BPL internal validation found that RSID™-Urine is not specific to human urine.

Mixture Study

Mixture studies are performed to assess the performance of the test method when samples containing mixtures of similar or different body fluids are tested. Samples were prepared as follows:

- Urine/vaginal secretions: a previously prepared vaginal swab (see specificity study) was saturated with approximately 100 µL urine.
- Urine/menstrual blood: previously prepared menstrual blood swab (see specificity study) was saturated with approximately 100 µL urine.
- Urine/fecal: previously prepared fecal swab (see specificity study) was saturated with approximately 100 µL urine.
- All other mixtures: prepared in a 50/50 ratio and approximately 100 µL of the mixture was applied to each swab.

Urine/perspiration samples were not tested in the mixture study, as a urine on skin sample was tested in the mock casework study.

Sample	Result
Urine/Semen	+
1:2 Urine Dilution/Semen	N
Urine/Blood	N
1:2 Urine Dilution/Blood	N
Urine/Fecal	+
Urine/Saliva	+
Urine/Vaginal Secretions	+
Urine/Menstrual Blood	N

When mixed with blood (peripheral or menstrual), RSID™-Urine was unable to detect urine. When mixed with other body fluids, RSID™-Urine was able to detect urine; however, dilute urine was not detected when mixed with other fluids. This finding was in line with the test's limited sensitivity.

Mock Casework Study

Mock casework studies are performed on samples that mimic or simulate a range of casework sample types. This may include laboratory-created or proficiency test samples. BPL does not plan to test items that can reasonably be expected to contain urine (e.g. toilet bowl); therefore, these items were not directly evaluated. Urine deposited on various fabrics, but not laundered or exposed to heat or the environment, were previously tested in the contamination study.

Samples were prepared as follows:

- Urine on fabric (laundered): Neat urine was applied to a shirt. The sample was allowed to dry and subjected to a wash and dry cycle in residential laundry machines. A cutting of the stained area was used for testing.
- Exposure samples were prepared by depositing approximately 100 µL neat urine onto cotton swabs.
    - The heat/sun exposure sample was placed on the dashboard of a vehicle on a summer day in Boca Raton, FL and left for 24 hours.
    - The environmental exposure sample was affixed to a tree on a non-rainy summer day in Boca Raton, FL and left for 24 hours.
- Substrate samples (e.g. skin and concrete): Neat urine was allowed to dry completely on the substrate and collected with a swab moistened with sterile water.

Sample	Result
Neat Urine on Fabric (Laundered Post Deposition/Drying)	N
Neat Urine Left in Car for 24 Hours (Heat/Sun Exposure)	+
Neat Urine Left Outside for 24 Hours (Environmental Exposure)	+
Urine on Skin	+
Urine on Concrete	N

RSID™-Urine could detect urine when exposed to heat and sun as well as to the elements. Urine could not be detected when it was washed and dried in home laundry machines, which was unsurprising given the test's limited sensitivity. Urine could be detected on skin, but was unable to be detected from concrete, likely due to the diffusion of the urine into the non-porous substrate and subsequent difficulty recovering the urine onto cotton swabs for testing.

PRODUCT IMPRESSIONS

The RSID™-Urine tests were provided from the manufacturer in boxes of 5 or 10 test cassettes, each with one bottle of RSID™-Urine buffer. The test cassettes are marked with a manufacturer lot number and expiration date and must be retained at room temperature. The buffer is marked with a different manufacturer lot number and expiration date and must be retained refrigerated. However, the product was shipped from the vendor without refrigeration (i.e. no cooler or ice packs). Disposable transfer pipettes and microcentrifuge tubes are not included with the test kits.

The test procedure was relatively easy to conduct but requires two pipetting steps and a sample tube for each test (all required materials not provided by the manufacturer). During a previous validation study that evaluated an Independent Forensics product, RSID™-Saliva, all analysts involved in the BPL internal validation experienced hesitancy recording results from the test at various times during the validation. In that study, the RSID™-Saliva test cassettes appear to have deep sample wells which resulted in possible "shadow bands" in the test area, making it difficult to read results. Multiple samples were recorded as inconclusive during the validation study (consensus between analysts) due to the uncertainty reading the test results. Deep sample wells and resultant shadow bands were not observed during the RSID™-Urine validation. Analysts did not note any uncertainty in interpreting test results.

SAFETY/DISPOSAL CONSIDERATIONS

RSID™-Urine does not require additional personal protective equipment or laboratory safety equipment beyond what is routinely used at BPL.

Refer to the SDS for RSID™-Urine Buffer for disposal considerations.

COST ANALYSIS

The cost per test was evaluated for RSID™-Urine.

Sample	10-Pack	5-Pack
Cost Per Package	$156	$81
Number of Tests Per Package	10 tests	5 tests
Cost Per Test	$15.60/test	$16.75/test

It is more cost effective to purchase RSID™-Urine in packs of 10; however, the product expires very quickly. Given the infrequency of projected RSID™-Urine use, it may be advisable to purchase as a 5-pack for casework purposes.

LIMITATIONS

- A negative result may be observed if urine is present at a level below the detection ability of the test.
    - The demonstrated limit of detection using the RSID™-Urine test procedure was a 1:2 urine dilution.
    - The Independent Forensics developmental validation indicated the limit of detection is 10 µL urine.
- Cross-reactivity was not observed with other human body fluids; however, the presence of blood interfered with the ability to detect urine.
- Exposure to bleach and/or laundering may diminish the ability to detect urine.
- Urine may not be collected from hard, porous substrates in adequate quantity to be detected with RSID™-Urine.
- THP levels are variable between donors and throughout a single day.
- RSID™-Urine is not human-specific. It is a presumptive test method for human urine.

SUMMARY

This validation study demonstrated the quality and robustness of the results obtained using RSID™-Urine and the limitations of the method. Based on the totality of the results obtained, the RSID™-Urine method will be implemented for urine testing in casework at BPL; however, it's limited sensitivity should inform casework acceptance guidelines.

COMPETENCY

All currently employed analytical employees at BPL were directly involved in sample preparation and validation testing under observation using the test method evaluated. Therefore, no additional competency testing will be required. All BPL employees will complete a supplementary urine training module, consisting of reading and answering questions prior to being authorized to conduct urine testing in forensic casework. Once authorized to conduct urine testing, employee proficiency will be regularly evaluated through the proficiency testing program. This training module will be incorporated into the BPL training program for future new employees.

REFERENCES

Independent Forensics (n.d.). *Developmental Validation of RSID™-Urine.*

Independent Forensics (2018). *Rapid Stain Identification of Urine (RSID™-Urine): Technical Information and Protocol Sheet.* Catalog #0400-05, 0400-10.

APPROVAL

Respectfully submitted for approval,

Kristen V. Archuleta — Sep 16, 2024

Kristen Archuleta, Biological Processing Laboratory Analyst — Date

Cayleigh Shufelt — Sep 16, 2024

Cayleigh Shufelt, Biological Processing Laboratory Analyst — Date

Caitlin Rogers — 09/16/2024

Caitlin Rogers, Biological Processing Laboratory Supervisor — Date

Amy Flagler — Sep 16, 2024

Amy Flagler (Sep 16, 2024 14:27 EDT)

Amy Flagler, Forensic Services Manager — Date

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