

# Independent Forensics

## Rapid Stain Identification of Urine (RSID™-Urine)

### Technical Information and Protocol Sheet for Cat #0400-05, #0400-10

#### INTENDED USE

RSID™-Urine is a new lateral flow strip test for the forensic detection of human urine. RSID™-Urine is designed for fast, easy, and reliable detection of urine from cotton swabs and a variety of fabrics encountered by forensic laboratories.

No other human body fluids tested cross react with RSID™-Urine, but consideration must be taken if the presence of blood is suspected on a piece of evidence since the presence of blood may inhibit the signal when using RSID™-Urine.

#### Introduction

RSID™-Urine is a lateral flow immunochromatographic strip test designed to detect the presence of the Tamm-Horsfall (THP) glycoprotein (sometimes called uromodulin). Tamm-Horsfall is the most abundant protein present in urine. It is secreted by the thick ascending limb of the loop of Henle, and then excreted into urine at a rate of 80-200 mg/day (Dulawa *et al.*, *Diabetologia* 28:827-830, 1985).

RSID™-Urine is specific for urine and does not cross-react with any other human bodily fluids. Current protocols for urine detection generally measure creatinine using Jaffe's test, which is neither specific for urine, nor particularly sensitive, nor easy to perform.

#### Principle of the Test

RSID™-Urine is an immunochromatographic assay that uses two rabbit polyclonal antibodies specific for Tamm-Horsfall. One of these antibodies is conjugated to blue latex beads and is deposited on a conjugate pad beneath the sample window. The other antibody is striped onto the "Test line" on a membrane, visible through the test window, and attached to the conjugate pad. The "Control line" on the membrane consists of anti-rabbit IgG antibody and is used as an internal control.

Following the addition of test liquid to the conjugate pad, sample and antibodies (complexed and free) are transported by bulk fluid flow to the membrane. The immobilized anti-THP antibodies on the test line capture the THP antigen-antibody-latex bead complexes, producing a blue line at the Test position. If no THP is present in the sample, no blue line will appear. A blue line should appear at the Control position on each strip. 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.

RSID™-Urine, IFI Cat # 0400, laboratory kit contains a single buffer for extraction of the sample and running of the RSID™-Urine tests. RSID™-Urine buffer is designed to efficiently extract THP from questioned stains and swabs, to dissolve the antibody-latex bead conjugate from the

conjugate pad, maintain an extract at the appropriate pH, and facilitate correct running of the test. Components of the buffer include buffer and salts (Tris, NaCl, KCl) for physiological stability, a chelating agent (EDTA) for stability, detergents and surfactants (Triton X-100 and Tween 20) for extraction efficiency and solubility maintenance, protein (BSA) for reducing non-specific adsorption and loss, and a preservative (sodium azide).

#### Reagents and Materials Provided

- i) Test cassettes: 5-10 cassettes individually wrapped and sealed in a moisture-proof foil (a silica gel desiccant pouch has been added for increased shelf life.)
- ii) 10 mL of RSID™-Urine Buffer

#### Protocol for Positive Control

Positive controls for RSID™-Urine can be produced from 100 µL of human urine deposited on a cotton swab. In order to produce a robust signal, the color intensity of the urine sample must be medium to dark yellow. Extract the urine swab in 300 µL of RSID™-Urine Buffer for 1-2 hours at room temperature with occasional vortexing and load 100 µL of this extract into the sample well. Use of a water bath sonicator for 15 min can improve extraction efficiency.

#### Protocol for Negative control

A negative control for RSID™-Urine can be produced from extracting a sterile cotton swab in the same manner as your samples. Alternatively 100 µL of RSID™-Urine Buffer may be used alone.

#### Suggested Extraction Protocol for Sample Analysis

Forensic samples obtained on fabric or cotton swabs should be extracted in minimal amount of RSID™-Urine Buffer for 1-2 hours. A portion of a swab may be used, and sufficient RSID™-Urine Buffer should be added to easily cover the sample. Stains on fabric or paper should be sampled by taking an 8 mm Harris punch or cutting (≈ 50 mm<sup>2</sup>) of the item. The punch or cutting should be extracted in ~120 µL of RSID™-Urine Buffer for 1-2 hours and the extract recovered. Use of a water bath sonicator for 15 min can improve extraction efficiency. Add 100 µL of the extraction solution to an RSID™-Urine cassette.

#### Strip Test Assay Procedure

Note: Assays should be performed at room temperature. It is recommended that a positive and negative control be included with every assay.

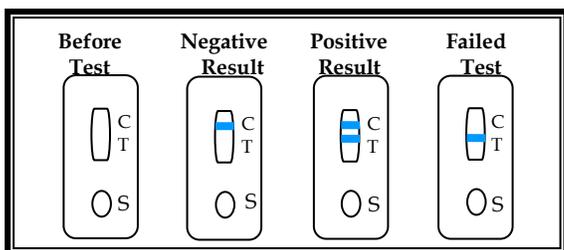
1. Remove cassette from the foil pouch. Discard silica gel desiccant.
2. Add 100 µL extract in RSID™-Urine Buffer to sample window and start timer.

3. At 15 minutes, score and record results as shown in the Scoring Results diagram shown below.

### Scoring Results

RSID™-Urine should be evaluated *exactly* 15 minutes after the addition of sample. Fig. 1 illustrates expected results:

- i) A visible blue line at the Control (C) position only indicates a negative result.  
*No Tamm-Horsfall detected.*
- ii) Visible blue lines at both the Control (C) and Test (T) positions indicate a positive result.  
*Tamm-Horsfall detected.*
- iii) A visible blue line at the Test (T) position only indicates a failed test.  
*Test failure, no conclusion possible.*



### Stability and Storage

RSID™-Urine cassettes are stable for 9 months and should be stored at room temperature. RSID™-Urine Buffer should be stored at 2-8°C. Do not use buffer or cassettes after the printed expiration date.

### Specificity

RSID™-Urine is specific for urine. No cross-reactivity with human saliva, semen, whole blood, vaginal fluid, or menstrual blood has been observed.

RSID™-Urine is body fluid specific but not species specific. RSID™-Urine tests positive with a subset of non-human samples (please refer to the RSID™-Urine validation summary for more details).

### Test Sensitivity

An important consideration when using RSID™-Urine is the normal physiological variation in the levels of Tamm-Horsfall protein in urine samples. Levels of Tamm-Horsfall vary widely from a single individual due to variation in hydration levels and diet. In addition, normal Tamm-Horsfall levels may vary greatly between individuals – by as much as three-fold. The detection limit for RSID™-Urine, used as suggested, is approximately 10 µl of human urine, however, the variation in THP levels between samples and individual prohibits a definitive limit of detection.

Undiluted urine should *not* be used with RSID™-Urine. The tested sample must first be deposited on a sterile cotton swab or fabric swatch and subsequently extracted with provided RSID™-Urine buffer and run in RSID™-Urine Buffer.

### High Dose Hook Effect

A high dose hook effect refers to a weak positive or false negative result seen with immunochromatographic strip tests when very high levels of target are present in the tested sample. Under these conditions, unbound target antigen can reach the test line *before* the latex bead-labeled antibody-bound antigen, occupying the test line antibody sites and resulting in a weak positive or false negative result.

We have observed a minor high dose hook effect on RSID™-Urine strip tests when very high amounts of urine are present in the sample (>50 µL urine). This effect results in a slight decrease in signal and no false negatives have been seen even when 100 µL of equivalent urine are tested.

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## Please Read This Section

### Known Cross-Reactivities

RSID™-Urine was developed against human THP and is specific for this protein which is found exclusively in urine. RSID™-Urine is a presumptive test for urine.

### Test Timing

Independent Forensics produces three other forensic specific lateral flow immunochromatographic strip tests: RSID™-Saliva, RSID™-Semen, and RSID™-Blood. These three tests have very similar testing protocols - RSID™-Urine requires a different buffer system (see below) and requires test evaluation at fifteen (15) minutes, NOT the ten (10) minutes required for RSID™-Saliva, RSID™-Semen, and RSID™-Blood.

### Limit of Detection Determination

RSID™-Urine detects the presence of THP. The production, secretion and final concentration of this protein in urine varies widely with physiology, hydration status, diet and personal genetics. This normal variation makes determination of a uniform experimental limit of detection difficult and laboratories should establish a standard extraction and testing protocol to address this issue. THP is a relatively insoluble protein prohibiting the use of purified THP to determine a limit of detection.

### Single Buffer System

RSID™-Urine uses a single buffer for both sample extraction and running of the test. This buffer is unique to RSID™-Urine and should not be used with any other RSID™ products. RSID™-Universal buffer cannot currently be used with RSID™-Urine.

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*Not for in vitro diagnostic use  
Manufactured by:*



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