

Independent Forensics
Rapid Stain Identification
Of Human Semen (RSID™-Semen)
Technical Information Sheet

INTENDED USE

The new **R**apid **S**tain **I**dentification (RSID™) test for semen is designed for fast, easy, and reliable detection of human semen from a variety of samples encountered by forensic laboratories including clothing, bedding, vaginal swabs, prophylactics, and stained surfaces.

The test will detect as little as 1 µl of human semen, and test results are complete within 10 minutes.

The detection protocol can be completely integrated into standard forensic laboratory procedures for DNA analysis, prior to STR analysis (see Provided Protocols).

The test sensitivity has been adjusted so that when semen is detected, sufficient biological material should be present to generate an STR profile (exceptions include low sperm count semen or semen from vasectomized men). Typical extraction protocols are provided for your convenience (see Provided Protocols).

This is the first commercially available confirmatory test for human semen. No other human body fluids tested cross react with this procedure (See Specificity below for fluids tested). The immunochromatographic strip test uses dual monoclonal antibodies specific for human semenogelin.

Introduction

Rapid Stain Identification of Human Semen (RSID™-Semen) is a lateral flow immunochromatographic strip test designed to detect the presence of human semenogelin. Semenogelin is a protein produced by the seminal vesicles that causes seminal fluid to form a coagulum subsequent to ejaculation. RSID™-Semen uses two anti-human semenogelin monoclonal antibodies in a lateral flow format, which detects the presence of semenogelin.

RSID™-Semen is specific for human semen and has numerous advantages over other methods for semen detection, including increased sensitivity, specificity, and speed. Current identification methods for semen are presumptive (provide a basis for continued analysis of the tested exhibit but are not specific for semen), and are therefore open to legal and scientific challenge.

Principle of the Test

RSID™-Semen is an immunochromatographic assay that uses two mouse monoclonal antibodies specific for human semenogelin. One of these antibodies is conjugated to colloidal gold and is deposited on a conjugate pad beneath the sample window. The other antibody is striped onto the "Test line" on a membrane attached to the conjugate pad. The "Control line" on the membrane consists of anti-mouse IgG antibody and is used as a test control.

Attached to the other end of the membrane is the wick, which absorbs the tested fluid and running buffer at the completion of the test thus preventing back-flow of the sample. Once the tested fluid is added to the sample window, the running buffer and sample diffuse through the conjugate pad, re-dissolving the gold-conjugated antibodies. If human semenogelin is present in the sample, an antigen-antibody conjugated to colloidal gold complex will form. Sample and antibodies (complexed and free) are transported by bulk fluid flow to the membrane phase of the strip test. The immobilized anti-semenogelin antibodies on the test line capture the semenogelin-antibody-gold complexes, producing a red line at the Test position. If no human semenogelin is present in the sample, then gold-conjugated antibody-antigen complexes do not form, and colloidal gold will not be accumulated at the Test line. The anti-mouse IgG on the control line captures any mouse antibodies flowing past the test line, 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.

Reagents and Materials Provided

- i) Test cassettes: 25 cassettes, individually wrapped and sealed in moisture-proof foil (a silica gel desiccant pouch has been added for increased shelf life.)
- ii) 5 ml of RSID™-Semen Running Buffer
- iii) 25 ml of RSID™-Semen Extraction Buffer (formulated for use with RSID™-Semen, certified STR free)
- iv) Suggested laboratory and validation protocols, optional validation summary form.

Protocol for Positive Control

Positive controls for RSID™-Semen can be produced from 50 µl of human semen deposited on a sterile cotton swab. The semen swab should be extracted in 1 ml of RSID™-Semen Extraction Buffer for 1-2 hours at room temperature; 5 µl of this extract should be diluted in 95 µl of RSID™-Semen Running Buffer (total volume 100 µl). Load all 100 µl into the sample well; this will give a strong positive signal.

Suggested Extraction Protocol for Sample Analysis

Forensic samples obtained on cotton swabs (e.g., vaginal swabs, prophylactics, and stained solid surfaces) should be extracted in 200-300 µl of RSID™-Semen Extraction Buffer for 1-2 hours. A 20 µl volume of this extract is added to 80 µl of RSID™-Semen Running Buffer for a total final volume of 100 µl. Stains on fabric or paper should be sampled by taking a punch or cutting (≈ 20 mm²) of the item. The punch or cutting should be extracted in 100 µl of RSID™-Semen Extraction Buffer for 1-2 hours. A 10 µl volume of this extract is then added to 90 µl of RSID™-Semen Running Buffer for a total final volume of 100 µl which is then loaded into the sample well on the cassette. The remainder of the extract can then be processed for STR analysis using one of the recommended protocols (see Provided Protocols).

Strip Test Assay Procedure

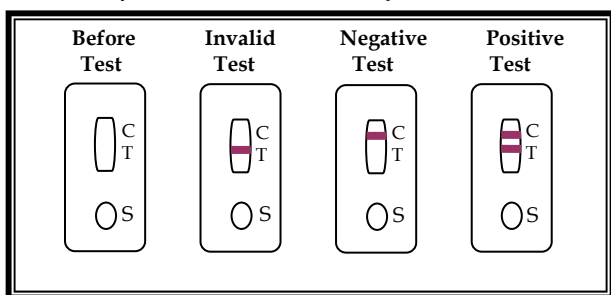
Note: Assays should be performed at room temperature, we recommend that a positive and negative control be included with every assay (see Provided Protocols).

1. Remove cassette from the foil pouch. Discard silica gel desiccant.
2. Bring sample to a final volume of 100 µl with supplied RSID™-Semen Running Buffer, typically in a disposable 0.6 ml microfuge tube.
3. Set a timer for 10 minutes.
4. Add sample in running buffer to sample window. Start timer.
5. At 10 minutes, score and record results as shown in the diagram.
6. Due to the high dose Hook effect, samples giving a weak positive or negative result should be diluted 1:20 and re-tested. For example: If 20 µl from a 200 µl swab extract gives a weak positive or negative result, 1 µl from the original extract should be added to 99 µl RSID™-Semen Running Buffer and analyzed on a new cassette (see High Dose Hook Effect below for details).
7. Discard cassette(s). Each cassette can only be used once.

Scoring Results

RSID™-Semen should be evaluated *exactly* 10 minutes after the addition of sample. Fig. 1 illustrates expected results:

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



Stability and Storage

RSID™-Semen cassettes should be stored at room temperature. RSID™-Semen Extraction and Running Buffers should be stored at 4°C. Do not use buffers or cassettes after the printed expiration date.

Specificity

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

No cross reactivity with animal semen has been observed. Species tested: dog, cat, mouse, cow, horse, pig, goat, and sheep.

Test Sensitivity

The detection limit for RSID™-Semen, used as suggested is 1 µl of human semen.

Undiluted semen should *not* be used with RSID™-Semen, as the viscosity of the sample prevents proper release of the conjugate from the conjugate pad. The tested sample should first be deposited on a sterile cotton swab, extracted in RSID™-Semen Extraction Buffer, and diluted as needed in RSID™-Semen Running Buffer before analysis with RSID™-Semen.

High Dose Hook Effect

A high dose Hook effect refers to weak positive or false negative results 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 colloidal gold-labeled antibody-bound antigen, resulting in a weak positive or false negative result.

We have observed weak positive and false negative results with RSID™-Semen when samples containing large amounts of human semen (≈ 3 to 50 µl) were analyzed. 20-fold dilution of these samples and re-testing with RSID™-Semen eliminated the weak positive and false negative results in all cases (see Validation Report for details).


User Note: Under standard laboratory testing, users of RSID™-Semen may observe weak positive or false negative results due to the high dose Hook effect. Therefore, any weak positive or negative result from RSID™-Semen should be confirmed by diluting the sample 1:20 and re-testing. If re-testing of the diluted sample results in a stronger positive signal, the original result was caused by the high dose Hook effect and a large amount of semen is present in the sample. If re-testing of the diluted sample is once again weakly positive or negative, the original result is confirmed.

Validation

A typical validation summary page and validation test description are supplied. Laboratories should adapt or edit these to suit their own requirements for technical validation.

Not for in vitro diagnostic use

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