JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, November 2012, Vol. 57, No. 6
doi: 10.1111/j.1556-4029.2012.02141.x
Available online at: onlinelibrary.wiley.com

TECHNICAL NOTE

CRIMINALISTICS

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False Positives Observed on the Seratec® PSA SemiQuant Cassette Test with Condom Lubricants

ABSTRACT: In the course of the validation of a new component of the prostate-specific antigen (PSA) SemiQuant Cassette Test marketed by Seratec®, a false-positive reaction was observed when testing samples collected from the surface of unused, lubricated condoms. A variety of personal lubricants and condoms were tested to determine the frequency of the false positive, as well as its potential source. Samples were extracted in both water and the manufacturer-provided buffer, and the test was performed according to the manufacturer's suggested protocol. The false positive was observed intermittently, but occurred consistently with samples containing nonoxynol-9, a strong detergent utilized as a spermicide. The reaction may be attributable to the combination of latex and nonoxynol-9. Because of the unreliability of the test to confirm the presence of PSA in samples collected from condoms, the PSA cassette is an unsuitable method for confirming the presence of seminal fluid in condoms.

KEYWORDS: forensic science, forensic biology, seminal fluid, prostate-specific antigen, Seratec®, false positive, condom lubricant, nonoxynol-9

Condoms are commonly submitted as an item of evidence to crime laboratories in cases of sexual assault and rape. To confirm the presence of seminal fluid in a condom, the most common test employed by the Forensic Biology field is a microscopic examination of the contents of the condom. Because spermatozoa are exclusive to seminal fluid and human spermatozoa possess unique conformational qualities, microscopic identification provides the highest level of confidence in identifying human semen (1,2). In some cases, a microscopic confirmation of the cellular constituent of seminal material is not possible; this may be due to a lack of spermatozoa present, or degradation resulting from the environment in which the liquid contents were contained. An alternative testing method to confirm the presence of seminal fluid through the identification of its constituents is to immunologically test for the presence of p30, also identified as prostate-specific antigen (PSA). p30 is not unique to seminal fluid. It has also been identified in male serum, male urine, and female breast tissue (3). p30 is, however, present in uniquely high levels in seminal fluid, making it an ideal marker for semen identification. The concentration of p30 ranges from 300 to 4200 μg/mL of semen, with an average level of 1200 μg/mL of semen (1,3,4). The method commonly employed in Forensic Biology laboratories to identify p30, or PSA, is an immunological cassette test. It is sensitive and efficient, providing accurate results with a less cumbersome protocol than other methods, such as double immunodiffusion and ELISA (5,6). The cassette test utilizes a mobile monoclonal anti-p30 antibody in the sample well of the test device. This dye-complexed monoclonal antibody will bind with the p30 antigen, creating a complex that migrates via capillary action through the membrane. An immobilized, monoclonal antibody, specific for a different epitope on the p30 molecule than the mobile monoclonal antibody, is present in the "test area" of the cassette membrane. The PSA of the dye bound, antibody–antigen complex will bind to the immobilized antibody. As the complexes bind, a pink line created by the aggregated dye can be visualized. The extract continues to migrate across the membrane, through a control area that contains an immobilized polyclonal antibody that recognizes the mobile anti-p30 antibody. This control area acts as a positive control to ensure that the test is operating correctly (6). In the course of validating the manufacturer-provided buffer, false-positive results were observed with samples of condom lubricants tested as contaminants. Condoms are submitted as items of evidence in the course of a sexual assault examination. The use of condoms in sexual assault cases has increased in the past several years (7). Condoms are often disposed of in a manner that does not protect the contents. Additionally, because of the physical nature of the item, it is often not conducive to the observation of spermatozoa; the moist environment can quickly lead to the degradation of cells present. In these cases, an alternative testing method to confirm the presence of spermatozoa, such as a p30 cassette test, would be utilized to confirm the presence of seminal fluid constituents.

Materials and Methods

Samples

A variety of personal lubricants, lubricated latex condoms, nonlubricated latex condoms, and lubricated polyurethane condoms, were donated for testing. The samples were collected to test the variability in personal lubricants and condom lubricants. The personal lubricants were deposited onto sterile cotton-tipped swabs in a

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Received 3 Mar. 2011; and in revised form 13 June 2011; accepted 14 Aug. 2011.

2012 American Academy of Forensic Sciences
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manner imitating collection in a sexual assault. The swabs were allowed to air-dry prior to testing. The condom lubricants were collected from the condoms by swabbing the surface of the condom with sterile cotton-tipped swabs. The swabs were then air-dried prior to testing. The condoms were sampled by cutting a 5×5 mm portion, independent of the previously swabbed area.

Seratec® PSA SemiQuant Cassette Test

The Seratec® PSA SemiQuant Cassette Test (Seratec® Postfach 3706 D-37027; Gottingen, Germany) is used for the confirmation of the presence of seminal fluid constituents. The test was utilized according to the manufacturer's suggested guidelines. The samples were extracted in both 250 μL of sterilized, ultra-pure water and 250 μL of the manufacturer-supplied buffer for comparison of the results (8). The pH of the remaining 50 μL of water and 50 μL of buffer was measured once using pH paper, to exclude pH variation as a potential source of the false positive (3).

Results and Discussion

An assortment of lubricants, collected on cotton-tipped swabs, was tested according to the procedure outlined in the Materials and Methods section. The initial testing of condom lubricant, which was utilized as a chemical insult while validating the manufacturer-provided buffer for use in casework, produced a false-positive result. Because of the positive result for condom lubricant, extracted by buffer, a second portion of the original sample was retested, and an additional condom lubricant also tested. The condom lubricants were sampled and tested in the same method described above. In this instance, both condom lubricant samples exhibited negative results for the water and buffer extraction methods (Table 1). Because of the irregularity of the results, it was then determined that a larger sampling group was required to preclude the possibility of a false positive due to the presence of condom lubricants. A larger selection of condoms was sampled for immunological testing, using the same sampling and testing method previously described (Table 2). Again inconsistent results were observed, and the test group was expanded to a more comprehensive grouping of condom lubricants, which were tested using the sample testing and sampling method described above (Table 3). The results obtained in the testing continued to be inconsistent in the occurrence of false-positive reactions. All samples were extracted in both water and buffer to determine whether the increase in sensitivity, resulting from the extraction efficiency of the manufacturer-provided buffer, contributed to the false-positive results observed with the condom lubricant. False-positive results were also observed in samples extracted in water, demonstrating that the false positive observed with condom lubricant is not a result of increased test sensitivity provided by the buffer extraction (Table 3).

In an attempt to determine the potential source of the false-positive reaction, further testing was pursued to separate the various elements that could cause the false positive. Cuttings of lubricated condoms, which were also swabbed for the collection of lubricants on the surface, and sources of noncondom latex were extracted using the method previously described to determine whether the latex was a contributing factor in the false-positive results (Tables 4 and 5). Similar false-positive results were not observed with noncondom latex sources. Additionally, personal lubricants were collected onto substrates and tested to determine whether similar false positives were observed because of components of the lubricants, independent of the condom material (Table 6). No false-positive reactions were observed. Several of the tests failed because of the viscosity of the lubricant present in the extraction, which prevented the sample from moving across the cassette membrane via capillary action. A literature search was conducted to determine whether similar samples had been tested in other studies. No studies performed in the United States reported samples collected from unused condoms being processed. A validation study of the BioSign™ PSA Membrane Cassette Test (Princeton BioMeditech Corporation, Princeton, NJ) produced similar results to the tests conducted using the Seratec® cassette. In the course of testing samples collected from condoms, false-positive results were observed with the BioSign™ PSA Membrane Cassette. In the paper resulting from the study, the

TABLE 1—*Effects of lubricants for the detection of p30.*

Sample	Buffer Result	Buffer pH	Water Result	Water pH
Lubricants				
Hand lotion (Curel Ultra Healing)	N	9	N	7
Vaseline	N	9	N	6
Condom lubricant (Trojan Pleasure Mesh)	+	9	N	6
Condom lubricant (Trojan Pleasure Mesh)—repeat	N	9	N	6
Condom lubricant (Durex Polyurethane)	N	9	N	6

+, positive; N, negative.

TABLE 2—*Effects of condom lubricants for the detection of p30.*

Sample	Buffer Result	Buffer pH	Water Result	Water pH
Condom lubricants				
Trojan Her Pleasure	N	9	N	6
Trojan Ultra Pleasure	+/N	9	N	6
Trojan Pleasure Mesh	N	9	N	6
LifeStyles Ultrasensitive	+/N	9	N	6

+, positive; +/N, weak positive; N, negative.

TABLE 3—*Effects of a variety of condom lubricants for the detection of p30.*

Sample	Buffer Result	Buffer pH	Water Result	Water pH
Condom lubricants				
Purex nonlubricated	N	9	N	6
Trojan Ultra-Ribbed Latex condom with spermicidal lubricant	N	9	N	6
Trojan Her Pleasure Latex condom with spermicidal lubricant	N	9	N	6
Trojan Ultra-Thin Latex condom with spermicidal lubricant	N	9	N	6
Trojan-ENZ Latex condom with spermicidal lubricant	N	9	N	6
Trojan Shared Pleasure Latex condom with *Warm Sensations* lubricant	N	9	N	6
Trustex Vanilla Flavored Lubricated Latex condom	N	9	N	6
Coronet gold Latex Condom	N	9	N	6
Reality Female Condom (with spermicidal lubricant)	N	9	+	6
Durex Ultimate Feeling Latex Condom	N	9	N	6
Durex Intense Sensation Latex Condom (with spermicide)	N	9	+	6
Durex Tropical Orange Latex Condom	N	9	N	6
Durex Performax Latex Condom (5% Benzocaine)	N	9	N	6

+, positive; N, negative.

TABLE 4—*Effects of a variety of condoms for the detection of p30.*

Sample	Buffer Result	Buffer pH	Water Result	Water pH
Condom cuttings				
Purex nonlubricated	N	9	N	6
Trojan Ultra-Ribbed Latex condom with spermicidal lubricant	N	9	N	6
Trojan Her Pleasure Latex condom with spermicidal lubricant	N	9	N	6
Trojan Ultra-Thin Latex condom with spermicidal lubricant	N	9	N	6
Trojan-ENZ Latex condom with spermicidal lubricant	N	9	N	6
Trojan Shared Pleasure Latex condom with *Warm Sensations* lubricant	N	9	N	6
Trustex Vanilla Flavored Lubricated Latex condom	N	9	N	6
Coronet gold Latex Condom	N	9	N	6
Reality Female Condom (with spermicidal lubricant)	N	9	N	7
Durex Ultimate Feeling Latex Condom	N	9	N	6
Durex Intense Sensation Latex Condom	N	9	N	6
Durex Tropical Orange Latex Condom	N	9	N	6
Durex Performax Latex Condom—5% benzocaine (with spermicide)	+	9	N	6

+, positive; N, negative.

TABLE 5—*Effect of noncondom latex and nonoynol-9 on the detection of p30.*

Sample	Buffer Result	Buffer pH	Water Result	Water pH
Latex glove	N	9	N	6
Sheet latex (tourniquet)	N	9	N	6

N, negative.

TABLE 6—*Effect of personal lubricants for the detection of p30.*

Sample	Buffer Result	Buffer pH	Water Result	Water pH
KY Jelly Personal Lubricant	Failed	9	Failed	5
Undercover Wear Bedroom Magic Silicone Based Lubricant	N	9	N	5
Undercover Wear Shunga Passion Fruit Warming, Water-Based Lubricant	N	9	N	5
KY Sensual Silk Personal Warming Lubricant	N	9	N	5
Undercover Wear Bedroom Magic Water-Based Lubricant	N	9	N	5
Undercover Wear Shunga Rasberry Warming, Water-Based Lubricant	Failed	9	N	5
Pure Romance Lickity Stiff Scented Flavored Massage Cream	N	9	N	5
Pure Romance Flavored Oral Stimulation Gel	N	9	Failed	5
KY Water-Based Jelly	N	9	N	5
KY Liquid	N	9	N	5
KY Intense Arousal Gel for Her	N	9	N	6
Pure Romance Sensations Edible Lubricant	N	9	N	5
Pure Romance Just Like Me Unscented, Unflavored Lubricant	Failed	9	Failed	6

Failed, extract would not absorb; N, negative.

testing laboratory attributed the false-positive reactions to the presence of nonoxynol-9, which is a harsh detergent commonly used as a spermicide by condom manufacturers (9). Antigens, proteins involved in the immune response, are subject to conformational change in the presence of harsh environmental factors. Latex antigens

TABLE 7—*Effect of noncondom latex and nonoynol-9 on the detection of p30.*

Sample	Buffer Result	Buffer pH	Water Result	Water pH
Latex with 28% nonoxynol-9 (14 days aged)	N	9	N	6
Latex with 28% nonoxynol-9 (1 day aged)	N	9	N	6
28% nonoxynol-9	N	9	N	6

N, negative.

are often utilized in immunological tests because of their high affinity for binding. In the presence of a strong detergent, such as nonoxynol-9, it is possible that the conformational structure of latex antigens could be sufficiently altered to bind with the antibodies utilized by the PSA cassettes available to the forensic community. It is also possible that the presence of nonoxynol-9 could act to break down the latex to release a greater quantity of latex antigens into the solution. Latex is common utilized in the serological and medical communities in the latex particle agglutination test for the identification of physiological fluids and diseases. The latex antigens could bind with the dye-bonded antibodies, creating the false-positive reaction observed. In communication, it was determined that the sampling method differed, which may account for the inconsistency of the results observed when testing the Seratec® PSA SemiQuant Cassette Test. Unlike the sampling method employed in this study, the samples tested in the course of the Biosign™ study were extracted directly in the condom through the addition of water to the condom. A portion of the extract was then pipetted directly into the cassette (9). Further testing was then performed using samples known to contain nonoxynol-9: 28% nonoxynol-9 contraceptive film and latex condoms with spermicidal lubricant (Table 7). The contraceptive film was tested separately from latex and was also combined with latex. All samples tested provided negative results. In the course of the study, we were unable to imitate the results obtained from condoms containing spermicidal lubricant with contrived samples composed of nonoxynol-9 contraceptive film and latex. As a result, we are unable to confirm the source of the false-positive reaction that was repeatedly observed.

Conclusion

Intermittent false-positive results observed when testing condom lubricants containing spermicidal lubricant nullify the ability to confirm the presence of seminal material through PSA testing of condoms containing bodily fluids. Because condoms received as evidence rarely contain brand or manufacturer information, it is impossible to determine whether the sample contains spermicidal lubricant, without chemical testing. The different methods of extraction, water or manufacturer-provided buffer, both produced false-positive results. Similarly, these results have been observed on multiple cassettes (i.e., Seratec® PSA SemiQuant Cassette Test and the BioSign™ PSA Membrane Test). A conservative approach is to restrict the confirmation of the presence of seminal fluid to the microscopic observation of spermatozoa and to maintain PSA cassette results as a presumptive test on samples obtained from condoms.

Acknowledgments

Thank you to the administration of the Allegheny County Office of the Medical Examiner: Janine Yelenovsky, Forensic Biology Manager, Robert Huston, Laboratory Director, and Dr. Karl E. Williams, Medical Examiner, for the opportunity to

perform this testing. The aid of Jacquelyn Bales in reviewing of the proposed article is deeply appreciated. The help of the members of the Forensic Biology Section, most especially Aaron Schneider and Thomas Meyers, is appreciatively acknowledged.

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