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A Comparative Analysis of Accuracy and Sensitivity in Semen Presumptive Testing: ABAcard P30™, RSID Semen™, and Seratec PSA™

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Table of Contents

List of Tables

List of Figures

Abstract

Semen presumptive tests are often used in forensics to detect the possible presence of semen on evidence from a crime scene. These tests work by identifying enzymes or proteins commonly found in semen, such as prostate-specific antigen (PSA) and semenogelin. However, presumptive tests can produce false positive results, as these biomarkers may also be present in other bodily fluids. Despite this limitation, some agencies still rely on presumptive test outcomes as a confirmation test for semen in legal proceedings.

This study evaluated and compared the accuracy and sensitivity of three rapid immunochromatographic test kits for semen detection: Rapid Stain Identification Series (RSID) Semen™, Seratec PSA™, and ABAcard P30™. The RSID Semen™ test detects semenogelin, while Seratec PSA™ and ABAcard P30™ detect PSA. Samples tested included serial dilutions of semen, as well as an array of bodily fluids and materials that could potentially cause false positive results. All samples were tested in triplicate with each kit.

The study found differences in sensitivity between the three test kits, with false positives occurring to some degree with all methods. RSID SemenTM, Seratec PSA™, and ABAcard P30™ all had issues detecting semen in a 1:10,000 dilution. Additionally, RSID Semen™ could not detect semen when it was mixed with dirt. There was an issue of non-specificity with all three of the test kits with various absorbent hygiene products. RSID SemenTM, Seratec PSA™, and ABAcard P30™ all had several false positive test results with tampons, menstrual pads with blood, and diapers with urine samples. Additionally, ABAcard P30™ had false positive test results with female urine samples.

These findings highlight the need for caution when using presumptive semen test results, especially as primary evidence in legal cases. These test kits should no longer be used as a

confirmatory test for semen in legal proceedings. The data generated will help forensics investigators determine which test kit may be most appropriate and reliable for detecting semen on different types of evidence, highlighting the potential for false results. Using precise testing methods is critical for drawing correct conclusions during criminal investigations. While Seratec PSA™ showed the highest sensitivity among the test kits evaluated, it had a concerning false positive rate of 12%, the highest rate observed. Of the three rapid semen detection kits compared, the ABAcard P30™ kit displayed the highest degree of accuracy. ABAcard P30™ had both the lowest false positive rate at 6% and the second-lowest false negative rate at 2% out of the kits tested, indicating superior sensitivity and specificity.

Introduction

When a crime occurs, investigators examine the scene for potential evidence. If they find a stain of interest while processing the scene, they will run a series of tests to determine if it is a biological stain. The first test in the series will be presumptive. Presumptive tests are used to determine the presence of a biological substance but cannot confirm the type of the substance. Presumptive tests are highly useful in forensic testing because of their high sensitivity. However, they are prone to false positives due to this sensitivity. Further testing must be done to verify the type of substance because presumptive tests can produce false positive test results. Assessing the validity of semen presumptive testing and the factors most likely to affect the results of these tests is important.

A current semen presumptive test investigators use is the Rapid Stain Identification Series, also known as RSID Semen™. RSID Semen™ is a lateral flow immunochromatographic test strip that can detect human-specific semen samples (Old et al., 2011). RSID Semen™ works to indicate the presence of semen by detecting semenogelin, a component of semen. It plays a crucial role in encapsulating spermatozoa, thereby shielding them from degradation within the female reproductive system, thus ensuring the integrity and viability of spermatozoa, which carry the male genetic material.

Furthermore, the process through which semenogelin facilitates coagulation involves an antigen-antibody reaction (Boward et al., 2013). This reaction, characterized by binding specific antigens present in semenogelin with corresponding antibodies, initiates the clotting of seminal fluid upon ejaculation. Consequently, the resulting coagulum aids in retaining and prolonging the presence of spermatozoa within the female reproductive tract, therefore increasing the chance of successful fertilization.

Another presumptive test that forensic investigators widely use is Seratec PSA™. Seratec PSA™ is a rapid immunochromatographic test that detects the prostate-specific antigen (Laux et al., 2006). This test operates on an antigen/antibody mechanism, similar to semenogelin role in semen coagulation. The antigen/antibody mechanism is utilized to specifically detect and identify the presence of target antigens, such as prostate-specific antigen in the case of the Seratec PSA ™. Prostate-specific antigen, or PSA for short, is a component of seminal fluid. PSA is needed to degrade the other components of semen to keep the semen sample from coagulating. The prostate mainly produces prostate-specific antigens, but they have been found in small quantities in other parts of the body. Small amounts of PSA have been detected in the bloodstream of both males and females. In males, PSA is typically found in low levels in the blood, but elevated levels can indicate prostate-related issues such as inflammation, infection, or prostate cancer. PSA has also been detected in female tissues; however, it is in significantly lower concentrations compared to males. For instance, studies have reported the presence of PSA in breast tissue, ovarian tissue, and even in the milk of lactating women (Laux et al., 2006). In past studies, researchers used different samples to test the sensitivity and selectivity of Seratec PSA™. Seratec PSA™, like RSID Semen™, also produced false positive test results at high rates (Bitner, 2012).

Forensic investigators also use the presumptive test ABAcard P30™ for the detection of seminal fluid on evidence. The ABAcard P30™ test kit uses PSA as an indicator for a possible semen sample just like Seratec PSA™. Similar to the other two test kits being studied, the ABAcard P30™ test kit relies on the interaction between antigens and antibodies. This mechanism allows for the detection of a specific marker, like PSA, offering a rapid and convenient method for preliminary analysis. However, because presumptive tests have a high

sensitivity and a low specificity, this test is suspectable to false positives. Previous research, as highlighted by Cooper (2008), has documented instances where the ABAcard P30™ test generated several false positive outcomes. These findings underscore the importance of exercising caution when interpreting results obtained from presumptive tests, particularly in forensic settings where accurate results are crucial. The heightened sensitivity of presumptive tests enables the detection of even trace amounts of target substances, but this must be balanced with the potential for false positives.

Research needs to be conducted regarding the semen presumptive tests Seratec PSA™, RSID Semen™, and ABAcard P30™ to test the accuracy and sensitivity of the test results. Moreover, the sensitivity and specificity limits of all the tests need to be evaluated. New studies should be done to test a variety of substrates and mixed samples to see how often false positive test results occur. Samples with no trace of semen should also be used to see if those samples produce false positive results.

Presumptive tests are crucial to crime scene investigations, so the test results must be as accurate as possible. False positives are paramount in forensics because they can affect how long the overall testing process takes or even result in incorrect results being used at trial. False positive presumptive test results may be reported and might give the jury a sense of misunderstanding regarding semen. In past studies, researchers have thoroughly studied the accuracy of Seratec PSA™, ABAcard P30™, and RSID Semen™ with semen dilution samples, but there has not been enough research done on mixed samples with various chemical components. Further research and testing must be performed to determine how accurate Seratec PSA™, ABAcard P30™, and RSID Semen™ are when the samples are “dirty” or mixed with other substances. Some samples should be mixed with antiseptic soap and some samples should

be mixed with dirt, to simulate crime scene samples. It is likely in a real crime scene that a semen sample will be combined with something else, so it is essential to know if the mixed sample will cause a false positive test result.

2. Semen Components

Semen is a liquid secreted by males, and it is produced inside the testes in the seminiferous tubules (Stratton, 2022). After sperm is produced, it is stored in a structure inside the testes called the epididymis (Stratton, 2022). Semen contains different components, including spermatozoa, prostate-specific antigens, and semenogelin. Semen also contains enzymes, free amino acids, zinc, fructose, citric acid, prostaglandin, potassium, and phosphorylcholine (Mandal, 2019). All these components together create the term we know as semen.

2.1 Prostate-Specific Antigen

Semen presumptive tests provide a chemical indication of the possibility of semen being present by detecting specific components of semen. Prostate-specific antigen, or PSA for short, is used in some presumptive tests. PSA is a seminal fluid component and glycoprotein (Tang et al., 2019). Glycoproteins are proteins with carbohydrate groups attached to a polypeptide chain (Tang et al., 2019). Glycoproteins are used as chemical markers to tag proteins that will be used outside the cell (Engelking, 2015). The KLK3 gene encodes the glycoprotein enzyme in humans (Wong, 2021). The purpose of PSA is to aid in the liquefaction of the seminal coagulum (Wong, 2021). Seminal coagulum is a gel-like structure composed predominantly of spermatozoa and seminal plasma proteins (Roan et al., 2011). PSA is secreted by the columnar epithelium of prostatic tissue (David& Leslie, 2022). PSA is initially synthesized as an inactive form known as a proenzyme. This proenzyme consists of 244 amino acids arranged in a sequence (Balk et al.,

2003). However, before PSA can carry out its intended functions, it must undergo a process called activation. This activation occurs through the cleavage, or cutting, of seven N-terminal amino acids from the proenzyme molecule. This cleavage is a crucial step in converting the inactive proenzyme into its active form, enabling it to perform its biological roles effectively (Balk et al., 2003). The activation of PSA is significant because it ensures that the enzyme is only activated when needed. PSA cleaves and then degrades semenogelin, another component of semen, to keep the semen sample from coagulating. PSA keeps the semenogelin and fibronectin proteins from coagulating by breaking down the large protein molecules into smaller peptides (David& Leslie, 2022). The prostate mainly produces prostate-specific antigens, but they have been found in small quantities in other parts of the body. PSA can be found in female urine, breast tissue, breast cancer tumors, breast milk, amniotic fluid, periurethral glands, and endometrium (Laux et al., 2006). The concentration of PSA is low for all other bodily fluids except during pregnancy. During pregnancy, the PSA levels are significant in breast milk and other tissues that play a role in pregnancy (Old et al., 2011). PSA has also been detected in amniotic fluid (Yu & Diamandis, 1995). Because PSA has been found in other parts of the body, it is a presumptive test and can be used to determine the possible presence of semen. However, it cannot confirm the presence of semen.

2.2 Semenogelin

Semenogelin is another component of semen that is used in semen presumptive tests. Semenogelin is the main component of the human semen coagulum and is highly concentrated in seminal vesicle fluid (De Lamirande, 2007). Semenogelin creates a gel-like substance that encases the male sex cells also known as spermatozoa, to prevent them from breaking down in

the female body (De Lamirande, 2007). The role of semenogelin is to clot the seminal fluid as the semen is ejaculated (Boward et al., 2013). The high concentration of semenogelin is degraded by PSA (De Lamirande, 2007). Semenogelin must be degraded to liquefy the semen. The PSA turns the semen from a gel-like substance to a more liquid form. This is essential for sperm motility and fertilization. Although semenogelin is predominately produced as a component of human-seminal fluid, it has also been detected in the blood serum of cancer patients as well as the semen of some primates (Old et al., 2011). Because semenogelin has been located in the serum of male cancer patients and other species, it can only be used to determine the possible presence of semen and is should not be used as a confirmatory test.

3. The Detection of Semen

In the context of confirming the presence of semen, spermatozoa or sperm cells play a vital role. Sperm cells are produced in the testes and are released into the semen during ejaculation. Therefore, the presence of sperm cells is a confirmation of semen in sexual assault cases, detecting semen is critical, but it is often hard to confirm if spermatozoa are absent. Cases where spermatozoa are absent or scarce are often difficult to solve for forensic investigators. There are various reasons an individual might lack spermatozoa in their semen sample including if a male had a vasectomy. If there are no sperm cells present, investigators cannot confirm the presence of semen. It is also hard for investigators to confirm a semen sample if the sample came from someone who is oligospermic. That means the individual has a low sperm count. A person who is oligospermic has a sperm count of less than 15 million sperm/mL (Castañeda et al., 2018). An average sperm count is typically more than 15 million sperm per milliliter of semen.

Another reason a person might lack spermatozoa in their semen sample is if they are aspermiac. An individual who is aspermiac will not release semen cells when they ejaculate (Yoshida et al., 2018). An individual who has azoospermia will also lack spermatozoa in their semen sample. Azoospermia is a condition in which there is no sperm in the ejaculate (Koukouvinos et al., 2017). This condition is present in 1% of men in the general population and in 15% of men with infertility (Department of Urology, 2019). The absence of sperm cells in a semen sample makes forensic testing more challenging. As multiple conditions can cause this, it makes it more feasible that a sample from a crime scene might lack spermatozoa. In these instances, semen cannot be conclusively reported, only presumptively indicated.

3.1 Semen Presumptive Testing

Presumptive tests play a critical role in the detection of semen in samples that do not contain sperm cells. They also play an important role in determining if confirmation testing should be conducted. Presumptive tests are fast and inexpensive, so they are often done first. To confirm that the sample is semen, analysts must determine if there are sperm cells present. To do this they use staining techniques, such as Nuclear Fast Red-Picroindigocarmine (NFR-PIC), which is commonly used to confirm sperm cells under a microscope (Allery et al., 2001). The NFR-PIC staining, also known as the Christmas tree staining technique, is a valuable tool in forensic investigations for confirming the presence of sperm cells in suspected semen samples. It utilizes two dyes: Nuclear Fast Red, a red dye that stains the sperm heads and nuclei of cells, and Picroindigocarmine, which is a green dye that stains the sperm tails and cytoplasmic components, including epithelial (skin) cells (Auger, 2010). This distinctive staining pattern, where the sperm heads appear red and the tails appear green, resembling a Christmas tree, allows

forensic analysts to identify and confirm the presence of human sperm cells based on their characteristic morphology. The confirmation of human sperm relies significantly on the unique characteristics of the sperm head.

4. Seratec PSA™

Seratec PSA™ is a semen presumptive test commonly used in forensic science. Seratec PSA™ is a rapid immunochromatographic test that detects prostate-specific antigen or PSA (Laux et al., 2006). The test uses PSA because it is a biomarker of seminal fluid (Tang et al., 2019). Seratec PSA™ was originally developed for medical purposes as a blood test that would detect concentrations of PSA for prostate cancer screening (*Seratec PSA Semiquant,* 2011). The test was later adapted for forensic science due to its sensitivity to concentrations of PSA. Seratec PSA™ detects PSA in concentrations ranging from 2 ng/mL PSA to 100 µg/ml of PSA (*Seratec PSA Semiquant,* 2011).

Seratec PSA™ has become widely popular among forensic investigators because the test can be used in the laboratory and the field. The test is simple, quick, and easy to use without the need for special training. To run a Seratec PSA™, the sample must be diluted. Seminal fluid should be diluted to a 1:500 concentration before use (*Seratec PSA Semiquant,* 2011). Once the sample is diluted, investigators take one of the test cassettes and add roughly 120 µl or three drops to the well (*Seratec PSA Semiquant,* 2011). After 10 minutes, results are recorded, and any results after 10 minutes are invalid. The dilution buffer used at the beginning of the Seratec PSA™ test is compatible with DNA extraction and typing (*Seratec PSA Semiquant,* 2011). If test results are positive for semen, investigators can use the dilution buffer for further DNA testing. Most laboratories will use a tiny piece of evidence or 1/8 of a swab for presumptive testing and use the remainder for DNA analysis.

4.1 False positives of Seratec PSA™

Presumptive tests cannot be considered confirmatory testing because they can produce false positives. False positive test results indicate the presence of a particular substance, when that substance is not actually present in the sample. Seratec PSA™ is designed to detect an antigen found in semen called PSA. However, small concentrations of PSA can also be detected in other substances, and this can cause a false positive on the testing device. False positives can result from household products, bodily fluids, or personal products. In forensics, false positives are a major issue, especially if they are not identified and the results are taken to trial.

False Positives Reported with Seratec PSA™
1.Condom lubricants
2.Personal lubricants
3.Nonoxynol-9
4.Male urine
5.Sodium hydroxide (caustic soda)
6.Female urine
7.Lactic acid
8.Citric acid

Table 1. Reported False positives with Seratec PSA™

4.2 False Positives with Condoms

Forensic investigators widely use Seratec PSA™ to detect the possible presence of semen at a crime scene. However, since it can produce false positives, several researchers have studied what can cause those results. It is essential to know what can affect your test results so that

adjustments can be made to the testing procedure. Bitner (2012) studied the effect of condom lubricant on the Seratec PSA™ semen test. Condoms are used regularly in sexual assault cases, so researchers wanted to determine how often condom lubricants produce false positives when tested with the Seratec PSA™. The researchers swabbed a variety of personal lubricants, lubricated latex condoms, unlubricated latex condoms, and lubricated polyurethane condoms. The swabs were then tested with the Seratec PSA™ test kit. The study produced inconsistent results, so the researchers increased the sample size of the study. After increasing the sample size, the results were still inconsistent, so the sample size was increased again. According to the researchers, they needed a larger sampling group to eliminate the possibility of a false positive test result from condom lubricants being mixed in with the sample (Bitner, 2012). Even after the sample size was increased twice, the results were too inconsistent in confirming the possible presence of semen. More research is needed to determine if condoms and lubricants cause false positives with semen presumptive test kits.

4.3 False Positives with Nonoxynol-9

In the same study, Bitner (2012) investigated nonoxynol-9, an ingredient in condom lubricant, as a cause of false positives. Results showed that nonoxynol-9 produced positive results even when there was no semen present. However, in casework, it may not be possible to identify the specific brand of condom used, and therefore there may be no way to determine if nonoxynol-9 was present in the condom. Future research needs to be done to determine if there is a way to tell if a condom has nonoxynol-9 present without knowing the brand.

Studies on vaginal lubrication have also found that nonoxynol-9 could potentially interfere with PSA detection when using semen presumptive tests (Snead et al., 2013). Snead et

al. conducted a study on vaginal spermicide gel. During that study, researchers observed that nonoxynol-9 interfered with the Seratec PSA™ presumptive test when the semen samples were collected inside condoms containing the spermicide (Snead et al., 2014). The vaginal spermicide studied contained nonoxynol-9. Future research also should be done to determine if nonoxynol-9 can affect other presumptive semen tests. If nonoxynol-9 does not affect other presumptive tests, then there must be some component specific to the Seratec PSA™ test kit that it inhibits.

4.4 False Positives with Lubricants

Snead et al. (2014) conducted a study to investigate the impact of condom lubricants and vaginal spermicide on the detection of prostate-specific antigens (PSA). Their findings revealed that certain lubricant brands, such as Gynol, Replens, Carbopol, and KY jelly, inhibited the detection of PSA. However, the study faced criticism due to the absence of both positive and negative control samples.

The researchers emphasized the importance of low semen concentration in minimizing false positives. Despite this, they acknowledged limitations in their study, particularly the lack of consideration for other potential inhibitors of PSA. They highlighted the necessity for further research to explore and eliminate additional factors contributing to false positives.

Although the researchers speculated that the lubricant Carbopol might have been responsible for PSA inhibition, they did not conduct subsequent investigations to confirm this hypothesis. Thus, further studies are required to elucidate the true cause of PSA inhibition by Carbopol or other potential inhibitors.

4.5 False Positives Caused by Urine

In the same study conducted by Sneed et al. (2014) regarding the effects of vaginal spermicide on Seratec PSA™, it was determined that substances like male urine and sodium hydroxide interfere with the detection of PSA (Snead et al., 2014). Future research needs to be done to determine what component in the urine and sodium hydroxide is causing the false positives. It is imperative to know what component in male urine is causing false positives because there is a good chance that male urine can be found at a crime scene with a male victim or assailant. If researchers can determine what is causing the false positives, improvements can be made to testing protocols.

Like males, some female urine samples can also generate false positives. This is because some females can have PSA in their urine (Schmidt et al., 2001). Schmidt et al. (2001) conducted a study in which urine samples from 217 females were analyzed for PSA. Out of all the samples, 11% contained positive levels of PSA (Schmidt et al., 2001). The researchers found evidence to suggest that age was a factor in PSA levels in female urine. They found that women who were younger than 50 that tested positive had a mean PSA level of 0.34 ng/mL (Schmidt et al., 2001). However, women over 50 had a mean PSA level of 0.23 ng/mL (Schmidt et al., 2001).

4.6 False Positives with Household Products

Research has been conducted on everyday household products to determine if they can cause false positives in semen presumptive tests. In a study conducted by Foley et al. (2020) lactic acid and citric acid-based products were evaluated as false positives for presumptive semen tests. These are common ingredients in household products. Lactic acid is a component of milk and is common in products like beer, milk, cottage cheese, legumes, and soy products. Citric acid is common in sodas, juices, limes, oranges, lemons, and cleaning products. Citric acid

is used in cleaning products because it kills bacteria, mold, and mildew. The results of this study showed that lactic acid and citric acid produced false positives. According to the researchers, this was “due to non-specific binding events in the presence of organic acids” (Foley et al., 2020, p. 2). Because these organic acids are so common in household products and food, forensic investigators need to consider the possibility of these types of substances producing false positives when conducting presumptive tests. Foley et al. (2020) emphasize the importance of acknowledging the potential for false positive results generated by organic acids when using immunochromatographic cartridges. They suggest that such awareness should be considered to avoid overstating the significance of results obtained through these assays.

4.7 Sensitivity and Specificity of Seratec PSA™

Knowing the sensitivity and specificity of presumptive tests is crucial for forensic testing. According to the Seratec PSA™ test pamphlet, the test showed no cross-reactivity with other proteins of the seminal fluid, seminal fluid of other mammals (dog, cat, horse, bull, pig) except for the seminal fluid of primates, and no cross-reactivity with blood serum (*Seratec PSA Semiquant,* 2011, pg. 1). Despite the website for Seratec PSA™ claiming the test does not cross-react with other substances, several researchers have tested whether Seratec PSA™’s claims are valid. In a sensitivity study, Laffan et al. (2011) tested the Seratec PSA™ device using different semen dilutions and mixed semen samples. They found Seratec PSA™ could detect PSA in seminal fluid up to a dilution of 1:1000 indicating that Seratec is sensitive to very low concentrations of semen. Laffan et al. also reported that Seratec PSA™ produced one false positive result. This occurrence highlights the impact of a small sample size on research outcomes. With the researchers' limited sample size, the likelihood of encountering false

positives was still evident. Increasing the sample size would have likely led to the detection of more false positive results. This highlights the importance of adequate sample sizes in research. In a similar experiment, Goncalves et al. evaluated the sensitivity of different semen presumptive tests and found Seratec to detect PSA at a dilution of 1:1000 (Gonçalves et al.,2017). In both studies, Seratec was able to detect PSA at a concentration lower than any other semen presumptive tests. Investigators need to be able to detect the possible presence of semen even at low concentrations, so knowing which type of presumptive test is the most optimal is important.

One positive observation of the Seratec study is that there were more true positive results than other presumptive semen tests that were being studied. According to the researchers, the Seratec PSA™ test is less susceptible to the high-dose hook effect (Laffan et al., 2011. High-dose hook effects are caused by high concentrations of antibodies or antigens affecting the antibodies' ability to form immune complexes. Immune complexes are macromolecules that are made up of immunoglobulins or antibodies, and they are bound to different antigens (Wilson & Hunt, 2002). If there is an excess amount of PSA in the sample, PSA will not be bound completely to the gold-labeled antibody. Reducing high-dose hook effects is essential in cases where investigators only have a small sample available. Nevertheless, since the test can produce false positives, it is important to do further testing to confirm the results.

5. Rapid Stain Identification Series (RSID)

One current semen presumptive test investigators use is the Rapid Stain Identification Series, or RSID Semen™. RSID Semen™ is an immunochromatographic assay that uses two monoclonal antibodies specifically to detect semenogelin (Old et al., 2011). A monoclonal antibody is an antibody that is composed of cells derived or made from a single cell. RSID

Semen™ tests utilize a color change reaction to show results. A sample is first added to the RSID Semen™ test strip, and specific semenogelin antibodies will then react with the sample (Old et al.,2011). If there are semenogelin antibodies, then the RSID Semen™ test will produce a colored line, indicating a positive test result as well as a control line (Old et al., 2011). RSID Semen™ is popular because the test is fast and easy. With RSID Semen™, results are shown within 10 minutes (*RSID™ field kit for human semen information page,* 2002*)*. According to the site that sells RSID Semen™ testing kits, it is "the first confirmatory test for human seminal fluid" (*RSID™ field kit for human semen information page,* 2002, pg. 1*)*. This is an incorrect statement for them to make. The only way to confirm seminal fluid is to locate sperm under a microscope. RSID Semen™ can only be used as a presumptive test. The makers of RSID Semen™ also claim that the test will not cross-react with other substances. However, researchers have reported RSID Semen™ to produce false positives (Table 2). It is essential to have an accurate test that does not react with other substances.

False Positives Reported with RSID Semen™
1. Lactic acid
2. Citric acid
3. Male urine
4. Female urine
5. Vaginal swabs

Table 2. Reported False positives with RSID Semen™

5.1 RSID Semen™ False Positives with Household Products

In a study conducted by Foley et al. (2020), everyday household products containing lactic acid or citric acid were tested to determine if they could cause false positive test results with presumptive semen tests, including RSID Semen™. The study showed that lactic acid and citric acid could produce false positive results in RSID Semen™ tests (Foley et al., 2020). RSID Semen™ false positives are a big problem because lactic acid and citric acid are common in cleaning and cosmetic products, which could potentially be found at a crime scene. The presence of organic acids in certain products might have interfered with the chemical bonds crucial for the functionality of RSID Semen™ tests. These organic acids can alter the pH or chemical composition of the sample, disrupting the specific interactions between antibodies and their target antigens in the RSID Semen™ test. However, further research needs to be conducted to determine if organic acids are the cause of the false positive results or if they are caused by another factor (Foley et al., 2020).

5.2 Sensitivity of RSID Semen™

Assessing the sensitivity of presumptive tests for semen is crucial, especially when dealing with samples collected at a scene that may be small or diluted. Various studies have been conducted to determine the sensitivity of different presumptive tests for semen, aiming to identify the most suitable option for fieldwork applications.

In one such study, Laffan et al. sought to assess the sensitivity and specificity of two semen presumptive tests: Seratec PSA™ and RSID Semen™. The researchers examined different semen dilutions, mixed semen samples, various substrates, post-intercourse samples, and washed stains to comprehensively evaluate the performance of these tests.

The results of the study revealed that RSID Semen™ exhibited lower sensitivity compared to Seratec PSA™ (Laffan et al., 2011). This finding contradicted the claims made by the manufacturers of RSID Semen™. According to the information provided on the RSID™ field kit for human semen, the test purportedly offers greater sensitivity and specificity than other commonly used presumptive tests such as PSA or AP, particularly when applied to actual case samples (RSID™ field kit for human semen information page, 2002).

This discrepancy between the study results and the manufacturer's claims underscores the importance of independent validation and rigorous evaluation of forensic testing methods. It highlights the need for researchers and forensic practitioners to critically assess the performance characteristics of presumptive tests, considering factors such as sensitivity, specificity, and reliability, to ensure accurate and reliable results in forensic casework.

Another study was conducted by Boward et al. (2013) in which the sensitivity of the semen presumptive tests RSID Semen™ and ABAcard p30™ were tested. Researchers tested aged semen stains, fresh post-vasectomy semen, frozen post-vasectomy semen samples, swabs collected after sexual intercourse, post-vasectomy semen samples mixed with saliva, blood, and urine, and non-semen samples (Boward et al., 2013). According to the study's results, "RSID™ showed low to moderate sensitivity when compared to the high sensitivity observed with ABAcard p30™" (Boward et al., 2013). The study results indicate that RSID Semen™ is not as sensitive of a test as the makers claim it is. There needs to be further research conducted comparing the sensitivity of RSID Semen™ to other semen presumptive tests to properly test RSID Semen™ claims of being the "more sensitive and specific" semen test compared to others on the market (*RSID™ field kit for human semen information page,* 2002, pg. 1*)*.

5.3 Accuracy of RSID Semen™

There is currently a backlog of evidence, particularly DNA evidence. Assessing the most accurate presumptive tests for detecting biological fluids helps to streamline the investigation process. There was a study conducted by Rodriguez et al. at the Institute for Bioengineering of Catalonia, to test the accuracy of semen presumptive tests as well as confirmatory tests (2019). The researchers used an alternative light source to locate bodily fluids and then tested the accuracy of the RSID Semen™ presumptive test across various samples. After that, the researchers tested the same semen samples with the RSID Semen™ test kit. When they got positive test results with the RSID Semen™ test, they tested the sample to see if they could obtain a DNA profile. This study was done to show how a combination of methods could be done at a crime scene to speed up the investigation. This study showed that all three testing methods produced effective results (Rodriguez et al., 2019). The study also showed that RSID Semen™ did not produce any false positive test results (Rodriguez et al., 2019). Note, that all the tests were done using semen samples from only five men, ranging in age from 19 to 26 (Rodriguez et al., 2019). The semen samples used for the tests do not represent the population as a whole. Because the samples were taken from only young men, there is not a wide range of semen concentrations. As men age, their sperm count loses quality and quantity, and this could affect presumptive testing results (David& Leslie, 2022). Further research needs to be conducted to account for the population as a whole and to accurately determine if false positives could be present using RSID Semen™. In another study conducted by Chang et. al., the accuracy of RSID Semen™ was tested and it was found that female and male urine produced several false positive test results with the RSID Semen™ test kit (Chang, 2011). In the same study, RSID Semen™ also produced false positives with semen-free vaginal swabs (Chang, 2011).

5.4 Accuracy of RSID Semen™ With Mixtures

In a study focused on the sensitivity of RSID Semen™ to mixed samples, Martinez et al. (2015) tested different semen dilutions and mixed substances The results indicated that the RSID Semen™ test successfully detects semen when mixed with other biological fluids, including vaginal fluid, blood, and urine. Similarly, in the study by Laffan et al. (2011), the sensitivity of semen presumptive tests with mixed samples results showed that the RSID Semen™ test produced no false positives with other substances. The researchers of both studies included a variety of sample types to evaluate the sensitivity of semen presumptive tests. However, each experiment lacked an adequate sample size. The researchers should have had utilized a larger sample size to determine if the mixed samples could cause false positive test results.

Another study was conducted by Old et al. (2011) to test the accuracy of semen presumptive tests when samples are mixed. The researchers evaluated three tests to compare the accuracy of the tests: Acid Phosphatase, Seratec PSA™, and RSID Semen™. The researchers also tested potential reactions between the test kits and other body fluids as well as different types of animal semen (Old et al., 2011). The researchers conducted a mock case where they tested semen samples mixed with contraceptives, sexual lubricants, different fabrics, and vaginal swabs from females who had just had intercourse with and without condoms. Results showed that RSID Semen™ does not cross-react with any of the other human body fluids tested or with any of the types of animal semen tested. According to the study, RSID Semen™ is more sensitive to samples containing mixtures of vaginal secretions and semen than other commercially available semen presumptive tests (Old et al., 2011, p. 1). The researchers used several different types of samples to compare the accuracy of the three tests, but they did not have many samples.

Some experiments tested upwards of 60 samples, but some only had six trials (Old et al., 2011). There needs to be further research done to test how accurate RSID Semen™ is with mixed samples with multiple trials.

6. ABAcard P30™

The ABAcard P30™ test was developed for the detection of p30 or PSA in forensic investigations for the detection of semen. The test kit features a mobile monoclonal antihuman PSA antibody (NFSTC, 2001). Monoclonal antibodies are laboratory-produced molecules designed to mimic the immune system's ability to fight off harmful pathogens (Van Wauwe et al.,1980). These antibodies are highly specific, recognizing and binding to a single target molecule, known as an antigen. This antibody is a fusion of a human antibody and a small portion of a rat monoclonal antibody, as described by Van Wauwe et al. (1980). Designed to react with any prostate-specific antigen (PSA) present in the sample, this glycoprotein is a constituent of seminal fluid and is primarily produced in the prostate gland, as highlighted by Maguire (2013). Importantly, PSA secretion occurs independently of spermatozoa production within seminal fluid (Maguire, 2013). Hence, PSA serves as a reliable marker for identifying seminal fluid during presumptive semen testing. However, it's essential to note that there remains a possibility of false positive test results.

False Positives Reported with ABAcard P30™
1. Male urine
2. Bovine semen
3. Tampon

Table 3. Reported False positives with ABAcard P30

6.1 False Positives with other Body fluids using ABAcard P30™

One reason ABAcard P30™ tests are susceptible to false positives is that PSA is found in other parts of the body. PSA can be found in feces, breast milk, urine, and sweat (Cicetti, 2010). Validation studies over ABAcard P30™ revealed that the test has specificity issues (Simich et al., 1999). In the study conducted by Chang (2011), the sensitivity and specificity of semen testing were compared, and it was found that male urine caused false positive test results with the ABAcard P30™ test (Chang, 2011). In another study, bovine semen produced invalid test results with the ABAcard P30™ test kit (Cooper, 2008). Bovine semen shares some biochemical similarities with human seminal fluid, which may cause cross-reactivity with certain components targeted by semen presumptive tests.

6.2 False Positives with Personal Products using ABAcard P30™

Bodily fluids are not the only cause of false positive test results with the ABAcard P30™ test kit. In a study by Cooper (2003), tampons were found to cause false positives (Cooper, 2008). However, there have not been many studies conducted to compare different products to see how they affect the accuracy of the ABAcard P30™ test.

6.3 Sensitivity of ABAcard P30™

Sensitivity issues were seen during validation studies over the ABAcard P30™ test (Simich et al., 1999). In another study by Cooper (2008), false negative results were reported when researchers tested semen samples that were on different substrates. Those materials included tissue, condoms, and black and blue denim. These results were likely caused by a high-dose hook effect. This occurs when the concentration of the target analyte in the sample becomes

extremely high, leading to interference with the assay's detection system (Min, 2012). When the substance concentration gets too high, it overwhelms the test, making it unable to properly bind. As a result, it can lead to false positives. In the same study, false negative results were also observed when semen samples were mixed with blood, breast milk, and urine (Cooper, 2008).

7. Significance of Continued Research

As the incidence of sexual assault cases continues to escalate, it is paramount that investigators have access to diagnostic testing methods that exhibit unsurpassed accuracy and efficiency in the identification of potential semen evidence. Past research into semen presumptive testing has shown issues with false positive test results. Issues with these tests may impede an investigation and result in a misuse of time and resources. However, an even bigger problem is if these results are not caught and are used in a trial to convict an innocent person. Presumptive semen results should never be used in the courtroom, but that doesn't mean they haven't been and continue to be used.

Additional research on the reliability of presumptive semen detection assays is imperative to strengthen forensic investigations. Microscopic sperm confirmation, while confirmatory, is not always feasible, as azoospermic and vasectomized individuals still produce other seminal fluid components. Thus, investigators rely heavily on screening tests targeting prostate-specific markers to identify likely semen stains. However, potential cross-reactivity with non-semen substances can produce false positive results, underscoring the need for rigorous specificity testing. Unfortunately, few studies have thoroughly evaluated the accuracy of common semen identification assays against an extensive panel of potential contaminants. Comprehensive analyses of diverse sample sets are essential to delineate the limitations of current semen tests

and curtail improper evidentiary conclusions. Methodical accuracy assessments will enable the development of more selective semen presumptive techniques, upholding the standards of reliability expected of forensic science. Robust validation is critical to elicit the full utility of presumptive testing in sexual assault and other probative investigations.

7.1 Future Research into Seratec PSA™

While initial studies have explored the utility of Seratec PSA™ for semen identification, more comprehensive validation is needed to firmly establish its reliability as a presumptive screening test. Previous analyses utilized limited sample sizes, curtailing the generalizability of their findings. Preliminary research indicates concerning false positive rates when testing Seratec PSA™ against various household products, intimate lubricants, and bodily fluids. Further evaluation using expanded validation matrices is imperative to fully delineate the scope of potential cross-reactivity issues. Additional non-semen sources containing compounds that could mimic PSA, including diverse all-purpose cleaners, antiseptic soaps, carbolic acid, and lactic/citric acid products warrant testing.

Furthermore, research on PSA expression in forensically relevant biological fluids is currently lacking. PSA has been detected in female urine, breast milk, and endometrial tissue, but the concentrations present and their implications for presumptive semen testing remain unclear (Laux et al., 2006). Therefore, this thesis study will assess female samples to determine if they could confound PSA-based screening approaches. Thorough validation across a spectrum of possible contaminants and fluid sources will help establish the strengths and limitations of PSA-targeting assays for sexual assault evidence. Comprehensive accuracy assessments are imperative to instill confidence in new presumptive semen tests for casework applications.

7.2 Future Research into RSID Semen™

While promising, past validation of RSID Semen™ has been limited, with concerning reports of false positive results emerging. Only a handful of studies have thoroughly evaluated the accuracy of this immunochromatographic assay on realistic forensic matrices. Further testing against an expanded panel of case-relevant samples is needed to delineate its true reliability. Experiments should assess common personal lubricants, condoms, lotions, soaps, and other products that may be encountered in sexual assault evidence. Varying semen deposition amounts on relevant substrates would also help establish the sensitivity limits of RSID Semen™ and its ability to detect trace seminal fluid. Robust validation is imperative to highlight the capabilities and limitations of rapid immunological semen detection techniques.

7.3 Future Research into ABAcard P30™

There have been a few studies conducted on the accuracy and specificity of the ABAcard P30™ test. There needs to be further research done into possible things that can cause false positives with the test. There were a few studies done in the past to compare ABAcard P30™ to other semen presumptive testing; however, the tests did not test out that many factors. In one past study, the researchers observed multiple instances where the test produced false negatives. Further research needs to be done to determine if false negatives have a chance of occurring when semen is mixed with other samples. There also needed to be further testing done with samples taken from female subjects.

7.4 Accuracy of Semen Presumptive Testing

Semen presumptive tests are critical in sexual assault cases because it is essential to have the best and most accurate evidence to take to trial. Presumptive tests are used to determine the possible presence of a biological fluid. However, inaccurate results could lead to missed forensic opportunities or the misdirection of investigative resources. Unfortunately, current semen confirmatory methods are possible with all semen samples. Thus, the reliability of presumptive testing carries such a profound weight. While assays like Seratec PSA™, RSID Semen™, and ABAcard P30™, have been tested in the past, the results are lacking variety. And no past test compared the accuracy of all three semen tests. Comprehensive accuracy assessments on forensically relevant samples including blood, breast milk, urine, and intimate products were needed to instill confidence in their performance. This study aimed to systematically evaluate the three semen presumptive tests against case-type mixtures to determine their capabilities and limitations.

The study also aimed to explore the potential for diapers, feminine hygiene pads, and tampons to generate false positive results with presumptive semen tests. These products are designed to absorb significant fluid secretions on a regular basis. Trace amounts of prostate-specific antigen (PSA) or other cross-reacting compounds could feasibly accumulate within the absorbent layers over time. Swabbing or extracting substances from the surface of used feminine products presents another avenue for contaminant collection. Comprehensive testing is needed to assess if PSA levels reach concentrations that confound common semen screening assays. Varying absorbency types, brands, and usage durations should be analyzed to fully evaluate the possibility of interference. In addition, chemical signatures of common diaper rash creams, vaginal antifungals, and spermicides require investigation, as these could produce misleading

responses. Forensic validation must thoroughly address these practical evidentiary sources to instill confidence in applying presumptive semen tests to sexual assault investigations where such products are encountered.

Methods and Materials

8. Specimens

A variety of human samples, household supplies, and personal items were tested to compare the accuracy of semen presumptive tests. The samples contained varying semen concentrations or no semen at all. Each sample was tested in triplicate using Seratec PSA™, ABAcard P30™, and RSID Semen™. Samples were then tested using different semen presumptive tests according to the test's instruction manual, which was provided by the manufacturer.

8.1 Body Fluids

All body fluid donors and research participants were healthy volunteers and were informed that they could withdraw from the study at any time per federal human subjects' protection policies. The research was approved by the University of Central Oklahoma Internal Review Board (UCO IRB #2023-064). Breastmilk was collected from lactating mothers who volunteered their sample. The breastmilk was kept in the refrigerator. Human male whole blood samples were obtained from the blood bank. Blood was donated for research purposes and was kept in the refrigerator. Menstrual pads containing menstrual blood were obtained from healthy individuals who didn't have the possibility of semen present in their sample. Tampons containing menstrual blood were obtained from healthy volunteers who didn't have the possibility of semen present in their sample. Diapers containing male or female urine were obtained from healthy

volunteers who didn't have the possibility of semen present in their sample. Female and male urine was collected from volunteers, and the urine sample was collected mid-stream into a plastic cup. Saliva was collected in a plastic tube from a healthy volunteer. Menstrual products, diapers, and urine samples were tested immediately after collecting the sample. Semen was purchased from the Serological Research Institute (SERI), which is a non-profit laboratory, that is accredited by the ANSI National Accreditation Board (ANAB). ANAB is the largest accreditation body in the United States, and they accredit institutions and labs based on recognized international standards. This provides confidence in the quality and reliability of the services and products they provide. The semen sample came as freeze-dried seminal plasma. The semen sample was kept in the freezer. The full list of products to be tested can be found in Table 4.

8.2 Personal Products

A range of personal lubricants, vaginal products, spermicides, and various condom types were obtained for analysis. The surfaces of the lubricated condoms were swabbed using sterile cotton-tipped swabs to collect samples of the condom lubricants. These swabs were then allowed to fully air-dry before testing, mimicking realistic forensic conditions. Personal lubricants were directly deposited in small quantities onto sterile cotton swabs and similarly permitted to dry. Boric acid vaginal suppositories were collected, and the inside of the suppository was transferred to a plastic vile. The powder was then mixed with DI water to dissolve it. The mixer was then swabbed with a sterile cotton swab. The yeast infection cream was deposited onto a sterile cotton swab and allowed to air dry. Anti-aging products were collected, and the creams were swabbed with a sterile cotton swab, the swabs were allowed to air-dry. Products were air-dried because

that is likely how they would be found in actual case work. The full assortment of intimate products analyzed in this study can be found in Table 4.

8.3 Household Products

Various common household products were obtained from commercial vendors to evaluate the potential for false positive results with presumptive semen testing. Each product was swabbed utilizing a sterile cotton swab, and the swabs were permitted to fully air-dry before further analysis. The specific products acquired for testing are detailed in Table 4. This controlled sampling of household items allowed for systematic assessment of possible interferences and cross-reactivity with forensic semen screening assays. Adherence to sterile swabbing and drying protocols ensured optimal sample collection and preservation for downstream comparative analysis.

8.4 General Protocol

For samples collected using a swab, a sterile swab was taken and used to collect the desired test sample. The swab was allowed to completely air dry. Once dried, a clean scalpel was used to carefully cut only the cotton tip of the swab into three equal portions, being careful not to cut too close to the inner stick as sample material was unlikely to have migrated that far down the swab. Each of the three cotton portions were transferred into separate, sterile 2ml microcentrifuge tubes for further processing and analysis.

For samples that could not be swabbed, a small cutting was taken. A fresh scalpel or scissors was used to cut out only the minimum amount of sample required, taking care to

preserve the overall integrity of the sample during this process. The cutting was transferred to a sterile 2ml microcentrifuge tube.

ABAcard P30™	RSID Semen™	Seratec PSA™
Vasectomized male semen.	Vasectomized male semen.	Vasectomized male semen.
Boric acid powder	Boric acid powder	Boric acid powder
Summer's Eve™ cleansing douche.	Summer's Eve™ cleansing douche.	Summer's Eve™ cleansing douche.
Yeast infection cream (7-day™ vaginal cream)	Yeast infection cream (7-day™ vaginal cream)	Yeast infection cream (7-day™ vaginal cream)
Retinol Collagen cream	Retinol Collagen cream	Retinol Collagen cream
VCF Contraceptive Gel™ (contains Nonoxynol-9)	VCF Contraceptive Gel™ (contains Nonoxynol-9)	VCF Contraceptive Gel ™ (contains Nonoxynol-9)
Trojan ENZ Latex Condoms™ (contains Nonoxynol-9)	Trojan ENZ Latex Condoms™ (contains Nonoxynol-9)	Trojan ENZ Latex Condoms™ (contains Nonoxynol-9)
Breastmilk	Breastmilk	Breastmilk

Breastmilk with blood	Breastmilk with blood	Breastmilk with blood
Juvenile male urine (10-year-old)	Juvenile male urine (10-year-old)	Juvenile male urine (10-year-old)
Female urine (28-years-old)	Female urine (28-years-old)	Female urine (28-years-old)
Vaginal swab (with no chance of semen)	Vaginal swab (with no chance of semen)	Vaginal swab (with no chance of semen)
Menstrual pad with menstrual blood (28-year-old)	Menstrual pad with menstrual blood (28-year-old)	Menstrual pad with menstrual blood (28-year-old)
Panty liner w/ menstrual blood	Panty liner w/ menstrual blood	Panty liner w/ menstrual blood
Diaper Liner with female urine (Bambo™ Brand)	Diaper Liner with female urine (Bambo™ Brand)	Diaper Liner with female urine (Bambo™ Brand)
Diaper Gel with female urine (Bambo Brand™)	Diaper Gel with female urine (Bambo Brand™)	Diaper Gel with female urine (Bambo Brand™)
Diaper Liner 1 with urine (Honest™ Brand)	Diaper Liner 1 with urine (Honest™ Brand)	Diaper Liner 1 with urine (Honest™ Brand)
Diaper Gel 1 with urine (Honest™ Brand)	Diaper Gel 1 with urine (Honest™ Brand)	Diaper Gel 1 with urine (Honest™ Brand)

Diaper Liner 2 with urine (Honest™ Brand)	Diaper Liner 2 with urine (Honest™ Brand)	Diaper Liner 2 with urine (Honest™ Brand)
Diaper Gel 2 (Honest™ Brand)	Diaper Gel 2 (Honest™ Brand)	Diaper Gel 2 (Honest™ Brand)
Diaper Liner 3 with urine (Honest™ Brand)	Diaper Liner 3 with urine (Honest™ Brand)	Diaper Liner 3 with urine (Honest™ Brand)
Diaper Gel 3 with urine (Honest™ Brand)	Diaper Gel 3 with urine (Honest™ Brand)	Diaper Gel 3 with urine (Honest™ Brand)
Diaper Liner 4 with urine (Honest™ Brand)	Diaper Liner 4 with urine (Honest™ Brand)	Diaper Liner 4 with urine (Honest™ Brand)
Diaper Gel 4 with urine (Honest™ Brand)	Diaper Gel 4 with urine (Honest™ Brand)	Diaper Gel 4 with urine (Honest™ Brand)
Diaper liner with female urine (Huggies™ newborn Brand)	Diaper liner with female urine (Huggies™ newborn Brand)	Diaper liner with female urine (Huggies™ newborn Brand)
Diaper gel with female urine (Huggies™ newborn Brand)	Diaper gel with female urine (Huggies™ newborn Brand)	Diaper gel with female urine (Huggies™ newborn Brand)
X	Diaper with male whole blood (Pampers Swaddlers™ size 2)	Diaper with male whole blood (Pampers Swaddlers™ size 2)

X	Diaper liner with DI water (Pampers Swaddlers™ size 2)	Diaper liner with DI water (Pampers Swaddlers™ size 2)
X	Diaper gel with DI water (Pampers Swaddlers™ size 2)	Diaper gel with DI water (Pampers Swaddlers™ size 2)
X	Bleach	Bleach
X	Mrs. Myers™ all-purpose cleaner (Lemon Verbena scent)	Mrs. Myers™ all-purpose cleaner (Lemon Verbena scent)
X	Saliva (28 years old)	Saliva (28 years old)
X	Whole blood (male)	Whole blood (male)
X	Semen mixed with dirt.	Semen mixed with dirt
X	X	Semen mixed with antiseptic soap.
X	X	Female urine taken during the menstrual cycle (28-year-old)
Menstrual pad with DI water	X	Menstrual pad with DI water

Menstrual pad with menstrual blood heavy flow (28-year-old-day 1 of cycle)	X	Menstrual pad with menstrual blood heavy flow (28-year-old-day 1 of cycle)
X	X	Menstrual pad with menstrual blood heavy flow pad 2 (28-year-old-day 1 of cycle)
X	X	Menstrual pad with menstrual blood heavy flow pad 3 (28-year-old-day 1 of cycle)
X	X	Menstrual pad with menstrual blood medium heavy flow (28-year-old-day 2 of cycle)
Menstrual pad with menstrual blood medium flow (28-year-old-day 3 of cycle)	X	Menstrual pad with menstrual blood medium flow (28-year-old-day 3 of cycle)
X	X	Menstrual pad with menstrual blood light flow (28-year-old-day 4 of cycle)

X	X	Menstrual pad with menstrual blood light flow (28-year-old-day 5 of cycle)
X	Tampon with menstrual blood	Tampon with menstrual blood
X	X	Tampon with no blood
X	X	Incontinence pad with urine
X	X	Incontinence pad 2 with urine

Table 4. Possible factors affecting semen presumptive testing.

9. Seratec PSA™ Test

For the Seratec PSA™ test, all test components were brought to room temperature according to the testing protocol (Seratec PSA Semiquant, 2011). This was because low temperatures could lead to a decrease in sensitivity in the test. After the components were at room temperature, around 3 drops of the sample (approximately 120 µl) were added to the sample well with a plastic pipette (Seratec PSA Semiquant, 2011). Dry samples were extracted in 250 µL of sterilized, ultra-pure water. The results were then read after 10 minutes at room temperature. A positive test result was indicated by 2 lines, one for the test and one in the control line, as seen in Fig. 1. A negative result would only have a pink line beside the control. If there was not a line by the control the test was considered invalid (Seratec PSA Semiquant, 2011). The Seratec PSA™ test was an immunochromatographic assay that relied on the binding of antibodies to the prostate-specific antigen to generate the result lines. Inside the test device, two

monoclonal murine anti-PSA antibodies were conjugated to colored particles and immobilized on the test line (Seratec PSA Semiquant, 2011).

When the semen sample was added to the well, any PSA present would bind to the mobile anti-PSA antibody conjugates. This PSA-antibody complex then migrated along the test strip via capillary action. When the complex reached the test line, the anti-PSA antibodies would bind to the immobilized anti-PSA antibodies, forming the visible pink test line through the accumulation of the colored conjugates. The intensity of this test line correlated with the concentration of PSA in the sample.

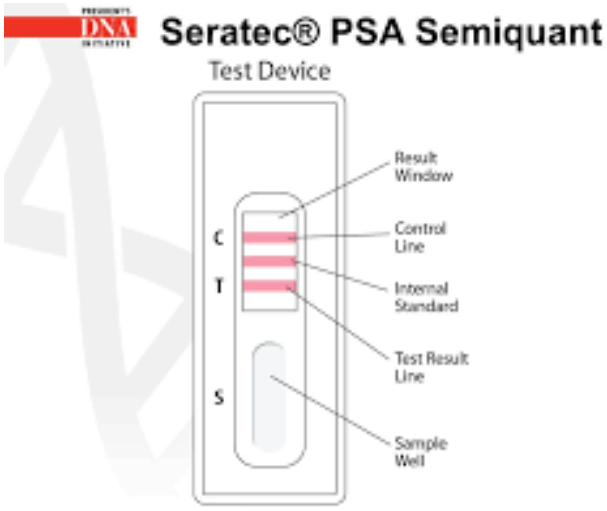

Figure 1. Seratec PSA™ Test Kit

10. RSID Semen™ Semen Presumptive Testing

The RSID Semen™ test was an immunochromatographic assay that detected semenogelin, a protein specific to seminal fluid, see Fig. 2. It used monoclonal antibodies targeted against semenogelin that were conjugated to colloidal gold particles.

To perform the test, a sample extract was added to the sample well, allowing it to migrate via capillary action along the test strip. The conjugated antibodies and sample extract flowed

laterally along the test strip via capillary action. If semenogelin was present in the extract, it would bind to the conjugated anti-semenogelin antibodies. This semenogelin-antibody complex continued moving across the test strip until it reached the test zone, which contained immobilized anti-semenogelin antibodies. The immobilized antibodies captured the semenogelin-antibody complex, resulting in the accumulation of the gold conjugates and the generation of a visible pink line in the test zone (Independent Forensics of IL, 2002). The test strip also contained a control zone with immobilized antibodies that bound excess conjugate, producing a pink control line to demonstrate the proper function of the test. Therefore, the presence of two pink lines - one in the test zone and one in the control zone - indicated a positive result for the presence of semenogelin and thus semen in the sample. A single pink control line without a test line indicated a negative result.

The RSID Semen™ tests were performed per the manufacturer's protocol. The recommended protocol for the RSID Semen™ test began with the removal of the cassette from the foil pouch followed by proper labeling. 95 microliters of refrigerated buffer solution were pipetted into a 2ml microcentrifuge tube. Next, one-third of the swab sample or a small cutting was added to the buffer and vortexed for thorough mixing. Using a disposable pipette, the entire liquid extract was then transferred to the test window on the cassette. After an incubation period of 10 minutes at room temperature, the test result could be interpreted (Independent Forensics of IL, 2002).

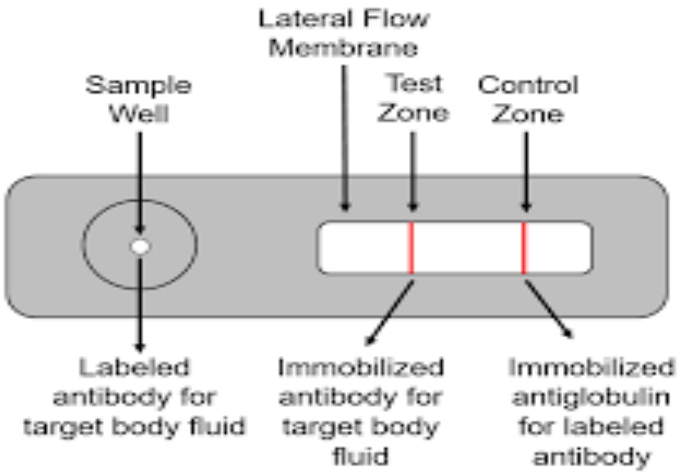

Figure 2. RSID Semen™ Semen Presumptive Test Kit

11. ABAcard P30™

The sample was added to a 2ml microcentrifuge tube along with the buffer from the test kit, followed by a tedious 2-hour incubation period. The samples had to be incubated in the refrigerator. All the ABAcard P30 test components were brought to room temperature. Then 8 drops of the sample (approximately 200 µL) were added to the sample well labeled with an "S" (DNA Analyst Training Laboratory Training Manual, 2001). The results were read after 10 minutes.

A positive result was indicated by the presence of two pink lines in the test window - one line for the control and one line for the test line. The test line formed through an antigen-antibody interaction. The ABAcard P30 test detected the presence of p30, a protein found in an abundant amount in semenogelin seminal vesicle-specific antigen, which is unique to human seminal plasma.

The test strip contained mobile monoclonal anti-p30 antibodies conjugated to colored particles. The formation of the pink test line relied on the specific antigen-antibody interaction between the p30 antigen (if present in the sample) and the anti-p30 antibodies used in the test.

When the sample was applied, the liquid migrated through the strip by capillary action (DNA Analyst Training Laboratory Training Manual, 2001). If p30 antigen was present in the sample, it would bind to the mobile anti-p30 antibody conjugates as the sample moved across the strip. This antigen-antibody complex was then captured by immobilized anti-p30 antibodies at the test line position, leading to the accumulation of colored particles and the formation of a pink test line (DNA Analyst Training Laboratory Training Manual, 2001).

A negative result only had a single pink line beside the control line position. The control line was formed by the capture of the mobile antibody conjugates, verifying that the test had been performed correctly and the components were functioning properly. If there was no line visible at the control line position, the test was considered invalid, indicating that the procedure may not have been followed correctly or that the test components were non-functional.

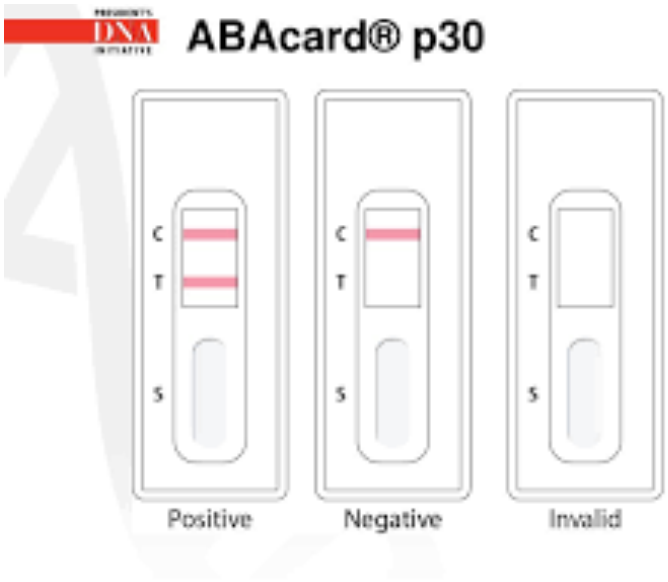

Figure 3. ABAcard P30™

Results

The accuracy and sensitivity of RSID Semen™, Seratec PSA™, and ABAcard P30™ were comprehensively evaluated to assess their effectiveness in semen detection, providing crucial insights into their performance within forensic contexts. The results of these evaluations are presented in the tables below.

*Note: X indicates that a test was not run, due to supply constraints

12. RSID Semen™ Results

SAMPLE	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4
Negative Control	Negative (-)	Negative (-)	Negative (-)	X
Positive Control	Positive (+)	Negative (-)	Negative (-)	Positive (+)

Table 5. RSID Semen™ Control Results

SAMPLE	TRIAL 1	TRIAL 2	TRIAL 3
1: 1000 Dilution	Positive (+)	Positive (+)	Positive (+)
1:10,000 Dilution	Negative (-)	Positive (+)	Positive (+)
1:100,000 Dilution	Negative (-)	Positive (+)	Positive (+)

Table 6. RSID Semen™ Dilution Results

SAMPLE	TRIAL 1	TRIAL 2	TRIAL 3
Vasectomized male semen	Positive (+)	Positive (+)	Positive (+)
Semen mixed with dirt	Negative (-)	Positive (+)	Positive (+)
Boric acid powder	Negative (-)	Negative (-)	Negative (-)
Summer's Eve™ cleansing douche	Negative (-)	Negative (-)	Negative (-)
Yeast infection cream (7-day™ vaginal cream)	Negative (-)	Negative (-)	Negative (-)
Retinol Colligan cream	Negative (-)	Negative (-)	Negative (-)
VCF Contraceptive Gel™ (contains Nonoxynol-9)	Negative (-)	Negative (-)	Negative (-)
Trojan ENZ™ Latex Condoms (contains Nonoxynol-9)	Negative (-)	Negative (-)	Negative (-)
Breastmilk	Negative (-)	Negative (-)	Negative (-)
Breastmilk with whole blood	Negative (-)	Negative (-)	Negative (-)
Whole blood (male)	Negative (-)	Negative (-)	Negative (-)
Juvenile male urine (10-year-old)	Negative (-)	Negative (-)	Negative (-)
Female urine (28 years old)	Negative (-)	Negative (-)	Negative (-)
Menstrual pad with menstrual blood	Positive (+)	Positive (+)	Positive (+)

Panty liner w/ menstrual blood	Negative (-)	Negative (-)	Negative (-)
Tampons with menstrual blood	Positive (+)	Positive (+)	X
Vaginal swab (with no chance of semen)	Negative (-)	Positive (+)	X
Diaper liner with DI water (Pampers Swaddlers™ size 2)	Negative (-)	Negative (-)	Negative (-)
Diaper gel with DI water (Pampers Swaddlers™ size 2)	Negative (-)	Negative (-)	Negative (-)
Bambo™ Diaper Liner with female urine	Negative (-)	Negative (-)	Negative (-)
Bambo™ Diaper Gel with female urine	Positive (+)	Positive (+)	Negative (-)
Diaper Liner with female urine (Huggies™ newborn)	Negative (-)	Negative (-)	Negative (-)
Diaper Gel with female urine (Huggies™ newborn)	Positive (+)	Positive (+)	Negative (-)
Pampers Swaddlers™ with male whole blood	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Liner 1 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Gel 1 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Liner 2 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Gel 2	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Liner 3 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Gel 3 with urine	Positive (+)	Negative (-)	Negative (-)
Honest™ Diaper Liner 4 with urine	Negative (-)	Negative (-)	Negative (-)

Honest™ Diaper Gel 4 with urine	Negative (-)	Negative (-)	Negative (-)
Saliva (28 years old)	Negative (-)	Negative (-)	Negative (-)
Bleach	Negative (-)	Negative (-)	Negative (-)
Mrs. Myers™ all-purpose cleaner (Lemon Verbena scent)	Negative (-)	Negative (-)	Negative (-)

Table 7. RSID Semen™ Test Results

13. Seratec PSA™ Results

SAMPLE	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4
Negative Control	Negative (-)	Negative (-)	Negative (-)	Negative (-)
Positive Control	Positive (+)	Positive (+)	Positive (+)	Positive (+)

Table 8. Seratec PSA™ Control Results

SAMPLE	TRIAL 1	TRIAL 2	TRIAL 3
1: 1000 Dilution	Positive (+)	Positive (+)	Positive (+)
1:10,000 Dilution	Negative (-)	Positive (+)	Positive (+)
1:100,000 Dilution	Negative (-)	Positive (+)	Positive (+)

Table 9. Seratec PSA™ Dilution Results

SAMPLE	TRIAL 1	TRIAL 2	TRIAL 3
Vasectomized male semen	Positive (+)	Positive (+)	Positive (+)
Semen mixed with dirt	Positive (+)	Positive (+)	Positive (+)
Semen mixed with antiseptic soap	Positive (+)	Positive (+)	Positive (+)
Boric acid powder	Negative (-)	Negative (-)	Negative (-)
Summer's Eve™ cleansing douche	Negative (-)	Negative (-)	Negative (-)

Yeast infection cream (7-day™ vaginal cream)	Negative (-)	Negative (-)	Negative (-)
Retinol Colligan cream	Negative (-)	Negative (-)	Negative (-)
VCF™ Contraceptive Gel (contains Nonoxynol-9)	Negative (-)	Negative (-)	Negative (-)
Trojan ENZ™ Latex Condoms (contains Nonoxynol-9)	Negative (-)	Negative (-)	Negative (-)
Breastmilk	Negative (-)	Negative (-)	Negative (-)
Breastmilk with blood	Negative (-)	Negative (-)	Negative (-)
Whole blood (male)	Negative (-)	Negative (-)	Negative (-)
Juvenile male urine (10-year-old)	Negative (-)	Negative (-)	Negative (-)
Female urine (28 years old)	Negative (-)	Negative (-)	Negative (-)
Female urine taken during menstrual cycle (28 years old)	Negative (-)	Negative (-)	Negative (-)
Incontinence pad with urine	Negative (-)	Negative (-)	Negative (-)
Incontinence pad 2 with urine	Negative (-)	Negative (-)	Negative (-)
Menstrual pad with DI water	Negative (-)	Negative (-)	Negative (-)
Menstrual pad with menstrual blood (28-year-old)	Negative (-)	Negative (-)	Negative (-)
Menstrual pad with menstrual blood heavy flow pad 1 (28-year-old-day 1 of cycle)	Positive (+)	Positive (+)	Positive (+)
Menstrual pad with menstrual blood heavy flow pad 2 (28-year-old-day 1 of cycle)	Positive (+)	Positive (+)	Positive (+)

Menstrual pad with menstrual blood heavy flow pad 3 (28-year-old-day 1 of cycle)	Positive (+)	Positive (+)	Positive (+)
Menstrual pad with menstrual blood medium heavy flow (28-year-old-day 2 of cycle)	Negative (-)	Positive (+)	Positive (+)
Menstrual pad with menstrual blood medium flow (28-year-old-day 3 of cycle)	Positive (+)	Positive (+)	Positive (+)
Menstrual pad with menstrual blood light flow (28-year-old-day 4 of cycle)	Negative (-)	Negative (-)	Negative (-)
Menstrual pad with menstrual blood light flow (28-year-old-day 5 of cycle)	Positive (+)	Negative (-)	Negative (-)
Panty liner w/ menstrual blood	Negative (-)	Negative (-)	Negative (-)
Tampons with menstrual blood	Negative (-)	Negative (-)	Negative (-)
Tampons with no blood	Negative (-)	Negative (-)	X
Vaginal swab (with no chance of semen)	Negative (-)	Negative (-)	Negative (-)
Diaper liner with DI water (Pampers Saddlers™ size 2)	Negative (-)	Negative (-)	Negative (-)
Diaper gel with DI water (Pampers Saddlers™ size 2)	Negative (-)	Negative (-)	Negative (-)

Diaper Liner with female urine (Huggies™ newborn)	Negative (-)	Negative (-)	Negative (-)
Bambo™ Diaper Liner with female urine	Negative (-)	Negative (-)	Negative (-)
Bambo™ Diaper with female urine	Negative (-)	Positive (+)	Positive (+)
Diaper Gel with female urine (Huggies™ newborn)	Negative (-)	Positive (+)	Positive (+)
Pampers Swaddlers™ with male whole blood	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Liner 1 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Gel 1 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Liner 2 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Gel 2	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Liner 3 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Gel 3 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Liner 4 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Gel 4 with urine	Negative (-)	Negative (-)	Negative (-)
Saliva (28-years-old Female)	Negative (-)	Negative (-)	Negative (-)
Bleach	Negative (-)	Negative (-)	Negative (-)
Mrs. Myers™ all-purpose cleaner (Lemon Verbena scent)	Negative (-)	Negative (-)	Negative (-)

Table 10. Seratec PSA™ Test Results

14. ABAcard P30™ Results

SAMPLE	TRIAL 1	TRIAL 2	TRIAL 3
Negative Control	Negative (-)	Negative (-)	Negative (-)
Positive Control	Positive (+)	Positive (+)	Positive (+)

Table 11. ABAcard P30™ Control Results

SAMPLE	TRIAL 1	TRIAL 2	TRIAL 3
1: 1000 Dilution	Positive (+)	Positive (+)	Positive (+)
1:10,000 Dilution	Negative (-)	Positive (+)	Positive (+)
1:100,000 Dilution	Negative (-)	Positive (+)	Positive (+)

Table 12. ABAcard P30™ Dilution Results

SAMPLE	TRIAL 1	TRIAL 2	TRIAL 3
Vasectomized male semen	Positive (+)	Positive (+)	Positive (+)
Boric acid powder	Negative (-)	Negative (-)	Negative (-)
Summer's Eve™ cleansing douche	Negative (-)	Negative (-)	Negative (-)
Yeast infection cream (7-day™ vaginal cream)	Negative (-)	Negative (-)	Negative (-)
Retinol Colligan cream	Negative (-)	Negative (-)	Negative (-)
VCF™ Contraceptive Gel (contains Nonoxynol-9)	Negative (-)	Negative (-)	Negative (-)
Trojan ENZ™ Latex Condoms (contains Nonoxynol-9)	Negative (-)	Negative (-)	Negative (-)
Breastmilk	Negative (-)	Negative (-)	Negative (-)
Breastmilk with blood	Negative (-)	Negative (-)	Negative (-)

Juvenile male urine (10-year-old)	Negative (-)	Negative (-)	Negative (-)
Female urine (28 years old taken during menstrual cycle)	Positive (+)	Positive (+)	Negative (-)
Menstrual pad with DI water	Negative (-)	Negative (-)	X
Menstrual pad with menstrual blood (28-year-old)	Negative (-)	Negative (-)	Negative (-)
Menstrual pad with menstrual blood heavy flow (28-year-old-day 1 of cycle)	Negative (-)	Negative (-)	Positive (+)
Menstrual pad with menstrual blood medium flow (28-year-old-day 3 of cycle)	Negative (-)	Positive (+)	Positive (+)
Panty liner w/ menstrual blood	Negative (-)	Negative (-)	Negative (-)
Vaginal swab (with no chance of semen)	Negative (-)	Negative (-)	Negative (-)
Bambo™ Diaper Liner with female urine	Negative (-)	Negative (-)	Negative (-)
Bambo™ Diaper Gel with female urine	Negative (-)	Negative (-)	Negative (-)
Diaper liner with female urine (Huggies™ newborn)	Negative (-)	Positive (+)	Negative (-)
Diaper gel with female urine (Huggies™ newborn)	Negative (-)	Negative (-)	Negative (-)
Honest Diaper Liner 1 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Gel 1 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Liner 2 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Gel 2	Negative (-)	Negative (-)	Negative (-)

Honest™ Diaper Liner 3 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Gel 3 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Liner 4 with urine	Negative (-)	Negative (-)	Negative (-)
Honest™ Diaper Gel 4 with urine	Negative (-)	Negative (-)	Negative (-)

Table 13. ABAcard P30™ Test Results

15. Overall Results

	Total Tests	False Positives	False Negatives
ABAcard P30™	101	6 (6%)	2 (2%)
RSID Semen™	119	11 (9%)	4 (3%)
Seratec PSA™	161	19 (12%)	2 (1%)

Table 14. Overall Semen Presumptive Test Kit Results

16. Statistical Analysis

This study compared the accuracy, sensitivity, and false positive/negative rates of three rapid immunochromatographic test kits for detecting semen: RSID Semen™, Seratec PSA™, and ABAcard P30™. Chi-square tests of independence were conducted to evaluate whether there were significant differences in performance between the kits. The chi-square ($\chi 2$) test of independence is used to determine if there is a significant relationship between two categorical variables such as semen or not, menses or not, and diapers or not. This study examined whether the test kit used (RSID Semen™, Seratec PSA™, ABAcard P30™) was independent of or associated with the accuracy outcomes observed across different test conditions.

16.1 Sensitivity Detection

The association between the brand of test kit and false negative results was analyzed (Table 20). The chi-square test indicated no statistically significant association ($\chi 2(2, N=127)$, $p > 0.05$). Furthermore, there was no significant difference in inaccuracy rates among the different test kit brands ($\chi 2(N=127)$, $p > 0.05$) (Table 24).

16.2 Interference from Other Substances

The false positive rates for semen samples did not significantly differ between the test kits ($\chi 2(N=127)$, $p > 0.05$) (Tables 16 & 17). However, menstrual blood significantly affected accuracy across all kits ($\chi 2(N=127)$, $p < 0.001$) (Table 31-33). This indicates that menstrual blood interferes with kit performance, leading to increased inaccuracies. The calculated Phi coefficient effect size of 0.3 indicates a moderate association between the binary variables under examination. This effect size suggests a discernible relationship between the variables, though it falls short of a strong or large effect. Similarly, the presence of diapers influenced accuracy ($\chi 2(N=127)$, $p =< 0.05$) (Table 33-36), suggesting that diaper components may compromise testing accuracy.

When focusing on semen detection versus other substances (Table 27), semen samples showed a significantly higher inaccuracy rate of 46.7% compared to 16.1% for non-semen samples, suggesting inferior semen detection performance. Analysis of inaccuracy rates concerning menstrual blood and products (Table 30) revealed a significant difference ($\chi 2(N=127)$, $p < 0.001$), with a higher rate of 52.9% for menstrual samples compared to 14.5% for non-menstrual samples. The calculated Phi coefficient effect size of 0.3 indicates a moderate association between the binary variables under examination. This effect size suggests a

discernible relationship between the variables, though it falls short of a strong or large effect. Additionally, the presence of diapers influenced test accuracy (Table 34), with higher rates of inaccuracy when diapers were present but improved accuracy when absent.

Interestingly, the test kits demonstrated optimal performance (92.5% accuracy) when none of the target substances (menses, diapers, semen) were present in the sample. However, accuracy significantly dropped (to 28.4%) when any of these substances were detected (Table 38). Further analysis focusing on menses and semen specifically (Table 42) revealed a slight tendency towards inaccuracy (53.3%) when semen was present, but higher accuracy (52.9%) for menstrual samples.

In conclusion, while no statistically significant differences were observed among the brands, variations in performance were evident. Seretec™ tended to exhibit higher inaccuracies compared to the other two test kits. Importantly, all brands struggled with accurate detection when absorbency products were present, emphasizing the need for caution when utilizing these test kits.

Discussion

17. Purpose of comparing RSID Semen™, Seratec PSA™, and ABAcard P30™

Identifying and confirming the presence of semen in forensic samples is crucial in sexual assault investigations. Several commercially available tests have been developed for this purpose, each targeting different biomarkers found in seminal fluid. However, past research has not comprehensively compared the performance of three widely used semen identification tests: RSID Semen™, Seratec PSA™, and ABAcard P30™.

The need for a comparative study arose due to the following reasons:

- **Varying target analytes:** Each test kit targeted a different biomarker present in seminal fluid. RSID Semen™ detected semenogelin, Seratec PSA Semiquant detected prostate-specific antigen (PSA), and ABAcard P30™ detected the p30 antigen. Comparing their performance provided insights into the reliability and specificity of each biomarker for semen identification.
- **Forensic casework applicability:** Semen presumptive tests are routinely used in forensic laboratories for sexual assault cases. A comparative study was needed to provide valuable information to forensic practitioners on the most suitable test(s) for different sample types and scenarios encountered in casework.
- **Evaluation of Sensitivity and Specificity**: It was essential to assess the sensitivity and specificity of each test to determine their effectiveness in correctly identifying the presence or absence of semen. Comparing these results helped in understanding the reliability of each test kit in differentiating between semen and non-semen samples.
- **Detection Limits:** Investigating the detection limits of each test kit was crucial to determine the minimum amount of semen required for reliable detection. Understanding the detection thresholds provided insights into the practical applicability of these tests, especially when dealing with trace amounts of biological material. Which is commonly encountered in forensic samples.
- **Cross-Reactivity**: Assessing the potential for cross-reactivity with substances commonly encountered in forensic samples was essential to evaluate the specificity of each test kit. Cross-reactivity from products or biological fluids at the crime scene could lead to false-

positive results, compromising the accuracy of forensic investigations. Comparing the cross-reactivity of different non-semen samples with each of the test kits helped in understanding their susceptibility to interference from substances other than semen.

18. The Flawed Use of Presumptive Tests as Confirmatory Evidence in Courtrooms

In the past, presumptive tests for the detection of semen held a prominent position in forensic investigations and court proceedings, often being presented and marketed as confirmatory evidence. These tests, created to offer presumptive screening results, were initially portrayed as definitive indicators of the presence of semen. When in reality, they only confirm the possibility of a product that is found in semen.

These tests were thought of as conclusive evidence because of several factors. Firstly, presumptive tests were developed to target components of semen, such as PSA or semenogelin. The tests were designed to produce color change reactions when they came into contact with the byproducts of semen.

Secondly, presumptive tests were frequently relied upon due to how simple they are to use and the time it takes to get results. Unlike more complex confirmatory tests, which often require specialized equipment and training to perform, presumptive tests require minimal training and setup. This made them particularly appealing for use in forensic evidence screening.

Furthermore, the historical context in which these presumptive tests because wrongfully confused as a confirmatory for seminal evidence. During earlier periods of forensic science development, there was a strong belief that scientific methods were without fault. As a result, presumptive tests were highly regarded as a reliable testing method. This then led to their widespread acceptance as evidence.

Over time, advancements in forensic science and an increased understanding of the limitations of presumptive tests have challenged this perception. However, it's important to acknowledge that this evolution hasn't entirely altered the perspective of the entire forensic science community. This could stem from a variety of factors, including historical precedent, institutional practices, or individual perspectives shaped by years of experience. Continued education and dialogue are essential for a widespread understanding of the sensitivity and specificity issues for each of the three kits evaluated, within the forensic science community.

19. Comparison of Sensitivity

This study resulted in concerning rates of false negative results with the three semen presumptive test kits that were evaluated:

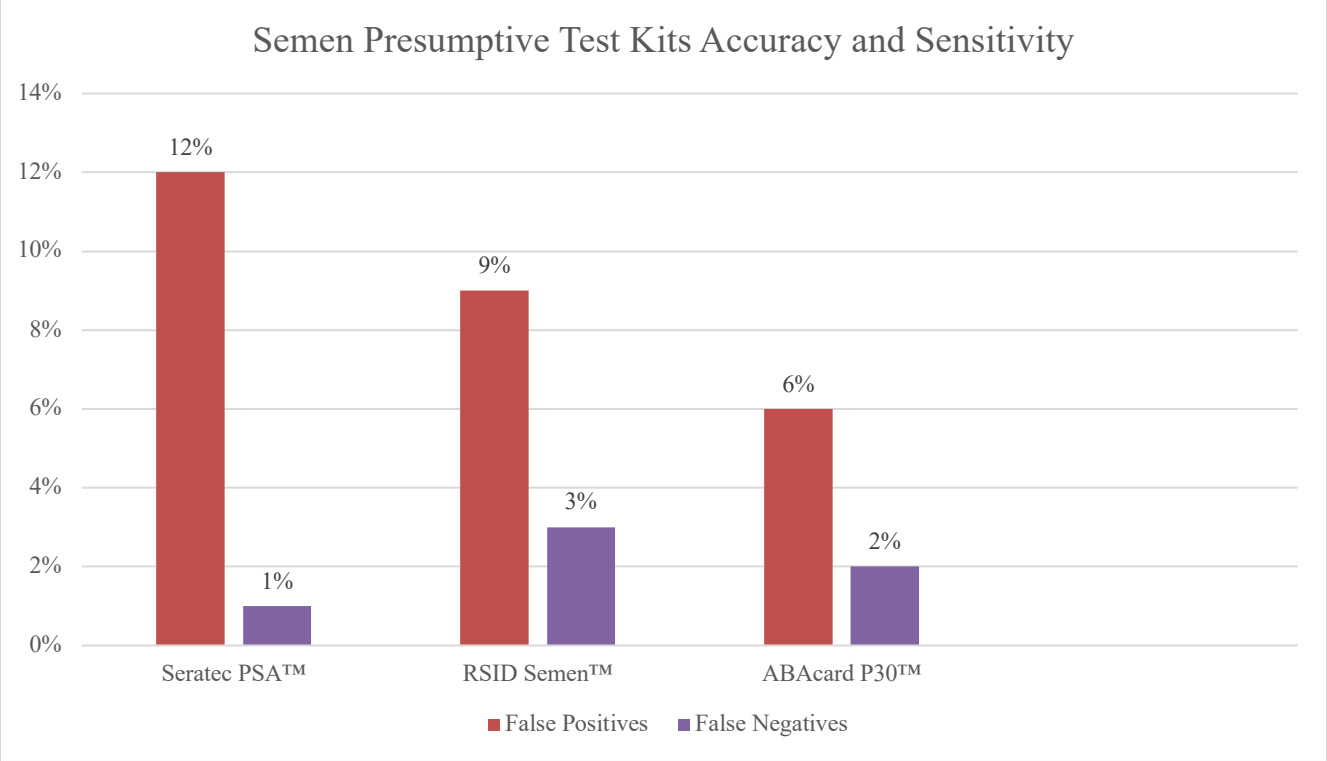

Figure 4. Semen Presumptive Test Kits False Positive and False Negative Rates

While these percentages may appear low, false negative results in forensic casework can have severe consequences and should not be overlooked. It is also, important to note that the total tests evaluated for each kit were 101 for ABAcard P30™, 119 for RSID Semen™, and 161 for Seratec PSA™. The rate of false negatives might increase as the sample increases. However, even a low percentage of false negatives can have significant consequences. For example, if each of these three test kits is used to analyze only 100 samples containing semen, the observed false negative rates could result in 1 to 3 samples being incorrectly reported as negative and potentially leading to missed evidence or the incorrect elimination of a suspect (Figure 4).

In forensic analysis, particularly in cases involving sexual assault or other crimes where the presence of semen is crucial evidence, the semen presumptive tests need to be highly sensitive and capable of detecting even a minuscule amount of semen. Failure to detect semen at such low concentrations can undermine the integrity of the forensic analysis and may impact the outcome of legal proceedings.

RSID Semen™ failed to detect semen in 2 of the positive controls. In Trial 2 and Trial 3, the positive control sample gave a negative result, when it should have tested positive (Table 5). This is a significant problem, as the positive control contained semen and therefore should have resulted in a positive with this presumptive semen test. Since the positive control failed 2 out of 4 trials, it calls into question the reliability and accuracy of the entire test.

When testing the semen sample that was mixed with dirt, there was a false negative with the RSID Semen™ test. This might have occurred because the presence of dirt molecules could have interfered with the processes required for the test kit to detect the semenogelin antigen, which is the target molecule for this test.

19.1 Heavy and Light Chains

The RSID-Semen™ test works by using antibodies that specifically bind to semenogelin, a protein found in semen. Antibodies are immunoglobulin proteins produced by the immune system to recognize and bind to specific antigens (foreign substances). They are Y-shaped proteins composed of four polypeptide chains: two identical heavy chains and two identical light chains. These chains are held together by disulfide bonds and non-covalent interactions (Chailyan et al., 2011).

Heavy chains are larger polypeptides with a molecular weight ranging from 50 to 70 kDa (Chailyan et al., 2011). Each heavy chain consists of one variable domain (VH) and three and sometimes four constant domains (CH1, CH2, CH3, and sometimes CH4). These domains are responsible for recognizing antigens as well as helping with the binding (Chailyan et al., 2011). Whereas light chains are smaller polypeptides with a molecular weight of approximately 25 kDa (Chailyan et al., 2011). Like heavy chains, light chains have one variable domain (VL). However, they only have one constant domain (CL). But like heavy chains, these domains are responsible for the recognition and binding of antigens (Chailyan et al., 2011).

The variable domains of both the heavy and light chains contain hypervariable regions, also known as complementarity-determining regions (CDRs) (Polonelli et al., 2008). The CDRs are responsible for the recognition and binding of the antibody to its target antigen. The CDRs of the heavy and light chains work together to form the antigen-binding site (Polonelli et al., 2008). The site is a three-dimensional structure that complements the shape and chemical makeup of the antigen (Polonelli et al., 2008). The variable regions of the heavy and light chains are encoded by different gene segments that undergo rearrangement and somatic hypermutation during B-cell development, resulting in a vast diversity of antibodies capable of recognizing a wide range of

antigens (Polonelli et al., 2008). The specific effector functions of an antibody depend on the isotype and the corresponding constant region of the heavy chain.

The proper interaction and folding of the heavy and light chains are crucial for the correct formation of the antigen-binding site and the overall structure and function of the antibody molecule. Disruptions with this interaction can potentially affect the antibody's ability to recognize and bind to its target antigen, as was possibly the case with the RSID Semen™ test when the semen sample was mixed with dirt.

It is possible that the dirt molecules interfered with the proper folding or structure of the antibodies used in the RSID Semen™ test, compromising their ability to bind to semenogelin. This interference could have led to a false negative result, where the test failed to detect the presence of semen in the sample.

The failure of the RSID Semen™ kit to detect semen when mixed with dirt highlights a crucial concern in forensic analysis: the susceptibility of the tests to interference from the makeup of the sample and contamination. In real cases, biological evidence that has been collected from a crime scene is often mixed with various substances like dirt. This type of complex sample poses a significant risk for forensic investigators, as it can potentially lead to false negative results.

19.2 Sensitivity Issues with Dilutions

All three semen presumptive test kits showed limitations in detecting semen at lower dilutions, with negative test results occurring around the 1:10,000 dilution range (Table 6, Table 9, and Table 12). It is noted that the kits do not explicitly mention the ability to detect a 1:100,000 dilution of semen. Samples were only tested at that dilution to see if any of the test kits could detect semen after it had been diluted to almost nothing. All three test kits detected

semen at a 1:100,000 dilution, however, they were not able to detect it in every sample tested (Table 7). The inability of all three kits to reliably detect semen at dilutions of 1:10,000 or higher raises significant concerns about their effectiveness in cases where only trace amounts of semen may be present on evidence. This limitation could lead to false negative test results. Which in return could lead to missed evidence and issues with the investigation.

19.3 Sensitivity Issue Impact on Forensics

The decreased sensitivity observed with these semen presumptive tests has important implications for their use in forensic investigations:

- **Missed Evidence:** Failure to detect semen at lower concentrations could lead to crucial evidence being overlooked or missed. This is especially important to consider in cases involving trace amounts of semen.
- **Impact on further DNA analysis**: DNA analysts are not able to test every piece of evidence from every case. So, investigators must pick the evidence that they think will get the best results. And this is where the importance of accurate presumptive testing comes into play. If a semen sample gets missed during the presumptive testing stage, the evidence might not make it to DNA testing.
- **Risk of investigative assumptions:** False negative results due to the low sensitivity of the tests may lead investigators to incorrectly assume that there isn't semen on the evidence. This can have serious consequences, as it may misdirect investigative efforts away from crucial leads or suspects.

Overall, forensic scientists must be aware of the limitations in sensitivity for all three semen presumptive tests studied and take appropriate precautions to mitigate the risk of false negatives.

These findings emphasize the importance of not relying solely on presumptive test results, even when they are negative. Negative results from presumptive tests should be treated with caution and should not be used to rule out the presence of semen definitively. Instead, additional confirmatory testing should be conducted to ensure that no evidence has been overlooked.

20. Evaluation of Specificity

The specificity of each test kit was evaluated by observing the rate of false positives associated with each respective test kit (Figure 4). The Seratec PSA™ kit had the highest false positive rate at 12% (19 out of 161 total tests), with positive results obtained for:

- Menstrual pads with varying menstrual blood flow (13 out of 21 trials)
- Bambo™ Diaper with female urine (2 out of 2 trials)
- Diaper Gel with female urine (Huggies™ newborn) (2 out of 2 trials)
- Bambo Diaper with female urine (1 out of 2 trials)
- Diaper Gel with female urine (Huggies™ newborn) (2 out of 2 trials)
- Diaper liner with female urine (Huggies™ newborn) (1 out of 2 trials)
- Menstrual pad with menstrual blood heavy flow pad 1 (28-year-old-day 1 of cycle) (3 out of 3 trials)
- Menstrual pad with menstrual blood heavy flow pad 2 (28-year-old-day 1 of cycle) (3 out of 3 trials)
- Menstrual pad with menstrual blood heavy flow pad 3 (28-year-old-day 1 of cycle) (3 out of 3 trials)
- Menstrual pad with menstrual blood medium heavy flow (28-year-old-day 2 of cycle) (2 out of 3 trials)

- Menstrual pad with menstrual blood medium flow (28-year-old-day 3 of cycle) (3 out of 3 trials)
- Female urine (28-years-old taken during menstrual cycle) (1 out of 3 trials)

The RSID Semen™ test kit had a false positive rate of 9%. (11 out of 119 total tests). False positives were observed with the following samples:

- Menstrual pad with menstrual blood (3 out of 3 trials)
- Tampons with menstrual blood (2 out of 2 trials)
- Vaginal swab with no chance of semen (1 out of 2 trials)
- Bambo™ Diaper Gel with female urine (2 out of 3 trials)
- Diaper Gel with female urine (Huggies™ newborn) (2 out of 3 trials)
- Honest™ Diaper Gel 3 with urine (1 out of 3 trials)

The ABAcard P30™ test had the lowest false positive rate and it was 6% (6 out of 101 total tests):

- Female urine taken during menstrual cycle (2 out of 3 trials)
- Menstrual pad with heavy menstrual blood flow (1 out of 3 trials)
- Menstrual pad with medium menstrual blood flow (2 out of 3 trials)
- Diaper liner with female urine (Huggies™ newborn) (1 out of 3 trials)

21. False Positives in Absorbent Products

A particularly concerning finding was the high rate of false positives observed with various absorbent hygiene products across all three presumptive semen test kits evaluated. The

data showed multiple instances of positive test results for semen with various diaper brands (Pampers Swaddlers ™, Bambo™, Huggies ™, and Honest ™) when only urine was present, as well as with menstrual pads containing blood. Absorbent hygiene products like diapers and menstrual pads are commonly encountered items in forensic investigations, especially in cases involving sexual assault or abuse. However, the presence of these products can complicate forensic testing due to the materials they contain and their potential to interfere with testing procedures. One concern that has been raised is the potential for false positive results when testing absorbent products, such as feminine hygiene products and diapers, for the presence of semen.

Ingredients like sodium polyacrylate present in absorbent hygiene products may be causing the false positive results observed. Sodium polyacrylate is a super-absorbent polymer widely used in disposable diapers, feminine hygiene pads/tampons, adult incontinence products, and other absorbency products (Abd Manan et al., 2021). It can absorb 100 to 1000 times its mass in water or any other liquid. While very effective for its intended absorbent purposes, the polymeric structure of sodium polyacrylate means it has a high molecular weight and contains carboxyl functional groups. These characteristics could potentially lead to non-specific binding interactions with the antibodies used in the lateral flow immunoassays that the semen presumptive tests are based on.

The RSID™ Semen, Seratec™ PSA, and ABAcard P30™ kits all use antibodies that target proteins found in seminal fluid - semenogelin for RSID and PSA for the other two test kits. If sodium polyacrylate or any of the other absorbent polymers used in absorbency products cause non-specific binding to these antibodies, it could trigger a false positive result by mimicking the presence of the semen biomarker.

22. False Positives Due to Possible PSA in Menstrual Blood

The possibility of false positives for semen detection due to the presence of PSA in menstrual blood is an important consideration in forensic analysis. In this study, false positives were observed in all three of the test kits. Of the three test kits, ABAcard P30™ and Seratec PSA™ use PSA as the biomarker for semen detection. These findings have significant implications for forensic analysis, particularly in cases where menstrual blood may be present as part of the evidence. The presence of elevated PSA levels during certain phases of the menstrual cycle could potentially have led to false positive results for semen detection.

A study by Nagar & Msalati was conducted to study the changes in serum PSA for women during a normal menstrual cycle. The results of the study indicated that, for healthy women who are not pregnant, PSA levels go up in the serum of their blood during two stages of a menstrual cycle. There was a large increase during the follicular phase between the 4th and 8th day of the cycle. The mean serum PSA was (0.009 ± 0.058) ng/ml (Nagar & Msalati, 2013). The second stage in which PSA levels went up was in the luteal phase. There was a smaller peak between the 16th and 20th day of the cycle (Nagar & Msalati, 2013). This study highlights, the differences in PSA levels in a woman's body during their menstrual cycle.

23. False Positive with Female Urine

The ABAcard P30™ kit also exhibited a false positive when testing female urine. This observation added another layer of complexity to the reliability of the test. There could likely have been urine in sexual assault cases due to the nature of the crime. So, it was important to note that the test kit could produce a false positive with urine. The false positives were likely due to PSA in the urine sample. In a study by Schmidt et al. (2001), they found that 11% of all the

women they studied had PSA in their urine. The mean value of PSA found in the urine samples was 0.29 ng/mL. In their study, they also found that women younger than 50 had a higher mean level of PSA. The mean was 0.34 ng/mL for women younger than 50 and 0.23 ng/mL for women over 50.

Even though male urine samples in this study did not exhibit false positives with any of the three tests analyzed, it was important to consider the possibility of it occurring. Iwakiri et al. (1993) analyzed the urine samples of 18 patients diagnosed with prostate adenocarcinoma, a common type of prostate cancer. There was an average concentration of 915.1 ng/ml of PSA in the urine samples and a range spanning from 21 to 2,853 ng/ml (Iwakiri et al., 1993). The study also found noted high PSA levels after the patients got their prostate removed (mean 21.4 ng/ml). In another study, by Sato et al. (2002), they found that the concentration of PSA in male urine was around least 800 ng/ml for healthy males and 629.67 ng/ml for males with illnesses. This study is important to note because it showed that male urine samples had a concentration of PSA in their samples. This was important to consider when testing real case samples.

24. Implications of False Positives in Forensic Analysis

False positives in forensic analysis can have profound implications across various aspects of the criminal justice system and society. False positives can lead to the wrongful conviction of innocent individuals. This not only results in the miscarriage of justice but also undermines the credibility of the legal system. Individuals falsely implicated by forensic analysis may suffer irreparable damage to their reputations, even if later exonerated. Additionally, such errors could have wasted valuable resources, damaged public trust in forensic science and law enforcement, and perpetuated inequalities within the criminal justice system. All three test kits analyzed

produced multiple false positive test results. ABAcard P30™ had a 6% rate, RSID Semen™ had a 9% rate, and Seratec PSA™ had a 12% rate of false positives. These rates are concerning regarding the sensitivity and accuracy of the tests. They also highlight the need for confirmation testing when using these test kits.

25. Comparison of Testing Methodology

Semen presumptive tests are widely acknowledged for their ease of use, making them invaluable tools in forensic investigations. However, the methodologies of the three semen presumptive test kits evaluated in this study presented potential issues that could have led to errors or confusion.

25.1 RSID Semen™ Methodology

With the RSID Semen™ kit, the buffer solution required refrigeration. If the buffer was left out at room temperature, it could potentially have affected the results of the test. Some components of the buffer might have been sensitive to temperature, and leaving the buffer out at room temperature could have degraded the components. The temperature might also have affected the pH of the buffer. Buffer solutions are often formulated to maintain a specific pH level for a particular reaction. Differences in pH could have led to a different reaction than intended. Additionally, extended exposure of the buffer to room temperature could have increased the risk of microbial contamination. This could have potentially introduced extraneous biological material that interfered with the test reaction.

25.2 Seratec PSA™ Methodology

With the Seratec PSA™ kit, the testing methodology was simple and easy to use. However, the instruction sheet that came in the box with the test kits was printed with the instructions on the right side of the page. The other two test kits had the instructions on the left side of the page. Traditionally, in America, we read the left side of the page first. Because of this, Seratec PSA™, having the instructions on the right side of the page might have confused users. With Seratec PSA™, the sample had to be incubated for 10 minutes with the buffer, and that step might have been affected if the instructions were not read correctly. Additionally, the method of indicating positive results, with three lines for positive and two for negative, differed from other kits, potentially confusing result interpretation. The other two kits used two lines for a positive result. So, if someone was used to seeing two lines as a positive, they might have incorrectly recorded a result.

25.3 ABAcard P30™ Methodology

The ABAcard P30™ kit had multiple methodological challenges. The first major challenge was the fact that the sample had to be incubated for 2-hours before testing in a refrigerator. This added a significant time constraint as well as required additional equipment needed for testing compared to the other two testing kits. Another major challenge was that the test kit itself also looked identical to the ABAcard Blood™ test kit. This created the issue where someone might have confused the two tests, potentially leading to incorrect test results, misinterpretation of data, or compromised forensic analysis. To address this issue, it would be helpful for the test to include a label indicating that it is ABAcard P30™, preventing possible confusion.

Overall, each of the three semen presumptive test kits had issues in their methodology that could lead to potential errors or confusion among analysts. Addressing these challenges, by implementing clearer instructions, ensuring proper storage conditions, and distinguishing between similar test kits, is essential for reliable semen presumptive testing in forensic investigations.

26. Limitations

There are several limitations to consider when reviewing the results of this study. One main concern is the lack of diversity in the menstrual products examined. Most of the pads and tampons assessed came from a single individual. This creates a limitation because it doesn't account for the possible differences in menstrual products from different individuals. Additionally, the study only evaluated a limited number of brands of absorbent hygiene products, which may not accurately represent the wider range of offerings available in the market. Several factors can contribute to this variability, including:

1. **Individual physiological differences:** The chemical composition and properties of menstrual fluid can vary among individuals due to factors such as hormone levels, age, and overall health status.
2. **Menstrual cycle variations:** The characteristics of menstrual fluid can change throughout the different phases of an individual's menstrual cycle, potentially affecting the interactions with the test kits.
3. **Product formulations:** Different brands and types of menstrual products may have varying compositions, additives, or absorbent materials, which could influence their reactivity with the semen presumptive tests.

4. **Manufacturing processes:** Even within the same brand or product line, there may be batch-to-batch variations in the materials or manufacturing processes used, leading to potential differences in test outcomes.

By relying primarily on menstrual products from a single individual, the study may have failed to capture the full range of potential interferents or cross-reactivity that could occur with products from diverse sources.

Additional limitations came from the number of test kits available for use. Due to funding only three to four boxes were purchased for each test kit. This resulted in differences in the number of samples tested across the three kits (RSID Semen™: 119, Seratec PSA™: 161, ABAcard P30™: 101). The differences came from the varying quantities of tests provided in each kit's packaging.

It's important also to acknowledge the differences between testing a sample in a controlled laboratory testing, as conducted in this study, and the analysis of actual casework samples encountered in forensic investigations. While laboratory settings offer controlled conditions conducive to rigorous experimentation, they often fail to fully replicate the dynamic and multifaceted nature of evidence collected from real crime scenes.

In the field, forensic samples are subjected to a multitude of environmental variables and potential sources of contamination that are difficult to replicate in a laboratory setting. Factors such as exposure to varying temperatures, humidity levels, and microbial activity can all impact the stability and integrity of biological evidence, including semen. Additionally, the presence of multiple interferents, such as bodily fluids, substances from the environment, or surface contaminants, further complicates the analysis process.

Moreover, evidence collected from crime scenes may undergo degradation over time due to exposure to external elements, which can compromise the reliability of forensic testing methods. As the evidence degrades it might affect the chemical makeup of the substance, which in turn might affect the results of the presumptive test. Therefore, while this study provides valuable insights into the performance of the three semen presumptive tests evaluated, it is important to note that results might vary with real casework.

Despite the noted limitations, the study still gives valuable information about the sensitivity and accuracy of three widely used semen presumptive test kits. Despite the sample constraints noted above, the findings offer a critical insight into the strengths and weaknesses of these test methods, illuminating areas that require caution and further improvement.

27. Future Research

While the studies conducted so far have provided valuable insights, further research is still needed to investigate and validate the reliability of semen presumptive testing thoroughly. Future research in the field of semen presumptive testing should include several key areas to advance the reliability of semen detection methods.

Forensic laboratories should prioritize ongoing research and development efforts to enhance the robustness and reliability of forensic detection methods, particularly in scenarios where samples are likely to be contaminated or mixed with other substances. This would help evaluate the effectiveness of semen detection methods across various sample types and conditions.

Given the widespread use of diapers and pads, particularly in cases involving sexual assault, researchers should assess the performance of semen detection methods on various brands

and types of absorbent products. The testing conducted so far has been limited to a small number of brands, and it is important to expand this research to include a wider array of absorbent products. This should encompass, but not be limited to, diapers, menstrual pads, and tampons. By thoroughly examining the performance of semen detection methods across different absorbent brands and products, researchers can gain a more comprehensive understanding of their reliability and applicability in forensic settings.

Conclusion

Our evaluation of the sensitivity of the three test kits uncovered serious limitations, as demonstrated by the high rate of false negative results obtained with semen-containing samples across a dilution series ranging from neat to 1:100,000 (Table 6, Table 9, Table 12). The false negatives were observed in the 1:10,000 range in all three test kits. Despite prior validation of these kits for forensic semen detection, they frequently failed to indicate positive results for the PSA and semenogelin biomarkers even in the presence of diluted semen. As shown in Figure 4, the RSID Semen™ kit had the most, with a 3% rate of false negatives, followed by ABAcard P30 ™ which had a 2% rate, and Seratec PSA™ with the smallest rate of 1%. Additionally, RSID Semen™ failed to detect semen when it was mixed with dirt (Table 7). Thus, Seratec PSA™ demonstrated the highest sensitivity, with the fewest false negative results, while RSID Semen™ had the poorest sensitivity, with the highest number of false negative findings.

Additionally concerning was the non-specificity of the kits, evidenced by frequent false positive results. Seratec PSA™ demonstrated the poorest specificity, with a 12% false positive rate, followed by RSID Semen™ which had a rate of 9%, and ABAcard P30™ with a rate of 6%. The presumptive positive results for semen were observed for various absorbent hygiene

products, including diapers with urine, and used menstrual pads, indicating poor specificity. While the cause of these false positives is currently undetermined, absorbent ingredients common to these items may be implicated. Ingredients such as sodium polyacrylate may be implicated as the cause of the false positive results. However, further testing needs to be conducted in the future to determine if this is the case or not. Problematically, positive test results were also obtained with biological fluids from a female with no possibility of semen content, including vaginal fluids and urine taken during a menstrual cycle. The high incidence of false positives with these various samples further indicates insufficient specificity in all three of the test kits for accurate forensic semen detection.

Taken together, these findings demonstrate significant limitations in both sensitivity and specificity. Highlighting the need for caution when applying these kits forensically and for further research to improve the reliability of the test kits.

In addition to the varying rates of false results, some potential methodological errors could occur with these semen detection kits. The RSID Semen™ kit requires refrigerated storage of the buffer solution; if left unrefrigerated for too long, the buffer could be compromised and lead to inaccurate test results. The Seratec PSA™ kit uniquely indicates a positive result with three test lines, unlike the two-line positive results of the other kits: this non-standard format risks confusion or misinterpretation of results by analysts. Finally, the ABAcard P30 ™ kit necessitates a 2-hour incubation period for samples in the refrigerator before testing can be run. While this kit had the highest accuracy, the lengthy incubation introduces delays and requires laboratory equipment. In summary, while the ABAcard P30 ™ kit showed the best test performance, its need for refrigerated sample incubation is a drawback compared to the quicker and simpler testing procedures of the RSID Semen™ and Seratec PSA™ kits. However,

laboratories with sufficient time and resources to accommodate sample incubation may favor the ABAcard P30 TM for its superior accuracy. In closing, forensic laboratories should be aware of the inability of these tests to accurately confirm semen and should not be reporting confirmatory semen statements in their reports.

Appendix

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
brand of test * false_pos	127	100.0%	0	0.0%	127	100.0%

Table 15. Brand of Test False Positive Case Processing Summary

brand of test * false_pos Crosstabulation

			false_pos		
			yes	no	Total
brand of test	RSID	Count	6	34	40
		Expected Count	5.4	34.6	40.0
		% within brand of test	15.0%	85.0%	100.0%
	Seretec	Count	8	45	53
		Expected Count	7.1	45.9	53.0
		% within brand of test	15.1%	84.9%	100.0%
	Abacard	Count	3	31	34
		Expected Count	4.6	29.4	34.0
		% within brand of test	8.8%	91.2%	100.0%
Total		Count	17	110	127
		Expected Count	17.0	110.0	127.0
		% within brand of test	13.4%	86.6%	100.0%

Table 16. Brand of Test False Positive Crosstabulation

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	.834[a]	2	.659
Likelihood Ratio	.898	2	.638
Linear-by-Linear Association	.560	1	.454
N of Valid Cases	127		

a. 1 cells (16.7%) have expected count less than 5. The minimum expected count is 4.55.

Table 17. Brand of Test False Positive Chi-Square Test

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.081	.659
	Cramer's V	.081	.659
N of Valid Cases		127	

Table 18. Brand of Test False Positive Symmetric Measures

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
brand of test * false_neg	127	100.0%	0	0.0%	127	100.0%

Table 19. Brand of Test False Negative Case Processing Summary

brand of test * false_neg Crosstabulation

			false_neg		
			yes	no	Total
brand of test	RSID	Count	4	36	40
		Expected Count	2.5	37.5	40.0
		% within brand of test	10.0%	90.0%	100.0%
	Seretec	Count	2	51	53
		Expected Count	3.3	49.7	53.0
		% within brand of test	3.8%	96.2%	100.0%
	Abacard	Count	2	32	34
		Expected Count	2.1	31.9	34.0
		% within brand of test	5.9%	94.1%	100.0%
Total		Count	8	119	127
		Expected Count	8.0	119.0	127.0
		% within brand of test	6.3%	93.7%	100.0%

Table 20. Brand of Test False Negative Crosstabulation

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.109	.470
	Cramer's V	.109	.470
N of Valid Cases		127	

Table 21. Brand of Test False Negative Symmetric Measures

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
brand of test * innacuracy	127	100.0%	0	0.0%	127	100.0%

Table 22. Brand of Test Inaccuracy Case Processing Summary

brand of test * innacuracy Crosstabulation

			innacuracy		
			yes	no	Total
brand of test	RSID	Count	10	30	40
		Expected Count	7.9	32.1	40.0
		% within brand of test	25.0%	75.0%	100.0%
	Seretec	Count	10	43	53
		Expected Count	10.4	42.6	53.0
		% within brand of test	18.9%	81.1%	100.0%
	Abacard	Count	5	29	34
		Expected Count	6.7	27.3	34.0
		% within brand of test	14.7%	85.3%	100.0%
Total		Count	25	102	127
		Expected Count	25.0	102.0	127.0
		% within brand of test	19.7%	80.3%	100.0%

Table 23. Brand of Test Inaccuracy Crosstabulation

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	1.270[a]	2	.530
Likelihood Ratio	1.267	2	.531
Linear-by-Linear Association	1.241	1	.265
N of Valid Cases	127		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.69.

Table 24. Brand of Test Inaccuracy Chi-Square Tests

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.100	.530
	Cramer's V	.100	.530
N of Valid Cases		127	

Table 25. Brand of Test Inaccuracy Symmetric Measures

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
semen or not * innacuracy	127	100.0%	0	0.0%	127	100.0%

Table 26. Semen or Not Inaccuracy Case Processing Summary

semen or not * innacuracy Crosstabulation

			innacuracy		
			yes	no	Total
semen or not	semen	Count	7	8	15
		Expected Count	3.0	12.0	15.0
		% within semen or not	46.7%	53.3%	100.0%
	everything else	Count	18	94	112
		Expected Count	22.0	90.0	112.0
		% within semen or not	16.1%	83.9%	100.0%
Total		Count	25	102	127
		Expected Count	25.0	102.0	127.0
		% within semen or not	19.7%	80.3%	100.0%

Table 27. Semen or Not Inaccuracy Crosstabulation

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.248	.005
	Cramer's V	.248	.005
N of Valid Cases		127	

Table 28. Semen or Not Inaccuracy Symmetric Measures

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
menses or not * innacuracy	127	100.0%	0	0.0%	127	100.0%

Table 29. Menses or Not Inaccuracy Case Processing Summary

menses or not * innacuracy Crosstabulation

			innacuracy		
			yes	no	Total
menses or not	menses	Count	9	8	17
		Expected Count	3.3	13.7	17.0
		% within menses or not	52.9%	47.1%	100.0%
	not menses	Count	16	94	110
		Expected Count	21.7	88.3	110.0
		% within menses or not	14.5%	85.5%	100.0%
Total		Count	25	102	127
		Expected Count	25.0	102.0	127.0
		% within menses or not	19.7%	80.3%	100.0%

Table 30. Menses or Not Inaccuracy Crosstabulation

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	13.730[a]	1	<.001		
Continuity Correction[b]	11.409	1	<.001		
Likelihood Ratio	11.234	1	<.001		
Fisher's Exact Test				<.001	<.001
Linear-by-Linear Association	13.622	1	<.001		
N of Valid Cases	127				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.35.

b. Computed only for a 2x2 table

Table 31. Menses or Not Inaccuracy Chi-Square Tests

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.329	<.001
	Cramer's V	.329	<.001
N of Valid Cases		127	

Table 32. Menses or Not Inaccuracy Symmetric Measures

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
diapers or not * innacuracy	127	100.0%	0	0.0%	127	100.0%

Table 33. Diapers or Not Inaccuracy Case Processing Summary

diapers or not * innacuracy Crosstabulation

			innacuracy		
			yes	no	Total
diapers or not	diapers	Count	5	37	42
		Expected Count	8.3	33.7	42.0
		% within diapers or not	11.9%	88.1%	100.0%
	not diapers	Count	20	65	85
		Expected Count	16.7	68.3	85.0
		% within diapers or not	23.5%	76.5%	100.0%
Total		Count	25	102	127
		Expected Count	25.0	102.0	127.0
		% within diapers or not	19.7%	80.3%	100.0%

Table 34. Diapers or Not Inaccuracy Crosstabulations

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.403[a]	1	.121		
Continuity Correction[b]	1.724	1	.189		
Likelihood Ratio	2.572	1	.109		
Fisher's Exact Test				.157	.092
Linear-by-Linear Association	2.384	1	.123		
N of Valid Cases	127				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 8.27.

b. Computed only for a 2x2 table

Table 35. Diapers or Not Inaccuracy Chi-Square Tests

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	-.138	.121
	Cramer's V	.138	.121
N of Valid Cases		127	

Table 36. Diapers or Not Inaccuracy Symmetric Measures

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
not menses, diapers, semen * innacuracy	127	100.0%	0	0.0%	127	100.0%

Table 37. Not Menses, Diapers, or Semen Inaccuracy Case Processing Summary

not menses, diapers, semen * innacuracy Crosstabulation

			innacuracy		
			yes	no	Total
not menses, diapers, semen	everything else	Count	4	49	53
		Expected Count	10.4	42.6	53.0
		% within not menses, diapers, semen	7.5%	92.5%	100.0%
	not menses, semen, diapers	Count	21	53	74
		Expected Count	14.6	59.4	74.0
		% within not menses, diapers, semen	28.4%	71.6%	100.0%
Total		Count	25	102	127
		Expected Count	25.0	102.0	127.0
		% within not menses, diapers, semen	19.7%	80.3%	100.0%

Table 38. Not Menses, Diapers, or Semen Inaccuracy Crosstabulations

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	8.476[a]	1	.004		
Continuity Correction[b]	7.210	1	.007		
Likelihood Ratio	9.342	1	.002		
Fisher's Exact Test				.003	.003
Linear-by-Linear Association	8.409	1	.004		
N of Valid Cases	127				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 10.43.

b. Computed only for a 2x2 table

Table 39. Not Menses, Diapers, or Semen Chi-Square Tests

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	-.258	.004
	Cramer's V	.258	.004
N of Valid Cases		127	

Table 40. Not Menses, Diapers, or Semen Symmetric Measures

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
semen or menses * innacuracy	32	25.2%	95	74.8%	127	100.0%

Table 41. Semen or Menses Inaccuracy Case Processing Summary

semen or menses * innacuracy Crosstabulation

			innacuracy		
			yes	no	Total
semen or menses	semen	Count	7	8	15
		Expected Count	7.5	7.5	15.0
		% within semen or menses	46.7%	53.3%	100.0%
	menses	Count	9	8	17
		Expected Count	8.5	8.5	17.0
		% within semen or menses	52.9%	47.1%	100.0%
Total		Count	16	16	32
		Expected Count	16.0	16.0	32.0
		% within semen or menses	50.0%	50.0%	100.0%

Table 42. Semen or Menses Inaccuracy Crosstabulation

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.125[a]	1	.723		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.126	1	.723		
Fisher's Exact Test				1.000	.500
Linear-by-Linear Association	.122	1	.727		
N of Valid Cases	32				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 7.50.

b. Computed only for a 2x2 table

Table 43. Semen or Menses Inaccuracy Chi-Square Test

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	-.063	.723
	Cramer's V	.063	.723
N of Valid Cases		32	

Table 44. Semen or Menses Inaccuracy Symmetric Measures

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