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TECHNICAL NOTE

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Identification of Prostate-Specific Antigen and Spermatozoa from a Mixture of Semen and Simulated Gastric Juice

ABSTRACT: The detection of prostate-specific antigen (PSA) and visualization of spermatozoa from forensic-type samples containing semen exposed to simulated gastric juice was investigated as a support for forensic practice. Samples of simulated gastric juice mixed with semen were prepared and incubated for up to 4 h at 37°C. Samples were deposited on cotton cloth and on ceramic plates and allowed to dry. The samples were examined for the presence of PSA using the Seratec® PSA Semiquant immunochromatographic membrane test. Microscope slides were prepared, stained, and analyzed for spermatozoa. Spermatozoa were detected in all samples, and PSA was detected on neat samples and on samples from the ceramic plate after incubation for up to 4 h. PSA was not detected in the samples deposited on cotton cloth at incubation times greater than 15 min. This may serve as a support for examinations performed when vomit or vomit-stained evidence is submitted for analysis.

KEYWORDS: forensic science, prostate-specific antigen, spermatozoa, simulated gastric juice, vomit, drug-facilitated sexual assault

Vomit and gastric juice are physiological fluids that can serve as forensic evidence (1). Requests for the forensic analysis of vomit samples collected from sexual assault investigations for the presence of semen is a rare but not unprecedented occurrence in the crime laboratory. The analysis of vomit or vomit-stained clothing for the presence of seminal fluid is sometimes necessary in cases of alleged oral sexual assault with ejaculation if the victim has subsequently vomited. In the case of possible drug-facilitated sex crimes, finding vomit at the crime scene is relatively likely, and is often the best remaining evidence of an oral assault (2). The presence of prostate-specific antigen (PSA) and/or the detection of spermatozoa have been well characterized and validated in the forensic science community as markers for the presence of seminal fluid (3,4). In this study, forensic-type samples were prepared from a semen standard that was incubated in simulated gastric juice at 37°C for up to 4 h. These samples were then analyzed to determine if PSA or spermatozoa could be detected.

Methods

Simulated gastric juice was prepared by adding pepsin (Acros Organics, Geel, Belgium) to deionized water for a concentration of 13.3 mg/L (5,6). This solution was adjusted to a pH of 1.5 (5,6) with molecular biology grade HCl (Sigma, St. Louis, MO) and preheated to 37°C.

A mixture of 2.5 mL of human seminal fluid and 97.5 mL of the simulated gastric juice was used to prepare forensic-type samples for analysis. This ratio roughly corresponds to a normal volume of ejaculate (7) mixed with a stomach's normal Gastric Residual Volume (8). A mixture of 2.5 mL of deionized water added to 97.5 mL of simulated gastric juice was used as a negative control. The experimental mixture and the negative control were kept at a constant 37°C throughout the experiment. Samples were drawn at nine incrementally increasing time intervals from immediately after preparation (T_0) to 4 h of incubation (T_8). This time range corresponds to the typical gastric emptying period (9). All experiments were performed in triplicate.

Three types of forensic-type samples were prepared at each time interval. Approximately 1 mL of both the experimental and control solutions were placed on 3 cm × 3 cm cotton squares and allowed to dry, simulating the forensic-type sample of vomit dried on clothing. Approximately 50 μL of each solution was placed on a clean ceramic plate and allowed to dry, simulating vomit in flake or solid form, and 50 μL of each neat solution was placed directly into a HEPES buffer extraction solution. At the first and last time intervals, microscope slides were made from both the experimental and negative control solutions using Christmas tree stain (SERI) in accordance with the vendor's technical information sheet.

An area of 6.5 mm × 7 mm, or about 5% of the total cotton swatch area, was extracted for 2 h in 210 μL of HEPES buffer and tested with a Seratec® PSA test cassette. Samples dried on ceramic plates were rehydrated with 200 μL of HEPES buffer for 25 min and tested with a Seratec® PSA test cassette. The 50 μL neat samples were added to 150 μL of HEPES buffer and tested immediately with a Seratec® PSA test cassette.

Results and Discussion

Spermatozoa were easily identified from the microscope slides produced at T_0 and T_8 of each experiment, with no obvious differences noted in respect to spermatozoon morphology (data not shown).

Table 1 shows the results of Seratec® PSA Semiquant tests performed on the three types of forensic-type samples produced at different incubation times.

A positive result corresponded to a test line that was equal or greater in intensity to the 4 ng/mL reference line on the Seratec® PSA Semiquant test. Positive assays less intense than the 4 ng/mL reference line are noted as such to show the relative strengths of

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TABLE 1—*Results of prostate-specific antigen testing.*

		Neat						Dish						Cotton					
		Exp. 1		Exp. 2		Exp. 3		Exp. 1		Exp. 2		Exp. 3		Exp. 1		Exp. 2		Exp. 3	
	Min	Exp	Neg	Exp	Neg	Exp	Neg	Exp	Neg	Exp	Neg	Exp	Neg	Exp	Neg	Exp	Neg	Exp	Neg
T_0	0	+	–	+	–	+	–	+*	–	+*	–	+*	–	+	–	+*	–	+*	–
T_1	5	+*	–	+*	–	+*	–	+*	–	+*	–	+*	–	+*	–	–	–	–	–
T_2	10	+*	–	+*	–	+*	–	+*	–	+*	–	+*	–	+*	–	–	–	–	–
T_3	15	+*	–	+*	–	+*	–	+*	–	+*	–	+*	–	+*	–	–	–	–	–
T_4	30	+*	–	+*	–	+*	–	+*	–	+*	–	+*	–	–	–	–	–	–	–
T_5	60	+*	–	+*	–	+*	–	+*	–	+*	–	+*	–	–	–	–	–	–	–
T_6	120	+*	–	+*	–	+*	–	+*	–	+*	–	+*	–	–	–	–	–	–	–
T_7	180	+*	–	+*	–	+*	–	+*	–	+*	–	+*	–	–	–	–	–	–	–
T_8	240	+*	–	+*	–	+*	–	+*	–	+*	–	+*	–	–	–	–	–	–	–

*Positive result less intense than the 4 ng/mL reference line.

results. Neat samples showed positive results at T_0, immediately after the seminal fluid was mixed into the simulated gastric juice. The results from all three sample types progressed from a positive result at T_0 to positive results less intense than the 4 ng/mL reference line from T_1 through T_8. Negative control tests demonstrated negative results.

One possible explanation for the difference in the results of the PSA assay on the cotton as opposed to the dried samples may be a reduced PSA extraction efficiency which has been documented when extracting from cotton substrates (10,11).

This study shows that it is possible to identify spermatozoa and to detect PSA from forensic-type samples exposed to simulated gastric juice for up to 4 h. This may serve as a support for forensic examinations performed on cases where vomit or vomit-stained sexual assault evidence is submitted for analysis.

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