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The Examination of Vaginally Inserted Plastic Tampon Applicators for Genetic Markers and Evidence of Prior Sexual Intercourse

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ABSTRACT: Vaginally inserted plastic tampon applicators were obtained from 42 female volunteers. The applicators were examined for the presence of ABH blood group substances, phosphoglucomutase (PGM), amylase, acid phosphatase, P30, and intact spermatozoa. Each applicator was accompanied by a control blood sample, a saliva specimen, a brief sexual and menstrual history, and method of birth control of the donor. Eight of the male sexual partners of the donors submitted blood and saliva samples. One male sexual partner submitted only a saliva sample.

ABH blood group substances corresponding to the donor were recovered from 36 of the 42 applicators. The remaining 6 applicators revealed a combination of the donor's and sexual partner's ABH substances. The female's PGM type was recovered from 34 of the applicators. The remaining 8 applicators failed to show PGM activity. Of the applicators, 15 indicated evidence of prior sexual intercourse by the detection of ABH substances not consistent with the applicator donor (6 samples), high levels of acid phosphatase (11 samples), or recovery of spermatozoa (8 samples) or some combination of these. All applicator samples failed to show the presence of either P30 activity or PGM factors foreign to the female.

KEYWORDS: forensic science, phosphoglucomutase, genetic typing, semen, vaginal secretions

In most legal jurisdictions, the insertion of a foreign object into vaginal, anal, or oral cavities is considered to be the equivalent of rape or penile penetration. We are not aware of any previous study pertaining to the recovery of genetic markers from objects inserted into the vagina. The reliable recovery of genetic markers from such objects would be useful in substantiating vaginal penetration and confirming the identity of the individual penetrated. The recovery of seminal fluid or genetic markers not consistent with the individual penetrated would be an indication of sexual intercourse before the insertion of that object.

Data gathered from this study showed that genetic markers were present on vaginally inserted objects and that they could be reliably detected and used to associate the object to the person penetrated. In those instances where semen was detected, the genetic markers often could be used to help identify the donor of the semen. Since the sexual history of each volunteer in this study was provided, the persistence of acid phosphatase activity, the presence of

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intact spermatozoa, and phosphoglucomutase (PGM) enzyme activity could be correlated to the time since last sexual intercourse.

Methods and Materials

All specimens used in this study were collected under the direction of Dr. Brad Randall at the Laboratory of Clinical Medicine, Sioux Falls, South Dakota. The volunteer specimen collection protocol was approved by the Human Subjects Committee of the University of South Dakota School of Medicine. Informed consent was obtained from each volunteer. All items were coded with an identification number to ensure the confidentiality of the specimens and data. Samples were analyzed by the Minnesota BCA Forensic Science Laboratory.

Each of the 42 female volunteers was provided with an instruction sheet, 2 cardboard mailers, 2 sealed tampons, a small sterile gauze pad, a red-top blood tube, and a questionnaire sheet. Each volunteer was asked to indicate her age, date, and time of specimen collection; date and time of last sexual intercourse (to be omitted if greater than 5 days); date of last menstrual period; and method of birth control, if any.

The volunteers were instructed to collect a vaginal sample any time except for the five days preceding or following their menstrual period by vaginally inserting the plastic applicator and tampon, rotating them approximately 360°, and then removing them. The tampon itself was to be discarded and the outer plastic applicator placed in the cardboard mailer and allowed to dry for 24 h before sealing the mailer with the screw top cover.

The control saliva sample was obtained by placing a sterile gauze pad in the mouth until it was saturated with saliva. The gauze was to be air-dried and returned to its original packet for storage. The blood control was obtained in a red-top vacutainer tube.

Control blood and saliva samples were requested from the male sexual partners of the donors whose plastic applicator indicated seminal fluid or foreign blood group substances or both. Of the 15 male donors asked to give control samples, only 8 chose to donate the requested blood and saliva, and 1 agreed to give only a saliva sample. The control specimens from the male sexual partners were collected in the same manner as previously described from the female donors.

Each of the 42 plastic applicators was swabbed with a water moistened cotton swab and the swab was air-dried. The swabs were cut into 2 equal parts, one half to be tested for ABH blood group factors, amylase, acid phosphatase, and P30, and the other half to be used for PGM enzyme typing. Each of the 42 plastic applicators was then swabbed with a 3- by 5-mm LKB applicator tab which was subsequently used for PGM enzyme typing and secretor grouping. The LKB applicator tabs, when analyzed for PGM type, were prerun at 5 W for 15 min to allow the PGM enzyme to be transferred from the tabs to the focusing gel. The tabs were then removed from the gel and tested for ABH substances by absorption inhibition. After being swabbed with the cotton swabs and LKB applicator tabs, each of the 42 plastic applicators was then washed with about 2 mL of saline. The washing was centrifuged and the supernatant was tested for amylase activity, acid phosphatase activity, and the presence of P30 and ABH blood group substances. The remaining residue after centrifugation was dried on a glass slide, stained, and microscopically searched for spermatozoa.

Samples from the applicators and saliva controls were tested for amylase activity using the starch-iodine technique [*1*]. The presence of seminal fluid was determined by qualitative analysis for acid phosphatase [*2*], crossed-over electrophoresis with anti-P30 obtained from SERI [*3*], and microscopically searched for the presence of spermatozoa on smears stained with the Kernechtrot and Picroindigocarmine technique [*4*]. Thin-layer isoelectric focusing (IEF) using 1% agarose gels and a pH 5 to 7 gradient was used for all PGM typing [*5*].

Results

The known blood samples were used to determine the ABO, Lewis, and PGM types of each donor. All of the control saliva samples tested positive for the presence of amylase.

Secretor groupings of the saliva samples were shown to be consistent with the ABO and Lewis types determined from the corresponding blood samples. Blood typing, secretor grouping, amylase results, and personal data are shown in Table 1.

The results of the analysis for semen, secretor grouping, and PGM typing on the three types of samples collected from the plastic applicators are given in Table 2.

Positive acid phosphatase reactions were found in 11 of the 42 washings from the applica-

TABLE 1—*Test results on the control blood and saliva samples from the 42 donors and a summary of their personal histories.*

	Blood Typing Results			Saliva Testing		Personal Data			
Donor	ABO	PGM	Lewis	ABH	Amylase	Age	DSLP[a]	HSLI[b]	BC[c]
1	O	1+	a+b−	NS[d]	+	24	55	...	N
2	O	2+	a−b+	H	+	26	11	59	B
3	O	1+1−	a−b+	H	+	35	18	119	C, V
4	A	1+	a+b−	NS	+	28	21	64	B
5	A	2+	a−b+	A, H	+	24	4	43	B
6	O	1+	a−b+	H	+	35	27	32	B
7	O	1+2−	a+b−	NS	+	27	8	22	B
8	O	1+	a−b+	H	+	35	8	...	V
9	A	1+2+	a−b+	A, H	+	24	18	...	B
10	B	1+	a−b+	B, H	+	28	9	46	V
11	O	1+	a−b+	H	+	27	14	...	N
12	A	1+	a+b−	NS	+	25	2	45	N
13	A	1+2+	a+b−	NS	+	36	9	22	V
14	O	1+	a−b+	H	+	22	9	...	B
15	A	1+	a−b+	A, H	+	29	18	17	V
16	A	1+2+	a−b+	A, H	+	28	24	58	T
17	A	1+1−	a−b−	A, H	+	25	27	47	B
18	A	1−2+	a−b+	A, H	+	26	22	112	D
19	A	1+	a−b+	A, H	+	26	28	24	B
20	A	1+2+	a+b−	NS	+	21	24	...	B
21	A	1+	a−b+	A, H	+	30	27	39	B
22	A	1+	a−b+	A, H	+	24	17	46	B
23	O	1+	a+b−	NS	+	43	20	...	N
24	O	1+	a+b−	NS	+	34	21	...	T
25	B	1+2−	a−b+	B, H	+	38	8	...	N
26	A	1+2+	a−b+	A, H	+	27	7	47	B
27	B	1+1−	a−b+	B, H	+	21	23	...	B
28	O	1+2−	a−b+	H	+	30	11	...	N
29	O	1+2+	a+b−	NS	+	23	9	...	N
30	O	2+2−	a−b+	H	+	20	9	...	N, C
31	A	1+	a−b+	A, H	+	26	17	...	B
32	B	1+	a−b+	B, H	+	28	312	14	N
33	B	1+	a−b+	B, H	+	30	16	...	B
34	O	1+2+	a−b+	H	+	30	5	9	T
35	B	1−2+	a+b−	NS	+	25	15	38	B
36	O	1+2−	a−b+	H	+	43	...	...	N
37	B	1+	a+b−	NS	+	40	14	44	V
38	A	1+	a+b−	NS	+	31	7	37	B
39	O	1+2+	a−b−	H	+	30	7	41	N
40	O	1+	a−b+	H	+	24	19	19	B
41	O	1+	a+b−	NS	+	31	300	...	N
42	A	1+	a−b+	A, H	+	35	...	...	N

[a]DSLP = Days since last period.
[b]HSLI = Hours since last intercourse.
[c]BC = Birth control method: N = none, B = oral contraceptive, C = condom, V = vasectomy, T = tubal ligation, D = diaphragm.
[d]NS = nonsecretor.

TABLE 2—*Test results obtained from 42 vaginally inserted tampon applicators.*

	Saline Washing After Cotton Swabs and LKB Tabs					Cotton Swabs		LKB Tabs	
Donor	Amylase	AP[a]	P30	Micro	ABH	ABH	PGM	ABH	PGM
1	−	−	−	−	NF[b]	NF	NA[c]	NF	NA
2	−	−	−	−	H	H	NA	H	2+
3	−	−	−	−	H	H	NA	H	1+1−
4	−	−	−	−	NF	NF	NA	NF	1+
5	−	−	−	−	A, H	A, H	NA	A, H	2+
6	−	+	−	+	A, H	A, H	NA	A, H	1+
7	+	−	−	−	NF	NF	NA	NF	1+2−
8	−	+	−	−	A, H	A, H	NA	A, H	1+
9	−	+	−	−	A, H	A, H	NA	A, H	1+2+
10	−	−	−	−	B, H	B, H	NA	B, H	1+
11	−	−	−	−	H	H	NA	H	NA
12	−	−	−	−	A, H	NF	NA	A, H	1+
13	−	−	−	−	A, H	NF	NA	A, H	NA
14	−	−	−	−	H	H	NA	H	1+
15	−	−	−	−	A, H	A, H	NA	A, H	1+
16	−	−	−	−	A, H	A, H	NA	A, H	1+2+
17	−	−	−	−	A, H	A, H	NA	A, H	NA
18	−	−	−	−	A, H	A, H	NA	A, H	1−2+
19	−	−	−	+	A, H	A, H	...	A, H	1+
20	−	−	−	−	NF	NF	...	NF	NA
21	−	+	−	+	A, H	A, H	...	A, H	1+
22	+	+	−	−	A, H	A, H	...	A, H	1+
23	−	−	−	−	NF	NF	...	NF	1+
24	−	−	−	−	NF	NF	...	NF	1+
25	−	−	−	−	B, H	B, H	...	B, H	1+2−
26	−	−	−	−	A, H	A. H	...	A, H	INC[d]
27	−	−	−	−	B, H	NF	...	B, H	1+1−
28	−	+	−	−	H	H	...	H	1+2−
29	−	−	−	−	NF	NF	...	NF	1+2+
30	−	−	−	−	H	NF	...	H	2+2−
31	−	−	−	−	A, H	A, H	...	A, H	NA
32	−	+	−	−	B, H	B, H	...	B, H	1+
33	−	−	−	−	B, H	B, H	...	B, H	1+
34	−	+	−	+	B, H	B, H	...	B, H	1+2+
35	−	+	−	+	NF	NF	. .	NF	1−2+
36	−	−	−	−	H	H	...	H	1+2−
37	−	−	−	−	NF	NF	...	NF	1+
38	−	−	−	+	A, H	A, H	...	A, H	1+
39	−	+	−	+	H	H	...	H	1+2+
40	−	+	−	+	H	H	...	H	1+
41	−	−	−	−	NF	NF	...	NF	NA
42	−	−	−	−	A, H	NF	...	A, H	1+

[a]AP = Acid phosphatase.
[b]NF = No ABH substances found.
[c]NA = No activity.
[d]INC = Inconclusive.

tors (Nos. 6, 8, 9, 21, 22, 28, 32, 34, 35, 39, and 40). Of the 11 washings with positive acid phosphatase reactions, 6 revealed the presence of intact spermatozoa (Nos. 6, 21, 34, 35, 39, and 40) and 1 came from a donor whose sexual partner was vasectomized (No. 8). Of the remaining 31 applicator washings, 2 (Nos. 19 and 38) yielded intact spermatozoa, but failed to give a positive acid phosphatase reaction. None of the 42 applicator washings yielded a positive reaction with anti-P30.

Intact spermatozoa and acid phosphatase activity were detected in washings from applicators inserted 9 to 46 h after sexual intercourse. In three instances, the donors claimed no sexual intercourse for at least five days before inserting the applicators, yet reactions indicated high levels of acid phosphatase. We have no explanation for the unexpected high levels of acid phosphatase indicated on these samples with postcoital intervals exceeding 120 h except that the donors had high levels of vaginal acid phosphatase. The criteria for calling a reaction positive for acid phosphatase were subjective, based on comparisons to known semen with attention to the amount of color change and the length of time with which the color change occurred. Two experienced analysts independently performed the acid phosphatase testing with concurring results.

Secretor typing results were in agreement from each of the forty-two LKB tab samples and its corresponding saline washing sample. Five of the applicator samples collected on cotton swabs (Nos. 12, 13, 27, 30, and 42) failed to reveal the presence of ABH substances that were detected on the LKB tab method and in the saline washing method. The failure to detect the ABH substances on these five cotton swabs was attributed to a failure to transfer sufficient material from the applicator to the swab.

The secretor type on 36 of the 42 LKB tabs and saline washings corresponded to the secretor type of the donor. Secretor typing of the 6 differing samples (Nos. 6, 8, 12, 13, 34, and 38) revealed the presence of ABH substances from the female applicator donor plus additional ABH substances. The presence of semen was indicated or confirmed in 4 of these samples (Nos. 6, 8, 34, and 38). Secretor typing of these four samples indicated the presence of ABH substances matching those of the female donor in combinations with her sexual partner (Tables 3 and 4). No semen was detected in samples from the applicators of Donors 12 or 13; however, ABH substances recovered from these samples were consistent with the donors (both nonsecretors) in combination with their sexual partners (both A secretors). The possibility that the A and H detected came from the donors having low-level A and H substance could not be eliminated; however, the strength of the reaction indicated the levels of A and H to be higher than one would expect from a nonsecretor. The other possibility is that A and H substances were present from the male partners and these factors persisted for a longer period of time than the semen was able to be detected.

PGM typing was performed on the cotton swabbings of the plastic applicators of Donors 1 through 18. Since no PGM activity was detected on these swabs, the remaining cotton swabs

TABLE 3—*Test results on the control blood and saliva samples from eight of the sexual partners of the donors.*

	Blood Typing		Saliva Testing	
Sexual Partner[a]	ABO	PGM	ABH	Amylase
6	[b]	[b]	A, H	+
8	A	1+1−	A, H	+
12	A	1+1−	A, H	+
13	A	1+1−	A, H	+
19	A	1+	NS[c]	+
22	AB	1+	A, B, H	+
34	B	1+2+	B, H	+
38	A	1−2−	A, H	+
40	O	1−2+	H	+

[a]Sexual partner has same code number as the donor of the tampon applicator.

[b]No blood sample could be obtained from male sexual partner of applicator Donor 6.

[c]NS = Nonsecretor.

TABLE 4—*Comparison of secretor types of the donors of the tampon applicators and their sexual partners, with secretor types obtained from the plastic applicators.*

Sample	Male Partner Saliva ABH	Female Donor Saliva ABH	Presence of Semen	Cotton Swab ABH	LKB Tabs ABH
6	A, H	H	+	A, H	A, H
8	A, H	H	+	A, H	A, H
12	A, H	NS[a]	−	NF[b]	A, H
13	A, H	NS	−	NF	A. H
19	NS	A, H	+	A, H	A, H
22	A, B, H	A, H	+	A, H	A, H
34	B, H	H	+	B, H	B, H
38	A, H	NS	+	A. H	A, H
40	H	H	+	H	H

[a]NS = Nonsecretor.
[b]NF = No ABH substances found.

(Nos. 19 to 42) were not tested for PGM. All of the 42 LKB tab samples from the plastic applicators were tested for PGM. Of the 42 samples, 34 gave readable PGM results. The PGM types detected matched the PGM types of the donors of the applicators. On those 6 applicators where semen was indicated or confirmed to be present (Nos. 8, 19, 22, 34, 38, and 40) and blood controls were obtained from the donor's sexual partner, the PGM type of the donor's sexual partner was not detected. This was not unexpected since PGM activity has been reported to be detectable for only about 6 h after intercourse in semen samples collected from the vagina [*6*]. In 5 of the 6 cases, the applicator sample was collected more than 6 h after intercourse and in 1 case, the time was unknown. See Table 3 for the PGM type of the donors' sexual partners.

Discussion

The feasibility of examining vaginally inserted objects for forensic purposes has been demonstrated. As shown in this study, the ABO type and, in most cases, the PGM type of the donor of the vaginal material can be determined. In 15 instances, examination of the applicators also indicated evidence of previous sexual intercourse. Evidence of prior intercourse was found in 13 of 24 cases (54%) where there was reported intercourse within 5 days before specimen collection. Positive male markers were seen relatively evenly distributed over a postcoital interval of 9 to 46 h. Thus, examination of vaginally inserted foreign objects may often reveal markers of previous intercourse. In 8 instances, the recovery of spermatozoa was definitive evidence of prior intercourse.

In this study, the recovery of typable material from the vaginally inserted applicators was found to be dependent on the method of removal of the material. The use of 3- by 5-mm LKB tabs was found to be more effective than cotton swabs in recovering vaginal PGM from the applicators. Washing the applicators with saline or swabbing the applicators with an LKB tab was more effective for recovering secretor ABH substances than was swabbing of the applicator with a cotton swab. The failure to detect P30 was probably a result of using excessively dilute samples (2-mL saline washing).

The method of birth control did not appear to affect the recovery of male or female markers from the applicators in this study. Nineteen of the donors reported using oral contraceptives, twelve were not using birth control, five were relying on partner vasectomy, three had tubal ligations, one used a diaphragm, one indicated condoms or no birth control, and one relied on partner vasectomy or condoms.

Conclusion

In this study, 42 vaginally inserted plastic tampon applicators were tested. It was found that the donor's ABH factors were detectable on all the applicators where the donor was a secretor. Caution should be exercised in interpreting secretor results since ABH factors may be detected from prior sexual intercourse even when semen is no longer indicated to be present on the inserted object. Samples from the majority of the applicators, 34 of the 42 (about 80%), revealed PGM factors of the same type as their donor. Since the applicator samples were taken 5 days before or after the donor's menstrual period, these PGM types are indicated to be from vaginal PGM and not from menstrual blood. The detection on a vaginally inserted object of PGM activity from semen deposited in prior sexual intercourse is unlikely if this intercourse occurred 6 h or more before the insertion of the foreign object.

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