P 30 and rape

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INTRODUCTION

The detection of sperm in swabs or stains is usually performed by using two methods : search for spermatozoa and for seminal acid phosphatase.The presence of spermatozoa provides valuable evidence except when the raper is azoospermic. Nevertheless, it is not a practical test for large scale screening because of ocular weariness due to microscopic examination. Furthermore, even with considerable experience, it is difficult to recognize morphologically abnormal spermatozoa. With regard to the second test, seminal acid phosphatase, the problem is that vaginal secretions may sometimes give false positive reactions.

For all these reasons, a really specific, easy and sensitive marker of human seminal plasma would be of great interest to forensic medicine.A prostate specific glycoprotein called P 30 has been described in the literature; its molecular weight is about 30,000 d (Sensabaugh 1978) and its concentration in normal semen plasma has a mean level of 1.55 mg/mL (Graves 1985). The aim of the present study is to perfect an ELISA test for sensitive P 30 detection, and to apply it to sexual assault evidence.

MATERIALS AND METHODS

Samples : Semen samples were obtained from the Urology Clinic. Vaginal secretions samples - collected with sterile cotton wool swabs - were obtained from the Gynecology Clinic and from volunteer donors as well. Forensic expert cases, from 1977 to the present, were included in the trial when the remaining materials (suspect stains and swabs) were available in sufficient amounts. Data concerning spermatozoa (always) and acid phosphatase (sometimes) analyses were obtained as required at the time of facts.

Extraction : A 5 mm long thread of stain or 4 mm^2 of swab were extracted overnight at 4° or 20° C in 250 μL of PBS-BSA-TWEEN buffer ($NaH_2PO_4.H_2O$ 0.52 g; $K_2HPO_4 3H_2O$ 3.67 g; NaCl 8.76 g; H_2O 10 L containing 0.3 % bovine serum albumine and 0.1 % Tween 20).

ELISA test : Polystyrene immunoplates (CEB) were coated overnight at 4° C. with 75 μl per well of rabbit Ig anti P 30 (DAKO) diluted (1/8000) in carbonate buffer (Na_2CO_3 0.1 M;

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$NaHCO_3$ 0.1 M pH9.5). Plates were then washed three times with PBS-BSA-TWEEN buffer. After drying, they were ready to receive the samples to be tested. 50 μL of sample in twofold dilutions in PBS-BSA-TWEEN buffer were kept 2 h at 37° C; after three further washings, 50 μL of the optimal dilution (in PBS-PSA-TWEEN buffer 1/8000) of mouse anti P 30 monoclonal antibody (Hybritech) were added to each well and incubated for 2 h at 37°C. After three further washings, fixation of the monoclonal antibody was revealed by using 50 μL of Peroxydase-conjugated rabbit Ig anti-mouse Ig (Dako) at 1/250 dilution. This reagent was left for 1 h at 37°C. and the excess removed by three washings with running water. 50 μL of substrate solution (OPD) were added to each well and the reaction immediately stopped by adding 150 μL 2N H_2SO_4. An orange coloration indicated positive samples whereas negative samples remained colorless . Absorbency values at 492 mm were measured by using a spectrophotometer (Multiskan, Titertek). The results are expressed in terms of the last dilution of the samples that still gives a significant absorbency value.

RESULTS

Cutoff Value : 11 whole blood, 14 sera, 9 saliva, 3 perspirations, 5 nasal secretions, 13 urina, 14 feces , 12 vaginal secretions and 2 menstrual blood samples were tested; they manifested no P 30 antigenic activity. The cutoff of the test calculated as the (mean + 2σ) of these negative controls was found to be equal to 0.26.

Variation Coefficient : The calculated variation coefficient ($CV = 100\frac{\sigma}{m}$) is 12 % for the absorbency of 1. (N Replicate = 9)

Experimental Study : Fifteen different liquid sperm samples were analyzed (among them 1 vasectomized, 2 medical azoospermic and 2 oligozoospermic). The last detectable dilution was around 1/1,000,000. Consequently the detection limit -estimated from the mean level of P 30 in normal sperm- is 1.5 ng/mL. Fifty-four experimental stains from different sperm samples have been analyzed (see Fig. 1).

Conservation in Vagina : 19 vaginal swabs (a) were collected from 19 women and the interval time after coitus is known. 80 vaginal swabs (b) were collected from 8 volunteer donors; each woman collected herself 10 vaginal swabs at different time intervals after coitus. Figure 2 shows the decay curves of P 30 detection in vagina as a function of time.The mean delay for P 30 detection is 24 h with a range from 10 1/2 h to 24 h.

Application to Forensic Caseworks : 98 analyses were carried out on 74 exhibits from 42 forensic cases (dating from 2 days to 10 years) and were compared with spermatozoa and acid phosphatase researches. The results are given in table 1.The sensitivity and the specificity of P 30 test compared to spermatozoa test are respectively 92.7 % and 95.3 %.

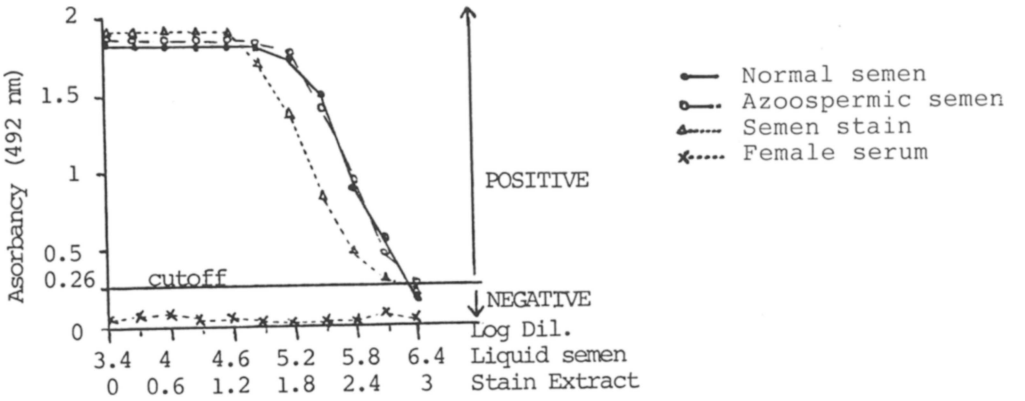

Fig. 1. P 30 detection (ordinate = absorbency at 492 nm) as a function of dilutions (abscissae = dil. log.)

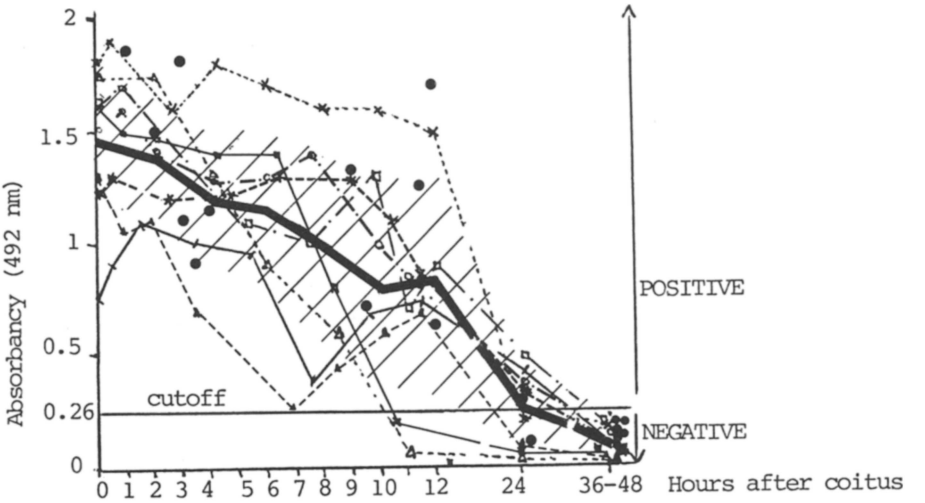

Fig. 2. P 30 detection in vagina (ordinate = absorbency at 492 nm) as a function of time after coitus (absissae = hours).
● : vaginal swabs from the 19 women (a)
eight curves : decay for each volunteer donor (b)
heavy line : mean decay for the 99 swabs (a and b)
hachured area : value of + and - one σ

Six discrepancies have been observed that need some comments :
(1) possible azoospermic semen ? . The presence of semen is in agreement with the history. (According to Willot (1982) the frequency of azoospermia is 1.9 % in sexual assault cases).
(2) Possible false positive result due to artefacts or contamination from the vaginal contents (Enos 1978).

Table 1. Results are presented separately for vaginal swabs, other swabs and stains on clothes, towels a.s.o.. The P 30 results are expressed by the last positive dilution. SPZ = spermatozoa and AP = acid phosphatase.

20 vaginal swabs

REF.	AGE	SPZ	P30	AP
ML1158	7 y	+++	1/4	+++
T 236	3 y	++++	1/256	+++
T 259	2.5 y	-	-	-
T 369	1 y	++++	1/32	+
T 385	7 m	+++	1/32	+
T 399†	7 m	-	-	-
T 482	6 m	-	-	NT
T 419	5 m	+	1/1	+++
T 414	5 m	+++	1/256	+++
T 426	4 m	++	1/32	+++
T 433†	3 m	-	1/16	+(1)
T 440	3 m	+++	1/32	+++
T 441	3 m	-	-	++
		-	-	-
T 457	1 m	+	1/256	-
T 461	1 m	++++	1/1024	NT
T 462	2 w	-	-	++
		-	-	NT
T 496	1 w	++	1/16	NT
T 470	2 d	-	-	NT

†dead body

5 anal swabs
2 buccal swabs

REF.	AGE	SWAB	SPZ	P30	AP
ML1158	7 y	anal	+	-	-(2)
		buc.	+	-	-(3)
T 94†	5 y	anal	-	-	-
T 207	3 y	anal	-	-	-
T 399†	7 m	anal	-	-	-
		buc.	-	-	-
T 434	5 m	anal	-	-	-

†dead body

47 diverse clothes, towels and so one

REF.	AGE	TYPE OF STAINS	SPZ	P30	AP
ML 972	10 y	preservatives	+++	1/4	-
		slip	-	-	NT
ML1158	7 y	menstrual strip	+++	1/8	+++
T 40	6 y	slip	+	1/32	+++
T 61	5 y	slip	-	-	-
T 73	5 y	pyjama	+++	1/64	+++
T 83	5 y	kleenex	+++	1/32	+++
T 120	4.5y	dress	+++	1/16	+++
T 102	4.5y	handkerchief	+	1/2	+++
		handkerchief	+	-	- (4)
		slip	+++	1/32	+++
T 143	4 y	slip	-	-	-
T 170	3.5y	slip	+++	1/64	+++
T 173	3.5y	slip	-	-	NT
T 207	3 y	handkerchief	+++	1/32	+++
		slip	+	1/2	-
T 209	3 y	trousers, slip	-	-	-
T 236	3 y	handk.,wrapper	++++	1/256	+++
T 220	2.5y	slip	+++	1/16	+++
T 233	2.5y	slip	+++	1/128	+++
T 232	2.5y	bust-bodice	+++	1/64	+++
		slip	-	-	-
T 240	2.5y	slip	++	1/128	+++
		kleenex	+	1/8	++
		glove	++	1/32	+++
T 286	2 y	towel	+++	1/1024	+++
T 340	1.5y	slips,handk.	-	-	-
T 347	1 y	slip	-	-	-
T 369	1 y	slip	+	1/16	-
T 376	1 y	slip	-	-	-
T 487	1 m	slip	-	-	NT
		kleenex	-	1/8	NT(1)
T 462	2 w	prostitute's clothing	+	-	++(5)
		slips	-	-	NT
T 466	1 w	mantle	-	-	NT
T 496	1 w	slip	++	1/64	NT
		kleenexes	+++	1/256	NT
T 470	2 d	towel	+++	1/128	NT

(3) According to Enos (1978) : " it is possible to identify spermatozoa in oral smears up to 6 hours after the attack, despite brushing teeth and drinking". In this case, buccal

swab has been collected 8 h after the rape on a woman alive; there was certainly no seminal plasma left. Note that acid phosphatase tests were also negative.

(4) The semen material was very small because it had been used for the analyses required by the court during judicial proceedings.

(5) It is perhaps an old semen stain which was cleaned. It may be presumed that spermatozoa persisted (Cordonnier 1922) whereas the seminal fluid itself did not.This fact is surprising but it has been reproduced experimentally in our laboratory.

CONCLUSION

The detection of seminal P 30 glycoprotein by ELISA testing is QUICK (results are obtained within 24 h with a short effective working time), EASY (large scale analyses till 30 tests a day with one technician), REPRODUCIBLE (variation coefficient : 12 %), SENSITIVE (threshold of P 30 detection : 1.5 ng/mL), SPECIFIC (no cross reaction observed with other human biological fluids or secretions).

In short, this test should be regarded as a very helpful complementary analysis to spermatozoa research in forensic expert cases. It shows better specificity and sensitivity than seminal acid phosphatase testing. We now apply it routinely in our laboratory.

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