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Evaluation of MHS-5 in detecting seminal fluid in vaginal swabs

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Abstract Vaginal swabs taken in 211 cases of alleged sexual assault were examined for seminal vesicle-specific antigen (SVSA) using an MHS-5-ELISA (SEMA kit). The results were compared with those obtained by sperm detection by means of light microscopy and the acid phosphatase reaction (ACP), using Phosphatesmo-KM papers. Especially in fresher samples (duration of storage between 10 days and 2 ½ months), a high degree of correlation was observed between the results of light microscopic and MHS-5 methods. Several cases with positive MHS-5 showed concurrent positive ACP reactions, even though no spermatozoa had been seen microscopically. The results are displayed in the light of time elapsed between the alleged assault and examination of the swabs. The longest time span after the alleged assault in which MHS-5 yielded a positive result was 47 h; in this case spermatozoa were also seen microscopically. SVSA is not totally stable in vaginal swabs stored over long (9 months to 5 ½ years) periods of time. Furthermore, results in eight penile swabs are reported. MHS-5 is a useful tool for medico-forensic semen detection in vaginal swabs, probably even in cases of azoospermia or aspermia.

Key words Semen detection · MHS-5-ELISA · Seminal vesicle specific antigen (SVSA) · Stored vaginal swabs · Penile swabs

Introduction

The monoclonal mouse anti-human-sperm antibody no. 5 (MHS-5) was first described by Herr et al. 1986 [11]. This antibody is directed against the SVSA (seminal vesicle-

specific antigen) protein [7, 11]. Therefore, SVSA belongs to the group of semen markers that are also positive in cases of azoospermic or aspermic semen. It has since been found [14] that SVSA is a substrate of p30 [17]. In the light of experimental data there can be no doubt that SVSA is human and organ specific; only primate semen samples showed cross reactions [11]. The use of SVSA proved to be feasible for the forensic detection of seminal fluid [20]. MHS-5 is commercially available as the SEMA kit (Humagen, Charlottesville, USA). Exhaustive examinations pertaining to the utilization of MHS-5 for semen detection in vaginal swabs in the forensic field have not yet been undertaken. Our intention was to compare the results of the MHS-5-method with microscopical findings and the ACP reaction.

Materials and methods**Vaginal swabs**

Vaginal swabs were taken in 211 cases of alleged sexual assault. According to the victims, complete penetration of the penis took place in all cases, however, intravaginal ejaculation could not be confirmed with certainty by any of the victims. The time elapsed between assault and vaginal swabbing and the time since the last elective sexual intercourse were noted and taken into consideration. Of the swabs, 55 (age span of victims 5–60 years, 3 victims under the age of 15) had been air dried and subsequently stored at room temperature for between 10 days and 2½ months. The remaining 156 swabs (age span of victims 7–60 years, 7 victims under the age of 15), which had first been deep frozen and later thawed and air dried, were stored at room temperature for between 9 months and 5 ½ years.

Penile swabs

Swabs were taken from the glans penis of eight men suspected of prior sexual assault. All men denied the accusations of forced sexual intercourse and as also stated that they had not ejaculated after the alleged assault. The time between the alleged assault and penile swabbing was noted. Swabs were air dried and stored at room temperature for up to 2.5 months.

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MHS-5

Commercially available SEMA kits including MHS-5, positive and negative controls were used.

Phosphatesmo KM

Commercially available test papers (Machery-Nagel, Düren, Germany) for the detection of acid phosphatase reaction (ACP) were used. According to the manufacturer, vaginal fluid yields negative reactions with these papers.

Extraction

Two cotton pieces of 2 mm³ volume each were cut from the tip of each vaginal and penis swab, one was used for the ACP reaction and microscopic examination and the other for MHS-5-ELISA.

Acid phosphatase reaction

The pieces cut from the swabs were moistened with a few drops of PBS and pressed onto a Phosphatesmo-KM paper for several seconds. The reaction was recorded after 30 s.

Microscopic sperm identification

Smears of the samples used in the prior ACP reaction were stained by a modified hematoxylin-eosin method [19] to identify spermatozoa. The presence of at least one spermatozoon on the entire slide was judged as a positive result.

Reaction with MHS-5 in assaying SVSA

The cotton piece for the ELISA was eluted in 150 µl PBS (5 min). The SEMA kit is a solid phase immunoassay and the tests were carried out according to the manufacturer's instructions:

- Incubation (60 min) of 100 µl eluate of each swab in plastic wells. Positive cases: binding of SVSA to the polystyrol surface. Subsequent removal of eluate.
- Incubation with blocking buffer (10 min). Subsequent emptying of plastic wells.
- Washing with distilled water.
- Incubation (20 min) with 100 µl biotin-streptavidin-peroxidase-labelled MHS-5 antibody. Subsequent emptying of wells.
- Washing with distilled water (4 times).
- Addition of 100 µl of a mixture of ABTS (2,2'-azinobis(3-ethylbenzthiazolinesulfonic acid)) and H₂O₂ (1:1).
- Results were read visually after 10 min in comparison to the controls.

Results

Storage time of vaginal swabs

The results of vaginal swabs stored for shorter periods of time can be seen in Table 1. When spermatozoa were seen microscopically, ELISA was positive in all cases, partly in combination with a positive ACP reaction. Furthermore, 7 cases displayed MHS-5-reactivity in spite of negative microscopy and in 3 cases together with a positive ACP reaction. ACP was positive in 24 out of 32 swabs (75%) in which spermatozoa had been identified. Results obtained in swabs stored for a longer period of time are depicted in

Table 1 Results of testing for sperm in 55 vaginal swabs (storage time 10 days to 2 1/2 months)

Method	Results				
Micro	+	+	-	-	-
MHS-5	+	+	+	+	-
ACP	+	-	+	-	-
	24	8	3	4	16

Table 2 Results of testing for sperm in 156 vaginal swabs (storage time 9 months to 5 1/2 years)

Method	Results					
Micro	+	+	+	+	-	-
MHS-5	+	+	-	-	-	-
ACP	+	-	+	-	+	-
	46	12	13	13	13	59

Table 2. Spermatozoa were visible in 84 swabs. A positive MHS-5-reaction was only obtained in 58 (69%) of these 84 swabs, and 46 swabs showed a positive ACP reaction at the same time. The microscopic evidence of spermatozoa was supported only by ELISA in 12 cases and only by ACP in 13 cases. Another 13 cases yielded a positive "sperm" diagnosis only after microscopy. In another 13 cases, ACP was positive but neither ELISA nor microscopy confirmed the presence of seminal constituents. A total of 59 (70%) of the 84 spermatozoa positive cases yielded a positive ACP reaction.

Time elapsed between alleged assault and physical examination

Because the results of the 55 swabs stored for a shorter period of time appeared to be more reliable (on the grounds of a higher percentage of MHS-5 positive reactions in this group), only these cases were included in the time-related analysis. Figure 1 shows 39 of these 55 cases in which at least one test for semen was positive. A total of 7 cases were positive for MHS-5 although no spermatozoa could be demonstrated. Samples not more than 5 h old accounted for 4 of these MHS-5-positive cases, although the ACP reaction was also positive in 2 of them. The remaining 3 cases with positive MHS-5-reaction and negative "sperm" diagnosis were examined 19, 20 and 41 h after the alleged intercourse, with 1 case (19 h) also yielding a positive ACP reaction. The last elective sexual intercourse had taken place between 3 and 4 days prior to the alleged assault in 4 of these cases. In the remaining 3 cases it was stated that intercourse on a voluntary basis had last taken place 2 weeks or "even longer" before the event in question.

Fig. 1 Constellation of findings of semen parameters compared with time elapsed between assault and vaginal swabbing. Swabs of 39 cases with storage times between 10 days and 2 ½ months. Isolated MHS-5 reaction of case at 4 ½ hours – extravaginal ejaculation after prior intravaginal intercourse

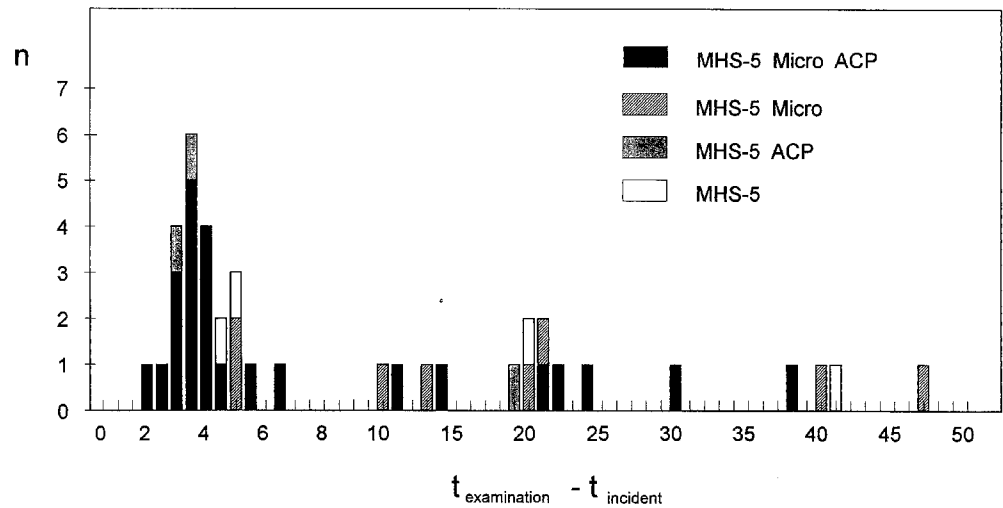
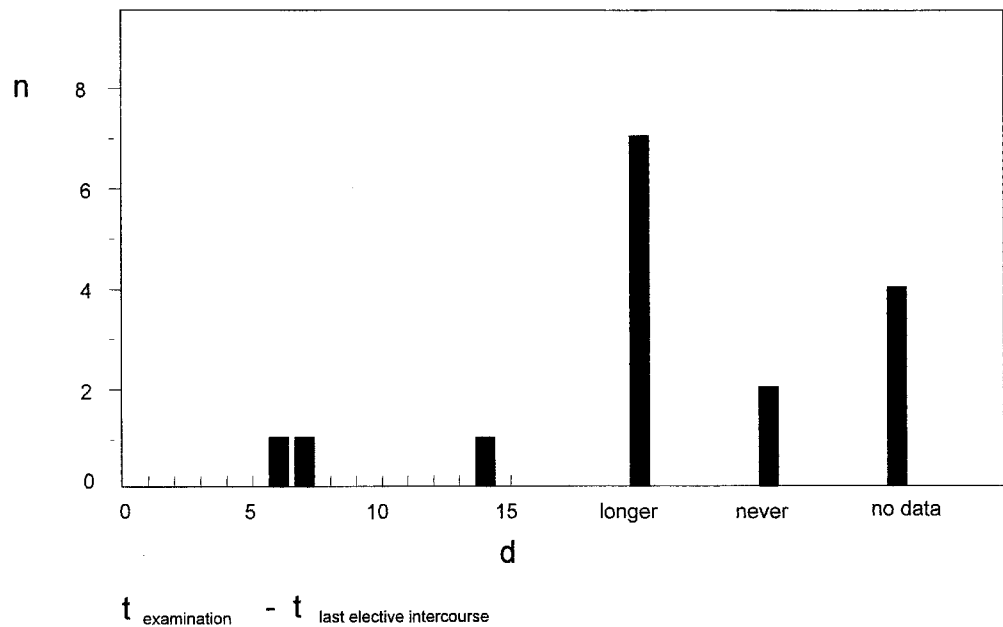


Fig. 2 Vaginal swabs from 16 women with all findings negative. Indicated time of last elective intercourse with ejaculation before swabbing (examination time = 0 on x-axis). Women presented with alleged sexual assault not more than 4 days before examination



Last elective sexual intercourse and negative seminal findings

Of the 55 swabs stored for a shorter period of time, 16 yielded negative results for seminal fluid in all tests (see Table 1). These cases were analysed with regard to the time when voluntary intercourse with subsequent ejaculation had last occurred (Fig. 2). According to the histories taken, the shortest period that had elapsed between elective intercourse with ejaculation and vaginal swabbing was 6 days.

Penile swabs

See Table 3 for results.

Table 3 Results of testing for sperm in 8 penile swabs from men taken into custody for suspected rape (storage time up to 2 ½ months)

$t_{\text{exam}} - t_{\text{incident}}$ (h)	Micro	MHS-5	ACP
3	+	+	-
3	-	-	-
3 ½	Inconclusive	+	-
4 ½	+*	+	-
6	-	-	-
9	-	+	-
17	Inconclusive	+	-
17 ½	+	+	-

*one head

Discussion

Results from the analysis of swabs stored for a shorter period of time show that the presence of SVSA can always be demonstrated when spermatozoa are found microscopically. In contrast, only 69% of the 84 swabs stored for longer times and demonstrating spermatozoa under the microscope yielded a positive MHS-5 result. This result makes a storage-related decay of the antigen plausible, with the degree of antigen decay not only being related to the duration of storage, but also to the variations in storage conditions. As expected, SVSA in vaginal swabs is not totally stable, but nonetheless displays a high degree of storage stability. In addition, positive MHS-5 reactions along with positive microscopical findings in samples taken up to 47 h after forced intercourse support the high stability of SVSA.

Of the swabs stored for shorter periods and yielding a positive MHS-5 reaction, 7 were negative for sperm when examined microscopically. Of these, 3 were positive for ACP. According to the manufacturer, the Phosphatesmo-KM test does not react with endogenous vaginal phosphatase [13]. A positive Phosphatesmo should thus be interpreted as a sign of the presence of seminal fluid, since MHS-5 does not react with vaginal fluid [11]. Conversely, it is indeed possible that a positive SVSA test in the absence of the other markers indicates the presence of semen, as in 4 of our cases (Table 1, Fig. 1).

On the whole, ELISA seems to be more sensitive than the ACP reaction. One of the 7 cases mentioned above (4 ½ h after alleged assault) should be noted in this context, since (according to the victim) penile penetration had taken place, but the ejaculation had finally taken place extravaginally (onto the abdomen). It appears possible that even sexual intercourse without ejaculation can lead to the deposition of sufficient amounts of seminal fluid in the vagina to be consistently detected by the MHS-5-ELISA. In contrast, the ACP reaction could be negative in such a case because of its lower sensitivity but further research in this direction is necessary. The fact that not only the vaginal but also the penile swabs yielded positive results for the ELISA more often than the ACP reaction or microscopic examination gives further support to this hypothesis.

To compare microscopic and ACP-reaction-based semen detection, multiple tests [3, 5, 8, 12, 15] have been carried out, with some authors [1, 2, 9, 18] taking the time between assault and medical examination into consideration. The problems of analysing ACP results (modification of the method, elevated endogenous vaginal ACP, chronology) are well known. Even so, the ACP reaction after sexual intercourse with ejaculation may occasionally be negative, in spite of the presence of spermatozoa. Furthermore, the possibility of a positive ACP reaction with concurrent negative microscopy findings exists. In this regard, our results and constellations of findings are consistent with data of other research groups [1, 2, 9, 18]. Therefore, we assume that the results we recorded means of the MHS-5-ELISA are valid.

Immunological detection of semen components is not yet an established part of forensic practice in Europe. The relatively large amount of time required for this method, especially when compared to the fast and simple method of microscopy, speaks against its routine employment. However, there has been an increasing demand for methods of detection of seminal fluid in the absence of spermatozoa, such as after vasectomy, which was performed in 25 000 cases in the former West Germany in the late 1980s [6]. This led to the addition of prostate-specific antigen [10, 17], prostate-specific phosphatase [15], LDH-X [16] and prostaglandin E [4] to the spectrum of semen markers. These markers are acknowledged to yield results that are sometimes superior to those obtained with microscopic methods. In view of the available results, it can be said that SVSA detected by means of MHS-5 represents another valuable semen marker, especially in swabs stored for quite short periods of time. It also seems that the MHS-5 reaction in vaginal swabs may even be positive when sexual intercourse without intravaginal ejaculation has taken place.

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