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Relationship of spermatoscopy, prostatic acid phosphatase activity and prostate-specific antigen (p30) assays with further DNA typing in forensic samples from rape cases

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ABSTRACT

In the forensic laboratory the biological analyses for rape investigation commonly include vaginal swabs as sample material combined to biochemical tests including sperm cytology (SC) and detection of acid phosphatase activity (AP) and prostate-specific antigen (PSA, p30) for the conclusive identification of semen components. Most reports comparing these tests relied on analysis of semen samples or donor swabs taken under controlled conditions; however their individual or combined efficacy under real live sampling conditions in different laboratories is largely unknown. We carried out SC, APA and PSA analyses in vaginal swabs collected from casework rapes submitted to Mexican Forensic Laboratories at Texcoco and Toluca. On the basis of positive and negative results from each assay and sample, data were classified into eight categories (I–VIII) and compared with those obtained in the two only similar studies reported in Toronto, Canada and Hong Kong, China. SC and APA assays had the higher overall positivity in Toluca and Texcoco samples respectively and otherwise PSA had a lower but very similar positivity between these two laboratories. When compared to the previous studies some similarities were found, namely similar frequencies (at a ratio of approximately 1 out of 3) of samples being positive or negative by all techniques (Categories I and VI respectively) and a comparable overall positivity of APA and SC but higher than that of PSA. Indeed the combined results of using SC, APA and PSA tests was considered as conclusive for semen detection from approximately 1 out of 3 cases (Category I) to approximately 1 out of 2 cases in a scenario where at least SC is positive, strongly presumptive in 2 out of 3 cases (with at least one test positive) and the remainder 1 out of 3 cases (Category VI) suggested absence of semen. By determining Y-STR polymorphisms (12-loci) in additional samples obtained at Toluca laboratory, complete DNA profiles were determined from all Category I samples, none marker was detected from all Category VI samples and mostly partial profiles were obtained from samples of other categories. These observations give an overview on the variability in efficacy of each test performed at different laboratories and provide a general notion about the *in praxis* contribution of SC, APA and PSA tests for further DNA typing in the forensic analysis of rape.

1. Introduction

In the forensic investigation of rape, the conclusive identification of semen is required to corroborate the alleged sexual assault as it is a usually unwitnessed crime. The medical examination of the complainant and the laboratory analyses of biological samples pursuit both detecting assailant semen and supplying legal resources for judicial consignation. Provided that women are mostly by far the victims [1,2] and vaginal swabs are the more reliable sample device [3], a number of sperm or seminal markers have been evaluated in these samples for forensic application. These include biochemical methods based either on detection of metabolites (free choline, Zinc, spermine, prostaglandin E) or enzyme activity (acid phosphatase [AP], gamma-glutamyl transpeptidase) as well as specialized immunological methods for specific proteins as are spermatozoid wall-specific antigens, seminal vesicle-specific antigens (semenogelins) and prostate-specific antigen (PSA, also called p30) [4–6]. Methodologies of more recent development based on detection of DNA (autosomal and Y-chromosome short tandem repeats [AS- and Y-STRs]) or RNA (semi-quantitative RT-PCR, Real Time PCR and microarrays) have been described for forensic purposes [7,8] and offer not only the

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potential benefit of higher sensitivity and specificity than biochemical ones but the possibility of assailant identification. To date, RNA-based protocols are still considered as supplementary to DNA-based techniques on the basis of the higher stability of DNA over RNA in casework material. In an increasing number of forensic laboratories DNA-based assays have already been introduced. In particular Y-STRs have earned acceptance over AS-STRs since for the latter ones the female DNA might mask the male DNA profile in samples containing gender-mixed materials and when more than a male DNA is involved AS-STR data may be inconclusive [9]. Likewise the use of multiplexed Y-STRs and specific loci sets for forensic purposes has been supported and recommended by the DNA Commission of The International Society of Forensic Genetics (ISFG) [10]. However in laboratories with limited support, e.g. in the developing world and emergent economies, this issue might deserve a longer time to be done. Moreover the conclusive demonstration of semen materials in rape samples by biochemical tests preceding a reliable male-specific DNA profiling still requires evaluations under real live sampling conditions.

The most recommended biochemical techniques for the routine forensic analysis of rape include sperm cytology (SC), AP activity (APA) and recently PSA detection [11]. SC is a 'gold standard' or confirmatory test; in cases of oligospermic, azoospermic or vasectomized individuals APA is a screening (presumptive) test because it can be found at lower concentrations in normal vaginal secretions [12] while PSA provides a more specific marker that has been detected with immunological techniques of increasing sensitivity [13]. Noteworthy, these markers have shown distinct stability when tested in vaginal fluid after intercourse: spermatozoa may be found up to four days [14] while PSA (as detected by ELISA) show a mean decay time of 27 h and for quantitative APA it is 14 h-post coitus [15]. As expected, a delay in sampling vaginal swabs (e.g. $\geq$16 h) adversely affect detecting these markers albeit their relative stability is kept the same [4,11].

In this context, a vast majority of reports on the comparison of SC, APA and PSA have relied on analysis of semen samples or donor swabs taken after known times after intercourse. However it was clear from comparative studies reported by Poyntz and Martin [16] that the efficacy of any given marker, e.g. PSA (as detected by crossed-over immunoelectrophoresis) is significantly higher in donor swabs (45 out of 52 cases) as compared to casework swabs (11 out of 59 cases). Several factors such as possible sampling errors, variable delays before sampling, environmental factors, artifact-derived sperm destruction and different handlings of casework samples from a laboratory to another account for and influence these results. Indeed the individual and collective contribution of these systematic factors to biased conclusions is largely unknown due to a lack of information on data obtained under real live sampling conditions. To date, only two detailed studies carried out at Toronto, Canada and Hong Kong, China compared the detection rates of these three markers in forensic casework samples and data were categorized on the basis of a positive result in one, two or the three tests performed in 54 and 144 vaginal swabs respectively [4,17].

The aim of this work was to carry out SC, APA and PSA tests in vaginal swabs collected from casework rapes submitted to two different Forensic Laboratories in Mexico. Data were grouped into eight categories and compared with those obtained and grouped similarly in the two previous studies aforementioned. Furthermore Y-STR profiling was carried out in representative samples from most of these categories. The present work gives an overview on the variability in efficacy of each test performed at different laboratories and provides a general notion about the individual and combined efficacy of these tests and its possible contribution for suitable DNA profiling in forensic analysis of rape.

2. Materials and methods

2.1. Specimens, fabric swabs and validation tests for PSA and APA

The semen samples of reference were obtained from the following donors: one normospermic (sperm counts: 65.5×10^6 cells/mL), one oligospermic (23.5×10^6 cells/mL), one azoospermic and one vasectomized donor. To determine the detection limit for PSA assay it was used one semen sample from a normospermic individual with known PSA concentration as determined with the commercial kit VITEK ImmunoDiagnostic Assay System for total PSA [VIDAS-TPSA™] following manufacturer's instructions. Samples of other bodily fluids and materials for validation of biochemical tests included: human urine (from males aged 5, 9, 10, 13, 14, 15, 31 and 80 years old and two additional samples from adults), sweat (from seven males), breast milk (from four women), blood (from two adults), fecal materials (from two men and one woman), vaginal secretions (from one woman that was sampled three times within a 2-day period) and saliva (from two men and two women).

Fabric swabs were prepared to validate sensitivity, specificity and interference by bodily fluids/materials in PSA and APA assays. In these, 200 μL of either diluted semen (sensitivity assays) or other bodily fluid/material (specificity assays), or a mixture of 200 μL of concentrate fluid/material plus 200 μL of a 1:10 dilution of semen (interference assays) were directly applied to intact swabs. These latter were processed in the same manner as described below for casework swabs.

2.2. Vaginal swabs

Two groups of samples were initially analyzed: the first composed by one-hundred and the second by forty-eight vaginal swabs collected by conventional technique [3] from the same number of alleged rape cases submitted to Laboratories of Forensic Chemistry of the Instituto de Servicios Periciales with venue at Toluca or Texcoco, in the State of Mexico, Mexico during February–June 2006 (Texcoco) and April 2006–March 2007 (Toluca). For DNA typing studies, 27 additional casework swabs were collected during a further 3-months period. Most swabs were analyzed on the same day of collection or after 48 h of storage. In few cases ($n = 8$) two swabs were collected simultaneously from the same case and tested separately, i.e. on the same day or after 5-day storage; this delaying in analysis did not affect results as assessed by the similar results (positive or negative) obtained in SC, APA and PSA assays carried out in the two swabs of all these cases. The same swab from each case was the initial material for detection of sperm, APA and PSA after a sequential processing. Briefly swabs were humidified with 750 μL SS (0.85% NaCl) within 15-mL centrifuge tubes and centrifuged at $650 \times g$ for 8 min. For PSA detection, 200 μL of supernatant were added to immunochromatographic membranes; for spermatoscopy the pellet was resuspended in a minimal volume of spent SS (approximately 30 μL) and 10 μL were deposited and dried on glass slides; in some cases the remaining volume of pellet suspension was used for Y-STR typing as described afterwards. For AP determinations the remaining pellet suspension was applied to the whole swab then this was used as described below.

2.3. Sperm cytology

This was carried out using the 'Christmas Tree' stain technique, reported as the most useful test when compared to hematoxylin–eosin and alkaline fuchsin [18]. Smears from each pellet suspension were prepared in glass slides, heat-dried, fixed in alcohol and ether, and stained with the nuclear fast red solution for 15 min in a humidified chamber. Slides were washed with deionized water, stained with picroindigocarmine solution for 30 s then bleached with ethanol and air dried. Preparations were microscopically screened for spermatozoa with an ×100 magnification and the total cell number was scored. The sample was considered as positive when at least 1 sperm (usually head) was unequivocally observed.

2.4. PSA (p30) detection

For this technique, a commercially available immunochromatographic membrane test assay was used. It was the One Step ABA card PSA™ (Abacus Diagnostics, West Hills, CA) with a reported sensitivity of detection for PSA as low as 4 ng/mL. The test consists of an all-inclusive single test device. Two hundred microliters of supernatant were placed in the sample window of the device and reaction was allowed to proceed for 10 min. The presence of a line formed by an antigen–antibody–dye sandwich in the reaction zone indicated a positive reaction. The device displays also an internal control line of reaction for monitoring device quality. This test has proven to be useful in previous studies [12,19].

2.5. Acid phosphatase activity

In this case the widely used qualitative technique based on the α-naphthyl phosphate (α-NP) substrate [20] was employed at Texcoco and Toluca laboratories with some differences. In brief, one whole swab was placed between two small pieces of filter paper then over a glass slide. Afterwards 150 μL of substrate solution (prepared in concentrations of 0.8% and 0.5%, w/v, at Texcoco and Toluca laboratories respectively) were added to the swab and it was left at room