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Preliminary investigations on the standardization and quality control for the determination of acid phosphatase activity in seminal plasma

Jin-Chun Lu [a,b,*], Fang Chen [a,c], Hui-Ru Xu [a,d], Yu-Feng Huang [a,*], Nian-Qing Lu [e]

[a] Department of Reproduction and Genetics, Jinling Hospital, School of Medicine, Nanjing University, Nanjing, Jiangsu 210002, China
[b] Department of Laboratory Science, Nanjing Hospital, Jiangsu Corps, the Armed Police Force, PLA, Nanjing, Jiangsu 210028, China
[c] Department of Microbiology and Immunology, Basic Medical College of Nanjing Medical University, Nanjing, Jiangsu 210029, China
[d] Life and Science College, Nanjing Normal University, Nanjing, Jiangsu 210097, China
[e] Jiangsu Family Planning Research Institute, Nanjing, Jiangsu 210036, China

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Abstract

Background: The determination of ACP in seminal plasma was considered as an appropriate biochemical marker to evaluate prostate function, as recommended by the WHO manual. However, few reports on the standardization and quality control for the determination of biochemical markers in seminal plasma have been documented.
Methods: Two frozen samples of seminal plasma with or without phenylmethylsulfonyl fluoride were determined for their acid phosphatase (ACP) levels. The ACP level and sperm concentration of each of 72 samples of seminal plasma obtained at 1000×*g* for 10 min or 3000×*g* for 15 min centrifugation were assayed. ACP activity in 10 samples of seminal plasma was measured immediately or standing for 30 min after dilution. The ACP levels in seminal plasma with or without chymotrypsin were also assayed.
Results: There was no significant difference of ACP levels ($P=0.166$) but of sperm concentrations ($P=0.000$) in seminal plasma obtained by centrifugation at different velocity. ACP activities in seminal plasma measured when standing for 30 min after dilution were significantly lower than those measured immediately after dilution ($P=0.001$). Both chymotrypsin and freezing–thawing had no apparent effect on the determination of ACP in seminal plasma.
Conclusion: The results indicated that standing time after dilution and centrifugation velocity should be standardized, and frozen seminal plasma could serve as the quality control products for the determination of ACP activity among different laboratories.

Keywords: Acid phosphatase; Centrifugation velocity; Chymotrypsin; Freezing–thawing; Quality control; Standardization

1. Introduction

In this age of Evidence-Based Medicine, there is nothing more important than the quality of laboratory test [1]. However, the reliability of semen analysis has been suspected for a lack of standardization, and a wide variation within the same laboratory and among different laboratories [2–6]. In recent years, more and more andrologists have put great emphasis on the standardization of semen analysis [7,8]. At present, quality controls for the determinations of sperm concentration, motility, morphology, and antisperm antibodies, have been emphasized widely [9,10]. However, few reports on the standardization and quality control for the determination of biochemical components in seminal plasma have been documented. Therefore, we attempted to establish the standardization and quality control for the determination of acid phosphatase (ACP) activity in seminal plasma.

The determination of biochemical markers in seminal plasma could be used to evaluate the functions of male accessory sex glands, which may contribute to investigating the cause and mechanism of male infertility [11]. ACP in semen was mainly synthesized and secreted by the prostate [12], which was

* Corresponding authors. Department of Reproduction and Genetics, Nanjing General Hospital, PLA, 305 East Zhongshan Road, Nanjing 210002, China. Tel.: +86 25 5299 9865.
E-mail address: jclu@jlonline.com (J.-C. Lu).

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regulated by androgens [13]. The determination of ACP in semen has been used for the forensic identification [14,15]. When the prostate suffered from the inflammation, the level of ACP in seminal plasma would decrease [16–18]. Furthermore, ACP level could be used as a marker for the diagnosis and therapy control of prostate cancer [13,19], and for the evaluation of the potential effects of various drugs or stimulators such as dichlorodiphenyltrichloroethane (DDT) and its metabolites, lead, male contraceptives, tobacco consumption, clomiphene citrate treatment and testosterone intake etc. on the prostate [20–26]. Therefore, the determination of ACP in seminal plasma was considered as the appropriate marker to evaluate prostatic function [27], which has also been recommended by the WHO manual [28].

Thus, the accuracy of the determination of ACP activity in seminal plasma was very important. To guarantee the accuracy of ACP determination and the consistency of the results obtained from different laboratories, the determination of ACP in seminal plasma should be standardized and quality control should be involved. Here, we reported our preliminary results for the effects of centrifugation velocity, standing time after dilution, freezing–thawing and chymotrypsin on the detection of ACP activity in seminal plasma.

2. Materials and methods

2.1. Reagents and instruments

Semen analysis was performed on a computer-assisted sperm analysis (CASA) system (WLJY-9000, Beijing, China). Spectrophotometer (Model 721, Shanghai Analysis Instrument Factory) and Low Speed Tabletop Centrifuge (KDC-1044 USTC Chuangxin Corporation Limited ZONKIA Branch) were employed. Commercial kits for the measurement of seminal ACP activity were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Chymotrypsin for injection (5 mg/4000 U), which was dissolved with 0.5 ml of normal saline to be 10 mg/ml of stock solution, was from the Department of Pharmacy, Nanjing Jinling Hospital. Phenylmethylsulfonyl fluoride (PMSF), was from the Nanjing Boquan Science and Techology Limited Corporation (Nanjing, China), was added into distilled water to be 0.1 mol/l of concentration for further use. Both low [$(18 \pm 2.5) \times 10^6$/ml] and high [$(35 \pm 5) \times 10^6$/ml] pre-calibrated standard latex bead solutions (Hamilton Thorne Biosciences, Beverly MA) were used as the quality control solution for sperm concentration.

2.2. Samples resource

Semen samples were collected by masturbation from the patients attending the Andrology clinic, Nanjing Jinling Hospital. After liquefaction, routine analysis (including semen volume, pH, sperm concentration, motility, and grade a and b motility) for each of semen samples was performed, and the rest of samples were applied to subsequent investigation.

2.3. The determination of ACP activity in seminal plasma

The ACP activity in seminal plasma was measured spectrophotometrically with commercial kits. ACP in seminal plasma could catalyze the degradation of disodium phenyl phosphate into free phenol and phosphoric acid, then the resultant phenol could react with 4-amido-pyrazoline in alkaline solution to produce red ramification of chinone under the condition of oxidization of ferricyanatum kalium. Thus, the resultant amount of red ramification of chinone could proportionally reflect the level of ACP activity in seminal plasma. In detail, seminal plasma was diluted for 10,000 times with normal saline, namely, 5 μl of seminal plasma was added into 495 μl of normal saline and vortexed vigorously, then 5 μl of resultant mixture was added into 495 μl of normal saline and vortexed again. Next, ACP activity in diluted seminal plasma was determined according to the instruction provided by the kit manufacturer. Briefly, 50 μl of diluted seminal plasma was added into the test tube containing 0.5 ml of balanced solution. Then, 0.5 ml of substrate solution was added. Diluted seminal plasma was replaced with standard phenol solution (0.1 mg/ml) to serve as standard and replaced with distilled water as blank. All tubes were incubated at 37 °C for 30 min. The reaction was terminated by adding 1.0 ml of alkaline solution, and subsequently 1.5 ml of developer solution was added. All tubes were kept at room temperature (20–25 °C) for 10 min, then the absorbance (A) of solution was read at 520 nm against the assay blank. ACP activity in seminal plasma was expressed as U/ml: A value of test tube / A value of standard tube × 10. One U of ACP activity equals to 100 mg of phenol produced by 1 ml of seminal plasma which reacts with substrate solution at 37 °C for 30 min.

2.4. Intraassay for the determination of ACP activity in seminal plasma

One liquefied fresh semen sample was randomly chosen, and centrifuged at 3000×*g* for 15 min, then the ACP activity in seminal plasma was detected. The test was repeated 10 times, and the coefficient of variation (CV) was calculated.

2.5. The determination of ACP activity in seminal plasma after freezing–thawing

Two well-liquefied seminal plasma samples were randomly collected, 2 ml of each of them divided into 2 aliquots equally, one stored at −20 °C directly, and the other stored at −20 °C after adding 0.1 mol/l of PMSF to seminal plasma with 0.1 mmol/l of final concentration. Two aliquots of seminal plasma were determined for ACP levels after thawing every day, and were refrozen for subsequent tests. The test was repeated 10 times during 10 days.

2.6. The determination of ACP activity and sperm concentration in seminal plasma obtained at different velocity centrifugation

Each of 72 samples of liquefied fresh semen was divided into 2 aliquots, one centrifuged at 1000×*g* for 10 min, the other at