Folia Medica Indonesiana Vol. 45 No. 1 January - March 2009 : 6-9

THE EFFECTIVENESS OF ACID PHOSPHATASE AND ZINC TESTS ON THE SEMINAL FLUID SPOT EXAMINATION AS THE PRIMARY IDENTIFICATION. A LABORATORY OBSERVATIONAL STUDY

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ABSTRACT

Spermatozoa in the vaginal tract are a definite sign of intercourse. However, there are times during microscopic examinations that none of the spermatozoa are detected or it turns out that the tests are false negative. One of the many factors that can affect this circumstance is no ejaculation in the vaginal tract. In addition, there are also other factors, such as oligo/azoospermia, vasectomy, time-degeneration of sperm, wrong sample taking, and improper storage. Therefore, it is necessary to have examinations on the other ejaculate components which are acid phosphatase, choline and spermine enzymes. The value of verification is lower when phosphatase, choline or spermine enzymes are compared to spermatozoa, because these three components are less specific. However, the concentration of phosphatase enzyme in the vaginal tract is lower compared to the seminal fluid from prostate gland. From this study, 192 samples had been taken from sperms/semen-spotted cloths, and were examined with acid phosphatase and zinc tests by using direct and indirect methods. Rinsing was performed in the first method on the first and seventh day and the third month, where each was later examined. Whereas the second method had rinsing and tests ran together on the first and seventh day and the third month. Rinsing was performed with seven different brands of detergents on four different sperm spot samples and also with water as a control. The result of the study showed that sensitivity value was lower (0.186) while the specificity was high. This showed that both tests had high specificity, where specific acid phosphatase test revealed the presence of phosphatase enzyme, while specific zinc test showed zinc contain in the semen. Phosphohydrolase-phosphate enzyme was easily disintegrated due to external factors such as temperature, humidity and also chemical substances, such as Sodium Douducyl Sulphate (SDS) that has the ability to catalyze the enzymes.The weakness of the acid phosphatase test is the result of the characteristics of an enzyme, which is easily disintegrated, partially or totally, due to the external factors such as temperature, humidity, heat and chemicals.

Keywords: *effectiveness, acid phosphatase and zinc tests, identification, seminal spot*

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INTRODUCTION

The identification of spermatozoa is critical in the evaluation of alleged sexual assault victims. One of the evaluation methods that have the most positive diagnostic value is a microscopy examination that can detect the apparent spermatozoa clearly from seminal fluid. Spermatozoa in a vaginal tract are a confirmed sign of sexual contact. However, there are times during microscopy examinations that spermatozoa are undetected or turned out to be false negative. This is due to many factors that could affect the outcome such as no ejaculation in vaginal tract. In addition, there are also other factors such as oligo/azoospermia, vasectomy, time-degeneration of sperm, improper sample taking and storage. Thus, the ejaculate components consisting of acid phosphatase, cholin and spermine require some examinations. The value of verification is lower because these three components are less specific compared to spermatozoa. However, the concentration of phosphatase enzyme in the vagina is lower than seminal fluid of prostate gland.

Currently, an evaluation method has been developed to identify the problems. The first examination is to identify the seminal fluid using an old physical method with naked eyes or with the help of ultra-violet. This method seems to be less accurate because it produces ultra-violet fluorescence, such as the synthetic fibers, paint, detergents and others. Chemical examination is an alternative option in the screening evaluation to confirm the appearance of the sperms. This chemical test is based on the concentration differences of the particles or enzymes in the seminal fluid, which is higher compared

to other parts of the body. Therefore, this chemical test does not depend on the spermatozoa found.

However, high enzyme concentration in seminal fluid is not easy to evaluate because it requires several methods of examination. There are several evaluating techniques that have been developed until recently, such as the Florence and Barberio tests that are used to investigate cholin or spermine concentration. Other tests, such as Acid Phosphatase test, is a spot test that is very sensitive (up to 64 x dilution) and simple based on the high concentration of acid phosphohydrolase-phosphatase, non specific enzymes that originating from the prostate. This acid phosphatase test has a weakness, which is less specific. Spots from plants such as cauliflower and vaginal discharge, cosmetic and spermicidal products can cause false positive reaction and decreasing activity due to duration or humidity of its surroundings.

In 1983, Suzuki et al. introduced a new method to identify seminal spot that is also known as zinc test. This test is based on the zinc concentration contained in the seminal fluid, which is higher than the body fluid or tissues from 5 to 23 mg/ 100 ml. Acid phosphatase and zinc tests have quite high sensitivity value as a screening in seminal spot examination. However, more than 40% of the sperm evidence is sometimes found deteriorating due to deliberate action, such as washing the evidence off or accidentally throwing it away or soaking in water. Until then, the effectiveness of the acid phosphatase and zinc tests examinations in identifying sperm spots in washing with a several brands of detergents are yet to be found.

MATERIALS AND METHODS

This was a laboratory observational study. The site of study was at Department of Medicolegal and Forensic Medicine Airlangga University, School of Medicine, Dr. Soetomo Teaching Hospital, Surabaya. It was conducted in October 2006 to March 2007. The size of samples for this study was using 192 sperm spots. The materials used in this study comprised of reagent for zinc test and reagent for acid phosphatase test. The reagent for zinc test consisted of 10 mg 1-(2-pyridylazol)-2-naphtol that was diluted in 2 ml Triton X-100 and mixed with 98 ml 0,5 M Tris solution (6 gram (hydroxyl-methyl) aminomethane in 100 ml aqua). Reagent was kept in a dark bottle in a refrigerator under the temperature of 4^0C. The reagent for acid phosphatase test was using as follows, 10 gram of NaCl, 0,5 ml Glacial acetic acid, 1.5 gram sodium acetate unhydrous, and 0.5 ml Teepol that were mixed into 90 ml aqua. 50 mg Sodium Naphthyl-ortho-phosphate was diluted in 25 ml buffer. 50 mg Diazo Blue B (O-Dianisidine) was diluted in 25 ml buffer. The sodium and Diazo Blue were mixed and added with the remaining buffer. This solution was kept after centrifugation in a dark bottle under the temperature of 4^0C. The brands of the rinsing materials (detergents) used in this study were Ultra-soklin, Surf, Rinso, Attack, Daia, Lux and Wings. The fresh seminal spots were applied on 192 pieces of 5 x 5 cm cotton wools. Each cloth was marked. Samples were dried in room temperature.

Samples management was performed into two methods. In method 1, on the first day, 32 pieces of cloths that had been applied with the seminal spot were rinsed with seven different brands of detergents where each was used to rinse four pieces of cloths and the rest were to be washed with plain water as a control subject. The rinsing method was by soaking the cloths for five minutes, scrubbed it for five times and rinsed it once with water. Then, the acid phosphatase and zinc tests were performed on the cloths by spraying it directly and indirectly through Whartman filter paper that had been attached on the rinsed cloths. The rinsing, direct and indirect examinations were performed with the same methods and total samples on the seventh day and third month later.

In method 2, on the first day, 96 samples of cloths were rinsed together with seven different brands of detergents where each detergent was used on 12 samples and the rest were rinsed with plain water. The rinsing method was to soak the cloths in five minutes, scrubbed it five times and rinsed it once with plain water. Then, 32 samples had been rinsed with seven brands of detergent and plain water. Each four samples were performed with the acid phosphatase and zinc tests by spraying it on the cloths directly and indirectly through Whartman filter papers that had been attached on the samples. The same methods and total of samples were performed on day seven and third month. The results of the test were recorded. In less than 30 seconds acid phosphatase test was positive when it turned purple seconds whereas zinc test was positive when it turned pink. In each test, a positive control was performed on a cloth containing sperm spots without rinsing and cloth without the sperm spots served as a negative control. Table 1 shows that zinc test was more sensitive than the acid phosphatase test on the sperm spots rinsed with several brands of detergents. Table 2 shows that the zinc test was more durable against the rinsing effect from the different brands of detergents in certain duration compared to acid phosphatase test. The data of sensitivity and specificity calculation results from both tests showed that the tests had low sensitivity values whereas the specificity value was high or definite. This was probably due to the differences in the screening

detection of the acid phosphate and zinc concentrations in the seminal fluid.

RESULTS

Table 1. The results of the acid phosphatase (TAF) and zinc (TZ) tests on sperm spots after drying under temperature room in different duration, and being rinsed with several brands of detergents (Method I)

Brands of Detergent	Rinsing and Evaluation Days											
	Day 1				Day 7				Third Month			
	TAF		TZ		TAF		TZ		TAF		TZ	
	D (pc)	I (pc)	D (pc)	I (pc)	D (pc)	I (pc)	D (pc)	I (pc)	D (pc)	I (pc)	D (pc)	I (pc)
Ultra-soklin	- (2)	- (2)	+ (2)	+ (2)	- (2)	- (2)	+ (2)	+ (2)	- (2)	- (2)	+ (2)	+ (2)
Surf	- (2)	-(2)	+(2)	+(2)	- (2)	- (2)	+(2)	+ (2)	- (2)	- (2)	+ (2)	+ (2)
Rinso	- (2)	-(2)	+(2)	+(2)	- (2)	- (2)	+(2)	+ (2)	- (2)	- (2)	+ (2)	+ (2)
Attack	- (2)	-(2)	+(2)	+(2)	- (2)	- (2)	+(2)	+ (2)	- (2)	- (2)	+ (2)	+ (2)
Daia	- (2)	-(2)	+(2)	+(2)	- (2)	- (2)	+(2)	+ (2)	- (2)	- (2)	- (2)	- (2)
Wing	- (2)	-(2)	+(2)	+(2)	- (2)	- (2)	+(2)	+ (2)	- (2)	- (2)	- (2)	- (2)
Lux	+ (2)	+ (2)	+ (2)	+ (2)	- (2)	- (2)	+ (2)	+(2)	- (2)	- (2)	- (2)	- (2)
Plain Water (control)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)
Total samples (pieces)	16	16	16	16	16	16	16	16	16	16	16	16

Notes: D : Direct Examination, I : Indirect Examination using Whartman filter paper
+ **:** < 30 seconds, - : > 30 seconds

Table 2. The results of the acid phosphatase (TAF) and zinc (TZ) tests on sperm spots after drying under temperature room in different duration, and being rinsed with several brands of detergents (Method II)

Brands of Detergent	Evaluation Day											
	Day I				Day 7				Third Month			
	TAF		TZ		TAF		TZ		TAF		TZ	
	D (pc)	I (pc)	D (pc)	I (pc)	D (pc)	I (pc)	D (pc)	TL (pc)	D (pc)	TL (pc)	D (pc)	TL (pc)
Ultra-soklin	- (2)	- (2)	+ (2)	+ (2)	- (2)	- (2)	+ (2)	+ (2)	- (2)	- (2)	+ (2)	+ (2)
Surf	- (2)	-(2)	+(2)	+(2)	- (2)	- (2)	+(2)	+ (2)	- (2)	- (2)	+ (2)	+ (2)
Rinso	- (2)	-(2)	+(2)	+(2)	- (2)	- (2)	+(2)	+ (2)	- (2)	- (2)	+ (2)	+ (2)
Attack	- (2)	-(2)	+(2)	+(2)	- (2)	- (2)	+(2)	+ (2)	- (2)	- (2)	+ (2)	+ (2)
Daia	- (2)	-(2)	+(2)	+(2)	- (2)	- (2)	+(2)	+ (2)	- (2)	- (2)	+ (2)	+ (2)
Wing	- (2)	-(2)	+(2)	+(2)	- (2)	- (2)	+(2)	+ (2)	- (2)	- (2)	+ (2)	+ (2)
Lux	- (2)	- (2)	+ (2)	+ (2)	- (2)	- (2)	+ (2)	+(2)	- (2)	- (2)	+ (2)	+ (2)
Plain Water (control)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)
Total Samples (Pieces)	16	16	16	16	16	16	16	16	16	16	16	16

Notes : D : Direct Examination, TL : Indirect Examination using Whartman filter paper
+ **:** < 30 seconds, - : > 30 seconds

DISCUSSION

A study on the effectiveness of acid phosphatase and zinc tests in screening evaluation of sperm/seminal spots until now was rarely performed. Thus, not much has been disclosed especially as identification materials in forensic cases. For the first time, Hoof P (1960) reported his study on comparison of acid phosphatase and zinc tests in primary identification of seminal spot as evidence. Suzuki et al (1983) also reported that the alternative examination on seminal spot by using a zinc test based on the zinc concentration in the sperm. In Indonesia, Atmaja DS et al (1990) also reported on the zinc (PAN) and acid phosphatase tests in identifying the seminal spot.

In this research, zinc test was more durable and sensitive against rinsing factor with several brands of detergent compared to the acid phosphatase test. The first method of the examination was to rinse after being dried in room temperature for one day, seven days or three months. The rinsing was using seven different brands of detergents in the market. The examination was performed directly on the rinsed cloth by spraying onto it. The indirect examination was applying a wet Whartman filter paper by attaching it onto the rinsed sample cloth that had not being used for direct examination. Whartman filter paper was then sprayed with acid phosphatase and zinc test. Whereas, the second method was a sperm-spotted cloth after being

dried in room temperature. Subsequently, the cloth was rinsed with several brands of detergents. The result of the rinsing was checked directly and indirectly on the first day, seventh day and third month.

The validity evaluation result in screening test was performed by comparing the acid phosphatase and zinc tests. The evaluation was performed with the concept of sensitivity and specificity. Sensitivity is in regard with the test accuracy to classify through positive results in the subjects. The higher the sensitivity value, the less the results to be false negative. In this research, the sensitivity result was very low (0.186). This was due to the differences in value or point of detection from both tests. Acid phosphatase test was used on sperm spots for its acid phosphatase concentration, whereas zinc test examined the zinc concentration in sperm. Therefore, the seminal fluid of azoospermia males will result in negative zinc test but the result of acid phosphatase test will be because the fluid does not contain any sperms.

Phosphohydrolase-phosphatase enzyme is a product of prostate gland. Therefore, any difference or disorder in the prostate gland will affect the quality and quantity of phosphatase acid in the seminal fluid. Besides that, phosphatase acid was also derived from the productions of the glands around the vagina, usually mixed with vaginal fluid. The activity of the seminal phosphatase acid within the vagina decreases quite rapidly because it is diluted with vaginal discharge, pH changes in the post-coital vagina and disintegration. Thus, the qualitative resolution of phosphatase acid is not sufficient to determine the existence of sperms in the vagina. Therefore, a quantitative resolution is required.

Phosphohydrolase-phosphate enzyme is easily degraded due to external factors such as temperature, humidity and chemical substance. Chemical effects consisting of Sodium Douducyl Sulphate (SDS) particles have the ability to catalyze the enzyme and also manipulate the particles of linear alkyl benzene suphonate (LAS) that contained two groups, alkyl (CH3) and sulphonate (SO_3H), each is hydrophilic and hydrophobic that could bind materials such as fat or oil because it emulsifies both substances. Whereas, zinc test is based on the zinc concentration or inorganic material containing in high concentration in the semen, especially in human sperms (140mg/ml) that seems more stable. Since inorganic material does not degrade over temperature, humidity or chemical effects, it binds firmly to the object. This zinc test does not produce false positive result that usually occurs in the acid phosphatase test but it can produce positive result from plants such as cauliflower and vaginal fluid. The reaction between 1-(2-pyridazol)-2-naphtol (PAN) with zinc will form pink chelation. Whereas, when the test was using reaction cylinder or performed in porcelain, the reaction will turn red. Practically, the acid phosphatase test is more superior because when the reagent in this test is sprayed onto the cloth or Whartman filter paper, it will develop a contrast. Whereas, spraying method in zinc test does not develop any contrast.

The specificity values in the zinc and acid phosphatase tests were both valid (100%). From the result obtained, increasing specificity will reduce the sensitivity of the screening test. This, in general, shows that the screening test in the subjects is either in normal or abnormal categories. However, there are subjects in the grey zone. Therefore, in dichotomous classification, borderline subjects can be categorized into normal or abnormal, depending on the cut-off point chosen. Concretely, when we loosen the positive criteria, the borderline subjects tend to be classified as true positive. In contrast, when strict rules applied, subjects in the grey zone tend to be classified as true negative.

CONCLUSION

In this study, the sensitivity of zinc and acid phosphatase tests have low values (0.186), whereas the specificity value is 100%. The weakness of acid phosphatase test is that the nature of an enzyme is easily degraded partially or totally due to external factors such as temperature, humidity, heat and chemical substance. Detergents can also cause enzyme degradation as a result of SDS and LAS concentration within.

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