

Comparison of Two Seminal Detection Methods, The Acid Phosphatase Test and The Zinc Test for Sensitivities in Sexually Assaulted Females Positive of Sperm

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ABSTRACT

Objective: To determine (1) the duration for which sperm, acid phosphatase, and zinc could be detected; (2) the sensitivities of the acid phosphatase and zinc tests; and (3) the preferred screening tests to provide evidence of sexual assault in females.

Methods: The results of acid phosphatase and zinc tests from cases over the last 10 years were retrieved if they met the following criteria: (1) results of acid phosphatase and zinc tests performed in females who were sexually assaulted by males; (2) sperm was detected in the genitals; and (3) the time between sexual assault and evidence collection was recorded. Data were analyzed using McNemar's test and chi-square with Bonferroni correction.

Results: The best chance of detecting sperm in females is within 24 h of assault. The sensitivities of the acid phosphatase and zinc test were greatest at 24 h, although the sensitivities were only 78.1% and 28.5%, respectively, at this time. The sensitivity of the acid phosphatase test was much greater than that of the zinc test.

Conclusion: This study revealed that the zinc test has a low sensitivity and is unsuitable as a screening test of sexual assault. The acid phosphatase test has a moderate sensitivity and is more suitable as a screening test of sexual assault. Other screening tests with greater sensitivities are needed to provide evidence of sexual assault. These results also suggest that the evidence collection should be performed within 24 h of sexual assault.

Keywords: Sexual assault, acid phosphatase test, zinc test, sensitivity

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INTRODUCTION

Sexual assault is a criminal act in Thailand and the detection of seminal components and sperm is important evidence in court.¹ Following sexual assault by males, the presence of sperm is considered pivotal evidence¹⁻³ and

relies on the detection of semen. Because acid phosphatase and zinc are the components of semen,⁴⁻⁸ tests have been introduced to detect acid phosphatase and zinc to provide evidence of semen and sexual intercourse.⁴⁻⁸

Unlike consensual sex, rape causes significant stress and changes in the female's body that affect the stability of semen. Most studies were performed following consensual sex for which the time and duration were known.⁹⁻¹² Fewer studies have investigated the stability of seminal components following sexual assault.¹³ In Thailand,

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the result of study by A. Vitoon et al,⁹ revealed that acid phosphatase from post coital vagina was reliable for 4 days. And P. Vichan et al,¹⁴ had discovered that sensitivity of acid phosphatase in raped case was 65.5%.

The study by Allery et al,¹² revealed that the acid phosphatase test had better performance than the zinc test based on the sensitivity, specificity, positive predictive value, and negative predictive value of each test. By contrast, Hooft et al,^{3,8} reported that the sensitivity, positive predictive value, and negative predictive value were greater for the zinc test than the acid phosphatase test. Another study by Hooft et al,¹⁵ suggested that the reliabilities of the acid phosphatase test and the zinc test were questionable. Accordingly, larger-scale analyses of approximately 1,000 cases might provide greater reliability and accuracy of the results, which was one of the purposes of the present study. In fact, the discovery and development of tests for prostate specific antigen and semenogelin in semen has devalued the acid phosphatase and zinc tests.¹⁶

In developing countries, acid phosphatase and zinc tests might be economical, especially compared with newer tests. The costs of the acid phosphatase and zinc tests at Siriraj Hospital is only about 30 Baht (US\$1) for each test. By contrast, the costs of the prostate specific antigen test and semenogelin test exceed 320 Baht (US\$10.6) and 460 Baht (US\$15.3), respectively. The objectives of this study were to determine (1) the duration for which sperm, acid phosphatase, and zinc could be detected; (2) the sensitivities of the acid phosphatase and zinc tests; and (3) the preferred screening tests to provide evidence of sexual assault in females in Thailand considering the expected costs.

MATERIALS AND METHODS

This study used the results of acid phosphatase and zinc tests for females who were sexually assaulted by males over a period of 10 years (B.E. 2546-2555). Once specimens were collected from the female's genitalia with a swab and dried for 12 h, they were transferred to the evidence laboratory in accordance with the chain of custody.

The specimens were then tested using the acid phosphatase test, the zinc test, and a sperm.^{17,18}

The acid phosphatase test used at Siriraj Hospital is performed in a similar manner to the method used by the Virginia Department of Forensic Science¹⁷ and is based on a direct method.^{19,20} As a quality control, the solution is added to a test solution with a seminal filter and a negative control filter before the test.

The zinc test is performed using Suzuki's modified method.¹⁸ As a quality control, the test solution is applied to a seminal filter and a negative control filter before the test.

To detect sperm, the entire specimen is prepared to obtain a supernatant and sediment. The sediment is observed by light microscopy to detect the presence of sperm. If sperm is detected, the sediment and all slides are transferred to the serology laboratory. For quality control the technician double-checks all slides.

The results were only used if sperm was detected in the female's genitalia. The results of the acid phosphatase and zinc tests were recorded as positive or negative. The time from sexual assault to evidence collection was retrieved from the case notes.

The results of the acid phosphatase and zinc tests were assessed using three methods: (1) the chance of detecting sperm within specific times after sexual assault; (2) the sensitivities of the acid phosphatase and zinc tests at specific times after sexual assault; and (3) the sensitivities of the acid phosphatase and zinc tests were directly compared. Data were analyzed using McNemar's test and Chi-square with Bonferroni correction, as appropriate.

RESULTS

Between B.E. 2546 (2002) and B.E. 2555 (2012), 4,320 cases of sexual assault were recorded at the Department of Forensic Medicine Siriraj Hospital. Sperm was detected in the genitalia of 943 females (21.82%). The tests for sperm, acid phosphatase, and zinc were mostly performed on specimens which were collected within 24 h of sexual assault. The sensitivities of the acid phosphatase and zinc tests for specific time-points

were compared using McNemar's test. The comparisons of the sensitivities of the two were statistically significant for all time-intervals, except at 120.1-168 h, as shown in Table 1.

The acid phosphatase test was positive in 627 cases (66.50%) while the zinc test was positive in 198 cases (21%) (Table 2). Overall, 20.1% of cases were positive for both acid phosphatase and zinc, 46.30% were positive for only acid phosphatase, 0.8% were positive for only zinc. Overall, 32.70% of cases were negative for both acid phosphatase and zinc.

The sensitivities of the acid phosphatase test and zinc tests according to the time interval between sexual assault and the test have been shown in Tables 3 and 4, respectively. Using chi-square with two-sided Bonferroni correction at a significance level of 0.05, the sensitivity of the acid phosphatase test decreased significantly from within 24 h. to increasing time interval. Similarly, the sensitivity of the zinc test decreased significantly from within 24 h. to increasing time interval, except at 120.1-168 h and >168 h.

The sensitivities of acid phosphatase and/or zinc tests according to the time since sexual assault have been shown in Table 5. Using Chi-square with two-sided Bonferroni correction at a significance level of 0.05, the sensitivities of acid phosphatase and/or zinc tests decreased significantly from within 24 h. to increasing time interval.

This maximum sensitivity of the acid phosphatase test was 78.10% within the first 24 h, and declined significantly from 24 h onwards. The overall sensitivity of the acid phosphatase test was 66.50%. The zinc test had a maximum sensitivity of 28.50% at 24 h, and declined significantly from 24 h to 120 h. The overall sensitivity of the zinc

test was 21%. The sensitivity for a positive result of either test was 79% at 24 h, with an overall sensitivity of 67.30%.

DISCUSSION

The analyses reported here revealed several interesting findings. First, sperm was most often detected within 24 h of sexual assault and its detection rate decreased with increasing time since assault. After a duration of 5 days since sexual assault, sperm was detected in 93.60% of cases.

The results of the acid phosphatase and zinc tests revealed that performing the zinc test did not increase the sensitivity of the acid phosphatase test compared with the acid phosphatase test alone. Moreover, the zinc test had very low sensitivity when it was used alone. These results imply that the zinc test is of limited use for the detection of semen after sexual assault. Although the acid phosphatase test had moderate sensitivity, it is not optimal for the detection of semen. Therefore, other tests, including the prostate specific antigen and semenogelin tests, were developed to overcome the limitations of existing tests. Nevertheless, because some male offenders have a zoospermia, it is still necessary to perform tests of seminal components. The present results indicate that the acid phosphatase is better test for this purpose and may be especially useful for economic reasons.

The sensitivities of acid phosphatase test in this study were nearly equal to those reported by P. Vichan et al.¹⁴ Also, the results of this study were similar to those reported by Allery et al.¹¹ However, the study of Allery et al,¹¹ had studied the cervicovaginal samples from consenting women who knew the actual time of intercourse

TABLE 1. Proportions of cases in which laboratory tests were performed at specific times after sexual assault.

Test	Time interval from sexual assault to sample collection (h)						Total
	<24	24.1-48	48.1-72	72.1-120	120.1-168	>168	
Sperm	590 (62.6)	161 (17.1)	65 (6.9)	66 (7)	20 (2.1)	41 (4.3)	943 (100)
AP	461 (78.1)	88 (54.7)	30 (46.2)	24 (36.4)	8 (40)	16 (39)	627 (66.5)
Zinc	168 (28.5)	13 (8.1)	6 (9.2)	5 (7.6)	2 (10)	4 (9.8)	198 (21)
p-value	<0.001	<0.001	<0.001	<0.001	0.031	<0.001	<0.001

Values are presented as the n (%), AP: acid phosphatase

so that was different from this study. The study of Allery et al,¹¹ mentioned positive predictive value and negative predictive value. This study was studied in sexual assaulted women, so it was impossible to find the negativity of intercourse. Moreover, that is why this study could not mention specificity and predictive values.

This study used the data recorded in case notes, as reported by the victims, which may result in unreliable data. Moreover, in some cases, consensual sexual intercourse may be falsely reported as sexual assault.

This study was performed in a single center, which might introduce some bias in terms of comparing the tests, the population distribution, and the behavior of the population, which might not reflect the national or international situation. Due to the retrospective design of this study, it was not possible to perform other newer tests.

In conclusion, the acid phosphatase test only had moderate sensitivity for detecting seminal components, which suggests that other tests should also be considered. However, for economic

TABLE 2. Results of the acid phosphatase and zinc tests.

Test result	Zinc test		Total
	Negative	Positive	
Negative	308 (32.7)	8 (0.8)	316 (33.5)
Positive	437 (46.3)	190 (20.1)	627 (66.5%)
Total	745 (79.0)	198 (21.0)	943 (100.0)

Values are presented as the n (%), AP: acid phosphatase

TABLE 3. Results of the acid phosphatase test according to the time from sexual assault to sample collection.

Test result	Time interval from sexual assault to sample collection (h)						Total
	<24	24.1-48	48.1-72	72.1-120	120.1-168	>168	
Negative	129 (21.9)	73 (45.3)	35 (53.8)	42 (63.6)	12 (60.0)	25 (61.0)	316 (33.5)
Positive	461 (78.1)	88 (54.7)	30 (46.2)	24 (36.4)	8 (40.0)	16 (39.0)	627 (66.5)
Total	590 (100)	161 (100)	65 (100)	66 (100)	20 (100)	41 (100)	943 (100)
p-value	<0.001*						

Values are presented as the n (%), *=Chi-square test and multiple comparison by Bonferroni method ≤ 24 hr. was significant difference all other period by significant level of 0.05

TABLE 4. Results of the zinc test according to the time from sexual assault to sample collection.

Test result	Time interval from sexual assault to sample collection (h)						Total
	<24	24.1-48	48.1-72	72.1-120	120.1-168	>168	
Negative	422 (71.5)	148 (91.9)	59 (90.8)	61 (92.4)	18 (90.0)	37 (90.2)	745 (79.0)
Positive	168 (28.5)	13 (8.1)	6 (9.2)	5 (7.6)	2 (10)	4 (9.8)	198 (21.0)
Total	590 (100)	161 (100)	65 (100)	66 (100)	20 (100)	41 (100)	943 (100)
p-value	<0.001**						

Values are presented as the n (%), **=Chi-square test and multiple comparison by Bonferroni method ≤ 24 hr. was significant difference all other period except at 120.1-168 and >168 by significant level of 0.05

TABLE 5. Proportions of cases who tested positive for the acid phosphatase test and/or zinc test according to the time from sexual assault to sample collection.

Test result	Time interval from sexual assault to sample collection (h)						Total
	<24	24.1-48	48.1-72	72.1-120	120.1-168	>168	
Negative	124 (21.0)	73 (45.3)	32 (49.2)	42 (63.6)	12 (60.0)	25 (61.0)	308 (32.7)
Positive	466 (79.0)	88 (54.7)	33 (50.8)	24 (36.4)	8 (40.0)	16 (39.0)	635 (67.3)
Total	590 (100)	161 (100)	65 (100)	66 (100)	20 (100)	41 (100)	943 (100)
p-value	<0.001***						

Values are presented as the n (%), ***=Chi-square test and multiple comparison by Bonferroni method ≤ 24 hr. was significant difference all other period by significant level of 0.05

reasons, the acid phosphatase test is still useful. Since the zinc test showed very low sensitivity for detecting semen, this test could be cancelled for economic reasons, and newer tests might be needed in some cases. The present results also highlight that specimens should be obtained within 24 h of sexual assault.

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REFERENCES

1. Thai Criminal Code. Available from: http://www.thailaw.com/thailaw2_4.pdf (Accessed 5 August 2014).
2. Wasserstrom A, Frimkin D, Davidson A, Shoitzen M, Herman Y, Gafny R. Demonstration of DSI-semen-A novel DNA methylation-based forensic semen identification assay. *Forensic Sci Int Genet.* 2013 Jan;7(1):136-42.
3. Hooft PJ, Van de Voorde HP. Bayesian evaluation of the modified zinc test and the acid phosphatase spot test for forensic semen investigation. *Am J Forensic Med Pathol.* 1997 Mar;18(1):45-9.
4. Davies A, Wilson E. The persistence of seminal constituents in the human vagina. *Forensic Sci.* 1974 Feb;3(1):45-55.
5. Schumann GB, Badawy S, Reglow A, Henry JB. Prostatic acid phosphatase. Current assessment in vaginal fluid of alleged rape victims. *Am J Clin Pathol.* 1976 Dec;66(6):944-52.
6. Schiff AF. Reliability of the acid phosphatase test for the identification of seminal fluid. *J Forensic Sci.* 1978 Oct;23(4):833-44.
7. Suzuki O, Asano M, Kido A, Oya M. Zinc test as a new tool for identification of human seminal stains. *Forensic Sci Int.* 1983 Aug-Sep;22(2-3):231-5.
8. Hooft PJ, Van de Voorde HP. The zinc test as an alternative for acid phosphatase spot tests in the primary identification of seminal traces. *Forensic Sci Int.* 1990 Oct;47(3):269-75.
9. Attanatho V, Fongsiripaibul V, Kiriwat O. The Acid Phosphatase Reaction on the Postcoital Vaginal Swabs. *Siriraj Hosp Gaz.* 1983;35:677-84.
10. Yu JS. Study on time limit of ACP and sperm positive-detected in mixed spot. *Fa Yi Xue Za Zhi.* 2001 Aug;17(3):157-9.
11. Astrup BS, Thomsen JL, Lauritsen J, Ravn P. Detection of spermatozoa following consensual sexual intercourse. *Forensic Sci Int.* 2012 Sep 10;221(1-3):137-41.
12. Allery JP, Telmon N, Blanc A, Mieusset R, Rougé D. Rapid detection of sperm: comparison of two methods. *J Clin Forensic Med.* 2003 Mar;10(1):5-7.
13. Hooft PJ, Van de Voorde HP. Evaluation of the modified zinc test and the acid phosphatase test as preliminary screening methods in sexual assault case material. *Forensic Sci Int.* 1992 Mar;53(2):135-41.
14. Peonim V, Worasuwannarak W, Sujirachato K, Teerakamchai S, Srisont S, Udnoon J, et al. Comparison between prostate specific antigen and acid phosphatase for detection of semen in vaginal swabs from raped women. *J Forensic Leg Med.* 2013 Aug;20(6):578-81.
15. Hooft PJ, Van de Voorde HP. Interference of body products, food and products from daily life with the modified zinc test and the acid phosphatase test. *Forensic Sci Int.* 1994 Jun 10;66(3):187-96.
16. Graves HC, Sensabaugh GF, Blake ET. Postcoital detection of a male-specific semen protein. Application to the investigation of rape. *N Engl J Med.* 1985 Feb 7;312(6):338-43.
17. Forensic biology section procedures manual II Presumptive and Confirmatory Tests for Biological Substances. Available from: <http://www.dfs.virginia.gov/wp-content/uploads/2014/07/210-D300-FB-PM-II-Presumptive-and-Confirmatory-Tests-for-Biological-Substances.pdf> (Last accessed 25 August 2014).
18. Hooft PJ, Van de Voorde HP, Van Dijk P. A more sensitive modification of the zinc test for seminal traced suitable for stable test paper strips. *Forensic Sci Int.* 1992 Mar;53(2):131-3.
19. Allard JE, Baird A, Davidson G, Jones S, Lewis J, McKenna L, et al. A comparison of methods used in the UK and Ireland for the extraction and detection of semen on swabs and cloth samples. *Sci Justice.* 2007 Dec;47(4):160-7.
20. Lewis J, Baird A, McAlister C, Siemieniuk A, Blackmore L, McCabe B, et al. Improved detection of semen by use of direct acid phosphatase testing. *Sci Justice.* 2013 Dec;53(4):385-94.