Comparable between Rapid One Step Immunochromatographic Assay and ELISA in the Detection of Prostate Specific Antigen in Vaginal Specimens of Raped Women

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Background: *Rape is a crime found in Thailand nowadays. The crime is often lacking of eyewitnesses. Therefore, examination for forensic biological evidence becomes quite important, especially investigating sperm and semen in vaginal specimens of the victim. Acid phosphatase test for semen is commonly used in Thailand but is just a presumptive test. Recently, confirmatory kit tests became available in Thailand for detecting the prostate specific antigen (PSA) from semen. This test is simpler and cheaper than ELISA.*

Objective: *To compare the rapid one-step immunochromatographic assay with ELISA for the detection of prostate specific antigen in vaginal specimens of raped women.*

Material and Method: *A diagnostic test was conducted on the vaginal specimens of raped women that were sent to the laboratory of the Pathology Department, Faculty of Medicine, Ramathibodi Hospital, Mahidol University during April-August 2006. One hundred vaginal specimens were examined for prostate specific antigen by rapid one step immunochromatographic assay and compared with ELISA.*

Results: *There were 85% and 83% of sensitivity, 85% and 85% of specificity, 85% and 85% of accuracy, 89% and 89% of positive predictive value, and 79% and 77% of negative predictive value from rapid one-step test kit and ELISA respectively.*

Conclusion: *The result showed that there was no difference on specificity, accuracy and positive predictive value between the two methods but sensitivity and negative predictive value of rapid one-step test kit was better than ELISA. The research team recommends that rapid one-step test kit for prostate specific antigen should be routine service in vaginal specimens of raped women.*

Keywords: *Rape, Prostate specific antigen, Semen detection*

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Rape is a crime found in Thai society nowadays. This type of crime is often lacking eyewitness that accidentally saw the wrongdoer. Thus, examination of forensic biological evidence becomes quite important in seeking for the rapist, especially the technique for investigating sperm and semen in vaginal specimens of the victim. Sperm investigation is done by the microscopic examination, which is defined as the gold standard of test[1-3] in examination. Semen investigation is done by detecting for biochemical, which are components of semen. Enzyme acid phosphatase is one example of investigation done in Thailand. This test, called Acid phosphatase test, is designed to find enzyme produced by the prostate gland. While this test is used often, it is just a presumptive test. Recently, a confirmatory test has become available for finding the prostate specific antigen (PSA)[4-12] from human semen.

Prostate specific antigen (PSA) was first described in 1971 by Hara, et al in terms of "Y-seminoprotien". In 1973, this term was called "E1" by Li and Beling[13]. Later, in 1978, Sensabaugh had termed it as "protein P30"[7,14] due to it was glucoprotein as its

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molecular weight was approximately 30 kg daltons. PSA is a test done mainly to screen and monitor for prostate cancer[15-18] by examining PSA level in serum. Later, the technique for finding PSA in semen had been developed as a quantitative analysis by the method of "enzyme linked immunosorbent assay (ELISA)"[7,19,20]. Then it was developed again to be a semi-quantitative analysis of rapid test[21,22]. This new developed test is fast, simple, not expensive, and widely used in USA, European countries, Japan and China, etc. Nowadays, in those countries, PSA is done mainly in semen, as it is a confirmatory test. In the past, PSA was significantly done based on quantitative analysis by ELISA[19,20]. This analysis method was costly, as it needed expensive tools and a specific technician. Later, this method was developed to be easier, cheaper and without the need for complicated tools or a specific technician. This new method is called "Rapid one-step immunochromatographic assay". It is a semi-quantitative analysis based on the principle of assay.

The rapid one-step PSA test is a colloidal gold enhanced immunoassay for the determination of prostate specific antigen in human serum, plasma, whole blood and semen. The nitrocellulose membrane was treated with mouse anti-human PSA Mc Ab in the test region. During the assay, the semen extract specimen is allowed to react with colored conjugate (antibody-colloidal gold conjugate); the mixture then migrates on the membrane chromatographically by the capillary action. If PSA is present in the specimen, the specific antibody-PSA-colored conjugate complex will form in the test region on the membrane. Absence of this colored band in the test region suggests a negative result. Assay procedures dispense 100 µL (3 drops) extracted fluid to the sample well and read the test result in 10 minutes. The negative shows only one colored band in the control region. In addition to the control band, a distinct colored band also appears in the test region, which is a positive result. This assay is designed to detect PSA at the cutoff level of 4 ng/ml. The cutoff level of ELISA is 0.5 ng/mL.

In Thailand, there is no report yet on employing the rapid test kit for use in routine service while a research team believes that the semi-quantitative test would improve the work capacity on investigating rape objects. For all the above reasons, the research is aimed to prove the quality of this test by comparing the accuracy of the rapid one-step prostate specific antigen test kit with the ELISA test. The finding would be beneficial for using the test widely in routine service.

Material and Method

The present research was done by the diagnostic test method on vaginal specimens of raped women, which were sent to be examined in the laboratory of the Pathology Department, Faculty of Medicine Ramathibodi Hospital, Mahidol University during April-August, 2006. The vaginal specimens sent to the routine laboratory would be examined for rapid one-step immunochromatographic assay method compared with ELISA. Both these methods employ the microscopic examination for sperm detecting in the gold standard. There are 100 specimens used as sample size calculated from the formula of $N = Z^2. P(1-P)/d^2$ at 96% expected sensitivity when "N" is equal to the number of vaginal specimens of raped women found with sperm. Z is set its value at alpha error two-tailed of 1.96.Probabiltity of expected sensitivity (P) is needed at 0.96 while "d" or acceptable probability of error is set at 0.05. The calculated value of "N" is 59 [(1.96^2) (0.96) (0.04)/(0.05^2)] while the previous study revealed that there was 67.5% could detect sperm from vaginal specimens Thus, there are at least 87 specimens needed to be samples. Then the research team made the decision to use 100 specimens as samples for the present study.

For vaginal specimens sent to the laboratory, if the specimens are in type of vaginal swab, the cotton part is cut off. But if the specimens are in type of filter paper, the area of paper where the vaginal secretion is found is cut to be a piece at size 0.3x0.3 cm. both cotton and paper parts would be put in a tube containing 2 mL distilled water. The tube would be centrifuged for 3 minutes and 100 microliters of centrifuged solution would be sucked from the tube by a pipette to be tested with the rapid test kit and ELISA. All specimens would be detected for sperm with microscopic examination. Each step of the examination uses a different specific technician and each of the technicians is not allowed to know the other's examination result.

PSA results from both methods are compared with the result from sperm detecting (gold standard) shown in a 2x2 Table. This step is done for comparing PSA by sensitivity, specificity, accuracy, positive predictive value and negative predictive value gained from each method.

Results

Results from sperm detected by microscopic examination in 100 vaginal specimens of raped women and semen detecting in prostate specific antigen by rapid one-step immunochromatographic assay kit and ELISA are shown in a 2 x 2 Tables 1, 2 as follows:

Table 1. Comparable between detection of PSA by rapid one step test kit and microscopic examination for sperm

		Sperm examination			
		Present	Absent		
Rapid one step test kit	Positive	51 (a)	6 (b)	57	Positive predictive value 89%, a / (a + b)
	Negative	9 (c)	34 (d)	43	Negative predictive value 79%, d / (c + d)
		60	40	100 (n)	

Sensitivity a / (a + c) 85%, Specificity d / (b + d) 85%, Accuracy (a + d) / n 85%

Table 2. Comparable between detection of PSA by ELISA and microscopic examination for sperm

		Sperm examination			
		Present	Absent		
ELISA	Positive	50	6	56	Positive predictive value 89%
	Negative	10	34	44	Negative predictive value 77%
		60	40	100	

Sensitivity 83%, Specificity 85%, Accuracy 85%

Discussion

Detection for sperm and semen from vaginal specimens quite an important step for finding evidence related to a rape case in terms of forensic science.

Sperm detection is usually done by microscopic examination[1-3] while semen detection is done by examining biochemical substance contained in semen, and produced from the prostate gland. The biochemical substance examining is provided in forms of Florence test, Barbeiro test, Choline test, Zine test[23-25] LDH isoenzyme method, Ammonium molybdate test, Creatinine phosphokinase test, and Acid phosphatase test[19,26-30]. However, the most widely used in Thailand nowadays is the Acid phosphatase test.

Recently, a rapid one-step PSA testing method for identification of the seminal fluids has been used worldwide among developed countries. Therefore, the researcher team is aimed to test for application of the rapid PSA test in the presented routine forensic works in Thailand.

In the present study, the results from PSA comparative examination between rapid one-step test kit and ELISA by microscopic examination for sperm on gold standard showed as follows:

There were 85% and 83% of sensitivity, 85% and 85% of specificity, 85% and 85% of accuracy, 89% and 89% of positive predictive value and 79% and 77% of negative predictive value from rapid one-step test kit and ELISA respectively. Comparative results from both methods showed that there were no different values of specificity, accuracy and positive predictive value but sensitivity and negative predictive value of rapid one-step test kit was a little bit better than ELISA. The result of one case is not correspondence may be caused by uncertainty in measurement of each method[31,32].

According to previous international studies, PSA rapid membrane test assays offer the same sensitivity as ELISA-based test for forensic identification of seminal fluids[7] and can be adequately replaced by the ELISA method of PSA detection in forensic laboratory work[33].

From the results of both methods, the research team believes that PSA examination of semen from vaginal specimen of raped women by rapid one-step test kit is an effective method. In addition, the team believes that the rapid one-step test kit should be the routine service since it is a simple method, does not required a dedicated technician, and its less expensive. Furthermore, this method is also a confirmatory test for semen investigation.

Conclusion

Research on PSA comparative examination in vaginal specimens of raped women by rapid one-step test kit and ELISA was provided in 100 specimens sent to the Pathology Department, Faculty of Medicine, Ramathibodi Hospital during April- August, 2006. The research result showed that there was no difference on specificity, accuracy and positive predictive value between the two methods but also sensitivity and negative predictive value of rapid one-step test kit was better than the ELISA method. Furthermore, it is not only a confirmatory test, but it is also a fast and safe method. With this finding, the research team recommends that the rapid one-step test kit be employed as routine service for PSA detection in vaginal specimens of raped women.

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การเปรียบเทียบการตรวจหา prostate specific antigen (PSA) ในวัตถุพยานกรณีข่มขืนกระทำชำเราด้วยชุดตรวจ rapid one step immunochromatographic assay กับวิธี ELISA

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ภูมิหลัง: การข่มขืนกระทำชำเราเป็นคดีที่พบได้บ่อยในประเทศไทยและการหาประจักษ์พยานจะกระทำได้ยาก หลักฐานวัตถุพยานทางการแพทย์จึงมีความสำคัญอย่างยิ่ง โดยเฉพาะการตรวจหาตัวอสุจิและการตรวจหาน้ำอสุจิจากช่องคลอดของผู้เสียหาย การตรวจหาน้ำอสุจิในประเทศไทยขณะนี้ใช้ตรวจด้วยวิธี acid phosphatase ซึ่งถือเป็นวิธีการตรวจขั้นต้นเท่านั้น แต่การตรวจด้วยวิธี prostate specific antigen เป็นวิธีการตรวจแบบยืนยัน สามารถตรวจได้ง่ายด้วยชุดตรวจสำเร็จและมีความจำเพาะสูง

วัตถุประสงค์: เพื่อเปรียบเทียบการตรวจหา prostate specific antigen ในวัตถุพยานกรณีข่มขืนกระทำชำเราด้วยชุดตรวจ rapid one step immunochromatographic assay กับวิธี ELISA

วัสดุและวิธีการ: ใช้วิธี diagnostic test จากวัตถุพยานที่ส่งตรวจ ณ ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดีในช่วงเวลา เมษายน - สิงหาคม พ.ศ. 2549 จำนวน 100 ตัวอย่าง โดยกำหนดให้การตรวจหาตัวอสุจิด้วยกล้องจุลทรรศน์เป็นวิธีมาตรฐาน นำผลมาวิเคราะห์โดยตาราง 2 x 2

ผลการศึกษา: จากผลการตรวจ prostate specific antigen ด้วยวิธี rapid one step immunochromatographic ได้ค่าความไว 85% ความจำเพาะ 85% ความแม่นยำของการทดสอบ 85% การทดสอบที่ให้ผลบวกและตรวจพบตัวอสุจิ 89% การทดสอบที่ให้ผลลบและตรวจไม่พบอสุจิ 79% ส่วนผลการตรวจหา prostate specific antigen ด้วยวิธี ELISA ได้ค่าความไว 83% ความจำเพาะ 85% ความแม่นยำของการทดสอบ 85% การทดสอบที่ให้ผลบวก และตรวจพบตัวอสุจิ 89% การทดสอบที่ให้ผลลบและตรวจไม่พบตัวอสุจิ 77%

สรุป: ผลการศึกษาพบว่าจากการตรวจหา prostate specific antigen ในทั้งสองวิธีมีค่าความจำเพาะ ความแม่นยำ และการทดสอบที่ให้ผลบวกและตรวจพบตัวอสุจิไม่แตกต่างกัน แต่ความไวและการทดสอบที่ให้ผลลบและตรวจไม่พบตัวอสุจิ จากการตรวจด้วยวิธี rapid one step test kit มีค่าดีกว่าวิธี ELISA คณะผู้นิพนธ์จึงเห็นสมควรให้ใช้วิธี rapid one step test kit ในการตรวจหา prostate specific antigen เป็นวิธีตรวจในงานบริการประจำจากตัวอย่างส่งตรวจกรณีถูกข่มขืนกระทำชำเรา เพราะเป็นวิธีตรวจได้รวดเร็ว ไม่ยุ่งยาก ค่าใช้จ่ายไม่สูง และไม่ต้องใช้บุคคลที่มีความชำนาญการเฉพาะ