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DNA QUALITY AND QUANTITY TEST OF SPERM IN FRESHWATER

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***Abstract*—Sperm is one of the body fluids that contain DNA and can be used as evidence or guidance to uncover various criminal cases. Evidence such as sperm can be found in various environments depending on the crime scene, such as being on a submerged cloth. The quality and quantity of DNA obtained from sperm is strongly influenced by the environment where the sperm are found. In this study, we tested the quality and quantity of DNA from sperm spots soaked in freshwater. The quality and quantity of DNA was measured using a spectrophotometer with a wavelength of 260nm and 280nm. The results showed an increase in the quality and quantity of DNA from sperm spots soaked on Day 1, 3 and 7. DNA ribbon also appeared on all samples when the DNA was amplified with STR primers at D21S11 and D13S317 locus.**

Keywords—Crime, Sperm, DNA, STR-CODIS

I. INTRODUCTION

Indonesia was ranked 62nd with 0.00567 per 1000 people or 5-6 per one million people in the case of rape. If the population of Indonesia is 250 million people, then there are 1,418 cases of rape that occurred. In terms of national average, Bandar Lampung Province is the province with the highest number of rape cases. In 2009, there were 106 rape cases in Bandar Lampung, followed by Jakarta with 90 cases, whereas Bali Province recorded only 19 cases of rape [6]. The many cases of rape that occur are always a difficult problem for the authorities to solve. Of course, in order to cultivate the scene there is also a time limit to assess the purity of any signs found on the scene.

In rape cases, evidence such as sperm is often found in the victim's body, especially the vagina or in media such as the condom or the clothes of the victims at the crime scenes. Sperm is the reproductive cell of the male body studied first in 1677 by Stephen Ham, one of the students of Antonie van Leeuwenhoek [1]. All the cells and the living system, including spermatozoa contained in sperm, contain (Deoxyribonucleic acid), a DNA [4]. Sperm is often used as evidence to solve cases of rape, especially in the identification of offenders.

Ejaculate fluid samples on the shirt / clothing at a crime scene or vaginal fluid can be recognized by external factors that can degrade DNA quality. Examples of factors are temperature, humidity and light, and media such as sperm on fabric, fresh water immersion, etc. Research conducted by Wahyuningsih (2008) about cotton cloth, concluded cotton fabric has properties that do not react strongly against heavy and high acid in the air, resulting in a high acid content in the fabric component.

Identifying the suspect in a rape case can be done by identifying the DNA contained in sperm spots which are found. In practice, primers such as STR-CODIS (Short tandem repeat combined DNA index) are needed to amplify very little DNA. STR is the shortest and most informative polymorphic piece of DNA based on PCR to try to individualize biological materials. STR-CODIS is often used in cases for human identification because it has been agreed by the World Forensic Institute and the FBI as an easy way to match samples at the scene with suspected victims or suspects [3].

Based on the above background, this research will discuss the effect of fresh water immersion on DNA quality of sperm spots that will be detected with STR-CODIS D13S317 and D21S11.

II. METHODS

A. Materials and Tools

The material used for this study is sperm spots from probandus. Materials used for the analysis of genetic variation include: (1) Fertmentas DNA isolation kit, namely: GeneJET Genomic DNA Purification Kit, 1.75% acrylamide gel, TBE 1X buffer, and etidium bromide (EtBr); and (2) primary : PCR Kit ®Fermentas (DreamTaqTM Green PCR Master Mix (2x)), ethanol 70%, PBS, akuabides (water nukleasefree), sterile aquades, aluminum foil, and ladder DNA (marker) 100 - 3000 bp ®Vivantis.

The tools used are glass, cloth, ultracentrifuge (Gyrozen Co., Ltd.), vortex mixer, refrigerator for storing samples, micro pipettes (eppendorf pippet) P10, P100, and P1000, Thermal cycler PCR Machine (Boeco), electrophorator Mini Run Gel Electrophoresis System GE-100), UV transilluminator (BioRad), ice box, microwave, incubator, autoclave and digital camera. The primers used in this study consisted of two of each locus studied, namely primary A (forward / upstream) and primary B (reverse / downstream) [5], in the following nitrogen base sequence:

1. D13S317 : 5’ATTACAGAAGTCTGGGATGTGGAGGA-3’

   5’-GGCAGCCCAAAAAGACAGA-3’

2. D21S11 : 5’-ATATGTGAGTCAATTCCCCAAG-3’

   5’-TGTATTAGTCAATGTTCTCCAGAGAC-3’

B. Sampling

Sperm is taken from probandus through glasses by masturbation. Then the sperm in the glass is dropped using a pipette on a 10 x 10cm square cloth. The next stage of fabric containing sperm spots is drying. Then all the samples are soaked in a container containing fresh water. Isolation of sperm DNA is started after sampling at each time period;day 1 as many as six samples, on the 3rd day of six samples and on the 7th day as many as six samples.

C. DNA Isolation

Isolation of DNA from sperm samples is by using GeneJET Genomic DNA Purification Kit ® Fermentas isolation kit with working procedure in accordance with the procedure of GeneJET Genomic DNA Purification Kit ® Fermentas.

D. Measurement of DNA Content and Purity with Spectrophotometer

The isolated DNA samples are taken as 10µl and added into the cuvette then mixed with 690µl of distilled water then vortex it. Then a blanko solution containing water is prepared and treated the same as the sample. The spectrophotometer device is activated and the wavelength regulated as (λ) 260 and 280. Each sample and blanko solution is measured at both wavelengths. After getting the result, the purity of the DNA is calculated with comparison of the result of absorbance ratio λ 260 / λ 280 [2].

E. PCR with STRs Primer

The isolated DNA samples are removed from the -200C freezer, then thawed by hand and then vortexed and spindowned. The DNA is used as a DNA template (Angel, 2005). The next step is to make the mixed mixture for PCR with the composition, as in following table below.

Table 1. Composition of PCR

The Composition of PCR Reaction	Volume
Master Mix dream Taq	12.5 µl
Primer STR (10pmol/µl)	1.5 µl
DNA *template* (0.2pg – 20ng/ µl)	1 µl
dH2O	10 µl
Total Volume	25 µl

All components are prepared in a PCR tube. After mixing it all, the PCR tube is vortexed and spindowned at 3000rpm. Then the PCR tube is inserted into the Thermal Cycler PCR Machine. The PCR step is performed according to the following table.

Table 2. Step of PCR

Stages	Temperature (^{0}C)	Time	Total Cycle
Pre-denaturation	95^{0}C	5 minutes	1
Denaturation	95^{0}C	45 seconds	1
Annealing	52-54^{0}C	1 minute	1
Elongasi	72^{0}C	2 minutes	30
Post Elongasi	72^{0}C	5 minutes	1
Endless	4^{0}C	∞	1

The result of this DNA amplification is stored at 40^{o}C until the next process. The total PCR cycle above is 35 cycles. After the PCR process is completed, the electrophoresis process is followed by using acrylamide 1.75% in 1x TBE buffer and using DNA ladder as reference of visualized DNA bands [2].

F. Buffer TBE 1x Fabrication

The equations are an exception to the prescribed specifications of this template. You will need to determine whether or not your equation should be typed using either the Times New Roman or the Symbol font (please no other font). To create multileveled equations, it may be necessary to treat the equation as a graphic and insert it into the text after your paper is styled.

G. Acrylamide Gel Fabrication

Acrylamide Bis / N, N'-Methylenebisacrylaide 4.5ml with added Temed / N, N, N ', N'-Tetramethylenedsiamine 15µl in a beaker glass, then add 100mg ammonium persulfate (APS) and pour it into a glass and shake it for a while. Pour the mixture into the electrophoresis mold then add the comb (mold well) and let the gel thicken [2].

H. Visualization of PCR Result with Electrophoresis

TBE 1x is poured into the electrophorator until the first limit. Then a thickened acrylamide gel is placed in the center of the kit. Then the result of PCR as much as 5µl is inserted into the acrylamide gel. The first well is filled with a DNA ladder as a marker and the next wells are filled with PCR results. TBE 1x is poured until the second limit of electrophorators. Subsequently, the gel is run with a 50V voltage for 60 minutes, then the electrophorator is turned off and the acrylamide gel was dyed [2].

After the electrophoresis process, the acrylamide gel is immersed in a drying solution consisting of 20% methanol, 2% glycerol, and 100ml distilled for five minutes. Then, it is fixed with 10% ethanol solution and 5% acetic acid in 100ml of distilled water for 15 minutes, then washed with distilled water three times and then painted with 0.1% AgNO3 in 100ml of distilled water for one hour. Wash three times with distilled water and soak in NaOH 1.5% and formalin 100µl in 100ml of distilled water, then it is visualized with the UV. Results obtained are directly photographed using a digital camera [2].

I. Analysis Data

In accordance with the design "control time series" stated above, the method of data analysis used in this study is one way anova test. The data is processed by SPSS 16.0 program, so it is not required to compare between the research results with statistics table, because from this program, the amount of Z value and significant level can be known at the end of all statistical techniques tested.

Table. 3 The Result of DNA Average

Fresh Water	DNA Level	DNA Level Average	DNA Purity
Day 1	98.66	40.43 ± 29.69	1.59
	26.38		1.65
	31.60		2.04
	18.97		1.53
	23.87		1.66
	43.08		1.81
Day 3	241.10	139.66 ± 65.43	1.94
	159.56		1.70
	176.93		1.80
	111.73		1.81
	71.90		1.81
	76.76		1.77
Day 7	246.34	151.38 ± 49.31	2.28
	163.92		1.94
	118.76		1.90
	132.15		1.93
	123.44		1.90
	123.69		1.89

The average of DNA level shows a significant increase each time. This is influenced by the characteristic of cotton cloth that can absorb liquid, even in wet conditions. In amplification, the DNA size of PCR product (bp) for the D13S317 locus is 169-201bp. The following figure shows the amplification of PCR locus D13S317 to the sperm spots sample soaked in fresh water from day 1, 3 and 7. From this figure, we can see the size of the DNA band of each sample is different.

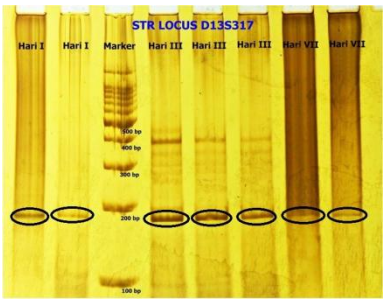

Fig 1. Results PCR locus D13S317

The DNA size of PCR product (bp) for the D21S11 locus is 203-259bp. The following figure shows the amplification of PCR locus D21S11 to sample sperm spots soaked in fresh water from day 1, 3 and 7. From this figure, at the same locus, namely D21S11 locus, the size of the DNA band in each sample is different.

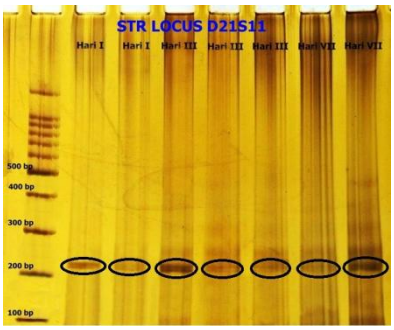

Fig 2. Results PCR locus D21S11

III. CONCLUSION

The conclusion obtained in this study is that the DNA isolation of sperm spotted on cotton cloth soaked for 1, 3 and 7 days has different grades and purity. The results obtained in this study about the levels and purity of DNA has increased significantly.

This happened because the media of sperm spots was a cotton cloth. Cotton fabrics have a strong feature in wet conditions, it increases by 25% and can absorb high water (hygroscopic). The longer the sperm spots sample is on the cotton clothes, the more components as well as DNA sample can be absorbed into the cotton fibers.

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