Jurnal Biosains Pascasarjana Vol. 20 (2018) pp

Spot Urine for Sex Determination Forensic Identifications with Amelogenin Locus and Y chromosomes (DYS 19).

Bercak Urin untuk Identifikasi Forensik Jenis Kelamin dengan Lokus Amelogenin dan Y Kromosom (DYS19)

Yeti Eka Sispita Sari, Mieke Sylvia, and Ahmad Yudianto
Departemen of Odontology Forensic
Faculty of Densitry, Universitas Airlangga
Surabaya – Indonesia
Email: yeti.eka-12@pasca.unair.ac.id

Abstract

Background: Amelogenin gene was a single copy gene located in an X chromosome and a Y chromosome. The location of amelogenin gene for identification of sex chromosome has good variability between the form and the shape of the X chromosome and the Y chromosome and between Amelogenin alleles among different populations. **Purpose**: To prove urine spot examination on the results of the sex determination through Deoxyribo Nucleid Acid (DNA) isolation using amelogenin and Y chromosome loci (DYS19). **Methods**: Spotting the microscopic examination of urine samples to determine the presence or absence of urethral epithelial cells, followed by isolation Deoxyribo nucleid Acid (DNA) in order to determine the extent and purity of DNA amplification. Then performed Polymerase Chain Reaction (PCR) amelogenin locus at 106bp - 112bp and Y chromosomes (DYS19) at 232 -268 bp. **Results**: in 9 samples of men from 3 families with 3 kinship of different regions shows the results of different tests, because Amel Y variation between individual and populations method of determining the sex of 100% was inaccurate. In some men Amel Y can be removed entirely. This research should be visualized one band on the Y chromosome (DYS19) and the Amelogenin two bands during electrophoresis occurs misidentification of the sample as a woman. **Conclusions:** Identification of sex using Amelogenin locus and Y chromosomes (DYS19) has six identical and ambiguous results because the two samples shown as the sign of men but visualized as women, another sample was not visualized because of the thick level and concentration of Deoxyribo nucleid Acid (DNA).

Keywords: Urine Spot, Sex Determination, Amelogenin, Y chromosome (DYS19).

Abstrak

Latar belakang: Amelogenin merupakan gen salinan tunggal yang terletak di kromosom X dan kromosom Y, antar alel Amelogenin berbeda setiap populasi dikarenakan Amelogenin tidak mengkombinasi kromosom Y yang secara efektif mengisolasi tekanan seleksi secara normal. **Tujuan:** Membuktikan Pemeriksaan bercak urin pada uji jenis kelamin

melalui analisis hasil isolasi Deoxyribo Nucleid Acid(DNA) menggunakan lokus Amelogenin dan Y kromosom(DYS19). **Metode:** Bercak urin sampel dilakukan pemeriksaan mikroskopik untuk mengetahui ada tidaknya sel epithel uretra, dilanjutkan dengan isolasi Deoxyribo Nucleid Acid(DNA) guna mengetahui kadar dan kemurnian DNA selanjutnya dilakukan Amplifikasi Polymerase Chain Reaction(PCR) lokus Amelogenin pada 106bp – 112bp dan Y kromosom(DYS19) pada 232 -268 bp. **Hasil:** pada 9 sampel laki-laki dari 3 keluarga dengan 3 kekerabatan dari daerah yang berbeda menunjukkan hasil uji yang berbeda, namun karena Amel Y variasi antara individu dan populasi metode penentuan jenis kelamin 100% tidak akurat. Dalam beberapa laki-laki Amel Y dapat dihapus seluruhnya. Penelitian ini seharusnya divisualisasikan satu band pada Y kromosom(DYS19) dan pada Amelogenin dua band selama elektroforesis namun terjadi kesalahan identifikasi dari sampel sebagai perempuan. **Kesimpulan:** Identifikasi jenis kelamin menggunakan lokus Amelogenin dan Y Kromosom(DYS19) enam identik dan dua terjadi hasil ambigu karena benar dari laki-laki namun divisualisasikan menunjukkan perempuan, satu sampel tidak tervisualisasikan dikarenakan terlalu pekat kadar dan konsentrasi Deoxyribo Nucleid Acid(DNA).

Kata kunci: Bercak Urin, Uji Jenis Kelamin, Amelogenin, Y Kromosom(DYS19).

INTRODUCTION

Forensic identification by DNA testing is now very believable for the identification of unidentified bodies, the bodies have decomposed, burned, and mass accident, the crime was planned or not, natural disaster or civil unrest resulting in the death, leaving very little evidence, identification forensic also plays a role in many other cases such as child abduction, Paternity. Forensic identification very important for an investigation step until a judgment step in the court.[1]

Sex identification very important to the investigation until sentencing on further rate until termination of the case in forensic DNA profiling test in judgment. DNA is responsible for storing all the genetic material and unique to each individual. DNA tests currently available has a high reliability and accepted as evidence in a court of law[2]. In this research to sex determine used DNA test Amelogenin gene and Y kromosom (DYS19), Amelogenin test is already available in the form of kit Polymerase chain reaction (PCR) which is marketed commercially. Amelogenin test most widely used to identify the sex of man who was

instrumental in solving forensic cases, prenatal diagnosis, DNA data base and storage of blood samples[3.] Amelogenin gene is a single copy gene located on the X chromosome and Y chromosome, the location of Amelogenin gene for identification of sex chromosome variability between the form and the shape of the X chromosome and the Y chromosome Amelogenin alleles among different populations due Amelogenin Y chromosome does not combine effectively isolate selection pressure is normal[4]. Corresponding microscopic analysis of urine sediment, epithelial cells in urine are classified according to the type and quantity. Quantities are given as number, moderate, little or a lot. Identify specific types of epithelial cells in the sample help to detect the cause of the disease in the medical condition. Although squamous cells found in the skin, on the outside of the vagina and urethra, the transitional located in the bladder, ureter and renal pelvis.

In the case of forensic evidence can not be ascertained what left at the scene, the forensic science whatever was left behind at a crime scene can be used as evidence. In search of the crime scene evidence is often referred to in any criminal case. Stands the scene of the crime is the place where the discovery of evidence, clues and evidence or scene of a crime or suspected crime according to a witness. Evidence is something that is valid for submission to the court to determine the truth of a case that is undeniable. Investigators should be able to identify, collect, and use of evidence in criminal cases in order to investigate the success of the investigation[5].

The sophistication of modern technology and the growing crime rate now makes criminals more quickly and cleanly in doing the crime, it is often difficult to make the investigator misled and obtain evidence of a crime,. The incident with the lack of evidence and has degraded evidence at the crime was the inspiration researcher wants to help find a solution for the identification of gender through urine spots, so far identification analysis

Gender through urine patches are rare[6]. Urine patches are often found at the crime scene in the case of crimes such suicide, Murder and other criminal cases. This research focused planned to prove spotting urine as an alternative material in gender identification through analysis Deoxyribo nucleid Acid (DNA) with Amelogenin gene. The results of this research can help solve various cases of crime and mass disaster involving the identification of sex.

MATERIALS AND METHODS

Samples were taken from urine patches 9 volunters large Samples. The cotton within urine spot from the volunteer had been cut and put in the falcon cylinder and sunk in the 5-10 ml destiled water (DW) taken for vortex-sonication process five times, and incubated for 24 hours. Then the sediment of this process was taken for microscopic examination in order to find the epithelia urethra cell. After that, the liquid was taken for centrifugation in 12000rpm for 10 minutes in the 4^0C, the Supernatant was removed, and the isolation DNA within DNA zol was done on the pellet. The purity and the level of DNA were measured, the DNA was also measured through UV- Spectrophotometer according to irradiation UV-light principle which reserved by nucleotide, protein, and the liquid. This measurement was done based on how much the UV-light which was reserved by the liquid of DNA and directly proportional with in how much DNA in the sample. The maximal reservation of UV-light by the protein was reached in (λ) 280 nm length of wave. DNA's amount determination (µg/ml) = the result was (λ 260) x retail factor x 50µl/ml. 1 OD (optimal density) = 50µg/ml for DS (double strand) DNA[7].

Samples great preformance of this study is 9 samples criteria Samples: spotting urine specimen taken from urine samples contained epithelial cells of the urethral tract, urine sample was dropped by 2 drops (30µl) on cotton fabric, Volunteers male sex, age 25-60 years, Levels of DNA typing results for the isolation of at least 20 ng / mL[10] and DNA purify

1-2 (Ideal 1.8 to 2) to allow PCR. Materials Research Insulation material / DNA extraction: DNAzol Reagent, Destiled water (DW), 100% ethanol solution and 75% Ethanol. PCR Mix primary Y chromosome (DYS19) (GAA TAT TCC CGC TCT CCG GA), Amelogenin (CCCT GGG CTC TGT AAA GAA), Marker 100bp Electrophoresis and painting materials: Bis Acrylamid, Agarose gel Polyacrylamid, Research tools PCR Cycle (Gene Amp PCR System 9700, Applied Biosystems), Spectrophotometer (UV-Visible Spectrophotometer, Shimatzu), electrophoresis, Whirlimixer (CE), Centrifuge (SCR Himac 20B, Hitachi), Micropipette White (0,5-10µl), yellow (10-100µl) and blue (100-1000µl), Tips micropipette white, yellow and blue, UV transluminator, camera, Transsonic 310 (Elma), Spinator (Millipore), 0,5cc Eppendorf tubes, 1,5cc da 2cc, Microwave (Imarflex), Scales Electric (Libror EB-3200B.Shimadzu), 15ml falcon tube.

The maximum UV absorption by proteins is achieved at a wavelength (λ) 280 nm. Determination of DNA concentration (ug / ml) = result read (λ 260) x dilution factor x 50µl / ml. 1 OD (Optimal density) = 50µg / ml to a double-stranded (DS) DNA and 40µg / ml for single-stranded (ss) DNA or RNA. DNA purity was determined by calculating the ratio of λ260: λ 280, results: 1-2, if greate 1 makes it possible to do PCR (ideally 1.8 - 2 for DNA) UV procedure Spectropotometer bari Eppendorf tube filled with distilled water plus 10µl 690µl DNA isolation, Read the UV Spectrophotometer results for λ 260 and λ 280.

PCR amplification for Amelogenin and Y kromosom The next step after the amplification composite agarose electrophoresis with polyacrylamide gel stained with silver staining and then viewed with a UV lamp until obvious. Data visualization electrophoresis of the DNA bands obtained from urine patches in the determination of the sex chromosomes in a particular locus are identical / consistent / matching.

RESULTS

Epithelial tissue is one of the main types of tissues in the human body. Although the skin is composed of epithelial tissue, mostly in the upper body cavity and organs are also lined by epithelial cells. This network is made of epithelial cells, of various types. Epithelial cells of the urethra begins as transitional cell after coming out of the bladder. Throughout the urethra composed by stratified columnar epithelial cells, then cell-rise flat near the outlet. epithelial cells in urine sample. This is because the regular shedding of cells from the bladder and external urethral. Epithelial cells of the kidney is usually not shed, but the increase in the number of epithelial cells in the urine can indicate health problems. The presence of an abnormal form of epithelial cells can also be a problem. The types of epithelial tissue can be divided into two based on the number of layers of its constituent cells. The second network is a single layer of epithelial tissue (simple epithelium) and multi-layered epithelial tissue (stratified epitellium). Research that the identification of the sex of the DNA isolation from urine patches through Amelogenin locus and Y chromosomes (DYS19) partly there and partly identical to the sample shows male and female visualized very clearly visible on the test results so basically spotting the urine can be a material alternative in forensic identification as other identifying material derived from blood, semen, hair, urine, sweat spots, spots of blood, vaginal secretions, cigarette butts, as well as from other sources. Sex determinant test using Amelogenin locus and Y chromosome (DYS19) DNA obtained by examination of urethral epithelial cells. Microscopic examination to look for urethral epithelial cells in urine spot samples, test results obtained 1-2 epithelial cells of the whole field of view then performed assay and purity of DNA with the results of the DNA content of

isolated patches of urine samples that vary greatly between the range of 4263 - 6520.5 ng / ml and purity of DNA from 1.135 to 2.570, Amelogenin test results and Y chromosomes (DYS19) in the 9 spot urine sample was only 8 are eligible to typing, based on measurements and DNA purity (range 1-2) .In case identification forensic anything found at a crime scene evidence while evidence from urine patches is evidence that was never considered to exist and be ignored by investigators from the attitude that too many cases incomplete or mistaken. Identification of urine patches are not separated from the epithelial cells of the urethra which is very rare in number and of identification urine spots can also be known metabolic activities of the past victims / patients of used clothing in the washing has not been performed.

DISCUSSION

Y chromosome in forensic DNA testing is only done on men. Gene SRY (sex-Determining region Y) determines masculinity. Because most crimes where DNA evidence is helpful, especially sexual abuse, involving men as perpetrators, the Y chromosome test, the results of which can be interpreted can be obtained in some cases where the test autosoma limited by evidence, such as the high level of female DNA in the presence of a minor amount male DNA. These situations include evidence of sexual persecution of men azospermic and vasectomized and blood mix-blood or saliva-blood where the absence of sperm prevent differential extraction for DNA isolation men. In addition the number of individuals involved in the 'gang rape' can be easily translated to the results of the Y chromosome STR mixture autosoma very complicated. Using PCR primers specific Y chromosome can increase the chance of detecting low levels of DNA actors in the background DNA tall female victims[4].

Every human has 23 pairs of chromosomes consisting of 22 pairs of somatic chromosomes and one pair of sex chromosomes. XX chromosomes determine the sex of a person with a female and XY for someone male sex. Chromosome is obtained from the

parents, half from the mother and half from the father. Because a mother fully lowered mitochondrial DNA to his son, and a father will inherit a Y chromosome to his son (because the Y chromosome only possessed male XY sex chromosomes). While the girls do not have a Y chromosome (XX female sex chromosome)[6]. To prove the family's relationship with the father's side can be done by comparing the Y chromosomes of a child with his biological father or with siblings from his father's side. Because the Y chromosome examination only for boys. Sex Determinant test patches is taking DNA from epithelial cells of the urethra which can be seen by microscopic examination, Results in figure 1.

Amelogenin test results and Y chromosomes in 9 patches urine sample was only 8 are eligible for submission as typing, based on measurements and DNA purity (range 1-2) then performed amplifikasi Amelogenin locus PCR with the following

Figure 2 shows the electrophoretic visualization Amelogenin locus on the spot urine samples were all true men, apparent differences in test results on samples showed identical 1,2,6,7,8,9 at 106 bp, 112 bp, the readout said laki- male (XY). Test results of samples 4.5 shows Identics at 106 bp, the women said reading (XX), 3 sample test results did not show because very liquid visualization and concentration levels of DNA.

Figure 3 shows the electrophoretic visualization Amelogenin locus on the spot urine samples were all true men, apparent differences in test results on samples 1,6,9 showed identical to 232-268 bp, the test results of samples 2, 7.8 faint band at 232-268 bp, 4.5 test results did not show the tape on the y chromosome test this. 3 sample test results did not show because too pekatnya visualization and concentration levels of DNA.

9 samples showed visualization on Amelogenin locus and Y chromosomes, 1,2,6,7,8,9 samples showed identical to the Amelogenin locus and Y chromosomes, the

sample 4.5 shows the identical women in Amelogenin and Y chromosomes showed no band when the sample is actually a man, sample 3 is not out tape.

In response to previous research trials using gene Amelogenin sex accurate in general, other Y chromosome markers such as SRY, STR, or 50f2 can be used for identification of ambiguous gender, the test results amelogenin male sample showed visualization 106bp said to be female (XX).

The Y chromosome is the result of a genetic mutation that results in the birth of SOX3 SRY (Sex - Determining Region Y), a new gene that controls the development of the testes and male genes - male. Over time, SRY gene also evoke nature - the nature of the other men that enables trouble combining the X and Y chromosomes during meiosis (one way undergo cell division - ed). As a result, the Y chromosome will be vulnerable to changes such as test results on samples that actually men showed no release of the tape on the Y chromosome test.

From the results of this research note that the isolation of DNA from urine patches on fabrics / garments in Used (In forensic cases the victim) may be an alternative material in forensic identification and purity DNA level despite low or even less, so the PCR amplification techniques are required average more than one-time amplification. In this study, the identification of sex using Amelogenin locus partly identical and two of them occurred ambiguous results because the samples are from men but test results are visualized showing women. Similarly, the identification of the sex of using y chromosome loci DYS19 on two samples amelogenin test results showed women in these two test samples y chromosome is not true when the tape out two samples of the male sample.

REFERENCE

1. Thangaraj K,Reddy AG,Singh L.2002.Apakah gen Amelogenin diandalkan untuk identifikasi jenis kelamin pada identifikasi forensic dan diagnose prenatal Int J Legal Med.116(2). 3-121.
2. Gatut S and Ujinaka T.2004.Pengaruh waktu terhadap kualitas dan kuantitas DNA Forensik : Sampel Whole Blood tanpa zat antikoagulan dalam bunga rampai Ilmu Kedokteran forensic&Medikolegal,Konas III PDFI Semarang 23-24 Juli 2004
3. Atmaja D S, 2005. Peranan Sidik jari DNA dalam bidang Forensik, Seminar Nasional Aplikasi DNA Finger Printing dalam bidang kedokteran,29 Agustus 2005, PS UGM.
4. Lattanzi, W., Di Giacomo, M. C., Lenato, G. M., Chimienti, G., Voglino, G., Resta, N., Pepe, G., Guanti, G. A large interstitial deletion encompassing the amelogenin gene on the short arm of the Y chromosome. Hum. Genet. 116: 395-401, 2005. [PubMed: 15726419, related citations]
5. Kusuma S E,Yudianto A, Sosiawan A. 2007. Pemeriksaan Identifikasi Forensik Molekuler (panduan laboratorium).Kelompok Studi Human Genetik TDC. Universitas Airlangga.
6. Syukriani Yoni. 2012. DNA Forensik.Sagung seto.Jakarta
7. Tilgner, Hagen, Knowles, David G., Johnson, Rory, Davis, Carrie A. Chakrabortty, udipto; Djebali, Sarah; Curado, João; Snyder, Michael, Gingeras, Thomas R. Guigó, Roderic. 2012 . "Deep sequencing of subcellular RNA fractions shows splicing to be predominantly co-transcriptional in the human genome but inefficient for lncRNAs". Genome Research 22 (9): 1616–25.

No Sampel	λ 260	λ 280	Kemurnian (λ 260 / λ 280)	Kadar (ng/µl) (Kemurnian x 50 x 70)
1	0,321	0,265	1,211	4238,5
2	0,325	0,253	1,284	4494
3	0,293	0,114	2,570	8995
4	0,371	0,228	1,627	5694,5
5	0,315	0,169	1,863	6520,5
6	0,285	0,251	1,135	3972,5
7	0,273	0,224	1,218	4263
8	0,222	0,128	1,734	6069
9	0,327	0,213	1,535	5372,5

Tabel 1, Concentration and purity of DNA spot urine sample.

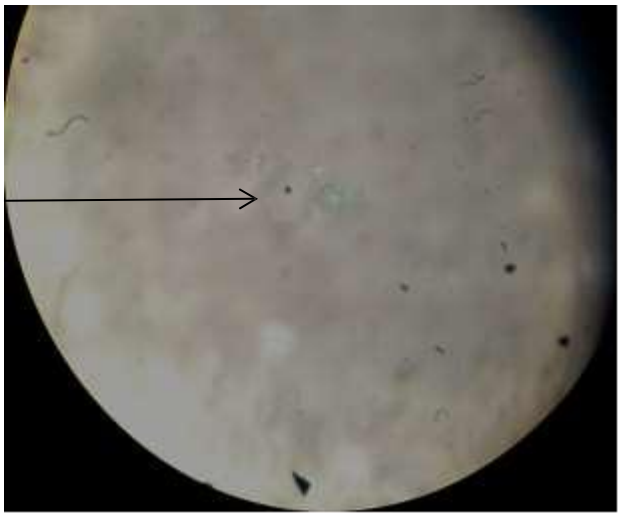

Figure 1. Epithel Squamous

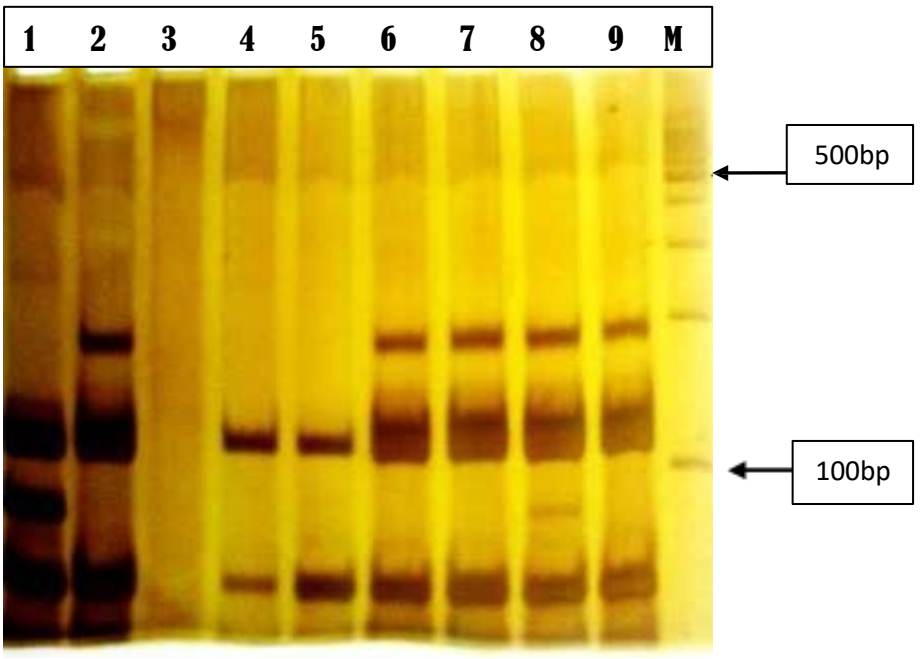

Figure 2. Results on the spot urine sample lokus Amelogenin

Specification:

M : Marker 100 bp

1,2,6,7,8,9 : Results lokus Amelogenin in Spot urine samples 106 bp, 112 bp =

Male (XY)

4.5 : Results lokus Amelogenin Spot urine samples 106 bp = Female (XX)

3 : Results lokus Amelogenin Sample Not out tape

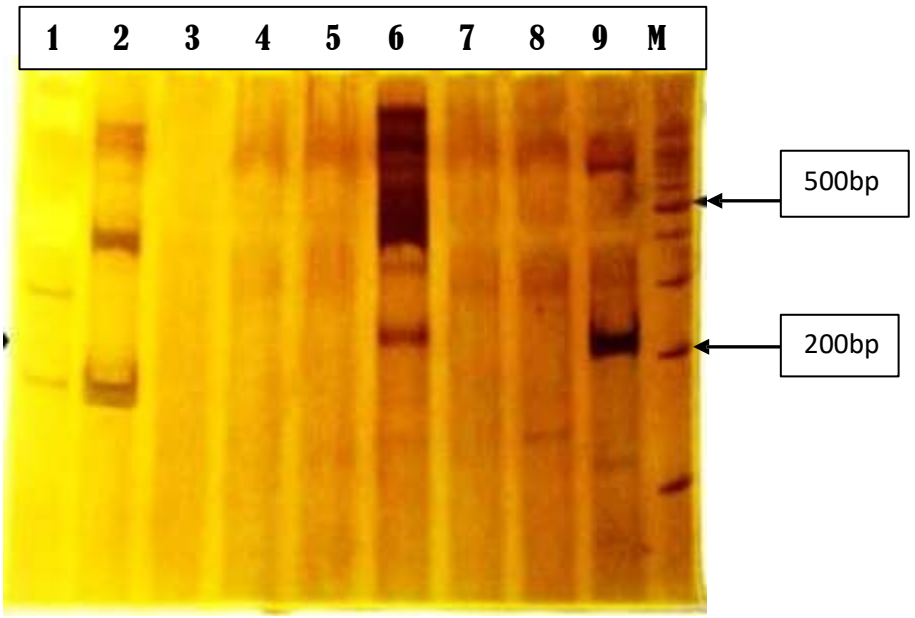

Figure 3. Results on the spot urine sample lokus Y Kromosom (DYS19)

Specification::

M : Marker 100 bp

DYS19 : 232 -268 bp

1, 6, 9 : Results lokus DYS19 in Spot urine samples 232-268 bp

2, 7, 8 : Results lokus DYS19 in Spot urine samples 232-268 bp

3, 4, 5 : Results lokus DYS19 Sample are not out tape