DNA ISOLATION FROM HUMAN URINE STAIN AS AN ALTERNATIVE MATERIAL FOR PERSONAL IDENTIFICATION EXAMINATION

Ahmad Yudianto[1,2,3], Yeti Eka Sispitasari[3]
[1]Department of Forensic Medicine & Medicolegal, Faculty of Medicine, [2]Human Genetic Group, Institute Tropical Disease, [3]Master Program in Forensic, School of Postgraduate Studies, Universitas Airlangga

ABSTRAK

Penentuan akurasi identitas pribadi sangat penting untuk penyelidikan sejak ketidaktepatan apapun dapat menyebabkan konsekuensi yang fatal dalam proses peradilan. Identifikasi melalui analisis DNA melibatkan kromosom somatik dan mtDNA. Setiap bagian dari tubuh manusia dapat diambil sebagai spesimen karena setiap sel berinti dalam tubuh seseorang memiliki urutan DNA yang identik. Untuk saat ini, sampel untuk identifikasi melalui analisis DNA yang diperoleh dari noda darah, air mani noda, tulang, swab vagina, swab bukal dll Dalam kasus tertentu, urin noda pada pakaian telah sering diabaikan. Sejauh ini, identifikasi pribadi melalui analisis DNA dengan menggunakan urin noda belum umum Dilakukan. Penelitian ini terdeteksi band di CSF1PO lokus, THO1, TPOX dan 106bp-112bp amelogenin di semua sampel divisualisasikan dari hasil Polymerase Chain Reaction (PCR) dengan pewarnaan Polyacrylamid Agarose Gel elektroforesis-perak untuk jangka waktu pemaparan dari 1, 7 dan 14 hari , Namun, untuk durasi paparan dari 20 hari (maksimum dalam penelitian), band yang hanya terdeteksi di THO1 lokus dan TPOX di semua sampel (100%), sedangkan lokus amelogenin CSF1PO dan 50% dipamerkan band yang jelas. Hal ini menunjukkan sumber bahwa analisis DNA dari noda urin melalui deteksi lokus STR CSF1PO, THO1, deteksi TPOX dipamerkan respon yang berbeda untuk jangka waktu eksposur yang berbeda ditugaskan untuk sampel urin noda. deteksi sukses Ulasan lokus ini didukung oleh perbedaan dalam produk amplikon dan konten GC pada setiap lokus. Lokus yang diteliti, rasio konten GC dari primer, diurutkan dari terendah, adalah sebagai berikut: lokus CSF1PO dari 42,6 1%, TPOX dari 56,25%, dan THO1 dari 63,83%. Kesimpulannya, lokus THO1 dan TPOX memiliki probabilitas yang sama sukses di STR pemeriksaan Dibandingkan dengan CSF1PO lokus. ***(FMI 2016;52:277-284)***

Kata kunci: *isolasi DNA, urin noda, identifikasi, STR*

ABSTRACT

Accurate determination of personal identity is crucial for an investigation since any inaccuracy may lead to fatal consequences in the judicial process. Identification through DNA analysis involves somatic chromosomes and mtDNA. Each part of the human body can be taken as a specimen since every nucleated cell in the body of an individual has identical DNA sequence. To date, samples for identification through DNA analysis are obtained from blood stains, semen stains, bones, vaginal swab, buccal swab etc. In certain cases, urine stains on the clothing have frequently been overlooked. So far, personal identification through DNA analysis by the use of urine stains has not been commonly carried out. The present study detected bands in the loci CSF1PO, THO1, TPOX and 106bp-112bp amelogenin in all samples visualized from the results of Polymerase Chain Reaction (PCR) with Polyacrylamid Agarose Gel Electrophoreses-silver staining for exposure durations of 1, 7 and 14 days. However, for exposure duration of 20 days (the maximum in the study), bands were only detected in the loci THO1 and TPOX in all samples (100%), whereas the loci CSF1PO and 50% amelogenin exhibited obvious bands. This indicated that DNA analysis of urine stains through detection of the locus STR CSF1PO, THO1, TPOX exhibited different detection responses for different exposure durations assigned to the samples of urine stain. Successful detection of these loci was supported by the differences in amplicon product and GC content at each locus. Of the loci studied, the ratio of GC content of the primers, sorted from the lowest, were as follows: locus CSF1PO of 42.6 1%, TPOX of 56.25%, and THO1 of 63.83%. In conclusion, the loci THO1 and TPOX had the same probability of success in the STR examination compared with the locus CSF1PO. ***(FMI 2016;52:277-284)***

Keywords: *DNA isolation, urine stains, identification, STR*

Correspondence: Ahmad Yudianto, Department of Forensic Medicine & Medicolegal, Faculty of Medicine, Universitas Airlangga, Jalan Prof Dr Moestopo 47, Surabaya 60131, Indonesia. E-mail: yudi4n6sby@yahoo.co.id

INTRODUCTION

Personal identification is a problem in civil and criminal cases. Determining the exact personal identity is critical to the investigation due to a wrong can be fatal in the judicial process (Idries 1997). When this method of identification has evolved towards molecular forensics. Deoxyribonucleotic acid (DNA) is the smallest unit of heredity and is present in all living organisms ranging from microorganisms to higher organisms such as

humans, animals and plants. According Notosoehardjo (2000), each network has a different DNA content depending on the structure and composition of the cell. Network with many nucleated cells and little connective tissue generally have high levels of DNA. Every part of the human body can be taken as a specimen because every nucleated cell in the body has the identical DNA sequence (Notosoehardjo 2003). During this specimen/ sample that is widely used in DNA testing to identify, are stains of blood/blood, staining sperm, vaginal swab, buccal swab and bone (Kusuma 2004). In one of the forensic medical examination of the investigation were very helpful examination of the evidence, that there is in the body of the victim/perpetrator and the crime scene (TKP). In the case of a trap or hang themselves, which generally obtain their pee or semen out of her genitals and dirt from her anus, it is the result of the state of suffocation (asphyxia). Urine that is attached to the pants or cloth around it or in other words urine stains are often overlooked in the examination.

In a previous study found that urine contains the nuclear DNA and mitochondrial DNA. The composition of urine containing a small amount of epithelial cells is the result of the release of the bladder and urethra regular from externally. With regard to the composition, then the urine stains the garments contain nucleated somatic cells that do DNA extraction. Until recently in Indonesia personal identification through stains of urine by the method of DNA analysis (DNA Profiling) has not been done, so this research may provide answers on matters related to the effectiveness of using stains of urine as a forensic identification.

MATERIAL AND METHODS

This type of research this study was an observational study to prove the identification through DNA isolation staining urine stored for 1 day, 7 days, 14 days, 20 days at the locus STR CODIS: CSF1PO, THO1, TPOX and amelogenin, with cross sectional design survey, conducted in the laboratory of Human Genetic Study Group, Institute of Tropical Disease, Airlangga University.

Primer: CSF1PO (5'-AACCTGAGTCTGCCAAGGA CTAGC-3 'and 5'-TTCCACACACCACTGGCCATCT TC-3'), THO1 (5'-CTGGGCACGTGAGGGCAGCGT CT-3 'and 5'-TGCCGGAAGTCCATCCTCACAGTC-3'), TPOX (5'-ACTGGCACAGAACAGGCATCTAG G-3 'and GGAGGAACTGGGAACCACACAGGT 5'-3 '), amelogenin X 106 bp; Y 112 bp (5'CTGATGGT TGGCCTCAAGCCTGTG-3' and 5'TAAAGAGATTC A TTAACTTGACTG-3 ').

DNA extraction with the DNA staining urine-Zol Reagent (Invitrogen Tech-Line sm):

Fabrics containing urine stains are cut, mixed with a conical tube inserted Water Free, further sonication for 2-3 hours. The generated pieces of cloth, which was deposited in liquid centrifuges 10,000 g for 10 minutes. Pellet taken mixed with 1 ml DNAZol. Both were mixed in a way vortexing Incubate for 5 minutes at room temperature. Centrifuged 10,000 g for 10 min at 4 °C. viscous supernatant then taken and put into a new tube. Add 0.5 ml of absolute ethanol (100%), inverted, and then incubated for 1-3 minutes.

Centrifuged 4,000 g for 1-2 minutes at a temperature of 4 ° C, then the supernatant was discarded carefully, so that the DNA did not go wasted. Wash pellet with 75% ethanol 0.8-1 ml, 2 times, and each time washed with 75% ethanol, back and forth for 3-6 times. Place the tube in an upright position for 0.5-1 minutes, after which the waste of 75% alcohol by means pippeting or decanting. Pellet dried by allowing the open tube for 5-15 seconds after ethanol 75% discharged. Dissolved pellet containing the DNA with NaOH solution as much as 0.2-0.3 ml 8mm. vortex taste, then stored at -20 ° C.

PCR amplification

DNA amplification by PCR amplification protocols do:

CSF1PO (Gene Ampr.PCR System 9700 Thermal Cycler, Promega Corp.2001): Phase I: Initial denaturation 96^0C for 2 minutes, phase II: Cycle 1 (10 times), comprising: Subsequent denaturation 94 0C during 1 minutes, 64^0C during Annealing 1 minute, Extension 1 minutes 70^0C for 30 seconds, cycle 2 (20 times) consisting:: 90^0C for 1 min denaturation, annealing for 1 minute 64^0C, 70^0C Extension for 1 minute 30 seconds, stage III: Hold step 4^0C

THO1 (Gene Ampr.PCR System 9700 Thermal Cycler, Promega Corp.2001): Phase I: Initial denaturation 96 0 C for 2 minutes, phase II, Cycle 1 (10 times) comprising: Subsequent denaturation 94^0C during 1 minutes, Annealing 64^0C for 1 minutes, Extension 1 minutes 70^0C for 30 seconds, cycle 2 (20 times) consisted of: denaturation 90^0C for 1 min, Annealing 64^0C for 1 min, Extension 70^0C for 1 minute 30 seconds, stage III: Hold step 4^0C

TPOX (Gene Ampr.PCR System 9700 Thermal Cycler, Promega Corp.2001): Phase I: Initial denaturation 96 0 C for 2 minutes, phase II, Cycle 1 (10 times) consisting of: Subsequent denaturation 94^0C for 1 min, annealing 640C for 1 min, Extension 1 minutes 70^0C for 30 seconds, cycle 2 (20 times), which consists of: denature-

ation 90^0C for 1 min, Annealing 64^0C for 1 min, Extension 70^0C for 1 minute 30 seconds, phase III Hold step 4^0C

Amelogenin (Promega Corp., .2001): Phase I: Initial denaturation 96^0C for 2 minutes, Phase II, Cycle 1 (10 times), which consists of subsequent denaturation 94^0C for 1 'Annealing 70^0C for 1' 30 '', Extension 64^0C for 1 ', Cycle 2 (20 times): consisting of denaturation 90^0C for 1', Annealing 60^0C for 1', Extension 70^0C during the 1'30' ', stage III: Hold step 4^0C.

Electrophoresis

Procedures Silver Staining Polyacrylamid Agarose Composite Gel (Edvotek, 2001) comprising: Drying: (methanol 20% + glycerol 2%) in 100C distilled water for 5 minutes, the fixation (ethanol 10% + acetic acid glycerol 5%) in 100 cc of distilled water for 20 minutes, wash/rinse with distilled water 1x quickly, staining: AgN03 0.1% in 100 cc of distilled water for 50-80 minutes, developing: (NaOH + 1.5% Formalin 100 ul) in 100 cc of distilled water, then it will be clearly seen.

RESULTS

DNA levels

In this study, the treatment begins with staining urine samples, the long exposure time. As for the length of time of exposure in this study: day 1, day 7, day 14 and day 20. Then proceed with the extraction/DNA isolation staining urine samples with methods DNAzol. The results of the sample DNA extraction followed by measurement of DNA samples using a UV-Visible Spectrophotometer at a wavelength of 260 nm (λ 260). The results of measurements of DNA after DNA isolation from urine stains on the fabric prior to PCR amplification (Polymerase Chain Reaction) is seen in Table 1. Table 1 shows the decreased levels of DNA from urine stains in samples exposed a long time. The longer described getting down the levels of DNA, which is the average on day 1: 32.5 µg/ml, day 7: 21 ug/ml, day 14: 16.5 ug/ml and a day to 20 : 14 ug/ml. The length of time the effects of exposure to DNA from urine stains in CODIS STR loci (CSF1PO, THO1, TPOX and amelogenin)

Table 1. Levels of DNA in urine stains

Samples		Level (µg/ml)	Mean level (µg/ml)
Day 01	1	35	32.5
	2	30	
Day 07	1	20	21
	2	22	
Day 14	1	15	16.5
	2	18	
Day 20	1	13	14
	2	15	

To determine the effect of the length of time of exposure to the DNA from urine stains in CODIS STR loci (CSFIPO, THO1, TPOX, amelogenin). Detection test results can be seen in Table 2. From Table 2, the entire sample in this study were examined by DNA profiling at the locus CSF1PO, THO1, TPX and amelogenin of DNA isolated from urine stains all detected, although sightings ribbon is not the same band for their treatment. In CSF1PO loci and amelogenin on day 20 only one sample showed the ribbon bands that are not visible/sketchy.

Visualization of PCR products by agarose composite polyacrylamide gel (PAGE) Fig. 1, showing all samples detected in all treatments (long time) against TPOX locus (range between 224bp - 252bp) and the locus Thoi (range between 179bp - 203bp). Visualization of PCR products by agarose composite polyacrylamide gel (PAGE) of Fig. 2, showing all samples detected in all treatments (long time) against TPOX locus (range between 224bp - 252bp) and against loksu Thoi (range between 179bp - 203bp).

Table 2. Results of the effects of long exposure time detection of DNA from urine stains in various long treatment time, detection at STR loci (CSF1PO, THO1, TPOX, amelogenin)

Length	CSFIPO		THO1		TPOX		AMELOGENIN	
	Detected	Not Detected	Detected	Not Detected	Detected	Not Detected	Detected	Not Detected
Day 1	2	0	2	0	2	0	2	0
Day 7	2	0	2	0	2	0	2	0
Day 14	2	0	2	0	2	0	2	0
Day 20	1	1	2	0	2	0	1	1

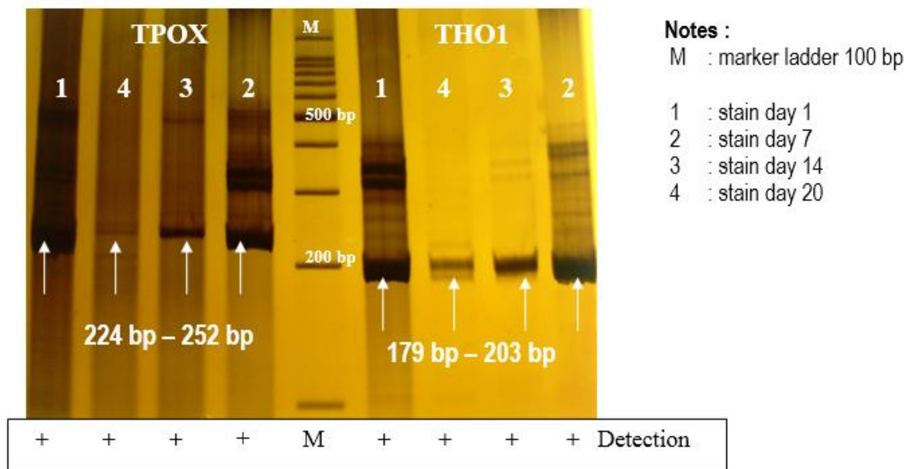

Fig. 1. Visualization TPOX locus PCR results and THO1 at staining the sample 1.

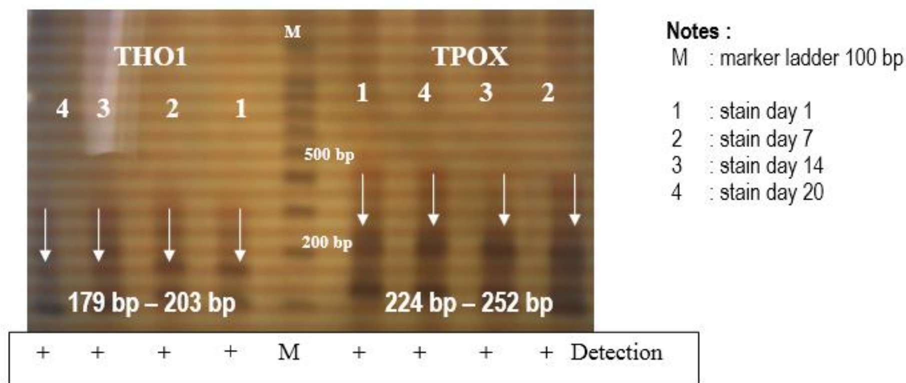

Fig. 2. Visualization TPOX locus PCR results and THO1 at staining the sample 2.

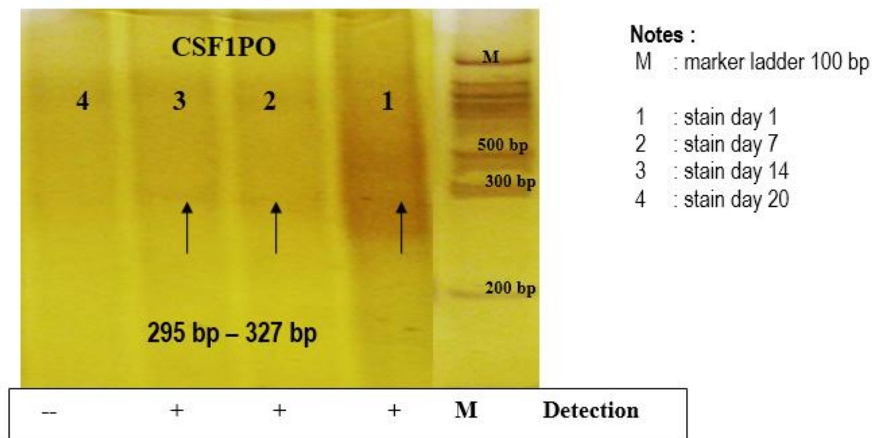

Fig. 3. Visualization CSF1PO locus PCR results in staining the sample.

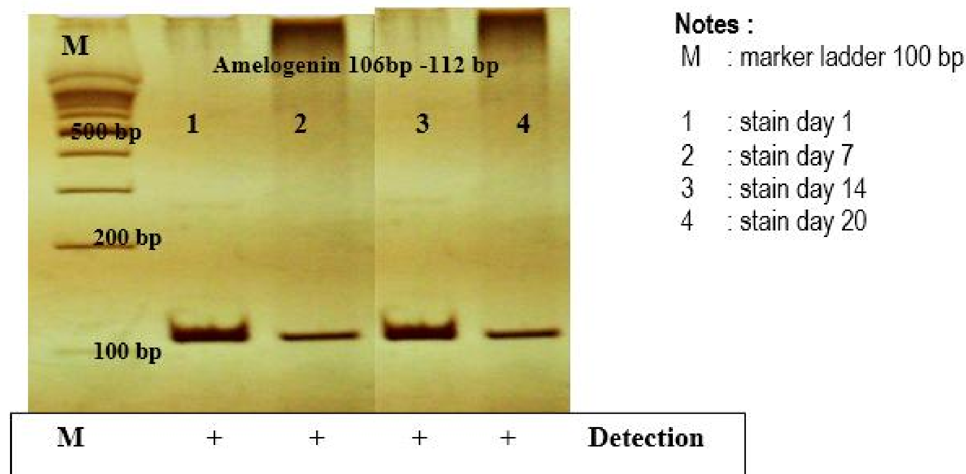

Fig. 4. Visualization amelogenin locus 106bp PCR-112 bp in the sample stains.

Visualization of PCR results with polyacrylamide agarose composite gel (PAGE) Fig. 3, showing almost all samples detected in all treatments (long time) against the locus CSF1PO (range between 295bp - 327bp) are just a sample staining days to 20 show a ribbon bands that are not visible/sketchy. Visualization of PCR results with polyacrylamide agarose composite gel (PAGE) Fig. 4, showed almost all samples detected in all treatments (long time) against the locus amelogenin (range between 106bp - 112bp), namely on the 1st, 7th, 14th and 20th out ribbon-band.

DISCUSSION

The principle of personal identification of the victims died in the field of forensic medicine is a series of actions data comparison results of examination of the bodies (data postmortem) with the data when the alleged victim was still alive (data antemortem). The data will then be compared with the data belonging to the alleged victim, in which the data can be obtained from the families, medical records, medical records dental, police data, and so forth. Compatibility between the data antemortem and postmortem of data, will narrow the number of alleged victims. Thus this will further reinforce the notion that the victim is actually the person who had been expected so far.

With the discovery of DNA fingerprinting by Sir Alec J Jeffreys in the mid-eighties, in which the present invention has brought about the development of DNA technology in the field of forensic medicine in the direction of encouraging progress, identification of the victim in the field of forensics is not a problem that is so big (NotoSoehardjo 2003).

It is given that a DNA examination on the victim unrecognizable, is no longer based on the physical characteristics of the victim, but on the region (locus) victim's DNA. This examination was based on a fact that turns human DNA is individual and specific. This implies that the composition of human DNA is unique for each individual. So it can be used to distinguish individuals from one another. However the examination of DNA as a tool in forensic identification process is not without drawbacks. It is based on the reality that the DNA will be damaged (damage), when exposed by exposures abnormal, which can cause DNA damage as well as chemicals, pH, temperature, and exposure to other (Farley et al, 1991, Melcher 2000). Quantity or concentration Deoxyribonucleid Acid (DNA) is an important factor in the examination of forensic DNA analysis that affect the success of STR genotyping the DNA samples. Decreased levels of DNA 1 ng potentially decreased ability to detect short tandem repeat (STR) to 95% (Bergen et al 2005).

According Notosoehardjo (1999) and Gatut (2004) DNA levels of the minimum required in forensic DNA examination of each of 50 ng and 20 ng, while Butler (2005), the levels of DNA in the examination of at least 0.5-2.5 ng STR. In this study, the levels of DNA obtained from samples of urine stains between the range of 35 -15 pg/ml, so it is sufficient for the examination of DNA analysis. A decrease in levels of DNA research shows the effect of the length of time of exposure is significant, resulting in the damage to the DNA structure. DNA damage caused by abnormal exposures ie high temperature according to Watson (1987), caused by damage to the DNA hydrogen bonds are irreversible. These conditions resulted in a couple of purine-primidin

damage to DNA, where the pair purine-primidin this is a major component in the structure of DNA.

From the results of this study environmental effects in terms of the length of time of exposure to the effects in this study prove their influence on the measurement of DNA contained. It is seen from the results of measurements of DNA by UV spectrophotometry showed decreased levels in samples of urine stains are stored in room temperature and places with high humidity from day 1, 7, 14 to day 20 there were any significant decline. But with the decline in those levels, not a constraint because the levels of DNA is left still for examination allowing DNA profiling which is at least 50 ng (Notosoehardjo 1999). Number of minimum levels of DNA that can be used in DNA analysis, in principle, depending on the needs and the type of examination performed. On examination of forensic DNA-based Restriction Fragment Length Polymorphism (RLFP) for example, it takes a relatively large levels of DNA which is about 100ng, still 'fresh' with a view to increasing the chances of success in the management of DNA profiling (Thomson et al 1999).

Besides the levels of DNA samples in a DNA test based on Polymerase Chain Reaction (PCR) are also needed sufficient quality DNA. The quality of the DNA is that DNA is used in the analysis must be in degraded condition. If the DNA is degraded lead to severe primary or annealing cannot stick to the target DNA to be duplicated (Muladno 2002, Rudin, 2002, Yuwono 2006). According Muladno (2002), in order to obtain adequate visualization needed for adequate purity DNA and DNA levels were adequate, so that DNA can be used as material including DNA examination in this case is the identification and paternity tests. So that good quality DNA into fundamental prerequisite for the success of the reaction Polymerase Chain Reaction (PCR) as a whole (Yamada et al 2002). According to Chung et al (2004) suggest that the sensitivity of the Polymerase Chain Reaction (PCR) is a function of the number of cycles and levels as well as the integrity of DNA.

This study used a sample of the urine stains. Urine is the liquid remaining excreted by the kidneys which will then be removed from the body through urination. Urine consists of water with dissolved material in the form of waste products such as urea, salt solvents and volatile organic compounds. On urine dipstick analysis to determine the presence of protein, blood and nitrite in urine as well as to determine the pH and heavy. While microscopic analysis is under microscopic study of urine, to detect the presence of bacteria, red blood cells, white blood cells and epithelial cells. The epithelial cells are the cells that make up the inner wall surface of the kidney and urinary tract (there are 3 types of epithelial cells: squamous, transitional and renal tubular cells). So normal when the cells were detected in the urine. Epithelial cells almost always present in the urine, especially coming from the bladder (urinary vesica), urethra and vagina.

On staining urine, the urine will be lost due to evaporation while these cells will attacg fibers fabrics. Staining urine is attached at room temperature for up to 20 days (according to the old Code of Criminal Procedure detention period in the investigating process). In this study indicate that environmental influences affect the levels of DNA. As is known environmental factors such as humidity and temperature environment is affecting the condition of DNA that are used as ingredients in the field of forensic DNA identification, as well as on other DNA testing in the field.

Environmental factors that cause DNA degraded or degraded DNA. This degradation can be fast or slow, it depends on factors that influence and time of exposure. DNA damages are divided into two types: the damage from the inside, eg caused by reactive oxygen species (ROS), and damage from external factors, such as temperature, humidity etc.

Free radicals, which are often called reactive oxygen species (ROS), also can be formed through metabolic or enzymatic. Oxygen-activated by the free radical-forming agents, such as ionic radiation, can induce DNA lesions that cause defects, mutations and even death. DNA damage is shown by sugar and base sections are easily oxidized, causing degradation and destruction of single-strand, as well as the protein cross-linking. Degradation of DNA bases will produce products such as 8-hidroksiguanin, hydroxymethyl urea, thymine, glycols, open chain thymine and adenine, and other saturated products. Group - the group easily oxidized such as aldehydes, ketones and hydroxyl or molecules that easily turns into a compound having one of these groups appears to be the target of the reactivity of free radicals. Therefore, nucleic acids such as DNA and RNA, which is known to contain many carbohydrates such as ribose (in RNA) and deoxyribose (in DNA), vulnerable to attack by free radicals.

As Watson, Melcher (2000), states that the DNA damage (DNA damage) caused by exposures such as X-rays, chemical agents, spontaneous instability, or by the extremely high temperatures or extreme temperatures, resulting in many types of damage, such as chain damage (strand) DNA (both double strand or single strand), base damage (damage to DNA bases), sugar damage, even the DNA-DNA crosslinks and DNA-protein crosslinks.

Single-strand breakage occurs because part of the sugar is oxidized by hydroxyl radicals. In the physiological condition of hydrogen peroxide and superoxide cannot cause damage to the strand, but in vivo toxicity together with metal catalyst to form Fenton reaction. Cross-linking of DNA by proteins is further hydroxyl radical attacks, which occur on DNA or protein. Although the cross-linking of DNA-protein less dangerous than a single-strand destruction, but its existence cannot be repaired and will probably cause cell death. Free radicals can also cause changes in the DNA, such as hydroxyl bases thymine and cytosine, purine and pyrimidine core opening and disconnection of DNA phosphodiesterase. The damage is not so severe it can be repaired by DNA repair system. However, if severe enough, for example, DNA chains were cut off in many places, the damage can not be repaired.

In the research shows the longer the exposure time that is days 1, 7 and 14 at the locus (CSF1PO, THO1, TPOX and amelogenin) all Ampel in the visualization results of Polymerase Chain Reaction (PCR) with silver staining Polyacrylamid Agarose Composite Gel visible band/band (detected). However, the length of time of exposure days to 20 (maximum in this study) obtained only locus THO1 and TPOX all samples (100%) appear ribbon-band (detected), whereas locus CSF1PO and amelogenin only each - each of the samples (50%) were evident band-band, the other samples did not appear ribbon-band undetected)/vague. This shows that the DNA testing of materials staining urine through the detection of STR loci (CSF1PO, THO1, TPOX) different detection responses obtained at different exposure time that has been given the urine stain samples.

The success of this locus detection is supported by the differences in amplicon product, GC content or guanine-cytosine bonds at each locus. According Muladno (2002), GC content have a high degree of stability against denaturation than the bond between adenine and thymine. The result of the calculation of the ratio of GC content have significant value. Of the loci were examined, the ratio of GC content in the primer sorted from the lowest CSF1PO locus is as follows: 42.61%, TPOX: 56.25%, THO1: 63.83%. So from the research showed that the locus THO1 and TPOX have the same possibility in the success of the examination compared to the STR loci CSF1PO. Also in previous studies that the research Sosiawan (2006) where the sequence of DNA loci success from small to large are TPOX, THO1 and VWA. Meanwhile, according to Mc Cord (2003) as for the success of the locus DNA sequence from small to large adal TPOX, THO1 and VWA. Besides, the use loci CSF1PO, THO1 and TPOX given that these loci is one of the first locus developed by the Forensic Science Service, as well as the loci that have a probability of a match with a ratio of 1 in 50 million (Butler 2003).

This study uses STR analysis (CSF1PO, THO1, TPOX and amelogenin X 106bp; Y 112bp), since most samples of DNA forensic examination, 40% are already experiencing degradation or contamination (Noto-soehardjo 1999b). So with the analysis of Short Tandem Repeat (STR), which has a core sequences of approximately 1 kb (kilobase) is very effective and the value of success is quite high, especially in the degraded DNA will terfragmented (fragmented) by generating short fragments.

Federal Bureau for Investigation (FBI) in synergism with the Combined DNA Index System (CODIS) has designed 16 STR loci for a recommendation in the examination, forensic or paternity identification (Kusuma 2004). The minimum requirements regarding the number of STR loci used for examinations until now there is no agreement. There are several laboratory requires a minimum of three loci for paternity checks or DNA identification, at the University of Airlangga do TDC 7-8 loci (Kusuma 2004), currently in Jakarta (Atmaja 2005) conducted 9 loci plus amelogenin locus in a paternity test. Nidom (2005), say 5 to 6 STR loci have a ratio of 1: 100 billion. So that in principle regarding the number of loci examined were more loci that are used the better value inspection accuracy.

Analysis STR loci in this study using CSF1PO, THO1 and TPOX. The accuracy of research on locusts - this locus has been reported in several studies, among others: the population of chromosomes and sequences of alleles at the locus THO1 (Van Oorshot 1996), the population in Thailand with 8 loci STR including THO1, TPOX, CSF1PO and VWA (Sueblinvong T et al 1999) as well as research Chang - En Pu (1998) resident population of china in Taiwan with STR, research A. Foreman et al (1998) genetic variation in Caucasia, Ching - in May et al (1999) examined the genetic variation in the population of citizens of the Philippines and Thailand were stay in Taiwan using 9 STR loci. While in Indonesia, Novita (2005) examined the pattern of alleles in a population THO1 Batak in Surabaya. STR loci typing method primarily THO1 a reasonable method, powerful and efficient so it is a useful method in forensic cases (Van Oorschot 1996).

While the determination of the gender of molecular biology with amelogenin locus. Amelogenin is a gene that encodes a protein that is found on the sex chromosomes (X and Y). In amelogenin gene are 6 bp deletion in intron 1 homologous X, so that when the PCR amplification of this area with the primer, resulting amplicon 106 bp and 112 bp of chromosome X and Y. It

takes specific observations on the results elektroforese to distinguish the location of the band X and Y only 6 bp, so it only appears to be a thick band (XY/men) and thin (XX/female). Due to the deletion of 6 bp on the X chromosome, so that product X chromosome itself plays a role as a positive control, because according Mannucci et al DNA woman is 100 times larger than the male DNA. While the use of the Y chromosome for forensic purposes is limited by the lack of polymorphic markers. Because the Y chromosome is inherited from father to son without recombinant, the Y chromosome is not variable among individuals and the outcomes of individual markers cannot be combined using the product rule (Buller 2001).

CONCLUSION

There are external factors that affect the environment and the long exposure quantity and quality of DNA from urine stains on clothes, however, still can be an alternative material in forensic identification. Found mapping pattern sequence of the success of STR loci are CSF1PO, TPOX and THO1. This is in accordance with the ratio of content GC respectively. This is consistent with the calculation of the ratio of GC content THO1 and TPOX have the same relative value of the TPOX: 56.25%, THO1: 63.83%., Compared with the 42.61% CSF1PO. There is the ability of STR loci (THO1 & TPOX) 100% (all samples) could still detect DNA in the urine stains until day 20, while locus amelogenin CSF1PO and 106bp-112bp only 50% of the samples were still able to detect DNA staining urine.

REFERENCES

Atmaja DS, 2005, Peranan Sidik jari DNA dalam Bidang Forensik,Seminar Nasional Aplikasi DNA Finger Printing dalam Bidang Kedokteran, 29 Agustus 2005, PS Bioteknologi UGM

Butler,JM,2001, STR Analysis for human testing,STR Typing.Current protocols in Human Genetic Unit.14.8,pp 1-37

Butler,JM, 2003, Forensic DNA Typing, Academic Press, Sandiego-Florida, page 28-30, page 59-96.

Cooper M.G, Hausman Robert E, 2004, The Cell A Molecular Approach, Third Edition, ASM Press Washington, DC.

Fawcet DW,2002, Histology, Edisi 12 Penerbit Buku Kedokteran, EGC Jakarta

Gibson, J.2003, Modern Physiology and Anatomy For Nurses, 1st ed. Published by Arrangement with Blackwell Science Limited, Oxford, page 238-239.

Hammond HA and Caskey CT,1994.Human DNA fingerprinting using STR loci, Methodes Mol.Cell.5:78-86

Henry A.E, 1992, PCR Technology, Principles and Applications for DNA Amplification, W.H.Freeman and Company, New York

Idries, A.M, 1997, Pedoman Ilmu Kedokteran Forensik, Edisi pertama, Jakarta, Penerbit Binarupa Aksara.

Jackson M Kelly, 1990, The Polymerase Chain Reaction, Simposium DNA Probing: Teknik dan Penerapan Klinis, HKKI.

Jusuf M, 2001, GENETIKA I Struktur & Ekspresi Gen, Sagung Seto, Jakarta

Mark A.F, 1991, Forensik DNA Technology, Lewis Publishers inc.

Murray et al, 1997, Biokimia Harper, edisi 24, EGC, hal 366-370

Muladno, 2002, Seputar tehnologi rekayasa genetik, Edisi pertama, Bogor, Pustaka.

Notosoehardjo I, Kuntaman,2002, Teori dasar dan penerapan Praktis PCR,TDC Universitas Airlangga, hal 1-11.

Peter P.B, 2004, Teri's Find a forensic study in authentication, Biro Fine art restoration and forensic studies in art. Pp 23-25.

Watson et.al, 1986, Molecular Biology of The Gene, 4nd.Ed. Cummings Publishing Company,Inc.

Westwood SA & Werret DJ,1990, An evaluation of the Polymerase Chain Reaction method for forensic application, Elsevier Science International, pp 201-215.

Watson D. J.et al, 1988, DNA Rekombinan,Penerbit Erlangga, 1988.

Zainuddin M, 2000, Metodologi Penelitian, Kumpulan kuliah Pascasarjana UNAIR