

Comparative Study of Sperm DNA Isolation Method for Forensic Analysis

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ABSTRACT

Forensic is a multi-discipline science that is used to obtain evidence of various criminal cases, such as rape. DNA analysis on sperm specimen is needed to identify the rapist. However, the success of this analysis depends on the DNA isolation method used. Several methods of DNA isolation from human sperm have been developed, but no method has been proven effective for the forensic analysis need. This study aimed to determine the effective sperm DNA isolation method for forensic analysis. In this study, the DNA of sperm specimens was isolated using three methods: Boiling Water, modified TRIzol, and Chelex-100. The DNA isolation result was visualized using agarose gel electrophoresis method. The concentration and purity of isolated DNA were measured using a Nanodrop by comparing the absorbance of DNA at λ 260 nm and protein at λ 280 nm. The effectiveness of the sperm DNA isolation method was determined based on the concentration and purity of DNA, the specimen volume, the implementation time, and the costs involved. The result showed that the successful methods for isolating sperm DNA were TRIzol and Chelex-100. The quantity of DNA isolated using the modified TRIzol method was 1,5 times higher than Chelex-100 but required 120 times more specimen volume than Chelex-100. From 25 μ l sperm specimens, the concentration of DNA isolated using the Chelex-100 method was 612.6 ng/ μ l with a purity of about 1.7. Therefore, it can be concluded that the Chelex-100 is the most effective method for isolating sperm DNA for forensic analysis.

Keywords:

boiling water; chelex-100;

DNA isolation; sperm;

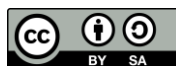
TRIzol

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INTRODUCTION

Forensic is a series of disciplines assisting the criminal justice system ranging from the investigation, prosecution, and court (Roux et al., 2012). Forensic is used to obtain valid evidence in handling various cases such as airplane accident, murder, domestic violence, and rape (Narejo and Avais, 2012; Sandwinata, 2019).

Rape is sexual violence affecting the victim physically and mentally. Authorities are often hard to identify the perpetrator or victim and determine the rape timing to solve this problem (M.S. Lanang et al., 2013). Investigators need to collect physical evidence and laboratory examinations of various biological materials found at the Crime Scene. The biological material often used as evidence of rape cases is sperm (Narejo and Avais, 2012; Jehuda, 2013; M.S. Lanang et al., 2013).

Microscopic examination is a general laboratory test on sperm specimens to check the mortality of spermatozoa. This test is useful to estimate the copulation time, but it cannot prove the identity of the rape perpetrator. Therefore, the identification method using DNA analysis is necessary (Sandwinata, 2019). The forensic expert can compare DNA profiles of samples and suspects through DNA analysis, such as Restriction Fragment Length Polymorphism (RFLP) and Polymerase Chain Reaction (PCR) (Biswas et al., 2017). The success of both methods depends on the quantity and quality of DNA isolated from the sperm specimen.

Various methods to isolate DNA have been developed using several types of human somatic cells, but ineffective for sperm cells. The structure between sperm cells and somatic cells is quite different. The chromatin structure of sperm cells is six times denser than somatic cells (Alarcón-Zúñiga et al., 2016; Darbandi et al., 2018). The sperm chromatin is denser because histone proteins in the nucleus are replaced by protamine to form disulfide bridges (Anvar et al., 2015; Wu et al., 2015). Several methods reported to isolate human sperm DNA are rarely applied in

forensic analysis.

Manuja (2010) reported that Chelex-100 can be used to isolate DNA from buffalo sperm specimen. The method used proteinase K to lyse cells, Dithiothreitol (DTT) to reduce protein, and Chelex-100 to inhibit DNA degradation by DNase and other contaminants affecting the further analysis. The Chelex-100 requires few specimens but has not been applied in human sperm.

Darbandi et al. (2018) successfully modified the TRIzol, so that it is effective to isolate DNA from human sperm specimens. Cell lysis in this method used proteinase K. Then, the Trizol reagent was useful for separating DNA from RNA and protein in acidic conditions. It reported that the TRIzol could produce a good quality and quantity of sperm DNA but required more specimens.

On the other hand, the Boiling Water offered various advantages, such as simple, fast, and reproducible. Moreover, it is not expensive, needs a small specimen volume, and does not require special equipment (Silva et al., 2012). The Boiling Water only uses heat for cell lysis without reagents for DNA purification. Besides, this method has not been applied to human sperm. Therefore, this study aimed to compare these three methods and determine the most effective method to isolate DNA for forensic analysis.

MATERIALS AND METHODS

Instruments

The equipment used in this study included (Innova® 42 New Brunswick Scientific), refrigerator (Panasonic), micropipette (BioRad), microtube (Biologix), nanodrop (Thermo Scientific, Nanodrop 2000 Spectrophotometer), a set of electrophoresis equipment (Mupid-EXU), centrifuge (Eppendorf 5810 R), UV transilluminator (Avegene), vortex (Thermolyne), and water bath (Julabo).

Materials

Then, the materials used were agarose (Thermo Scientific), buffer TAE (BioRad), ddH₂O (Thermo Scientific), DTT (Thermo Scientific), ethanol (Merck), HCl (Sigma-Aldrich), chloroform (Sigma-Aldrich), ladder (Thermo Scientific), loading dye (Thermo Scientific), MgCl₂ (Invitrogen), NaCl (Invitrogen), Na Citrate (Invitrogen), proteinase k (Qiagen), SYBR Safe (Thermo Scientific), tris Base (Invitrogen), TRIzol (Sigma-Aldrich).

Population and Research Samples

Sperm specimen were obtained from one man who was willing involved in this study. The sperms were collected in a clear, free detergents and preservatives container. Then, DNA isolation was done immediately after collecting the sperm specimens.

Research Procedure

Sperm DNA were isolated using three methods and repeated three times. The initial specimen volume used for all methods was 25 μ L, but only the Chelex-100 was successfully isolated the DNA. Therefore, the next process used different specimen volumes according to the procedure described by Manuja (2010), Silva et al. (2012) and Darbandi et al. (2018).

Boiling Water was the first method used. 100 μ L sperm specimens were mixed with 100 μ L ddH₂O in a 1.5 ml microtube. The suspension was homogenized using vortex and heated at 95°C for 15 minutes and centrifuged at 12,000 rpm for 10 minutes. Then, pellets were dissolved with 30 μ L TE Buffer.

TRIzol was the second modified method by Darbandi et al. (2018). 3 ml sperm specimens were allowed to dilute at 37°C for 30 minutes and centrifuged at 3400 rpm for 5 minutes. Then, pellets were resuspended with 1000 μ L sterile phosphate. 1000 μ L lysis buffer was added to the suspension and incubated at room temperature for 15 minutes. The suspension was centrifuged at 3400 rpm for 10 minutes. Pellets were mixed with 500 μ L TRIzol and 50 μ L proteinase-K.

After incubating at 56°C overnight, 500 μ L chloroform was mixed into the suspension and reincubated at 4°C for 15 minutes. Then, it was centrifuged with full speed at 4°C for 15 minutes. 400 μ L supernatant was added with 800 μ L of cold ethanol and 40 μ L of 3M sodium citrate. After incubation at -20°C for 1 hour, the solution was centrifuged with maximum speed at 4°C for 20 minutes. Then, pellets were washed using 600 μ L of 70% ethanol. Next, pellets were dried at room temperature overnight and dissolved in 100 μ L ddH₂O.

The Chelex-100 was the third method used, with the DNA isolation procedure by Manuja (2010). 25 μ L sperm specimens were added with 200 μ L of 5% Chelex-100, 5 μ L proteinase K, and 31 mM of DTT. The suspension was homogenized with vortex and incubated at 56°C for 45 minutes. Furthermore, the suspension was boiled in the water bath for 8 minutes and centrifuged at 10,000 rpm for 3 minutes. The supernatant was separated into new microtubes.

The DNA isolation result using these three methods were visualized by electrophoresis with 1% agarose. The concentration and purity of DNA were measured using the nanodrop. The absorbance of DNA at λ 260 nm was compared with that of protein at λ 230 and 280 nm. The study procedure has received approval from the Health Research Ethics Commission of Stikes Jenderal Achmad Yani with the ethical number 80/KEPK/V/2019.

Data Analysis

In this study, these three methods were compared to one another. Five parameters used determined the effectiveness DNA isolation method, including the concentration and purity of DNA isolated, specimen volume, implementation time, and cost required.

RESULT AND DISCUSSION

DNA isolation from the sperm specimens used three methods included Boiling Water, modified TRIzol, and Chelex-100. The electrophoresis result showed DNA bands in rows 3 and 4. These indicated that sperm DNA failed to be isolated using Boiling Water but was successfully isolated using the other two methods (Figure 1). The electrophoresis results showed that the DNA sperm isolated using the modified TRIzol and Chelex-100 had a large molecular weight and had not degraded.

Although both methods were successfully used, there were differences in quantity and quality of isolated DNA (Table 1). Sperm DNA isolated using the modified TRIzol had a higher concentration (λ 260 nm) than Chelex-100. Furthermore, DNA isolated using the modified TRIzol was purer than Chelex-100, both at ratio λ 260 nm/ λ 280 nm and λ 260 nm/ λ 230 nm.

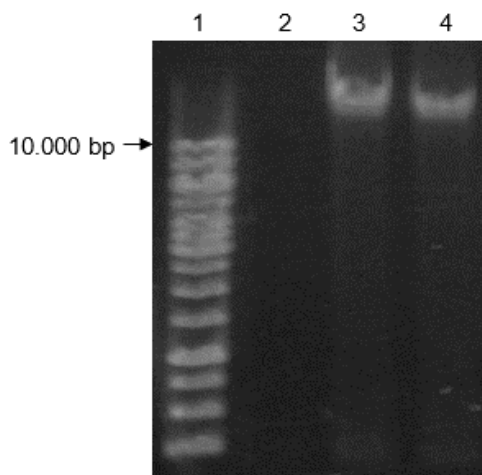


Figure 1. Electropherogram of DNA isolated from sperm specimens.

Ladder 1: Ladder Thermoscientific 1Kb. Ladder 2: Boiling Water. Ladder 3: Modified TRIzol. Ladder 4: Chelex-100.

Besides the concentration and purity of the isolated DNA, this study also compared specimen volume, implementation time, and the cost required of all methods. From these parameters, the Chelex-100 was better than the

modified TRIzol. DNA specimens can be isolated from a small volume using Chelex-100, but it cannot using the TRIzol. Besides, the time and cost involved were also fewer using the Chelex-100.

It is a challenge to determine the appropriate DNA isolation method for sperm specimens. The procedures used in cell lysis and purification during the DNA isolation process from sperm cells are different from somatic cells because of cell structure differences. During spermatogenesis, the sperm cell lost most cytoplasm. Also, it has motile tail development (Darbandi et al., 2018), and protamination (Anvar et al., 2015).

Protamination is the modification of 90-95% histone into protamine that makes sperm chromatin structure six times denser than other cells (Alarcón-Zúñiga et al., 2016; Darbandi et al., 2018). Protamination allows the compaction of genetic information needed for sperm motion and helps the genome protection from oxidation and harmful molecules in the female reproductive tract (Alarcón-Zúñiga et al., 2016).

The presence of disulfide bridges between protamine and outer membrane of sperm cell makes it resistant to chemicals commonly used in somatic cell lysis phase. Besides, at the sperm cell lysis stage, DNA damage can occur due to hyaluronidase. It is found in the acrosome of spermatozoa. This enzyme functions to attack hyaluronic acid when in contact with the ovum. DNA damage during cell lysis can also occur because of mitochondrial spermatozoa (Alarcón-Zúñiga et al., 2016).

In the DNA purification stage, a researcher must notice the fraction composition of non-cellular ejaculatory. This fraction contains zinc protecting the condensation of sperm chromatin, copper, glycogen, and several lipids that function as an energy source in the ejaculation process (Manuja, 2010; Alarcón-Zúñiga et al., 2016). DNA isolated must be free from these components because of inhibiting the PCR process.

Table 1. The Comparison of DNA Isolation Methods from Sperm Specimen

Parameter	Methods		
	<i>Boiling Water</i>	<i>TRIZol Modifikasi</i>	<i>Chelex-100</i>
DNA Concentration (ng/ μ l)	0	907.22 \pm 5.67	612.6 \pm 9.1
DNA Purity (λ 260 nm/ λ 280 nm)	0	1.87 \pm 0.03	1.7 \pm 0.06
DNA Purity (λ 260 nm/ λ 230 nm)	0	2.01 \pm 0.1	1.95 \pm 0.08
Number of Specimens (μ l)	100	3.000	25
Implementation time (hour)	0.5	48	1
Cost required per sample (IDR)	15000	70000	45000

The Boiling Water in this study cannot isolate the sperm DNA even though it was an effective method for bacteria cells (Queipo-Ortuño et al., 2008; Dashti et al., 2009) and yeast (Silva et al., 2012). Boiling Water is the simplest method because it only uses temperature at 95°C to damage membranes and cell walls. This method was ineffective for sperm cells because of the disulfide bridge. Ugale et al. (2015) reported that the disulfide bridges could be broken at a minimum temperature of 100°C without chemical compound help.

The modified TRIZol could isolate sperm DNA with good results. This method used guanidinium thiocyanate, phenol, and chloroform (Rio et al., 2010). Guanidinium thiocyanate and phenol function to dissolve biological materials and denature proteins. Moreover, chloroform is for the separation phase. The addition of proteinase K and incubation time during the test could improve the quality of DNA isolated (Darbandi et al., 2018).

Sperm DNA could be isolated with this method, but it required more specimen volumes, at 3 ml. It becomes an obstacle when doing forensic analysis. Furthermore, the forensic laboratory examination uses the remaining specimens at the crime scene. So finding 3 mL of sperm in the victim's body or crime scene has a small probability.

On the other hand, the Chelex-100 can isolate sperm DNA with a few specimen volumes. It uses proteinase K to break the peptide bonds, DTT to destruct protein disulfide bonds in sperm, and Chelex-100 to protect DNA because it has a high affinity for polyvalent metal ions (Manuja,

2010). Walsh et al. (2013) reported that Chelex-100 has many advantages, such as simple, fast, and does not involve organic solvents.

The concentration of DNA isolated by Chelex-100 was lower than that of modified TRIZol, but it was still sufficient for the PCR. Lorenz (2012) reported that the common concentration of mammalian genomic DNA used for PCR was 100 – 250 ng/ μ l. The purity of DNA isolated using Chelex-100 was also lower than that of the modified TRIZol, but the purity value was still relatively good. For PCR test, DNA with ratio value λ 260 nm/ λ 280, at 1-2 (ideal 1.8-2) (Sandwinata, 2019) and ratio value λ 260 nm/ λ 230 nm above 1.9 (Schiebelhut et al., 2017).

CONCLUSION

Based on these three methods comparisons, the Chelex-100 was the most effective DNA isolation method from human sperm specimens for forensic analysis. From 25 μ l of sperm specimens, the DNA concentration isolated by Chelex-100 was 612.6 ng/ μ l with a purity of about 1.7. The Chelex-100 has several advantages, including little specimen volumes, fast processing time, and effective cost.

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