

## **Analysis of Deoxyribonucleic Acid (DNA) Purity a Specimen of Blood from Menstrual Blood Specimens in Women of Reproductive Age**

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### **Abstract**

Menstrual blood is multiple materials fluid consisting of blood, vaginal fluid, and endometrial cells from the uterine wall that flows out during menstruation. All cells with a nucleus contain nucleic acid genetic material. Nucleic acids have an essential function in organisms' proliferation and cellular development. The most common nucleic acids are deoxyribonucleic acid and ribonucleic acid. Twente menstrual blood samples were obtained from women of reproductive age. Menstrual blood was collected by a specially designed menstrual blood collector using filter paper. The DNA specimens were extracted using QIAamp DNA Mini Kit (Cat No: 51304; Qiagen manufacture). The purity of the DNA extract was measured with the Thermo scientific Nano-Drop microvolume Spectrophotometer instrument. The statistical data analysis used univariate, and the frequency distribution data and average purity score were performed in this study. We found that the mean  $\pm$  SD DNA concentration is  $122.34 \pm 32.30$ , the purity value at the  $\text{\AA}260/280$  wavelength is  $1.90 \pm 0.06$  and  $\text{\AA}260/230$  is  $1.87 \pm 0.69$ . Menstrual blood samples collected in special feminine sanitary napkins made from filter paper are effective in storing DNA molecules. Furthermore, the DNA extraction method in this study produces optimal concentrations and purity.

**Keywords:** Deoxyribonucleic Acid, DNA, Menstrual blood, Nucleic Acid.

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### **1. Introduction**

Menstrual blood is a potential specimen for finding biomarkers for reproductive disorders [1]. Cells with nuclei contain genetic material. Nucleic acids regulate the biological

development of all cellular life forms [2]. The most common nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) [3].

DNA molecules are removed from the nucleus using appropriate isolation techniques [4]. Retrograde menstruation in endometriosis disease is the implantation of physiological endometrial cells to another place retrogradely through the fallopian tubes into the pelvic cavity and attack as well as multiply in the surrounding tissue [5-8]. It's a consideration for using menstrual blood as a sample [9-12].

In addition, there were differences in mRNA expression of genes encoding pain in the endometrium between people with endometriosis and those without endometriosis [13]. Furthermore, a study found that there were variations in the mRNA expression of the Hemoglobin Alpha (HBA), Matrix Metalloproteinase 7 and 11 (MMP 7 and 11) genes between menstrual blood and peripheral blood [14]. Menstrual blood collections have been utilized in genomic, proteomic, and imaging [15]. There are several ways to collect menstrual blood, such as using a menstrual cup, which is a bowl-shaped menstrual blood collection device that is inserted into the vagina during menstruation [16]. In addition, a previous study reported the utilization of tampons to gather menstrual blood for proteomic analysis [17]. Nevertheless, the previous research didn't offer a DNA extraction process using menstrual blood specimens obtained on filter paper. In general, there are three basic requirements for DNA isolation, namely lysing cell members to expose DNA; separation of DNA from molecular and other substances such as RNA, lipids, proteins, and carbohydrates; and DNA recovery [18-23]. Nucleic acid isolation is said to be optimal if the procedure carried out produces pure and intact nucleic acid [23-24]. This research aims to purify the purity of menstrual blood DNA extract collected on filter paper using a modified DNA isolation technique in the lysis and purification process.

## 2. Materials and methods

### 2.1. Materials

Feminine sanitary napkins are made from filter paper and cotton cloth. The blotting paper or absorbent paper on the sanitary napkins is of the Whatman number 1 type. The menstrual blood collected in the sanitary napkins is menstrual blood on the second day of the menstrual phase. 1.5 ml collection tube, micropipette, tips, centrifuge, tissue crusher, and other tools used for the DNA extraction process.

### 2.2. Sample Collection

Menstrual blood samples were collected from 20 reproductive-age women who were menstruating. Participants were asked to collect menstrual blood on the second or third day of the menstrual phase. A feminine sanitary napkin made from filter paper is placed on the underwear for 1 hour. The first layer of sanitary napkins containing menstrual blood is cut into small pieces with a diameter of around 0.5 cm, then stored in a sterile 1.5 ml tube at -20°C.

### 2.3. The DNA extraction

Weigh approximately 150 g of filter paper containing menstrual blood. The filter paper crushed used a homogenizer along with cell lysis fluid. DNA extraction was performed using the QIAamp DNA Mini Kit (Cat No: 51304; manufacturer Qiagen, Germany). The cell lysis process was carried out in 2 stages, namely the mechanical and enzymatic stages using proteinase K (Qiagen, Germany). The Ocktariyana et al., 2024

centrifugation speed used in the lysis stage was 8,000 rpm. Then, the purification stage is carried out following the kit protocol with a centrifugation speed of 13,000 rpm. DNA concentration is calculated using the formula:  $[DNA] = \frac{A_{260}}{x} \times 50 \times \text{dilution factor}$  Note:  $A_{260}$ : Absorption value at 260 nm 50: a solution with an absorption value of 1.0 is equivalent to 50µg of double-stranded DNA per ml.

### 2.4. Purity measurement

The purity Measures of deoxyribonucleic acid samples using absorbance fluorescent-labeled spectrophotometry. DNA concentration and purity measurements were carried out using NanoDrop. The NanoDrop tool used is the Thermo scientific Nano-Drop microvolume Spectrophotometer. The principles of spectrophotometry techniques are applied in the NanoDrop tool. The level of purity of nucleic acids can be estimated by determining the ratio of  $\lambda_{260}$  to  $\lambda_{280}$ . DNA purity values usually range from 1.8-2.0 in  $1 \times 10^{-6}$  liters (1µL) microliter of DNA extract solution.

### 2.5. Statistical analysis

We determined DNA purity and concentration by univariate analysis. Data was shown using tabulation of the average score of concentrations and the purity score.

### 2.6. Ethical approval

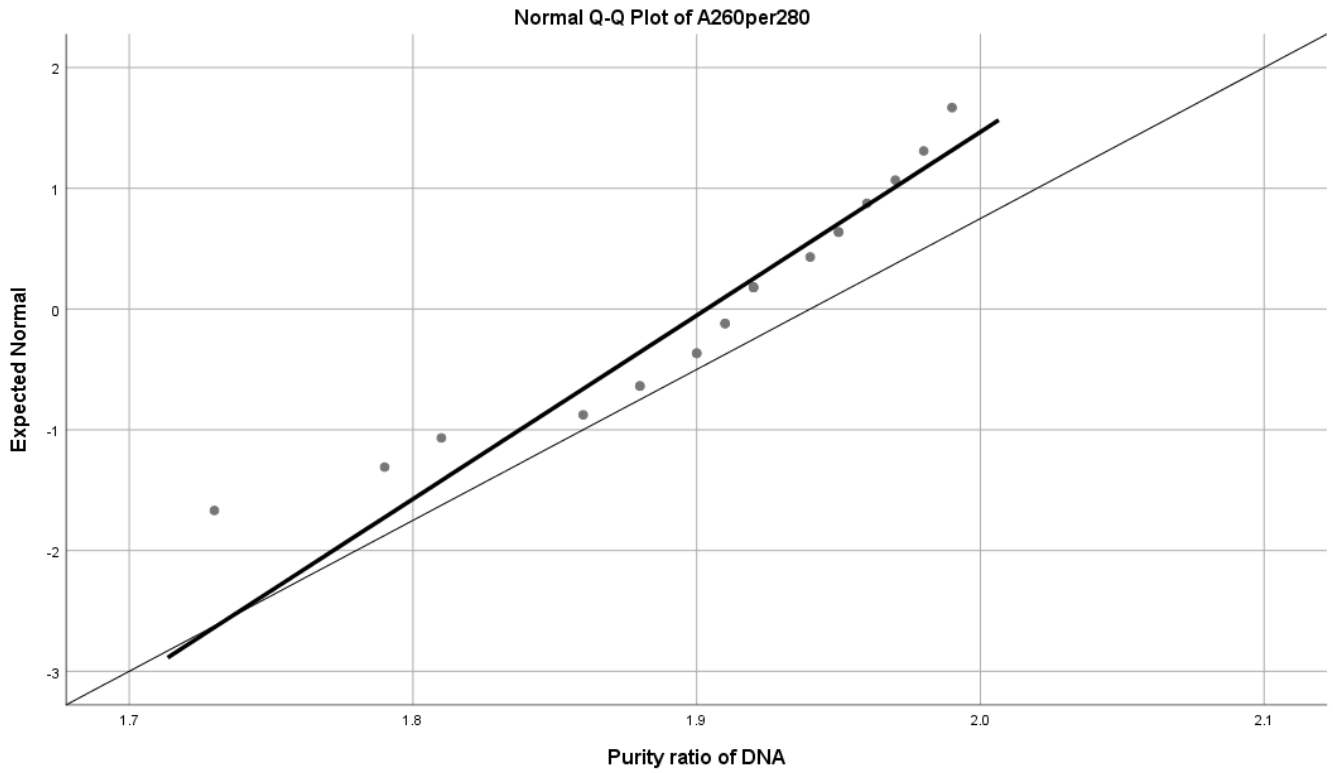
This study was approved for ethical exemption from Dr. Mohammad Hoesin Palembang Centre of Hospital of Ministry of Health with No.DP.04.03/D.XVIII.6.11/ETIK/94/2023. All participants agreed as respondents in this research by signing the informed consent form.

## 3. Results and Discussions

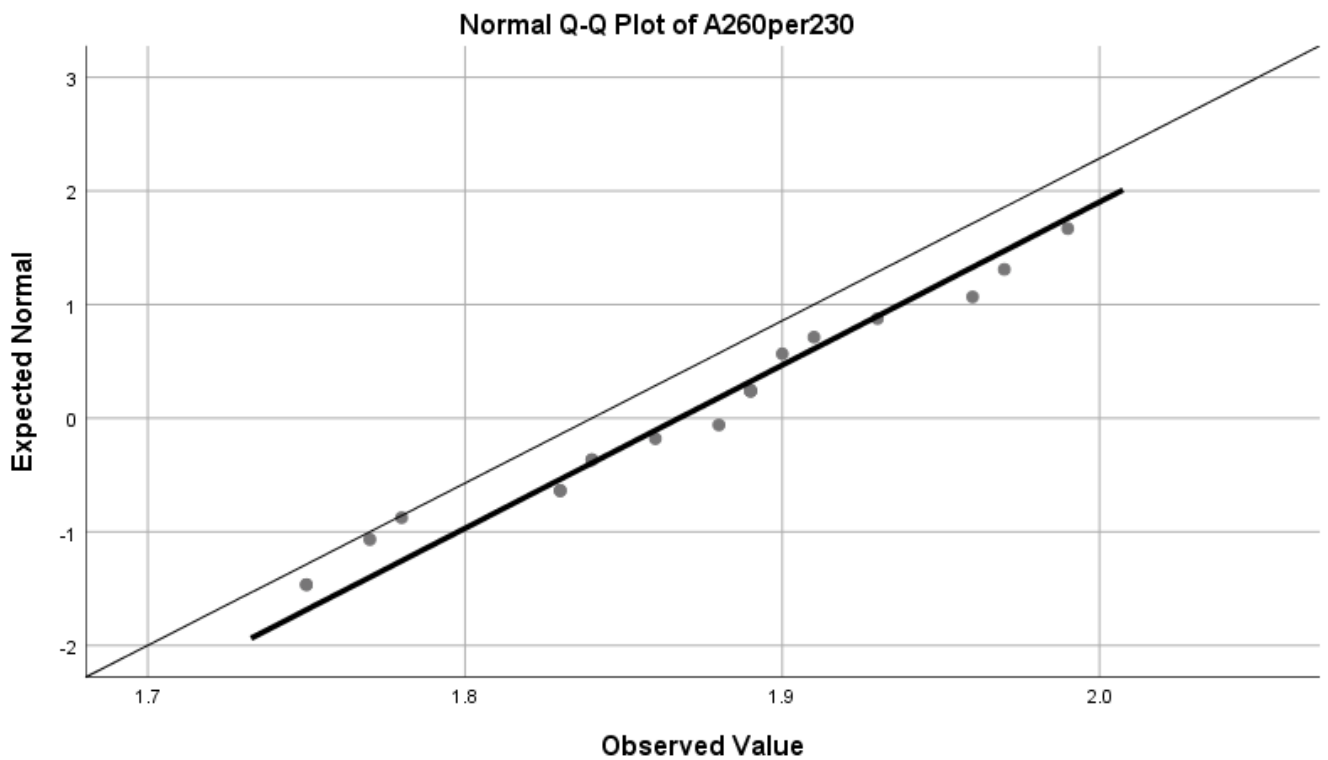
This study found the DNA concentration and purity values of menstrual blood specimens collected on filter paper (Table 1). Justification for DNA purity results includes DNA protein contamination if the purity is lower than 1.7; Pure DNA if the purity is 1.7 to 2.0; and DNA contaminated with RNA if above 2.0. DNA is the genetic material in humans and almost any other organisms [25]. DNA analysis plays an important role in understanding the mechanisms of life and the diseases that arise [3]. In this study, we used filter paper to absorb menstrual blood. The function of using filter paper is to maintain the stability of the specimen so that it remains in good condition until it reaches the laboratory. Based on Table 1, it is known that the mean  $\pm$  SD DNA concentration is  $122.34 \pm 32.30$ , the purity value at the  $A_{260}/280$  wavelength is  $1.90 \pm 0.06$  and  $A_{260}/230$  is  $1.87 \pm 0.69$ . In addition, in Figure 1 (a) & (b), the results of the data normality test using the Shapiro-Wilk test showed that the purity value of the menstrual blood DNA specimen has a homogeneous purity at wavelengths of  $A_{260}/280$  and  $A_{260}/230$  ( $p = 0.056$  and  $p = 0.498$ , respectively). The Shapiro-Wilk test for normality of data is declared homogeneous if the p-value is  $> 0.05$ . Using filter paper in this research was effective in obtaining adequate DNA concentrations. This is thought to be due to the filter paper's optimal absorption ability, and it protects blood cells and remains stable even when stored at room temperature. Petrini (2012) stated that the first use of filter paper occurred more than 50 years ago. Prior (1990) in his research carried out a simple method of collecting and storing whole blood samples using filter paper.

**Table 1:** Concentration and purity value of DNA in menstrual blood specimen.

Samples	Concentration (ng/μl)	Purity value	
		Å260/280	Å260/230
1	153.00	1.99	1.89
2	190.20	1.91	1.93
3	98.20	1.91	1.89
4	129.50	1.92	1.90
5	55.60	1.92	1.77
6	73.20	1.95	1.88
7	117.20	1.90	1.75
8	130.40	1.81	1.83
9	143.00	1.88	1.91
10	157.90	1.92	1.78
11	136.10	1.94	1.99
12	123.10	1.96	1.84
13	61.40	1.95	1.97
14	143.60	1.88	1.75
15	115.00	1.86	1.89
16	109.10	1.79	1.84
17	116.50	1.73	1.83
18	131.10	1.97	1.89
19	139.80	1.90	1.86
20	122.80	1.98	1.96
mean ± SD	122.34 ± 32.30	1.90 ± 0.06	1.87±0.69
Median (Min-Max)	126.27 (55.60 - 190.20)	1.91 (1.71-1.99)	1.88 (1.75-1.99)



**Figure 1 (a):** The homogenous curve of DNA purity value  $\text{A}260/280$  in menstrual blood specimens.



**Figure 1 (b):** The homogenous curve of DNA purity value  $\text{A}260/230$  in menstrual blood specimens.

Hollegaard et al., (2011) stated that blood collected on filter paper could be stored at room temperature and is easy to transport to the laboratory with a low risk of biohazard can be applied in areas that do not have adequate infrastructure [26-27]. Filter paper contains more than 90 percent cellulose fiber which has high absorption capacity [28]. Whatman number 1 is commonly used to separate nucleic acids from whole blood specimens in molecular diagnostics of malaria [29]. Several previous studies stated that there have been no reports regarding the use of filter paper to collect menstrual blood. In line with Wasniewski et al., (2014), they used filter paper as a blood transport medium to assess the effectiveness of the oral rabies vaccine by detecting antibody titers in fox and raccoon-lying animals [30]. In addition, Choi et al (2014) have developed the use of filter paper for biobanking [31]. Wijayanti et al., (2019) stated that filter paper is quite good to use as an alternative blood transport medium for examining rabies antibody titers [32]. In this study, we added cell lysis solution, and destroying it with a homogenizer aims to return the blood components absorbed on the filter paper back into liquid form at a stable pH and help break down the cells. Wang (2014) stated that filter paper has the hydrophobic nature of the paper so that the liquid flow on the paper can be patterned towards hydrophilic flow channels and is ultimately able to remove the components it absorbs [33]. In addition, adding absolute ethanol at the extraction stage aims to precipitate DNA in the form of precipitates and clean nucleic acids from salt flakes that come from the buffer due to the extraction process [34]. Furthermore, we added a purifying or washing solution in this invention aimed at purifying nucleic acids from cell extracts and other impurities. The purification step is carried out several times and is followed by centrifugation at maximum speed (13,000 rpm) for 30 seconds to produce pure nucleic acid [35].

#### 4. Conclusions

Menstrual blood collected on filter paper with adequate DNA extraction and appropriate modification processes can produce optimal DNA concentration and purity that meets standards.

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