International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

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

Ocktariyana[1,2], Refai[2], Asmarinah[3,4], Andon Hestiantoro[5], Anisah Nida'ul Haq[6], Jackson Mandala[7], Irsan Saleh[8], Fatimah Usman[9], Yusuf Effendi[9], Adnan Abadi[9]

[1]Department of Midwifery, Poltekkes Kemenkes Palembang, Palembang, Indonesia.

[2]Department of Medical Laboratory Technology, Poltekkes Kemenkes Palembang, Palembang, Indonesia.

[3]Medical Biology Department, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.

[4]Doctoral of Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.

[5]Department of Obstetrics and Gynecology, Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

[6]Magister Program of Biomedicine, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia.

[7]Residency Program Of Obstetrics And Gynecology, Faculty of Medicine Universitas Sriwijaya, Palembang, Indonesia.

[8]Department of Pharmacology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia.

[9]Departement of Obstetrics and Gynecology, Faculty of Medicine Universitas Sriwijaya, Palembang, dr. Mohammad Hoesin General Hospital, Palembang, Indonesia.

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 ± SD DNA concentration is 122.34 ± 32.30, the purity value at the Å260/280 wavelength is 1.90 ± 0.06 and Å260/230 is 1.87 ± 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.

Full length article **Corresponding Author*, email: ocktariyana@gmail.com

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^{o}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 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] = Å260 x 50 x dilution factor Note: Å260: 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 λ260 to λ280. DNA purity values usually range from 1.8-2.0 in 1 x 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 Å260/280 wavelength is 1.90 $\pm$ 0.06 and Å260/230 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 Å260/280 and Å260/230 ($p = 0.056$ 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.

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 $\pm$ SD	122.34 $\pm$ 32.30	1.90 $\pm$ 0.06	1.87$\pm$0.69
Median (Min-Max)	126.27 (55.60 - 190.20)	1.91 (1.71-1.99)	1.88 (1.75-1.99)

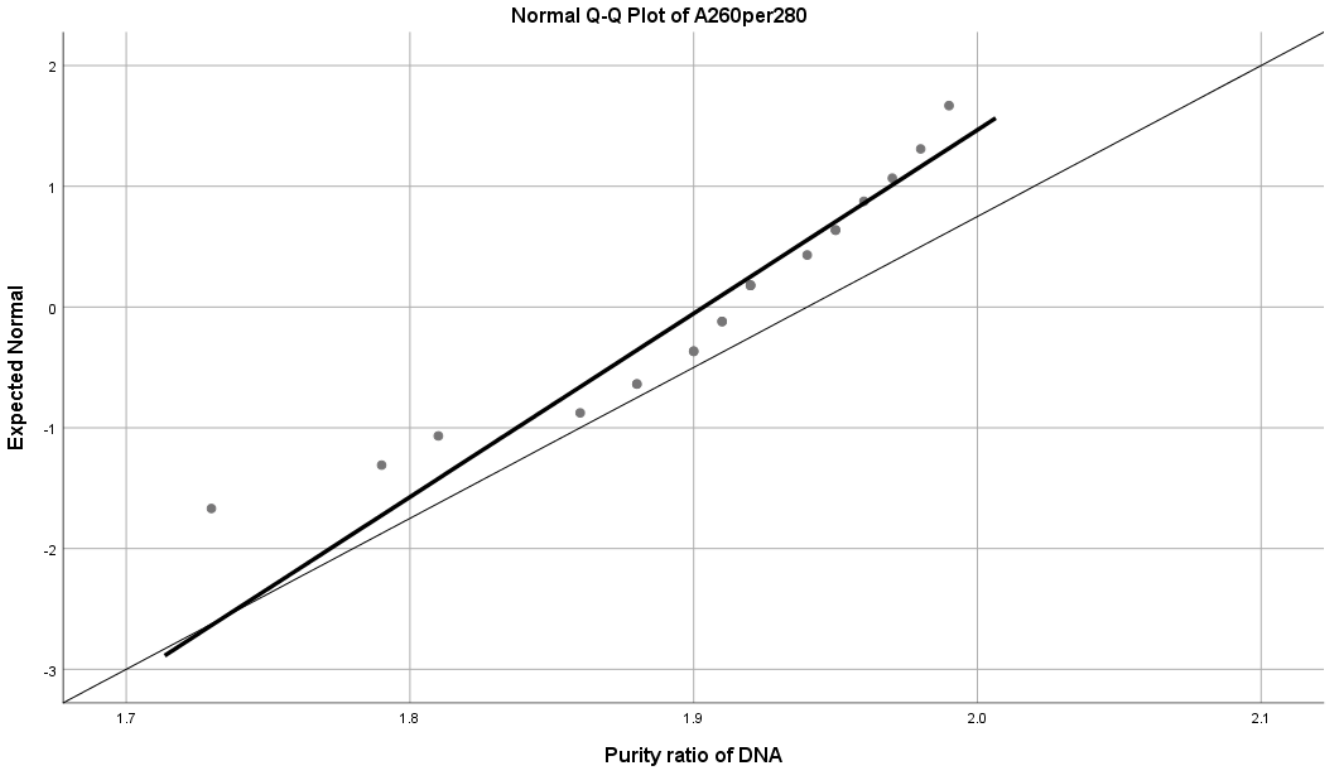

Figure 1 (a): The homogenous curve of DNA purity value Å260/280 in menstrual blood specimens.

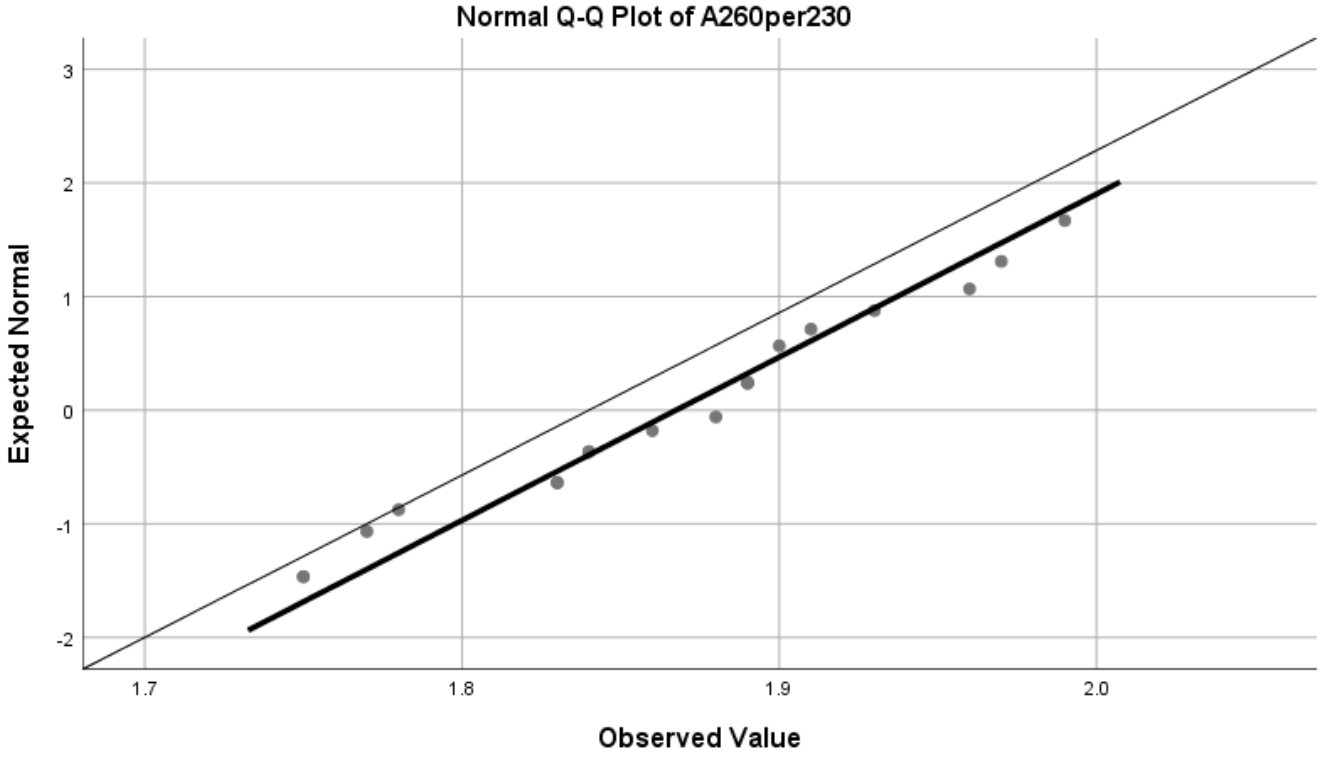

Figure 1 (b): The homogenous curve of DNA purity value Å260/230 in menstrual blood specimens.

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. Conclusion

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

References

[1] R. Chodankar, H. O. D. Critchley. (2019). Biomarkers in abnormal uterine bleeding. Biology of Reproduction. 101 (6): 1155–1166.

[2] A. C. Rowat, J. Lammerding, H. Herrmann, U. Aebi. (2008). Towards an integrated understanding of the structure and mechanics of the cell nucleus. Bioessays. 30 (3): 226–236.

[3] H. M. James. (2016). Overview of Deoxyribonucleic Acid (DNA). INOSR Scientific Research. 2: 1–6.

[4] S. Minchin, J. Lodge. (2019). Understanding biochemistry: structure and function of nucleic acids. Essays in Biochemistry. 63 (4): 433–456.

[5] J. A. Sampson. (1927). Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. American Journal of Obstetrics and Gynecology. 14 (4): 422–469.

[6] Y. B. Aznaurova, M. B. Zhumataev, T. K. Roberts, A. M. Aliper, A. A. Zhavoronkov, C. Meuleman, C. Tomassetti, M. G. D. V. Magro, B. V. Cleynenbreugel, A. D'Hoore, T. D'Hooghe, C. Chapron, A. Bourret, N. Chopin, B. Dousset, M. Leconte, D. Amsellem-Ouazana, D. Ziegler, B. de Borghese, P. Datta. (2014). Molecular aspects of development and regulation of endometriosis. Reproductive Biology and Endocrinology. 12 (1): 50.

[7] Y. Afshar, J. Hastings, D. Roqueiro, J. W., Jeong, L. Giudice, A. T. Fazleabas. (2012). Changes in eutopic endometrial gene expression during the development of experimental endometriosis in the baboon. Fertility and Sterility. 98 (3): S216.

[8] R. O. Burney, L. C. Guidice. (2012). Pathogenesis and Pathophysiology of Endometriosis. Fertility and Sterility. 98 (3): 1–19.

[9] M. Al-Jefout, G. Dezarnaulds, M. Cooper, N. Tokushige, G. Luscombe, R. Markham, I. Fraser. (2009). Diagnosis of endometriosis by detection of nerve fibres in an endometrial biopsy: a double-blind study. Human Reproduction. 24 (12): 3019–3024.

[10] A. G. Obstet, S. Ping, C. Ma, P. Liu, L. Yang, X. Yang. (2015). Molecular mechanisms underlying endometriosis pathogenesis revealed by bioinformatics analysis of microarray data. Archives of Gynecology and Obstetrics.

[11] A. Zahrah, R. Muharam, S. M. ML, I. E. Deraya. (2021). MRNA expression and DNA methylation level of the MMP-2 gene in peritoneal endometriosis. Journal of the Pakistan Medical Association. 71 (2): S112–S115.

[12] I. E. Deraya, A. Hestiantoro, R. Muharam, M. L. Marwali, A. S. As, A. K. Harzif, G. Pratama, A. Zahrah. (2020). Analysis of mRNA expression and DNA methylation level of RAC1 gene encoding focal adhesion molecule in endometrial and peritoneal endometriosis. Asia-Pacific Journal of Molecular Biology and Biotechnology. 28 (2): 43–49.

[13] N. Hikmawati, A. Hestiantoro, R. Muharam, M. L. Marwali, A. Surur, T. Aninditha, G. Pratama, A. Zahrah, N. F. Naura. (2021). Analysis of DNA methylation level and mRNA expression of transient receptor ankyrin member 1 (TRPA1) in endometriosis-associated pain. Asia-Pacific Journal of Molecular Biology and Biotechnology. 29 (3): 1–10.

[14] A. M. Moawad, H. S. zaghlol, M. H. Abdelsalam, A. Abdelfattah, D. Sabry, A. Atef. (2018). Differential genes expression biomarkers for menstrual and peripheral blood stains analysis. Egyptian Journal of Forensic Sciences. 8: 1–6.

[15] V. A. Duong, J. M. Park, H. J. Lim, H. Lee. (2021). Proteomics in forensic analysis: Applications for human samples. Applied Sciences. 11 (8): 3393.

[16] A. M. V. Eijk, G. Zulaika, M. Lenchner, L. Mason, M. Sivakami, E. Nyothach, H. Unger, K. Laserson, P. A. Phillips-Howard. (2019). Menstrual cup use, leakage, acceptability, safety, and availability: a systematic review and meta-analysis. The Lancet Public Health. 4 (8): e376–e393.

[17] H. Yang, B. Zhou, M. Prinz, D. Siegel. (2012). Proteomic analysis of menstrual blood. Molecular & Cellular Proteomics. 11 (10): 1024–1035.

[18] R. Markham. (1955). Nucleic acids, their components, and related compounds (pp. 246-304). Springer Berlin Heidelberg.

[19] K. S. Kirby. (1964). Isolation and fractionation of nucleic acids. Progress in nucleic acid research and molecular biology. 3: 1-31.

[20] A. Milling, N. C. Gomes, M. Oros-Sichler, M. Götz, K. Smalla. (2004). Nucleic acid extraction from environmental samples. In Molecular microbial ecology (pp. 17-36). Taylor & Francis.

[21] N. Ali, R. D. C. P. Rampazzo, A. D. T. Costa, M. A. Krieger. (2017). Current nucleic acid extraction methods and their implications to point-of-care diagnostics. BioMed research international, 2017.

[22] A. Fleck, H. N. Munro. (1966). The determination of nucleic acids. Methods of biochemical analysis. 14: 113-176.

[23] G. Lucena-Aguilar, A. M. Sánchez-López, C. Barberán-Aceitun, J. A. Carrillo-Ávila, J. A. López-Guerrero, R. Aguilar-Quesada. (2016). DNA Source Selection for Downstream Applications Based on DNA Quality Indicators Analysis. Biopreservation and Biobanking. 14 (4): 264–270.

[24] S. C. Tan, B. C. Yiap. (2009). DNA, RNA, and protein extraction: The past and the present. Journal of Biomedicine and Biotechnology, 2009.

[25] A. Mashaghi, A. Katan. (2013). A physicist's view of DNA. ArXiv Preprint ArXiv:1311.2545.

[26] B. G. Winkel, M. V. Hollegaard, M. S. Olesen, J. H. Svendsen, S. Haunsø, D. M. Hougaard, J. Tfelt-Hansen. (2011). Whole genome amplified DNA from stored dried blood spots is reliable in high resolution melting curve and sequencing analysis. BMC Medical Genetics. 12.

[27] M. V. Hollegaard, J. Grove, J. Grauholm, E. Kreiner-Møller, K. Bønnelykke, M. Nørgaard, T. L. Benfield, B. Nørgaard-Pedersen, P. B. Mortensen, O. Mors, H. T. Mors, Z. B. Harboe, A. D. Børglum, D. Demontis, T. F. Ørntoft, H. Bisgaard, D. M. Hougaard. (2011). Robustness of genome-wide scanning using archived dried blood spot samples as a DNA source. Springer. 12.

[28] H. T. Sahin, M. B. Arslan. (2008). A Study on Physical and Chemical Properties of Cellulose Paper Immersed in Various Solvent Mixtures. International Journal of Molecular Sciences. 9 (1): 78–88.

[29] S. A. Al-Harthi, M. B. Jamjoom. (2008). PCR assay in Malaria diagnosis using filter paper samples from Jazan region, Saudi Arabia. Journal of the Egyptian Society of Parasitology. 38 (3): 693–706.

[30] M. Wasniewski, I. Almeida, A. Baur, T. Bedekovic, D. Boncea, L. B. Chaves, D. David, P. De Benedictis, M. Dobrostana, P. Giraud, P. Hostnik, I. Jaceviciene, S. Kenklies, M. König, K. Mähar, M. Mojzis, S. Moore, S. Mrenoski, T. Müller, F. Cliquet. (2016). First international collaborative study to evaluate rabies antibody detection method for use in monitoring the effectiveness of oral vaccination programmes in fox and raccoon dog in Europe. Journal of Virological Methods. 238: 77–85.

[31] E. H. Choi, S. K. Lee, C. Ihm, Y. H. Sohn. (2014). Rapid DNA Extraction from Dried Blood Spots on Filter Paper: Potential Applications in Biobanking. Osong Public Health and Research Perspectives. 5 (6): 351–357.

[32] R. Wijayanti, R. Damayanti, S. Murtini, B. K. Pertanian, K. Pertanian, J. R. H. No, J. Selatan, D. I. P. H. K. M. V. F. K. Hewan, I. P. Bogor, J. R. Dramaga. (2017). Filter Paper as Blood Media Transport for Detection of Antibodies to Rabies.

[33] J. Wang, M. A. Sc (2014). Printing and characterization of inks for paper-based biosensors (Doctoral dissertation).

[34] S. Shaomianah. (2020). Variations in solubilization precipitation in RNA extraction using the trizole method and their effect on RNA purity.

[35] M. Faatih. (2009). Isolation and digestion of chromosomal DNA. 10 (1).