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Comparison of DNA Yield and Quality From Two Blood Extraction Kits

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Abstract: A high-quality isolated sample of deoxyribonucleic acid (DNA) is essential for the success and reliability of various molecular genetic analyses. This paper presents the results of a comparative analysis of the concentration and quality of extracted DNA from 200 samples of human blood using two different commercial kits: Qiagen DNeasy Blood & Tissue Kit and High Pure PCR Template Preparation Kit – Roche Diagnostics**.** Concentration and quality of isolated DNA were measured by spectrophotometric (NanoDrop One) and fluorometric methods (Qubit 2.0). The average concentration of extracted DNA by the Qiagen kit was 45.06ng/µl±17.75, while with the Roche kit it was 20.97ng/µl±8.8. The quality of DNA was presented as the ratio of absorbance at 260nm and 280nm. The obtained mean value of $A_{260/280}$ for the Qiagen kit was 1.8, and for the Roche kit, $A_{260/280}$ was 1,66. The concentration obtained by the Qiagen kit was 2.15 times higher than the concentration obtained by the Roche kit. Concentration measured by NanoDrop was 2,33 times higher than values obtained by Qubit for the same samples. Both kits showed good quality and concentration of isolated DNA, but the Qiagen kit has superior performance in both analyzed categories. Measurement with Qubit was more specific than measurement with NanoDrop.

Keywords: DNA isolation, Roche, Qiagen, DNA quality.

INTRODUCTION

Purity and concentration of DNA isolates are key parameters for the success of experimental and diagnostic techniques such as PCR, sequencing, quantitative PCR analysis, and CGH array. A properly performed isolation process directly affects the purity of the isolated DNA as well as the concentration of DNA in the isolate. A careful isolation process reduces the presence of unwanted molecules and substances that can interfere with the accuracy of the results. This becomes especially important in experiments where high specificity of identification and analysis of target nucleic acids is required (Lojo-Kadrić, 2018). Maintaining the integrity of the molecule during isolation is another important factor. A properly preserved structure of the DNA molecule is crucial for preserving the genetic information during further experimental steps. This is especially important to the optimization of polymerase chain reactions (PCR), where quality samples contribute to increasing the efficiency and reliability of these reactions. The quality and concentration of the isolate are necessary to achieve consistency and reproducibility of results and the validity of scientific research. Overall, achieving a high standard of isolation of nucleic acids plays a key role in providing a reliable foundation for various molecular analyses. There are several techniques available for DNA extraction, including organic extraction, the salting-out method, extraction using silica-based columns, and automatic isolation. In most clinical laboratories, silica column-based methods are the preferred approach due to their convenience and efficiency. The selection of an extraction method depends on the nature and quantity of the samples, as well as the specific molecular assays planned for downstream analysis (Lojo-Kadrić, 2018). Despite differences among methods, all DNA isolation protocols share common steps: cell lysis, separation

of DNA from debris and proteins, precipitation, purification, and finally resuspension in a suitable buffer. Silica column-based techniques utilize kits that contain columns filled with a silica matrix, which selectively binds DNA under specific conditions. Other cellular components are washed away, resulting in a purified DNA sample suitable for further analysis. This method is used to isolate DNA from various samples (Mardan-Nik, 2019; Psifidi, 2015; Sarnecka, 2019; Tagliaferro, 2021; Tolosa, 2007).

MATERIALS AND METHODS

Patients

In this work, data on concentrations and purity of isolated DNA taken from the protocols and databases of the Medical Genetics Department of the University Clinical Center of the Republic of Srpska (UCCRS) in Banja Luka were used. Consent for the use of data for the preparation of this paper was given by the Ethics Committee UCCRS under number 01-19-498-2/23. Personal data of all patients were anonymized according to the recommendations of the ethics committee.

The study included a total of 200 patients, all of whom were healthy individuals with normal leukocyte counts. DNA isolation was performed using two different commercially available extraction kits to allow for comparative analysis.

Isolation of DNA

Isolation of DNA was performed from venous blood obtained by venipuncture of 2 ml of blood in vacutainers with 3.8% Na-citrate as an anticoagulant. Genomic DNA was isolated from the patients' lymphocytes with two different commercial kits. In the first hundred analyzed patients (Group A), DNA isolation was performed with Qiagen DNeasy® Blood & Tissue Kit (Qiagen, 2023), and in the second 100 (Group B) with High Pure PCR Template preparation Kit - Roche diagnostics (Roche, 2023) according to the manufacturer's recommended protocols (Qiagen, 2023, Roche 2023). Isolation procedures of both kits are presented in **Table 1**.

Table 1. Isolation procedures by Qiagen DNeasy® Blood & Tissue Kit and ROCHE High Pure PCR Template preparation Kit

Qiagen	Roche
200 µl blood	200 µl blood
20 µl proteinase K	40 µl proteinase K
200 µl AL buffer, vortex	200 µl BB, vortex
Incubation 56°C 10 minutes	Incubation 70°C 10 minutes
200 µl ethanol (96-100%), vortex transfer to the filter tube	100 µl isoprpanol, vortex, transfer to filter tube
CF, 1 min. 8000 rpm	CF 1min. 8000 rcf
500 µl AW1 buffer	500 µl Inhibitor removal buffer
CF, 1 min. 8000 rpm	CF 1min 8000 rcf
500 µl AW2 buffer	500 µl Wash buffer
CF 3 min 14000 rpm	CF 1 min. 8000 rcf
200 µl AE buffer	500 µl Wash buffer
Incubation 1 min.	CF 1 min. 8000 rcf
CF, 1 min. 8000 rpm	CF 10 sec. 13000 rcf
Purified Template DNA	200 µl Elution Buffer
	CF 1min 8000 rcf
	Purified Template DNA

In the isolation process, all recommendations for good laboratory practice have been implemented.

Quantification of DNA

The concentration of DNA in the isolate was determined using both spectrophotometric and fluorometric methods.

NanoDrop One, Thermo Scientific, was used for spectrophotometric measurement of DNA concentration. DNA concentration was obtained by measuring the absorbance at 260 nm, while the purity of isolated DNA was shown through the absorbance ratio $A_{260/280}$ and $A_{260/230}$. DNA concentration is expressed in ng/μL.

Samples with an $A_{260/280}$ ratio of 1.8-2.0 were considered pure, while ratios below 1.6 may indicate the presence of contaminants like proteins and phenol. Additionally, the $A_{260/230}$ ratio can also be used to assess purity, with values greater than 1.5 generally indicating a pure sample. Lower values indicate the presence of unwanted organic compounds like Trizol, phenol, and Guanidine in the sample.

To measure the DNA concentration by the fluorometric method on the Qubit 2.0 Fluorometer, Life Technologies, Invitrogen, a kit for measuring the concentration of double-stranded DNA with high sensitivity (Qubit™ dsDNA HS) was used. This kit enables highly specific binding of the probe to double-stranded DNA so that the absorbance obtained from individual nucleotides and RNA molecules is not detected in the measurement.

Analysis of results

Microsoft Office Excel 2021 was used for data analysis, including the calculation of mean values and standard deviations, as well as for generating graphical representations of the results.

RESULTS AND DISCUSSION

Concentration of DNA isolated from blood with a commercial kit - Qiagen DNeasy® Blood & Tissue Kit

This group of 100 patients (Group A) had DNA isolated from whole blood with a commercial Qiagen kit. Isolation was performed for genomic DNA sequencing using the next-generation sequencing method (NGS). Sequencing requires high concentrations and high-quality DNA.

In this group, there were 54 male subjects and 46 female subjects. The median age was 39 years.

The average concentration of DNA in the isolate obtained from peripheral blood leukocytes was 45.06 ng/μL, with a standard deviation of 17.75. The lowest value obtained was 20.3 ng/μL, and the highest value of the isolate was 134.6. The purity of the $A_{260/280}$ sample is 1.8, while the $A_{260/230}$ is 1.37.

Observed by gender, the average concentration of DNA in the isolate obtained with the Qiagen kit is 43.11 ng/μl for men and 47.05 ng/μl for women.

The mean value of the DNA concentration in the isolate obtained with the Qiagen kit, observed by age distribution and gender, measured by NanoDrop, is shown in **Table 2**.

Table 2. The mean value of the DNA concentration in the isolate obtained in Group A (Qiagen), observed by age and sex

Sex \ Age	0-10	11-20	21-40	>41
Women	50.5 ng/μL	45.7 ng/μL	42.8 ng/μL	48 ng/μL
Men	42.15 ng/μL	43.88 ng/μL	43.58 ng/μL	45.75 ng/μL

Concentration of DNA isolated from blood with a commercial kit - High Pure PCR Template Preparation kit (Roche Diagnostics)

In this group of 100 healthy patients (Group B), DNA was isolated with a commercial Roche kit for routine diagnostics and examination of certain gene variants.

In this group, there were 14 male respondents and 86 female respondents. The median age was 35.3 years. The main study participants were women of reproductive age tested for inherited thrombophilia.

The average concentration of DNA in the isolate obtained from peripheral blood leukocytes was 20.97 ng/μL, while the standard deviation is 8.8. The lowest value obtained was 6.9 ng/μL, and the highest value of the isolate was 48.3 ng/μl. The purity of the $A_{260/280}$ sample is 1.66, while the $A_{260/230}$ is 1.25. Although the purity of samples obtained with the Roche kit is lower than recommended, in clinical routine diagnostics, it has been shown that there is no influence on the results of routine genotyping.

The mean concentration of DNA in the isolate obtained with the Roche kit, observed by patient gender, is 20.88 ng/μL for women and 20.12 ng/μL for men.

Observed by age groups and by sex, the determined DNA concentrations, measured by NanoDrop, are shown in **Table 3**.

Table 3. The mean value of the DNA concentration in the isolate obtained in Group B (Roche), observed by age and sex

Sex \ Age	0-10	11-20	21-40	>41
Women	12.35 ng/μL	25 ng/μL	22.1 ng/μL	17.3 ng/μL
Men	/	/	23 ng/μL	19.33 ng/μL

Comparative quantitative and qualitative analysis of isolated DNA from two different kits

To assess which commercial kit provides a higher yield and greater purity of the DNA isolate, the results have been summarized and presented in **Table 4.** The analysis was performed by gender as well as by age groups to determine whether gender and age have an influence on the yield of DNA in the isolate.

In comparing the results obtained by DNA isolation with the Qiagen and Roche High Pure PCR Template Preparation kits, significant differences are observed in the mean values of DNA concentration and isolate purity. The Qiagen kit showed a significantly higher average DNA concentration (45.06 ng/μL) compared to the Roche kit (20.97 ng/μL), suggesting its greater efficiency in isolating DNA from peripheral blood. Also, the purity of the isolate was higher with the Qiagen kit, which is an indication of less contamination with unwanted materials in the sample. Analysis of the results by gender and age revealed variations in DNA isolate concentrations. For instance, when using the Qiagen kit, women generally exhibited higher average DNA concentrations across most age groups compared to men; however, these differences were not statistically significant. In contrast, the Roche kit showed less pronounced gender-based differences.

Table 4. Comparison of DNA concentration and purity (A260/A280, A260/A230) between Qiagen and Roche kits, stratified by gender and age

	Mean DNA conc. (ng/μL)	Mean $_{260/280}$	Mean $A_{260/230}$	Women (ng/μL)	Men (ng/μL)
Qiagen	45.06±17.75	1.8	1.37	47.05	43.11
ROCHE	20.97±8.8	1.66	1.25	20.88	20.12

In Group A higher value for DNA concentration was noticed for women than for men, but the calculated p-value was above 0.05 (p=0.262), so that does not indicate statistical significance. In group B, there are no differences in DNA concentration between men and women. This suggests that biological sex differ-

ences do not affect the quality or quantity of DNA isolated from male and female samples.

In **Figure 1**, the distribution of concentration between patients is shown. There are more homogeneous results in group A than in group B. Only a few samples from Group B are near the mean value from Group A, and more than 10% of samples were under 10 ng/μL. In group A, there were no samples with values under 20 ng/μL, but there were a significant number of samples above 50 ng/μL.

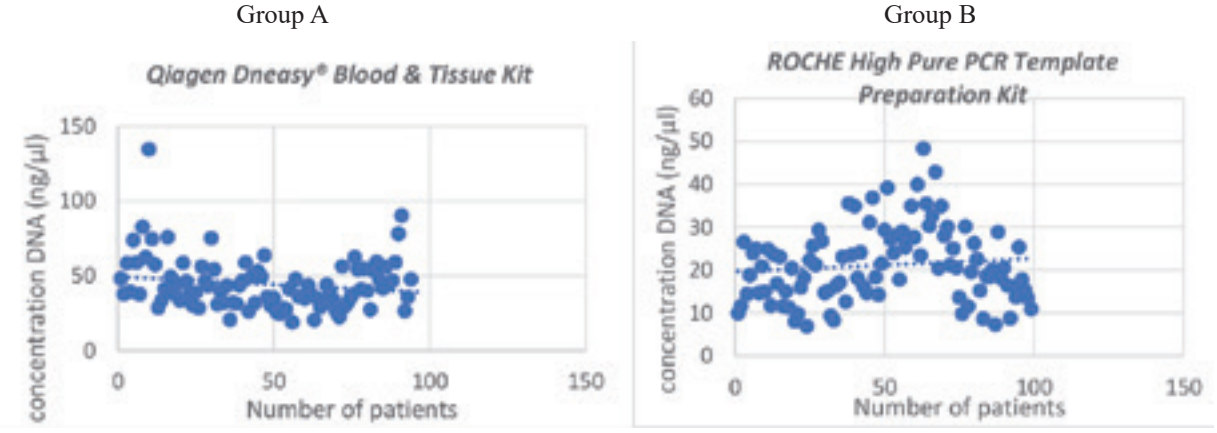

Figure 1. Comparative Analysis of Isolation Methods: Distribution of concentration of DNA in Group A and in Group B

Comparative analysis of measured concentrations of isolated DNA from blood on a spectrophotometer and a fluorometer

Since DNA concentration was measured using two different devices (each based on a distinct measurement principle), the results for the same samples were compared, and the coefficient of difference was calculated to quantify the discrepancy in measurements between the two devices.

In this section, 30 DNA samples isolated from blood using the Roche kit were randomly selected. Samples obtained by Roche kits used for routine diagnostics in the Department for Medical Genetics were saved for 5 years in a refrigerator at -20 °C. Samples isolated by the Qiagen kit were sent to the cooperating laboratory and were not available for measuring by Qubit 2.0 during this study. The average DNA concentration measured with the Qubit 2.0 fluorometer was 7.47 ng/μL, whereas the same samples measured with the NanoDrop One spectrophotometer showed an average concentration of 17.28 ng/μL. These results are summarized in **Table 5.**

Table 5. Comparative presentation of measured concentrations from the same samples by spectrophotometric (NanoDrop) and fluorometric methods (Qubit 2.0)

	Mean DNA conc. (ng/μL)
NanoDrop One	17.28
Qubit2.0	7.47

The coefficient of difference between the concentrations measured by Qubit and NanoDrop is 2.33. The formula for the obtained conversion factor is:

$$k=C_N/C_Q=2,33$$

k - conversion factor
C_N – Mean value of concentration measured on the NanoDrop One spectrophotometer
C_Q - Mean value of concentration measured on Qubit 2.0 fluorometer

The significant difference in concentration of DNA from the results obtained using different measurement methods (7.47 ng/μL – fluorimetry on Qubit 2.0 compared to 17.28 ng/μL –spectrophotometry on NanoDrop) which led to a coefficient difference of 2.33, indicates a mismatch between the two methods.

Fluorimetry, especially through devices like the Qubit system, provides a precise way to measure the concentration of DNA and RNA. This method uses fluorogenic dyes that selectively bind to nucleic acids and emit fluorescence. Concentration is determined based on fluorescence intensity, providing high accuracy, especially at lower concentrations. A Qubit system often allows specificity for DNA or RNA, improving measurement precision. Spectrophotometry, which includes devices like the Nanodrop spectrophotometer, uses light absorption by nucleic acids to determine concentration. DNA and RNA absorb light at characteristic wavelengths (eg, 260 nm). Measurement is performed directly on very small samples (eg, 1-2 μL), thus reducing the need for a large amount of sample. However, this method can be sensitive to contamination and requires careful sample preparation. Both fluorometry and spectrophotometry are reliable methods for measuring DNA and RNA concentrations. The choice between them depends on several factors, including the required sensitivity, the specific goals of the experiment, and the availability of laboratory equipment.

CONCLUSION

Both tested commercial kits (Qiagen DNeasy® Blood & Tissue Kit and High Pure PCR template preparation kit - Roche diagnostics) showed high quality of the obtained DNA isolate. The concentrations and purities of the isolates were satisfactory for further genomic analyses. The commercial kit Qiagen DNeasy® Blood & Tissue Kit showed better performance in terms of the values of the measured concentrations and the purity of the isolates compared to the High Pure PCR template preparation kit - Roche diagnostics. The values of the measured concentrations with the Qiagen kit are 2.15 times higher than those of the Roche kit. Roche High Pure PCR Template Preparation Kit shows more consistent concentration values among individual samples.

The Qiagen kit is the better choice for sample preparation for sequencing and microarray processes, while the Roche kit, due to consistent concentrations, is the choice for routine genotyping in clinical practice. The concentrations measured by the fluorimetric method (Qubit 2.0) and the spectrophotometric method (NanoDrop) show a significant difference. NanoDrop shows higher concentrations compared to those measured on Qubit 2.0. The conversion coefficient between the DNA concentration values measured using the Qubit 2.0 fluorometer and the NanoDrop spectrophotometer was calculated to be 2.33.

Conflict of interest
The authors declare no conflict of interest.

REFERENCES

DNeasy Blood & Tissue Handbook, Qiagen, 2023; *.https://www.qiagen.com/am/resources/download.aspx?id=68f29296-5a9f-40fa-8b3d-1c148d0b3030&lang=en.*

High Pure PCR Template preparation Kit, Method Sheet, ROCHE, 2023. https://lifescience.roche.com/global/en/products/others/high-pure-pcr-template-preparation-kit-3684811.html

Lojo-Kadrić N, Pojskić N, Pojskić L. Laboratorijske tehnike u molekularnoj biologiji. Univerzitet u Sarajevu – Institut za genetičko inženjerstvo i biotehnologiju, Sarajevo, 2018; ISBN 978-9958-083-08-2.

Mardan-Nik M, Saffar Soflaei S, Biabangard-Zak A et al. A method for improving the efficiency of DNA extraction from clotted blood samples.

J. Clin. Lab. Anal. 2019; 33(4):e22892. doi:10.1002/jcla.22892.

Psifidi A, Dovas CI, Bramis G et al. Comparison of eleven methods for genomic DNA extraction suitable for large-scale whole-genome genotyping and long-term DNA banking using blood samples. PloS ONE 10(1), 2015;10(1):e0115960. doi:10.1371/journal.pone.0115960.

Sarnecka AK, Nawrat D, Piwowar M, Ligęza J, Swadźba J, Wójcik P. DNA extraction from FFPE tissue samples – a comparison of three procedures. Contemp Oncol (Pozn) 2019; 23 (1): 52–58. doi:10.5114/wo.2019.83813

Tagliaferro SS, Zejnelagic A, Farrugia R, Wettinger SB. Comparison of DNA extraction methods for samples from old blood collections. BioTechniques, 2021;70(3):153-158. doi:10.2144/btn-2020-0170.

Tolosa JM, Schjenken JE, Civiti TD, Clifton VL, Smith R. Column-Based Method to Simultaneously Extract DNA, RNA, and Proteins from the Same Sample. BioTechniques, 43:799-804, 2007. doi:10.2144/000112597

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