Comparison of the Effect of Two Methods Used for DNA Purification from FFPE (Formalin-Fixed, Paraffin Embedded) Cancerous Tissue, Manual Method And Automated Magnetic Bead-based Method, on the Accuracy of DNA Purification Carried Out

Research Question:

To what extend does the method used for DNA purification from FFPE cancerous tissue (manual method and automated magnetic bead-based method) affect the accuracy of DNA purification carried out, measured by absorbance ratio (absorbance at 260 nm to that at 280 nm) values of the purified DNA samples?

Subject: Biology

Word Count: 3989

Table of Contents

1. Introduction

Analysis of DNA derived from cancerous mammalian tissues is a commonly studied topic in cancer biology and personal cancer treatment strategies (Sarnecka et al., 2019).

In conducted research, it is critical to preserve the DNA in a well-insulated area to prevent any potential damage. Formalin-fixed, paraffin embedded (FFPE) blocks are highly preferred to preserve the extracted DNA samples for long periods since they are practical and efficient (Ludyga et al., 2012; Mathieson et al., 2019). However, DNA samples must be isolated from these blocks first to conduct targeted molecular oncology tests and other applications (Berensmeier, 2006). Since it is challenging to extract DNA from preserving blocks (Einaga et al., 2017), it is essential to use an efficient extraction method to obtain high quality DNA and reliable test results (Sarnecka et al., 2019). Many alternative methods for DNA purification from FFPE cancerous tissues, including de-waxing and silica membrane-based extraction, have developed progressively (Flagstad et al., 1999). This study focuses on two methods: manual and magnetic bead-based method.

Being a newly developed method in DNA purification, there is limited number of studies made about magnetic bead-based method. In this study, the effect of these two methods on the accuracy of DNA purification is investigated by comparing the absorbance ratio (absorbance at 260 nm to that at 280 nm) of extracted DNA samples obtained by these two methods and evaluating their DNA purity according to the comparisons made.

1.1 Background Information

1.1.1 DNA Purification

DNA purification is a method of isolating any type of DNA from a biological material, including living organisms or conserved tissues. Firstly done by Miescher in 1869 and lately developed by Meselson and Stahl in 1958, it is known as one of the most crucial methods in molecular medicine because it allows the scientists to analyze the DNA structure (Brown, 2020; Tan and Yiap, 2009).

1.1.2 Importance of DNA Purification in Cancer Cases

In cancer research, it is important to analyze DNA accurately to develop effective and personalized diagnostics strategies and the understanding of cancer biology. Reliable DNA analysis in cancer requires uncontaminated and high quality of DNA, which are achieved through successful DNA purification (Diefenbach, 2018).

1.1.3 Manual DNA Purification Method

Manual method is a traditional method that has been used for a long time. It is based on six main steps: removal of paraffin from sample, sample lysis, heating and incubating THE sample, DNA binding, sample washing, and DNA eluting. Sample lysis step generally requires an organic solvent, such as ethanol, which are highly toxic. Since most of these steps are done manually, it is more likely for human errors to occur compared to automated methods, which could reduce the reliability and accuracy of the results. Requiring large number of steps also increases the degradation risk, sample loss or cross-contamination of samples (Berensmeier, 2006).

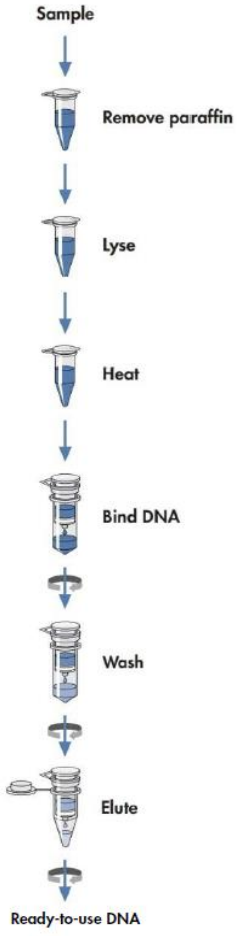


Figure 1. Procedure for DNA purification by manual method. Retrieved from "QIAamp DNA FFPE Tissue Handbook", 2020, *QIAGEN*.

1.1.4 Magnetic Bead-Based DNA Purification Method

Magnetic bead-based method is an automated method that has been developed due to technological advances in laboratory medicine. In this method, first, paraffin is removed from the samples like in manual method. After necessary heating and incubation steps, samples are put into an automated nucleic acid purification device. Sample lysis, magnetic separation, sample washing, and DNA eluting steps are done automatically. The main difference of magnetic bead-based method from manual method is the paramagnetic bead-based working principle: After sample lysis step, paramagnetic beads are added to the sample, which bind to DNA magnetically. External magnet in the device creates a temporary magnetic field and magnetically attracts the paramagnetic beads. Paramagnetic beads move to this side, so does the DNA. Consequently, DNA is separated from the sample. During sample washing process, all other cell materials, including proteins and lipids, are removed, which remains DNA and paramagnetic beads in the sample only. DNA is separated from paramagnetic beads during DNA elution step, and DNA purification process ends.

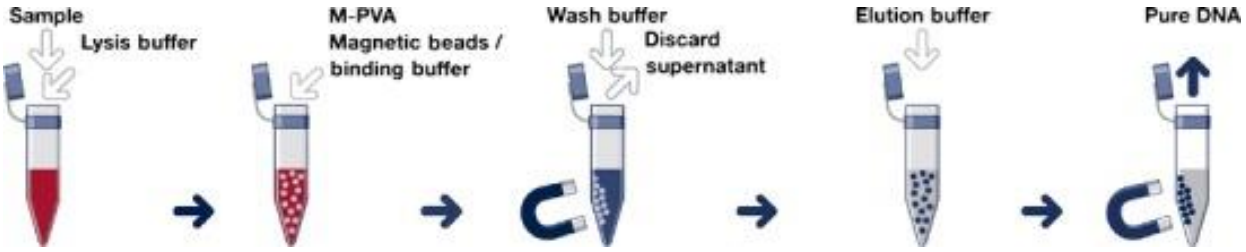


Figure 2. Schematic procedure for DNA purification by magnetic bead-based method. Retrieved from "Magnetic particles for the separation and purification of nucleic acids," by S. Berensmeier, 2006, *Applied microbiology and biotechnology*, *73*(3), 495–504.

1.2 Research Question

To what extend does the method used for DNA purification from FFPE cancerous tissue (manual method and automated magnetic bead-based method) affect the accuracy of DNA purification carried out, measured by absorbance ratio (absorbance at 260 nm to that at 280 nm) values of the purified DNA samples?

1.3 Aim of Study

The quality of purified DNA from FFPE cancerous tissues is critical in cancer research since it affects the reliability and accuracy of the results, so is important to conduct an efficient DNA purification method. This study aims to investigate the effect of two methods (manual and magnetic-bead based) used for DNA purification from FFPE cancerous tissue on the accuracy of DNA purification by comparing the absorbance ratio (A_{260}/A_{280}) of extracted DNA samples and evaluating their DNA purity according to the comparison made. The ultimate goal is to develop a better understanding of magnetic bead-based method and interpret whether this method has reliable results to be an alternative method for DNA purification processes from FFPE tissues.

1.4 Hypothesis

1.4.1 Null Hypothesis

H_0: There will be no statistically significant difference in the accuracy of DNA purity between the extracted DNA samples obtained by manual method and those obtained by automated magnetic bead-based method. Method used for DNA purification does not affect the accuracy of DNA purification carried out.

1.4.2 Alternative Hypothesis

H_A: There will be a statistically significant difference in the accuracy of DNA purity between the extracted DNA samples obtained by manual method and those obtained by automated magnetic bead-based method. Method used for DNA purification affects the accuracy of DNA purification carried out. DNA samples extracted by automated magnetic bead-based method will have a higher accuracy in obtaining a high quality of DNA purity than those extracted by manual method (Flagstad et al., 1999; Lehmann et al, 2006). Quality of DNA purity is classified as high for the absorbance ratio (A_{260}/A_{280}) range between 1.7 and 2.0 (Lucena et al., 2016).

1.5 Variables

1.5.1 Independent Variable: Method used for DNA purification. Two different methods are used for this process: manual method by using QIAamp® DNA FFPE tissue kit, and automated magnetic bead-based method by using Maxwell® RSC FFPE Plus DNA kit and Maxwell® RSC 48 Instrument.

1.5.2 Dependent Variable: DNA purity obtained by using each of the two methods mentioned, measured by absorbance ratio (A_{260}/A_{280}) values of the purified DNA samples.

1.5.3 Controlled Variables

Variable	Significance	How to Control?
Number of Trials	Unless the number of trials for each method are equal, the results of one method could be more accurate and reliable compared to the other one.	For each method, 25 trials were made with 25 FFPE DNA purification kits.
Nanodrop Spectrophotometer	Purified DNA is analyzed in this device. Type of nanodrop spectrophotometer affects the analysis results since different devices have different accuracies.	Thermo Scientific™ NanoDrop™ 2000 Spectrophotometer was used for each trial in manual method.
Centrifuge	Centrifuge is used to separate DNA from other cell materials, including proteins and lipids. Type of centrifuge affects the separation of DNA and the DNA purity results.	For each method, Thermo Scientific™ Sorvall™ Legend™ Micro 21R Centrifuge was used.
Thermomixer	Thermomixer is used to heat and incubate the DNA samples under controlled temperatures. Different thermomixers have different temperature accuracy and uncertainties. Unless same thermomixer is used, results may have different accuracy and reliability values than each other.	For each method, Eppendorf Thermomixer® was used.
Vortex Mixer	Vortex mixer is used to mix the samples. Different vortex mixers have different accuracy and precision values. Unless same vortex mixer is used, results may have different accuracy and reliability values than each other	For each method, Vortex Mixer VTX-3000 L was used.
Micropipette	Micropipette is used to accurately measure the volumes of solutions. Different micropipettes have different uncertainty and accuracy values. Unless same micropipette is used, results may have different accuracy and reliability values than each other.	For both methods, Microlit RBO-10000 micropipette was used.

Table 1. Table of controlled variables, their significance, and the strategy of how to control them.

2. Methodology

2.1 Materials and Apparatus

2.1.1 Manual Method

- 10 QIAamp® DNA FFPE tissue kits
- Thermo Scientific™ NanoDrop™ 2000 Spectrophotometer (± 0.001 AU)
- Thermo Scientific™ Sorvall™ Legend™ Micro 21R Centrifuge (± 0.1 rpm)
- Eppendorf Thermomixer® (± 0.1°C)
- Vortex Mixer VTX-3000 L
- Microlit RBO 10000 Micropipette (± 60 µl)
- 1 scalpel
- 1 dropper
- 10 x 200 µl QIAGEN's Deparaffinization solution
- 10 x 180 µl Buffer ATL (tissue lysis buffer)
- 10 x 1 mL xylene
- 10 x 20 µl proteinase K solution
- 10 x 200 µl ethanol
- 10 x 200 µl Buffer AL (lysis buffer)
- 10 x 500 µl QIAGEN Buffer AW1 (wash buffer 1)
- 10 x 500 µl QIAGEN Buffer AW2 (wash buffer 2)
- 10 x 40 µl QIAGEN Buffer ATE (elution buffer)

2.1.2 Automated Magnetic Bead-based Method

- 10 Maxwell® DNA FFPE Plus kits
- Maxwell® RSC 48 Instrument
- Thermo Scientific™ NanoDrop™ 2000 Spectrophotometer (± 0.002 AU)
- Thermo Scientific™ Sorvall™ Legend™ Micro 21R Centrifuge (± 0.1 rpm)
- Eppendorf Thermomixer® (± 0.1°C)
- Vortex Mixer VTX-3000 L
- Microlit RBO 10000 Micropipette (± 60 µl)
- 1 dropper
- 10 x 180 µl incubation buffer
- 10 x 20 µl proteinase K solution

2.2 Method Development

Selection of Tissue Samples: DNA was extracted from FFPE tissues of 25 randomly chosen archival cancerous tissue blocks for each method to avoid sampling error. The purpose of using 25 samples for each method is to increase the accuracy and reliability of the results and apply the t-test appropriately: t-test gives reliable results in which the sample size is at least 15 (Skaik, 2015).

Selection of FFPE DNA Purification Kits: High number of alternative kits for manual method is available but for magnetic bead-based method. Previous research was considered for the selection of the kits for both methods. Having highly reliable and accurate results in addition to having large number of studies made about them with high citations, QIAamp® DNA FFPE tissue kits were chosen for manual method, and Maxwell® DNA FFPE Plus kits were chosen for magnetic bead-based method.

Procedures for Manual Method and Automated Magnetic Bead-based Method: Each DNA purification method has highly serious standardized protocols that are required to be done the same to prevent any further consequences that could be faced. Therefore, published official protocols were followed exactly for both manual and magnetic bead-based methods: For manual method, protocol in "QIAamp® DNA FFPE Tissue Handbook (2020)" was used whereas for magnetic bead-based method, protocol in "Maxwell® RSC FFPE Plus DNA Kit (2021)" was used.

Determining the Efficiency of DNA Purification: Absorbance Method: The efficiency of DNA purification can be determined by absorbance method. The ratio of absorbance at 260 nm to that at 280 nm is one of the most common purity calculations used. If this ratio (A_{260}/A_{280}) is between 1.7 and 2.0 (preferably closer to 1.8), DNA is accepted as pure and well-qualified. Ratio between 1.6 and 1.7 indicates the DNA does not have a very good quality but still can be accepted as pure. If the ratio is lower than 1.6, it may indicate presence of proteins, phenol, or other contaminants since these contaminants absorb strongly at or near 280 nm (Lucena et al., 2016).

Range for the value of A_{260}/A_{280} ratio	**Indicator about the DNA quality and purity**
1.7 – 2.0 (preferably closer to 1.8)	pure and good qualified
1.6 – 1.7	not a very good quality but still can be accepted as pure
lower than 1.6	presence of proteins, phenol, or other contaminants

Table 2. Table showing the accepted approximate range for the value of A_{260}/A_{280} ratio and its indicator about the DNA quality and purity.

From A_{260}/A_{280} ratio, it can be determined whether DNA has a good quality and purity, which can be used to make interpretations about the accuracy of the DNA purification method.

2.3 Procedure

2.3.1 Manual Method

Remove Paraffin:

1. Using a scalpel, trim excess paraffin off the sample block.

2. Immediately place the sections in a 2 ml microcentrifuge tube, and add 1 ml xylene to the sample. Close the lid and vortex vigorously for 10 s.

3. Centrifuge at full speed for 2 min at room temperature (15–25°C).

4. Remove the supernatant by pipetting. Do not remove any pellet.

5. Add 200 µl QIAGEN's deparaffinization solution, and then entrifuge at 14,000 rpm for 2 min.

7. Remove the supernatant by pipetting.

8. Open the tube and incubate at room temperature or up to 37°C. Incubate until all deparaffinization solution has evaporated.

Lyse the Samples:

9. Add 180 µl Buffer ATL.

10. Add 20 µl proteinase K, and mix by vortexing.

Heat and Incubate the Samples:

11. Incubate at 56°C for 2 hours.

12. Incubate at 90°C for 1 hour.

Wash the Samples:

13. Add 200 µl Buffer AL to the sample, and mix thoroughly by vortexing. Then add 200 µl ethanol, and mix again by vortexing.

14. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.

15. Add 500 µl Buffer AW1, and then centrifuge at 14,000 rpm for 1 min.

16. Add 500 µl Buffer AW2, and then centrifuge at 14,000 rpm for 1 min.

Elute the DNA:

17. Centrifuge at 14,000 rpm for 3 min to dry the membrane completely.

18. Add 40 µl Buffer ATE to the center of the membrane, and then centrifuge at 14,000 rpm for 1 min.

Analyze the DNA Purity:

19. Drop 1 drop from the obtained DNA to the nanodrop spectrophotometer. It measures the absorbance of the purified DNA at different wavelengths.

2.3.2 Automated Magnetic Bead-based Method

Remove Paraffin:

1. Using a scalpel, trim excess paraffin off the sample block.

2. Immediately place the sections in a 2 ml microcentrifuge tube, and then centrifuge at 14,000 rpm for 15 s to collect the sample at the bottom of the tube.

Heat and Incubate the Samples:

3. Add 180 µl incubation buffer and 20 µl proteinase K solution.

4. Incubate at 70°C for 4 hours.

Put the Samples into the Maxwell® RSC 48 Instrument:

5. Put the samples into the Maxwell® RSC 48 Instrument, and run the device by touching the "start" button. The following steps are done automatically in approximately 23 minutes:

- Sample lysis by adding lysis buffer.
- Binding of nucleic acids to paramagnetic particles.
- Washing of the bound target molecules away from other cellular components.
- Elution of the DNA.

Analyze the DNA Purity:

6. After the process is done, take the samples from the device, and drop 1 drop from the obtained DNA to the nanodrop spectrophotometer. It measures the absorbance of the purified DNA at different wavelengths and the concentration of DNA in ng/ µl.

2.4 Evaluation of Ethical Issues and Risks

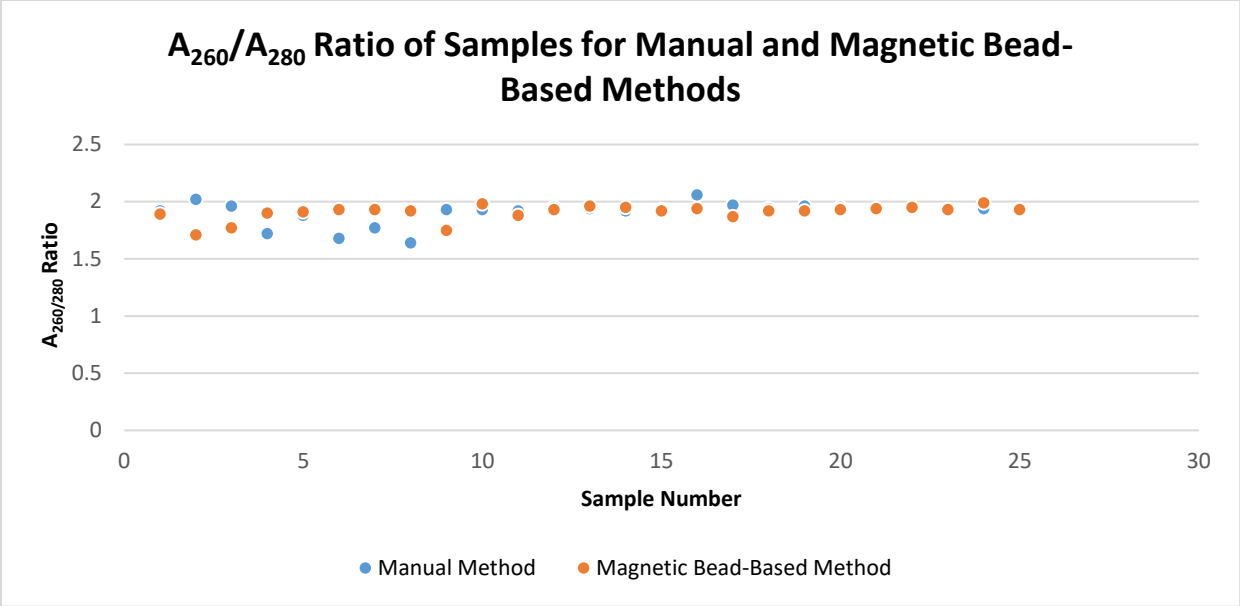
- I used gloves and goggles throughout the study to prevent any possible contamination.
- I received official permission from the laboratory prior to my study.
- I worked on existing FFPE cancerous tissues and took no additional DNA samples from the patients.
- I did not give any harm to any organisms throughout my study.
- Because the purification procedure and the instructions of the device used for magnetic bead-based method were highly technical, I received professional guidance to conduct my study.

3. Results

3.1 Raw Data

Concentration, A_{260}, A_{280}, and A_{260}/A_{280} Values for Purified DNA Samples, Derived by Manual Method and Magnetic Bead-based Method								
Purified DNA Samples	**Manual Method**				**Magnetic Bead-based Method**			
Sample Number	Concentration (±0.1 ng/ µl)	A_{260} (±0.001 AU)	A_{280} (±0.001 AU)	A_{260}/A_{280}	Concentration (±0.1 ng/ µl)	A_{260} (±0.001 AU)	A_{280} (±0.001 AU)	A_{260}/A_{280}
1	68.7	1.374	0.717	1.920	143.8	2.875	1.524	1.890
2	49.8	0.997	0.492	2.020	639.0	12.781	7.466	1.710
3	99.1	1.982	1.009	1.960	609.8	12.196	6.877	1.770
4	94.5	1.889	1.100	1.720	253.3	5.065	2.667	1.900
5	50.9	1.018	0.542	1.880	351.2	7.023	3.684	1.910
6	42.2	0.844	0.502	1.680	150.9	3.017	1.563	1.930
7	42.0	0.840	0.473	1.770	121.2	2.425	1.256	1.930
8	61.7	1.235	0.752	1.640	220.6	4.412	2.300	1.920
9	129.3	2.587	1.337	1.930	356.4	7.128	4.077	1.750
10	131.7	2.634	1.363	1.930	112.9	2.257	1.138	1.980
11	129.6	2.593	1.350	1.920	226.5	4.529	2.403	1.880
12	148.8	2.977	1.527	1.950	170.5	3.410	1.770	1.930
13	116.3	2.326	1.198	1.940	127.9	2.559	1.305	1.960
14	205.0	4.101	2.133	1.920	252.8	5.057	2.596	1.950
15	159.2	3.184	1.649	1.930	170.0	3.399	1.772	1.920
16	32.8	0.657	0.319	2.060	98.7	1.974	1.020	1.940
17	46.5	0.931	0.472	1.970	135.2	2.705	1.444	1.870
18	131.1	2.622	1.349	1.940	137.7	2.753	1.437	1.920
19	88.6	1.771	0.906	1.960	204.0	4.080	2.122	1.920
20	117.1	2.342	1.200	1.950	157.7	3.155	1.638	1.930
21	100.1	2.001	1.038	1.930	120.8	2.416	1.247	1.940
22	89.2	1.783	0.917	1.940	66.7	1.335	0.684	1.950
23	48.7	0.975	0.506	1.930	104.6	2.093	1.084	1.930
24	31.3	0.626	0.322	1.940	100.8	2.015	1.011	1.990
25	67.4	1.349	0.699	1.930	70.2	1.404	0.729	1.930

Table 3. Concentration, A_{260}, A_{280}, and A_{260} / A_{280} values for purified DNA samples, derived by using manual method and magnetic bead-based method



Graph 1. Scatter graph of $A_{260/280}$ ratio of samples for manual method and magnetic bead-based method.

3.2 Qualitative Data

Because the procedures for both DNA purification methods were highly technical, I focused on quantitative data only and used no qualitative data for my study.

3.3 Calculations

Mean:

Means of concentration, A_{260}, A_{280}, and A_{260}/A_{280} were calculated for samples derived by manual method and magnetic bead-based method by the given formula:

$$\bar{x} = \frac{\sum X}{N}$$

Range:

Range of concentration, A_{260}, A_{280}, and A_{260}/A_{280} were calculated for samples derived by manual method and magnetic bead-based method by the given formula:

$$Range = X_{max} - X_{min}$$

Standard Deviation:

Standard deviation of concentration, A_{260}, A_{280}, and A_{260}/A_{280} were calculated for samples derived by manual method and magnetic bead-based method by the given formula:

$$S = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x}_{max})^2}{n - 1}}$$

Paired T-Test:

Paired T-test was conducted for the concentration, A_{260}, and A_{280} values derived by manual method and magnetic bead-based method by the given formula:

$$t = \frac{\bar{d}}{s_d / \sqrt{n}}$$

3.4 Processed Data

Comparison of Mean, Range, and Standard Deviation (SD) Values of Concentration, A_{260}, A_{280}, and A_{260}/A_{280} Values of Manual and Magnetic Bead-based Methods						
	Manual Method			**Magnetic Bead-based Method**		
	Mean	**Range**	**SD**	**Mean**	**Range**	**SD**
Concentration (ng/ µl)	91.264	173.700	45.378	203.888	572.300	147.418
A_{260} (AU)	1.826	3.475	0.908	4.083	11.446	2.946
A_{280} (AU)	0.955	1.814	0.465	2.194	6.782	1.716
A_{260}/A_{280} (AU)	1.906	0.420	0.099	1.906	0.280	0.068

Table 4. Comparison of mean, range, and standard deviation (SD) values of concentration, A_{260}, A_{280}, and A_{260}/A_{280} values of manual and magnetic bead-based methods

P-Values For Concentration, A_{260}, and A_{280} Values Derived From T-Test	
Parameter	**P-Value**
Concentration (ng/ µl)	0.001117 ($p \leq 0.01$)
A_{260} (AU)	0.001085 ($p \leq 0.01$)
A_{280} (AU)	0.001826 ($p \leq 0.01$)

Table 5. p-values for concentration, A_{260}, and A_{280} values derived from t-test

Frequency Table for A_{260}/A_{280} Values in Manual and Magnetic Bead-based Methods		
A_{260}/A_{280} (AU)	**Manual Method**	**Magnetic Bead-based Method**
$A_{260}/A_{280} < 1.6$	0	0
$1.6 \leq A_{260}/A_{280} < 1.7$	2	0
$1.7 \leq A_{260}/A_{280} \leq 2.0$	21	25
$A_{260}/A_{280} > 2.0$	2	0
Accuracy for $1.7 \leq A_{260}/A_{280} \leq 2.0$ (%)	84	100

Table 6. Frequency table for A_{260}/A_{280} values in manual and magnetic bead-based methods

3.5 Statistical Analysis

Paired T-Test: T-test is a statistical test used to determine whether there is a statistically significant difference between the means of two groups (Mishra et al., 2019). In this study, since samples are same for both manual method and magnetic bead-based method, a paired t-test was applied to determine whether the difference in the concentration, A_{260}, and A_{280} values between two methods are significant. P-values for all three measurements being less than 0.01 ($p \leq 0.01$) indicated that the difference concentration, A_{260}, and A_{280} values between two methods were significant with a probability of 99.99%.

4. Discussion

The aim of this investigation was to develop a better understanding of magnetic bead-based method and interpret whether this method could be a promising alternative method for DNA purification processes from FFPE tissues. Hence, two methods for DNA purification from FFPE cancerous tissue, manual method and magnetic bead-based method, were used and the absorbance ratio (A_{260}/A_{280}) values of extracted DNA samples by these two methods were compared to draw interpretations about the efficiency of DNA purification.

According to *Table 4*, manual method had lower mean values for concentration, A_{260}, and A_{280} values than those for magnetic bead-based method, indicating that DNA samples extracted by magnetic bead-based method had a higher DNA concentration than those extracted by manual method. Alternatively, mean A_{260}/A_{280} values for both methods were extremely close to each other: a difference approximately 0.0004 was assumed to be negligible. Therefore, standard deviations (SDs) of two methods were considered to determine which method had more accurate A_{260}/A_{280} results. *Table 4* showed that manual method had a higher SD value of A_{260}/A_{280} than magnetic bead-based method. This could be also interpreted from *Graph 1*, in which A_{260}/A_{280}

ratio values for manual method were clearly seemed to be more dispersed than those for magnetic bead-based method. While this showed a higher human error for manual method than magnetic bead-based method, which was expected due to relatively high use of manual procedure in manual method, a lower SD for magnetic bead-based method indicated that this method had less distributed A_{260}/A_{280} values so did more accurate A_{260}/A_{280} results, supporting the H_A hypothesis.

Furthermore, *Table 6* highlighted that manual method had four A_{260}/A_{280} values out of the range between 1.7 and 2.0, two less than 1.7 and two more than 2.0, accounting for the accuracy percentage of 84%. Alternatively, magnetic bead-based method had A_{260}/A_{280} values for all samples in the range between 1.7 and 2.0, accounting for the accuracy percentage of 100%. Since ratio (A_{260}/A_{280}) value between 1.7 and 2.0 implies a high DNA purity and quality (Lucena et al., 2016), this frequency distribution indicated that DNA magnetic bead-based method was more accurate in obtaining well-qualified DNA samples, supporting the H_A hypothesis.

These results supported existing studies in the literature. Dairawan and Shetty highlighted that the use of magnetic beads magnetic-bead based method minimizes the risk of contamination of DNA samples while successfully purifying DNA, maximizing the efficiency of DNA purification (Dairawan and Shetty, 2020). Additionally, Chen et al. reported that especially for cases where DNA is effectively conserved, like in FFPE tissues, magnetic bead-based method produces more highly-qualified DNA extracts compared to other methods (Chen et al, 2020).

As a result, considering the limited research about the magnetic bead-based method for DNA purification in the literature, this investigation played a crucial role to improve the understanding of alternative methods for ensuring efficient DNA purification, which might be used in revolutionizing medical oncological diagnostics and developing effective personalized treatment strategies (Green and Sambrook, 2018).

5. Evaluation

Strengths	Reason(s) to be Considered as a Strength
Conducting my study under professional guidance.	This minimized random error. I ensured that I conducted my investigation precisely and properly.
Using standardized procedures for both of my DNA purification methods.	This increased the reliability of my results by allowing to compare my results with the results that were already found in the literature.
Using existing DNA samples for my study.	This eliminated any possible ethical issues that may have raised due to the patient rights or unnecessity of taking DNA samples from the patients.
Using absorbance method to make interpretations about the efficiency of DNA purification carried out.	Since absorbance method is currently accepted as the most reliable method to measure DNA quality, using absorbance method increased the reliability of my interpretations about the efficiency of DNA purification procedures.
Using highly technical and professional equipment for DNA purification procedure.	This minimized possible human error. Additionally, because professional equipment is very sensitive and has the minimum risk of systematic error, use of highly technical and professional equipment minimized systematic error.

Table 7. Table of strengths and the reasons to be considered as a strength

Limitations	Effect of Limitation on the Results of the Investigation	Suggested Improvement and the Reason
Only two methods, manual and magnetic bead-based method, were compared.	Focusing on two methods only limited the comprehensiveness of the study and narrowed down the interpretations about which method is more efficient for DNA purification.	Compare the accuracy of different methods, including de-waxing and silica membrane-based purification. This could give more reliable results about the accuracy of different methods in obtaining highly qualified DNA extracts.
Although exactly same procedures in official protocols were applied, some errors may have occurred during this process.	Human errors were more likely to occur in manual method since sample lysis, DNA washing, and DNA elution were also done manually in this method. This may have lowered the accuracy of the method results.	Implement automation in critical steps of DNA purification, especially for manual method, by using professional DNA purification equipment. This can minimize human error and increase reliability of data.
QIAamp® DNA FFPE tissue kits were used for manual method, and Maxwell® DNA FFPE Plus kits were used for magnetic bead-based method.	Although choice of these FFPE tissues were based on various previous research, use of other FFPE tissue kits may have been a confounding variable, leading to misleading interpretation.	Further research about trustworthiness of the use of alternative FFPE tissue kits in different DNA purification methods is required to use identical FFPE tissue kits for different methods.
A sample size of 25 was used due to the limited availability of existing DNA samples of cancer tissues.	Although this sample size was enough to apply t-test, it may have reduced the accuracy of results.	A bigger sample size, like a size of 50, may give more accurate results for the comparison of manual and magnetic bead-based methods.

Table 8. Table of limitations, their effect on the results of the investigation, and suggested improvements with reasons.

6. Conclusion

This study aimed to develop a better understanding about the cruciality of carrying out an efficient and accurate DNA purification from FFPE cancerous tissues method to obtain reliable and accurate test results for cancer research. The results in *Table 6* indicated that magnetic bead-based method were more accurate in obtaining high qualified ($1.7 \leq A_{260}/A_{280} \leq 2.0$) DNA extracts (100% accuracy for $1.7 \leq A_{260}/A_{280} \leq 2.0$) than manual method (84% accuracy for $1.7 \leq A_{260}/A_{280} \leq 2.0$). Additionally, t-test values being smaller than 0.01 for concentration ($p=0.001117$), A_{260} ($p=0.001085$), and A_{280} ($p=0.001826$) in *Table 5* suggested that DNA extracts of magnetic bead-based method had higher concentration and absorbance values than those of manual method. Both conclusions supported the H_A hypothesis, stating that DNA extracted by automated magnetic bead-based method had a statistically higher accuracy in obtaining a high quality of DNA than that extracted by manual method. Consequently, this study supported previous research findings about manual and magnetic bead-based methods (Chen et al, 2020; Dairawan and Shetty, 2020; Sarnecka et al., 2019).

Supporting that magnetic bead-based method is more accurate than manual method, this investigation suggested magnetic bead-based method, a newly developed method due to technological advances, was open to be a reliable and promising alternative method for DNA purification processes from FFPE tissues.

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8. Appendix

I – Official Permission To Study in the Laboratory For This Investigation

The student has performed her experiment in Mikrogen Genetics Laboratory, Department of DNA/RNA Isolation by herself with supervision of our staff.

Prof. Dr. Volkan BALTACI
Clinical Director

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Appendix I. The document of the official permission to study in the laboratory for this investigation