Indonesian Journal of Medical Laboratory Science and Technology

Open Access p-ISSN 2684-6748 | e-ISSN 2656-9825 IJMLST

Comparison of Purity and Concentration Values of *Mycobacterium tuberculosis* DNA Extraction Result from the Boiling and Spin Column Method

Intan Febriyanti[1], Ai Djuminar[1], Fusvita Merdekawati[1], Asep Iin Nur Indra[1]

[1]Departement of Medical Laboratory Technology, Polythecnic of Health Kemenkes Bandung, Cimahi Utara, Indonesia

Correspondence:
Intan Febriyanti,
Jl. Babakan Loa No. 10 A, Pasir Kaliki, Kec. Cimahi Utara, Kota Cimahi, Jawa Barat, Indonesia
Zip Code: 40514

Email: intanfebriyanti56@gmail.com

Received: July 24, 2023
Revised: October 1, 2023
Accepted: October 4, 2023
Published: October 27, 2023

DOI: 10.33086/ijmlst.v5i2.4771

Abstract

The Polymerase Chain Reaction (PCR) technique is extensively employed in molecular biology to precisely detect *Mycobacterium tuberculosis*. Prior to conducting PCR, extracting of high-quality genomic Deoxyribonucleic Acid (DNA) is crucial to ensure accurate and reliable results. The primary objective of this study is to conduct a comparative analysis of the purity and concentration of *M. tuberculosis* DNA acquired through the utilization of the boiling method and the spin column extraction methods. A descriptive comparative research design was employed, utilizing a sample of 16 sputum specimens that had previously been confirmed as positive for *M. tuberculosis* through Acid-Fast Bacteria (AFB) examination and Molecular Rapid Test (MRT). The extraction of DNA was carried out using the boiling method and the spin column method. Subsequently, the concentration and purity of the extracted DNA were assessed using the *NanoDrop Spectrophotometer*, and the results were compared. The obtained yield of *M. tuberculosis* DNA isolates through the boiling method ranged from 9.6 ng/µL to 1258.7 ng/µL, with an average purity value of 1.23. Conversely, for the spin column method, the concentration of *M. tuberculosis* DNA isolates ranged from 8.7 ng/µL to 207.8 ng/µL, with an average purity value of 1.83. In conclusion, there is a significant difference between the purity and concentration of *M. tuberculosis* DNA extraction results using the boiling method and spin column methods.

Keywords

Boiling, DNA Extraction, *Mycobacterium tuberculosis*, Spin Column.

Citation: Febriyanti I, Djuminar A, Merdekawati F, Indra AIN. Comparison of Purity and Concentration Values of Mycobacterium tuberculosis DNA Extraction Result from the Boiling and Spin Column Method. Indones J Med Lab Sci Technol. 2023;5(2):133–145. DOI: 10.33086/ijmlst.v5i2.4771

This is an open access article distributed under the Creative Commons Attribution-ShareAlike 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. ©2023 by author.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*, and Indonesia is among the countries significantly contributes to the global burden of TB cases (1,2). Based on data primarily gathered by World Health Organization (WHO) from national ministries of health during annual rounds of data collection, in 2022, there were 202 countries and territories reporting data on TB cases, representing more than 99% of the world's population (3).

Indonesia stands out as one of the countries contributing to the high increase in TB cases. It ranks third with 824 thousand cases of TB, following India and China, and records 93 thousand TB-related deaths annually, equivalent to 11 deaths per hour (4). The government's initiatives to reduce TB cases in Indonesia are outlined in the National Medium-Term Development Plan (RPJMN) for the health sector, spanning from 2020 – 2024. These initiatives are directed toward accelerating the elimination of TB by 2030. Hence, there is a pressing need for rapid and highly sensitive laboratory testing to identify TB cases early and ensure optimal treatment.

M. tuberculosis is a pathogenic Gram-positive bacterium that commonly infects the lungs. It can also infect other organ systems, such as the gastrointestinal, musculoskeletal, lymphoreticular, skin, liver, and reproductive systems (5,6). The Polymerase Chain Reaction (PCR) method is one of the molecular techniques used to detect *M. tuberculosis*, alongside microscopic examination and bacterial culture. The PCR technique exhibits very high sensitivity in detecting TB pathogens from sputum samples (7). PCR is a method recommended by the WHO for of examining *M. tuberculosis* infection due to its high sensitivity and specificity (8).

Prior to conducting identification via PCR, it is essential to extract genomic DNA in order to obtain pure DNA. This extracted DNA serves as a template during the PCR amplification process. Therefore, the quality of the extracted DNA becomes one of the factors that can influence the success of the PCR examination. Quantitative and qualitative tests of the isolated DNA are performed to assess its concentration and purity using a spectrophotometer (9). Optimal DNA purity is indicated by a λ 260/280 ratio between 1.8 to 2.0, reflecting high-quality standards (10).

There are several methods for extracting *M. tuberculosis* DNA, including conventional and kit-based extraction methods. The boiling method is a conventional DNA extraction method

that is simple, cost-effective, fast, and capable of extracting bacterial DNA from Gram-positive and Gram-negative bacteria at high temperatures. It has been proven to be efficient (11). According to research conducted by Fihiruddin et al. (8), the boiling method with heating at 95°C for 5 minutes was found to be the optimal temperature for DNA extraction from sputum samples. This study revealed an average DNA concentration of 0.94 ng/µL and a purity value of 1.23 for *M. tuberculosis* DNA using the boiling method at 95°C (8).

The solid-phase extraction method, also known as the spin column extraction, utilizes a silica membrane as the nucleic acid binder in the DNA extraction process. This method employs a kit, and the nucleic acid binding is optimized with specific buffer solutions, pH, and appropriate salt concentrations. Using silica particles in the spin column method ensures safety and minimizes the potential for cross-contamination (12,13).

According to the research by Ndhlovu et al. (13), it was demonstrated that *M. tuberculosis* DNA was successfully extracted from sputum samples using the spin column kit method, and it outperformed the modified Cetyl trimethylammonium bromide (CTAB) extraction method. The spin column method resulted in an average DNA concentration of 5.93 ± 0.94 µg/mL and an average purity of 1.69 ± 0.09. The purity value is lower than the modified CTAB method results, which is 1.73 ± 0.14. However, the modified CTAB method requires a longer time, approximately 7 hours, while the kit extraction method takes less than 1 hour. Based on the results of previous studies, both methods can be used to extract *M. tuberculosis* DNA and produce reliable DNA extraction results. However, no study has compared the performance of the two extraction methods for extracting *M. tuberculosis* DNA from sputum samples. Therefore, it is necessary to conduct research to determine the best extraction method for *M. tuberculosis* DNA from sputum samples (14). This study aims to compare the purity and concentration quality of *M. tuberculosis* DNA extraction results using the boiling method and the spin column method.

MATERIALS AND METHODS

This study employs a descriptive comparative research design, specifically utilizing a Post-test Only Control Group Design. Sixteen sputum samples were collected from patients diagnosed positive for Acid-Fast Bacilli (AFB) through microscopic examination and *M. tuberculosis* through the Molecular Rapid Test (MRT) from hospitals in the city of Cimahi, using purposive sampling. The research was conducted at the

Biosafety Level 2 Laboratory, Department of Medical Laboratory Technology, Health Polytechnic Kemenkes Bandung, during May and June 2023.

The sputum samples collected from tuberculosis patients in sputum pots underwent a washing process using a 1:1 ratio of 4% NaOH as a sputum dilution solution to removed mucus from the samples (15). The resulting sample mixture was vortexed at 2,500 rpm for 20 seconds and then centrifuged at 13,000 x 16,000 g for 2 minutes, followed by the removal of the supernatant.

The DNA extraction using the boiling method was conducted by pipetting 200 µL of processed sputum sample into a microtube, followed by the addition of 100 µL of 1x TE buffer and one µL of Proteinase K. The mixture was then frozen at -18°C for a minimum of one hour, heated at 60° C for one hour, and boiled for 5 minutes at 100°C. Subsequently, the resulting DNA extraction was centrifuged for 3 minutes, and the supernatant was transferred to a new microtube. The obtained DNA isolate was then analyzed for concentration and purity using the NanoDrop Spectrophotometer.

The DNA extraction using the spin column method was carried out with the *Geneaid Viral Nucleic Acid Extraction Kit II (VR300)*. Initially, 200 µL of processed sputum sample was pipetted into a microtube, followed by 400 µL of VB lysis buffer. The mixture was vortexed and incubated for 10 minutes. Subsequently, 450 µL of AD buffer was added and vigorously shaken. The lysate mixture was transferred into the silica column VB column (600 µL), and centrifuged at 14,000 x 16,000 g for one minute. The liquid passing through the silica membrane was discarded, and the VB column was placed back into the collection tube. The remaining lysate mixture was added back into the VB column and centrifuged at 14,000 x 16,000 g, with the liquid in the collection tube being discarded. Then, the VB column was transferred to a new collection tube, and 400 µL of W1 buffer was added and centrifuged at 14,000 x 16,000 g for 30 seconds. following this, 600 µL of Wash buffer was added and centrifuged at 14,000 x 16,000 g for 30 seconds. In order to desiccate the column matrix, the sample mixture was centrifuged at 14,000 x 16,000 g for 3 minutes, followed by the addition of RNase-Free Water to elute the DNA. The DNA isolates were then subjected to concentration and purity measurement using the NanoDrop Spectrophotometer.

RESULTS

The data obtained from the measurements using a *NanoDrop* Spectrophotometer consists of concentrations in ng/µL and purity values obtained from the absorbance ratio at λ 260 nm and 280 nm, as presented in Table 1.

Table 1. Data of Measurement Results for Concentrationa Purity of *M. tuberculosis* DNA Extraction Using Boiling Method and Spin Column Method

No Sample	Positivity	DNA Extraction Boiling Method		DNA Extraction Spin Column Method	
		Purity Value	Concentration Value (ng/µL)	Purity Value	Concentration Value (ng/µL)
1	AFB +1	1.0	479.7	1.8	20.8
2	AFB +2	1.15	895.6	1.8	8.7
3	AFB +2	0.51	9.563	1.9	207.0
4	AFB +2	1.5	530.4	1.9	47.0
5	AFB +3	1.1	1000.6	2.0	33.3
6	AFB +1	1.3	277.6	2.0	121.4
7	AFB +2	1.3	646.3	1.9	35.9
8	M. tb low detected	Error	Error	1.8	11.1
9	AFB +2	1.5	328.8	1.9	33.4
10	M. tb low detected	1.3	422.3	1.9	76.4
11	AFB +1	1.1	221.3	1.1	76.4
12	M. tb low detected	1.1	163.8	1.8	31.0
13	AFB +3	1.2	1131.4	1.9	190.3
14	AFB +3	1.1	1258.7	1.9	68.1
15	M. tb detected	1.8	184.8	1.8	36.9
16	M. tb detected	1.5	175.3	1.8	28.2

In Table 1, it is evident that there are sixteen positive sputum samples. Each demonstrating varying degrees of positivity, confirmed by the examination of Acid-Fast Bacilli (samples number 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 14), and positive *M. tuberculosis* sputum samples confirmed by the Molecular Rapid Test (samples number 8, 10, 12, 15, 16).

The boiling method yielded a higher DNA concentration compared to the spin column method, ranging from 9.6 ng/µL to 1258.7 ng/µL for the boiling method, and from 8.7 ng/µL to 207.8 ng/µL for the spin column method. However, an error was encountered when measuring the concentration and purity of the *M. tuberculosis* DNA isolates using the boiling method for sample number 8.

Analysis of the measurement results for *M. tuberculosis* DNA concentration using the boiling method, as outlined in Table 1, highlights sample number 3 as having the lowest DNA concentration. Conversely, the application of the spin column method reveals that samples number 1, 2, 4, 5, 7, 8, 9, 12, 15, and 16 present notably low DNA concentrations.

Moreover, examination of the results from the DNA purity measurements in *M. tuberculosis* using the boiling method, illustrated in Table 1. In Table 1, indicates that samples 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, and 16 exhibited relatively low DNA

purity values (with sample 8 resulting in an error). In contrast, the DNA purity measurements of *M. tuberculosis* using the spin column method reveal that only sample 11 demonstrates a notably low DNA purity value.

As presented in Table 2, the average purity value of DNA extracted from *M. tuberculosis* using the boiling method is 1.23, while the average purity value of DNA extracted using the spin column method is 1.83. This indicates that the DNA extraction results obtained using the spin column method are within closer proximity to the standard range of values, typically falling between 1.8 and 2.0.

Table 2. Results of DNA Purity Data Processing in *M. tuberculosis* Using the Boiling Method and the Spin Column Method

	Purity of the Boiling Method	**Purity of the Spin Column Method**
Mean	1.3	1.8
Minimum	0.5	1.1
Maximum	1.8	2.0
Average	**1.23**	**1.83**

The obtained data underwent rigorous comparative statistical analysis to discern any disparities in the purity and concentration values of DNA extraction results between the two distinct extraction methods. Initially, the sign test was conducted to compare the purity of the boiling method with the purity of the spin column method. The results suggest that the Sig value for DNA purity between the boiling method and the spin column method was < 0.05. It indicates a highly significant difference in purity between the Boiling Method and the Spin Column Method. This value suggests that the two methods produce significantly different levels of purity.

The Wilcoxon test was conducted to analyze the concentration of the Boiling Method in comparison to the concentration of the Spin Column Method. The results indicate that Z-score: -3.067b and Asymptotic Significance (2-tailed): 0.002. With a significance level (p-value) of 0.002 (< 0.05.), the results suggest a statistically significant difference between the concentrations obtained through the Boiling Method and the Spin Column Method. The negative Z-score (-3.067) indicates that the concentration of the Boiling Method is lower compared to the Spin Column Method.

DISCUSSION

The DNA extraction processes using the boiling method and the spin column method differ in principles and the reagents used. In the boiling method, the sputum sample is first treated with 4% NaOH and then subjected to centrifugation. Subsequently, it is supplemented with a TE buffer (Tris-EDTA) solution, which aids maintaining the solution's pH, dissolving the extracted DNA, and protecting it from degradation during storage (16). The addition of proteinase K

serves to degrade proteins in the nucleic acid. Cooling at -18°C and subsequent heating at 60°C and 100°C are intended to disrupt bacterial cells and tissues through physical means. Following this, the centrifugation process separates DNA, causing proteins, lipids, and other components, excluding DNA, to precipitated, thus enabling DNA concentration in the supernatant. The supernatant is then carefully transferred to a new sterile microtube. The addition of centrifugation in the final step of the boiling method is a modified procedure performed by researchers following the DNA extraction procedure of the boiling method described by Nurhayati and Darmawanti (17), as well as in the study conducted by Fihiruddin et al. (8).

The DNA extraction process using the spin column method is systematically executed in a microtube equipped with a silica membrane column (13). Reagents such as VB lysis buffer and AD lysis buffer are intriduced to facilitate the lyse of bacterial cell walls, enabling the release of cellular components. The lysis buffer solution serves to break down tissues, disrupt cell membranes, and eliminate contaminants, thus facilitating the extraction of genomic DNA. The addition of W1 buffer and Wash buffer serves the purpose of eliminating any residual proteins that might be attached to the DNA and eliminating prior buffer salts (18). Each addition of the reagents is followed by centrifugation, which applies centrifugal force to aid in the binding of DNA to the silica matrix, causing cellular components other than DNA to flow to the bottom of the tube. Subsequently, DNA is eluted using RNAse-free water to release the DNA bound to the silica matrix and collect it at the bottom of the tube. Additionally, RNAse-free water protects nucleic acids from degradation by nucleases, such as DNase or RNase (19). The extracted DNA samples are stored at a stable temperature of -20°C before being subjected to measurements using the NanoDrop spectrophotometer (18).

The conducted research highlighted a notable disparity between the purity and concentration of DNA obtained from *M. tuberculosis* using the boiling and spin column methods. Among the sixteen data points gathered from the purity results of DNA extracted using the boiling method from *M. tuberculosis*, only one sample (6.25%) fell within the range of pure values, specifically sample number 15. The average purity value of the extracted DNA from *M. tuberculosis* using the boiling method was 1.23. Conversely, from the data acquired in the purity results of DNA using the spin column method, 15 samples (93.75%) fell within the range of pure values, specifically 1.8 – 2.0. The average purity value of the extracted DNA from *M. tuberculosis* using the spin column method was 1.83. This observed purity outcome is line with a prior study by Fihirudin et al. (8), where the purity

values of DNA extracted using the boiling method at various temperatures (85°C, 90°C, and 95°C) were within the range of 1.18 – 1.23.

Significantly low purity results were obtained in the samples from the DNA purity measurements using the boiling method (samples number 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, and 16). This outcome may be attributed to the presence of various contaminants such as proteins, lipids, carbohydrates, and other cellular components in the extraction results, leading to compromised DNA purity (20,21). Notably, DNA and RNA are absorbed at λ 260 nm, while contaminants, including proteins and phenol, exhibit strongly absorbtion at λ 280 nm (22,23). Consequently, the heightened absorption of proteins at λ 280 nm can significantly impact DNA purity, as the assessment of DNA purity is reliant on the absorption ratio at λ 260/280 nm. The elevated levels of the contaminants ultimately contribute to diminished DNA purity. In the boiling extraction method, a particular step was altered to isolate DNA from contaminants or other components through a single centrifugation process follwong heating. Unlike certain other extraction methods, the boiling method does not incorporate washing and purification steps within the extraction process. This particular aspect presents a notable limitation of the method, potentially allowing contaminants to persist within the DNA extraction sample (24).

Regarding the sample of DNA isolate obtained through the boiling method, sample number 8 exhibited measurement errors in both concentration and purity assessments. Such error could potentially be attributed to several factors, including the sample possessing an absorbance value that falls beyond the detection range of the NanoDrop spectrophotometer instrument. The quality of the extracted DNA isolates from *M. tuberculosis* samples, which remained visibly concentrated and presented a reddish-brown color (likely due to the inclusion of sputum samples mixed with blood), may indicate the presence of contaminants. These contaminants can significantly interfere with the absorption of light transmitted through the sample during measurement, resulting in the inability to accurately detect absorbance values required for determining concentration and purity at specific wavelengths, thereby leading to errorneous results. Notably, the spectrophotometer instrument is designed with upper detection limits for dsDNA up to 3700 ng/µL, ssDNA up to 2400 ng/µL, and RNA up to 3000 ng/µL. In instances where the sample color is excessively concentrated, it may lead to concentrations surpassing the instrument's detection limits, ultimately resulting in error-prone measurement outcomes. Moreover, as outlined in the *NanoDrop* spectrophotometer

user manual, the lack of absorbance results can also be attributed to the sample or aliquot's lack of homogenity. Hence, it is imperative to ensure the homogenization of the DNA isolate sample before conducting measurements using the instrument to mitigate the likelihood of generating errorneous results (25).

Within the sample from the purity measurement of DNA using the spin column method, sample number 11 exhibited a notably low purity value. Such an outcome could be attributed to the presence of protein contaminants and other impurities within the extracted DNA isolate. Notably, the DNA extraction process carried out using the spin column method encompasses several intricate steps, including cell wall lysis, the separation of DNA from impurities and other cellular components, DNA binding to the silica column, as well as DNA washing and purification stages. While the utilization of the spin column method represents a more comprehensive process compared to the boiling method, it does not entirely preclude the possibility of the extracted DNA via the spin column method yielding lower purity levels. This potential disparity could arise from various factors, including potential errors during the analytical stages, inaccuracies during pipetting, and technical oversights during the measurements conducted using NanoDrop spectrophotometer.

Upon analyzing the samples using the boiling method for DNA concentration, notably higher concentration values were observed in the samples 1, 2, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, and 16. However, these samples exhibited comparatively lower purity values in contract to the DNA concentration results acquired through the spin column method. Similarly, in a particular case of an isolate obtained through the spin column DNA extraction method, specifically sample number 11. There was an observed high concentration value accompanied by a low purity value. This particular scenarion may be attributed to the presence of an additional type of nucleic acid, namely single-stranded DNA (ssDNA), within the sample, which was measured at λ 260 nm. As a result, it led to elevated DNA concentration (22).

Another form of nucleic acid that can be measured at λ 260 nm is RNA. However, if RNA contamination were presenst, the resulting purity values would exceed >2. Since there were no samples with purity values >2 from the purity measurements, the likelihood is that the measurable nucleic acid is ssDNA (single-stranded DNA). The presence of such contamination can significantly contribute to an increase in DNA concentration results. Notably, one of the potential contaminants impacting the samples could be the presence of DNA from other genomes, which might inadvertently

carry over through reagents, equipment, or various laboratory conditions. Therefore, it is crucial to pay attention to these factors. Additionally, the implementation of stringent controls measures is vital to ensure the integrity of the work conducted.

The DNA extraction results obtained using the spin column method from samples 3, 6, 10, 13, and 14 yielded high concentrations (>50 ng/µL) along with purity values falling within the range of 1.8 - 2.0. This outcome can be attributed to the efficient extraction of DNA molecules in substansial quantities, ensuring minimal contamination from proteins other impurities.

In the measurement of DNA extraction results using the spin column method, there are several samples, namely samples number 1, 2, 4, 5, 7, 8, 9, 12, 15, and 16, which exhibit low DNA concentrations (<50 ng/µL), but possess a high level of purity. The diminished DNA concentration in these instances can be linked to various factors, such as suboptimal processes related to cell wall lysis and the separation of DNA from cellular tissues, ultimately resulting in a limited amount of extracted DNA. Additionally, incomplete DNA binding during the DNA binding stage may contribute to an insufficient DNA yield. The likelihood of compromised DNA purity during measurements using the *NanoDrop* Spectrophotometer can be attributed to inadequate sample homogenization prior to the measurement process. The fundamental principle of spectrophotometry stipulates that the utilized sample should be clear and fully dissolved (22,26).

Another influential factor impacting the magnitude of DNA extraction concentration is the composition of the added lysis buffer, the specific physical treatment employed in each extraction method, as well as the buffer's capacity utilized in the DNA extraction process to effectively disintegrate bacterial cells (24).

CONCLUSIONS

The conducted research findings clearly delineate a significant disparity in the purity of *M. tuberculosis* DNA when employing the boiling method versus the spin column method. Specifically, the DNA purity attained from the boiling method is comparatively lower, with a recorded DNA purity level of 1.23. In contracts, the employment of the spin column method led to a notably higher DNA purity level of 1.83, aligning more closely with the average range value of 1.8 - 2.0. Discernible differences exist in the concentration values of *M. tuberculosis* DNA between the boiling method and the spin column method. Specifically, the concentration of *M. tuberculosis* DNA using the boiling method ranged from 9.6 ng/µL to 1258.7 ng/µL. In contrast, the utilization of the spin column method led to a concentration range for *M.*

tuberculosis DNA spanning from 8.7 ng/µL to 207.0 ng/µL.

Moving forward, it is recommended that further research should involve conducting PCR with *M. tuberculosis* specific primers, thereby enabling the visualization of DNA bands with a basepair length that matches the target gene of *M. tuberculosis*. Moreover, stringent control checks are imperative to ensure the accuracy of the extraction procedures and complete sterility of the utilized tools, thereby mitigating the occurrence of potential contaminations that could otherwise impact the concentration value and purity of the resulting DNA.

AUTHOR CONTRIBUTIONS

Intan Febriyanti: conceptualization, validation, formal analysis, investigation, resources, writing - original draft, writing - review & editing, funding acquisition. Ai Djuminar: methodology, validation, formal analysis, resources, data curation, writing - review & editing. Fusvita Merdekawati: methodology, validation, formal analysis, writing - review & editing, supervision, project administration. Asep Iin Nur Indra: methodology, validation, formal analysis, resources, writing - review & editing.

ACKNOWLEDGEMENTS

The author would like to express gratitude to all parties who have participated and assisted in the implementation of the research, as well as the respondent who have been involved in this study.

CONFLICT OF INTEREST

There were no conflicts of interest in this study.

REFERENCES

1. Maison DP. Tuberculosis pathophysiology and anti-VEGF intervention. J Clin Tuberc Other Mycobact Dis. 2022;27:100300. DOI: 10.1016/j.jctube.2022.100300
2. Iskandar D, Suwantika AA, Pradipta IS, Postma MJ, van Boven JFM. Clinical and economic burden of drug-susceptible tuberculosis in Indonesia: national trends 2017–19. Lancet Glob Heal. 2023;11(1):e117–25. DOI: 10.1016/S2214-109X(22)00455-7
3. World Health Organization. Global Tuberculosis report 2022 [Internet]. 2022. Available from: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022
4. Kementerian Kesehatan RI. Situasi TBC di Indonesia [Internet]. 2021 [cited 2022 Aug 5]. Available from: https://tbindonesia.or.id/pustaka-tbc/informasi/tentang-tbc/situasi-tbc-di-indonesia-2/
5. Jilani TN, Avula A, Gondal AZ, Siddiqui AH. Active Tuberculosis. In: NCBI bookshelf a service of the national library of medicine, national institutes of health [Internet]. StatPearls Publishing, Treasure Island (FL); 2023. p. 2. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513246/?report=reader
6. MacPherson P, Lebina L, Motsomi K, Bosch Z, Milovanovic M, Ratsela A, et al. Prevalence and risk factors for latent tuberculosis infection among household contacts of index cases in two South African provinces: Analysis of baseline data from a cluster-randomised trial. PLoS One [Internet]. 2020;15(3):1–14. DOI: 10.1371/journal.pone.0230376

7. Babafemi EO, Cherian BP, Ouma B, Mogoko *GM. Paediatric tuberculosis diagnosis using Mycobacterium tuberculosis* real-time polymerase chain reaction assay: a systematic review and meta-analysis. Syst Rev [Internet]. 2021;10(1):1–14. DOI: 10.1186/s13643-021-01836-w

8. Fihiruddin, Ilmi HF, Khusuma A. Variations in boiling temperature in amplification of the *M. tuberculosis* inHA gene PCR method. [Variasi temperatur boiling pada amplifikasi Gen inHA *M. tuberculosis* Metode PCR]. Titian Ilmu J Ilm MultiS ciences. 2022;14(2):57–62. DOI: 10.30599/jti.v14i2.1661

9. García-Alegría AM, Anduro-Corona I, Pérez-Martínez CJ, Corella-Madueño MAG, Rascón-Durán ML, Astiazaran-Garcia H. Quantification of DNA through the nanodrop spectrophotometer: methodological validation using standard reference material and sprague dawley rat and human DNA. Int J Anal Chem. 2020;2020: 8896738. DOI: 10.1155/2020/8896738

10. Bello GL, Morais FCL, Wolf JM, Gehlen M, Soares T dos S, Halon ML, et al. Improvement of *Mycobacterium tuberculosis* detection in sputum using DNA extracted by sonication. Brazilian J Infect Dis. 2020;24(5):398–404. DOI: 10.1016/j.bjid.2020.08.006

11. Junior JCR, Tamanini R, Soares BF, Oliveira AM De, Silva FDG, Fernandes F, et al. Efficiency of boiling and four other methods for genomic DNA extraction of deteriorating spore-forming bacteria from milk. Semin Ciências Agrárias, Londrina. 2016;37(5):3069–78. DOI: 10.5433/1679-0359.2016v37n5p3069

12. Shi R, Lewis RS, Panthee DR. Filter paper-based spin column method for cost-efficient DNA or RNA purification. PLoS One. 2018;13(12):1–14. DOI: 10.1371/journal.pone.0203011

13. Hidayat MT, Prasetyo EN. Modified Spin Column-Based RNA Extraction Methods of *Staphylococcus aureus* using PureLink® RNA Mini Kit and basic laboratory instrument. Indones J Med Lab Sci Technol. 2021;3(2):73–80. DOI: 10.33086/ijmlst.v3i2.1863

14. Ndhlovu V, Mandala W, Sloan D, Kamdolozi M, Caws M, Davies G. Evaluation of the efficacy of two methods for direct extraction of DNA from *Mycobacterium tuberculosis* sputum. J Infect Dev Ctries. 2018;12(12):1067–72. DOI: 10.3855/jidc.10592

15. European Centre for Disease Control. Handbook on tuberculosis laboratory diagnostic methods in the European Union [Internet]. Technical Report. 2018. 50–59 p. Available from: www.ecdc.europa.eu

16. Nasrollahzadehsabet M, Esmeilzadeh E, Shirmohammady N, Heidari MF. The Effect of EDTA buffer and temperature on DNA extraction from teeth for molecular forensic assessment. Ann Mil Heal Sci Res. 2021;19(2): e113043. DOI: 10.5812/amh.113043

17. Nurhayati B, Darmawati S. Cell and molecular biology. [Biologi Sel dan Molekuler]. Pusat Pendidikan SDM Kesehatan Badan Pengembangan dan Pemberdayaan Sumber Daya Manusia Kesehatan, Kemenkes RI.; 2017.

18. Afnan Uda MN, Parmin NA, Jambek AB, Hashim U, Uda MNA, Shaharuddin SNA. Evaluation and optimization of genomic DNA extraction from food sample for microfluidic purpose. IOP Conf Ser Mater Sci Eng. 2020;743(1). DOI: 10.1088/1757-899X/743/1/012031

19. Chauhan T. 4 Important functions of nuclease-free water [Internet]. Genetic Education. 2022. Available from: https://geneticeducation.co.in/4-important-functions-of-nuclease-free-water/

20. Setiaputri AA, Rohmad Barokah G, Alsere Bardian Sahaba M, Dini Arbajayanti R, Fabella N, Mustika Pertiwi R, et al. Comparison of DNA isolation methods in fresh and processed fishery products. [Perbandingan metode isolasi DNA pada produk perikanan segar dan olahan]. J Pengolah Has Perikan Indones. 2020;23(3):447–58. DOI: 10.17844/jphpi.v23i3.32314

21. Sophian A, Syukur A. Analysis of Purity and Concentration of Isolated DNA in making raw DNA of rat species. Erud Indones J Food Drug Saf. 2021;1(2):1–5. DOI: 10.54384/eruditio.v1i2.75

22. Dewanata PA, Mushlih M. Differences in DNA purity test using UV-Vis Spectrophotometer and Nanodrop Spectrophotometer in Type 2 Diabetes Mellitus patients. Indones J Innov Stud. 2021;15:1–10. DOI: 10.21070/ijins.v15i.553

23. Shen C-H. Quantification and analysis of nucleic acids. In: Diagnostic molecular biology (Second Edition) [Internet]. 2nd ed. Department of Biology, College of Staten Island, City University of New York, New York, NY, United States: Elsevier; 2023. Available from: https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/ultraviolet-spectrophotometry

24. Dayanti FG, Djuminar A, Dermawan A, Tantan A. Comparison of quantitative measurement values of *Salmonella typhi* DNA extraction results using the boiling method, NaOH, commercial kits. [Perbandingan Nilai pengukuran kuantitatif hasil ekstraksi DNA *Salmonella typhi* menggunakan metode boiling, NaOH, Kit komersial]. J Ris Kesehat Poltekkes Depkes Bandung. 2019;11(1):350–7. DOI: 10.34011/juriskesbdg.v11i1.762

25. Thermo Fisher Scientific Inc. NanoDrop 1000 Spectrophotometer V3 . 8 User ' s Manual. 2012;11(1):10. Available from: www.nanodrop.com

26. Dorado G, Besnard G, Unver T, Hernández Molina P. Polymerase chain reaction (PCR). 2019;1–3(6):473–492. DOI: 10.1016/B978-0-12-801238-3.08997-2