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OPEN PEER REVIEW

Comparison of Four Different DNA Isolation Methods from MGIT Culture for Long-Read Whole Genome Sequencing of *Mycobacterium tuberculosis*

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

2 comments · 23 May 2024 – 22 Jul 2024

Mohammad Hadi Fakoor Reviewer

MAJOR REVISION 23 May 2024

In the name of god
Dear Professor Zarei:
I have reviewed the manuscript, entitled "Comparison of four different DNA isolation methods from MGIT culture for long-read whole genome sequencing of Mycobacterium tuberculosis: Evaluation of input material quantity, concentration, purity, and integrity".
My comments are as follows:

Major and Minor Reviewer:

1. The title needs to be revised.
Comment 1. The title of the article is long, and there is no need for details in the title. It is better to make the title shorter.
2. The abstract of the article needs to be revised.
Comment 2. The abstract of the article is long. There is unnecessary content in the abstract that should be removed.
Comment 3. The keywords should be modified according to the protocol of the journal.
3. The background and objective are OK.
4. The materials and methods need to be revised.
Comment 4. The results, especially tables 1 to 3, should be checked statistically using software such as SPSS or GraphPad, and a section on statistical analysis should be added to the methods.
5. The results are OK.
Comment 5. In Table No. 1, DNA concentration is presented in ng/µl, while the text of the article in line 121 states that it is based on turbidity. Please correct it.
6. The discussion needs to be revised.
Comment 6. The discussion is weak. The authors should try to use references from the last 5 years in the discussion.
7. Conclusions need to be revised.
Comment 7. The authors should add a separate conclusion section to the article. A conclusion is required for this paper.
8. References need to be revised. The references should be corrected according to the protocol of the journal.

Decision: This manuscript needs revision to be suitable for publication.

Associate Editor

MAJOR REVISION 22 Jul 2024

Dear Authors
Title: It needs to be revised (shortened).
Abstract: It needs to be revised (improved), and keywords should be modified according to the protocol of the Journal.
Material and Methods: Two different quantities of pellets were prepared for each of the four DNA isolation methods.
At the end of the manuscript, the authors concluded that the Quick-DNA-Fecal/Soil-MK demonstrated the highest DNA yield and purity.

The amount of pellets, evaluated only by eye, did not indicate the bacterial cell count. The authors should calculate the cell count using the spectrophotometry method, and then the same cell concentration must be used to compare different DNA extraction methods.
Results: Tables 1 - 3, especially, should be checked statistically (using software such as SPSS or Graphpad).

In Table No. 1, DNA concentration is presented in ng/µl, while the text of the article in line 121 states that it is based on turbidity (correct it).
Discussion: It needs to be revised (use references from the last 5 years).
Conclusions: It needs to be revised, and a separate conclusion section should be added to the article.
References: They need to be revised (corrected according to the protocol of the journal).

My decision: Major Revision

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reviewers' Comments

First af all, I would like to thank your valuable and constructive suggestions. I try to reply your seggestions as I understand. I highligted the changed section with yellow colour in the revised manuscript.

Reviewer 1:

1: The Title need to be revised.

Comment: The title of the article is long and there is no need for details in the title. It is better to make the title shorter.

> **Answer:** The title was changed as '' Comparison of four different DNA isolation methods from MGIT culture for long-read whole genome sequencing of Mycobacterium tuberculosis''

2: The abstract article need to be revised

Comment 2: The abstract of article is long. There are unnecessary contents in the abstract that should be removed.

> **Answer:** All content of the abstract was revised to be shorten with focusing on unnecessary contents.

Comment 3: The keywords should be modified according to the protocol of the magazine.

> **Answer:** Keywords was modified according to protocol of the journal including
>
> • A list of 3-10 keywords must be provided for indexing purposes. All keywords should be provided according to the MeSH terms at: http://www.nlm.nih.gov/mesh/MBrowser.html.
>
> • The preferred expression is indicated by the label "MeSH Heading" and not "Entry Term". The desired terms may then be copied from the MeSH Browser. Another way of finding appropriate headings is to search PubMed to find articles on similar topics, and review the MeSH headings assigned tothose articles.
>
> The new keywords: Mycobacterium tuberculosis, Whole Genome Sequencing, Next Generation Sequencing, Nanopore Sequencing, DNA, Isolation and Purification

3. The backgrand and objectives are OK.

> The backgorund is not revised.

4. The material and Methods need to be revised.

Comments:The results, especially tables 1 to 3, should be checked statistically by software such as spss or graphpad, and section should be added in the methods for statistically performance.

Answer: You are absoulately right, I consulted a statistician taking your suggestion into account. Statistician suggestions that:

"There are two different types of pellets in the method. Pellets were prepared at different times for each method. At different times, one pellet was prepared for each method. In other words, three repeats were prepared for each method, but each repeat contains only one sample. One pellet was used in each repeat group.

Time 1 (group): Method 1 n=1 pellet

Method 2 n=1 pellet

Method 3 n=1 pellet

Method 4 n=1 pellet

Time 2 (group):

Method 1 n=1 pellet

Method 2 n=1 pellet

Method 3 n=1 pellet

Method 4 n=1 pellet

Time 3 (group):

Method 1 n=1 pellet

Method 2 n=1 pellet

Method 3 n=1 pellet

Method 4 n=1 pellet

To avoid bias, all pellets were assigned to the methods by a blinded individual who did not know which method was being used.

To determine whether there is a statistically significant difference between the methods, one-way ANOVA was considered. However, in the 4x2 repeated measures analysis (RMA), which is one of the conditions required for ANOVA, the interaction effect between subjects was examined. As a result of the analysis, the repeat X group effect was <0.001, indicating that there was an interaction effect between subjects. Therefore,

Statistician said that comparing and analyzing the data with non-parametric or parametric tests is not appropriate. The data conformed to homogeneous variance and were normally distributed. Although the initial one-way ANOVA analysis suggested a difference between the methods in terms of DNA quantity and purity, the post-hoc analyses showed inconsistent results between Tukey and Bonferroni tests. Therefore, due to the small sample size, the results were considered to be random

Since the bacterium is tuberculosis, it grows slowly, and many MGIT tube colonies are required to increase the pellet amount in each group. Unfurtanately, it was not possible to repeat the experiment. However, as a general comment, I believe the data obtained are informative, and this limitation has been mentioned in the conclusion and discussion sections As in discussion section '' Another significant limitation of the study is the small number of pellets of groups evaluated in each repetition, which may make restrict the generalizability of the results and making the statistical analysis.'' and as in the Conclusion section '' However, while this study provides guidance on selecting a suitable DNA extraction method for long-read sequencing of *M. tuberculosis*, a larger sample size would be necessary to generalize the findings and reach a definitive conclusion''

5. The results is ok.

Answer : ''Purity of DNA'' title ın The results section , the sentences on between 132-137 lines on orijinal article was removed beacuse od repeated sentences. It was mention the''Assessment of Genomic DNA quality'' title in the method section.

Comment 5: In the table NO 1; DNA concentration is presented based on ng/µl, While in the text of the article in line 121, it states that it is based on turbidity. Please correct it.

Answer: The Line 120-121 '' turbidity'' Word I was written incorrectly. In the study, Two types of pellet was used, one of the pellet was formed from 1 ml Mfc-2 bacterial suspensin adjusted by BD PhoenixSpec™ nephelometer and the other pellet named as ''collonial pellet'' was formed from collecting of all colonies from Two MGIT culture to give an idea in routine application. In the was revised as '' 1 ml Mcf-2 pellet''.

6. Discussion need to be revised.

Comment 6: The discussion is weak. The authors should try to use the references of the last 5 years in the discussion.

Answer: Articles from previous years before published 2019 have been excluded. The new articles were added. In the discussion section, the seperate paragraph including results of related article in the line between 180-195 lines. But the two article, (15 and 17.references) was published in the bioRvix and medRxiv, the two article was not accepted any offical journal, but the articles were related directly to DNA extraction methods and optimization of the methos in the next generation sequencing of *M. tuberculosis*

In my research and according to the publication guideline of publicationsThe bioRvix and medRvix, it is stated that such publications can be cited, which is why I used these types of articles.

7. Conclusion need to be revised.

Comment 7: Authors should add a separate conclusion section to the article. A conclusion is required for this paper.

Answer: the separate conclusion section was added tto he article as below

‘’ As detected from three repetitions of the Mcf-2 and colonial pellet extractions, It could be stated that Quick-DNA Fecal/Soil-MK kit yielded the highest DNA quantity and purity but lower DNA integrity compared to other methods. The kit needs adjustments for optimal results. As a starting material, the pellet composed of colonies from two MGIT cultures, which is equivalent to approximately 100 µl or more in a 1.5 ml microcentrifuge tube, is suitable for long-read WGS by this kit. However, while this study provides guidance on selecting a suitable DNA extraction method for long-read sequencing of *M. tuberculosis*, a larger sample size would be necessary to generalize the findings and reach a definitive conclusion. For long-read sequencing of challenging bacteria like *M. tuberculosis*, DNA extraction protocols need to be optimized to balance DNA yield, fragment size, and purity, ensuring efficient sequencing and accurate drug resistance analysis’’ in the between 205-215 lines of the revised manuscript.

8. References need to be revised. The references should be corrected according to the protocol of the journal.

Answer: All references were revised according to the journal, The references were revised with adding EndNOTE Brifelands style. The references of WHO were revised according to the articles related tuberculosis published in the Jundishapur Journal of Microbiology.

However, about ruled of citations the criteria about the references published in the the bioRvix and medRxiv in the Jundishapur Journal of Microbiology.

Reviewer 2:

The authors compared four DNA extraction methods: the CTAB method and three spin column methods, including the GeneJET-GenomicDNA-Purification Kit, Quick-DNA-Fecal/Soil- MicrobeKit, and Genematrix-Tissue/Bacterial-DNA-PurificationKit.

Two different quantities of pellets were prepared for each of the four DNA isolation methods. At the end of the manuscript, the authors concluded that the Quick-DNA-Fecal/Soil-MK demonstrated the highest DNA yield and purity. The value of pellets that were evaluated only by eye did not show the bacterial cell count. The authors should calculate the cell count using the spectrophotometry method, and then the same cell concentration must be used to compare different DNA extraction methods.

Answer: In the study, two types of initial pellet quantities were used. One type of pellet was formed by taking 1 ml of a bacterial suspension, adjusted

to McFarland 2 adjusted by BD PhoenixSpec™ nephelometer device, not by eye for standardizing the initial quantity. When we were adjusting the the MCfarland 2 solution, we do not do it by eye, we adjust it with the device. However, unfortunately, I was sorry that I did not realize that this detail was not provided during the manuscript control. The figure of the pellet was added in the manuscript. To provide insight for using of the methods in routine practice, the other pellet was prepared by centrifuging all the colonies grown in two MGIT tubes. To avoid bias, all pellets were assigned to the methods randomly by a blinded individual who did not know which method was being used. The figure of colonial pellet was shown in Figure 2. The initial quantity shown in Figure 2 was similar across the methods, and those with very small amounts, such as pellets corresponding to less than 100 microliters of water in a 1.5 ml microcentrifuge tube, were not selected for the methods.

I agree with you on the point that spectrometry would allow for more precise measurements. When planning the experiment, I also thought that spectrometry could provide better results. For this reason , BD PhoenixSpec™ nephelometer device was used to ajust McFarland two. However, no facility in our institution was available for OD measurement for tuberculosis. Therefore, I decided to standardize the initial quantity by adjusting the turbidity to the McFarland standard by using BD PhoenixSpec™ nephelometer device and taking 1 ml from there.

2 Revision 1

0 comments

No public comments in this round.