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OPEN PEER REVIEW

Effect of Propidium Monoazide in the Detection of Enterotoxigenic *Escherichia coli* in the Pediatric

Ahmad Nasser, Mohammad Mehdi Soltan Dallal, Abbas Rahimi Foroushani, Jila Yavarian

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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

4 comments · 12 Feb 2023 – 20 Mar 2023

Referee 1 Reviewer

MAJOR REVISION 12 Feb 2023

Dear Editor in Chief of APID,
The manuscript presented comparative results for the assessment of the application of Propidium monoazide (a viable dye) for the detection of viable ETEC by real-time PCR in stool samples. The results were compared with those of conventional culture and qRT-PCR. The study is mainly methodological and laboratory-based.

General comment:
The manuscript should be edited thoroughly to correct its syntax and grammar mistakes.

Specific comments:
Please correct the manuscript according to the following comments:
1. ABSTRACT:
The conclusion should present an overview and statements about the findings. Please delete general points (as depicted below) that are unrelated to the results:
"Monitoring of Escherichia coli (E. coli) pathotypes can lead to the identification of possible risks of their dissemination and the development of enteric disease. Identification of ETEC as one of major diarrheagenic agent is important. Children are sensitive to the loss of water and electrolytes caused by diarrheal disease. Accurate identification and the amount of bacteria have an important role in their treatment, because they reflect the degree of infection, so that a higher number of bacteria indicates a greater volume of bacteria inside the body. The number of bacteria in the dilution between 10-1 to a 10-9 is well distinguished in the present study. This method can reduce the time of detection from days to many hours. In addition, this method can only detect viable cell which can multiply and cause disease."

2. Methods:
Please correct the spelling of McConkey agar in the text and figure.
Please replace CT with Ct in all cases.

3. Results:
3.1. Please rewrite the following sentence. It is confusing:
"The result shows that was in the dilution that not treated with PMA, from 10-1 to 10-8 the CT numbers were 11.5, 13.5, 15, 19, 21.5, 24, 27, and 30 respectively. In the dilution 10-9 CT, the increased to 33.5, and finally in the dilution 10-10 the CT threshold was undetectable. These result are almost the same as the results of the standard curve."
3.2. Please use the past tense in the results section.
3.3. Please do not compare the results with results of other studies in the results section. Such comparisons should be transferred to the discussion section.
Example: "In previous studies the different dilutions of 10, 20, 40 and 100 µM of PMA were investigated, which showed good efficiency against free DNA and the lowest effect on the live cell. In this study we used a 50 mM dilution as the standard dilution (21-23). However, to treat a clinical sample, 50 mM of PMA should be used for optimal inhibitory effects.When a diluted sample was treated with PMA, the CT value increased due to the removal of DNA released by dead bacteria."
Discussion:
1. A comparison between the studied method and other methods that are available for the detection of viable pathogens should be presented in the discussion section. The LOD, sensitivity, and specificity of the methods

and their importance in clinical settings should also be described.
2. Vegetative is a term that is generally used for spore-forming bacteria. Please correct.

Figure:
While stool was described as the test sample in the study, milk was presented in the description line of Figure 1.

Referee 2 Reviewer

MAJOR REVISION 19 Mar 2023

Dear Editor
Thank you so much for considering me as a reviewer for your journal. This manuscript is about “Effect of propodium monoazide in the detection of enterotoxigenic Escherichia coli in the pediatric”. This manuscript needs major revision.
Comments
- The English of the manuscript needs improvement.
- The advantages and disadvantages of this method in contrast to conventional or usual Real-Time PCR should be discussed.
- The author should use up-to-date references.

Sincerely,

Associate Editor

MAJOR REVISION 19 Mar 2023

Dear Editor-in-Chief of APID,
The manuscript needs Major revision. Authors should consider the reviewers' comments.

Regards,
Reviewer 1:
- The English of the manuscript needs improvement.
- The figures were not visible in my reviewer profiles.
- The advantages and disadvantages of this method in contrast to conventional or usual real-time PCR should be discussed.
- The author should use up-to-date references.

Reviewer 2:
The study presents comparative results for the assessment of the application of Propidium monoazide (a viable dye) for the detection of viable ETEC by real-time PCR in stool samples. The results were compared with conventional culture and qRT-PCR. The study is mainly methodological and laboratory-based.
General comment:
1- Please consider the scope of the journal, since APID publishes studies with clinical relevance to the field of pediatric diseases. The decision about acceptance of methodological studies will be made by the Editor-in-Chief of APID.
2- The manuscript should be edited thoroughly to correct its syntax and grammar mistakes.

Please correct your manuscript according to the following comments:
1. ABSTRACT:
The conclusion should present an overview and statements about your findings. Please delete general points unrelated to your results, as shown below:
“Monitoring of Escherichia coli (E. coli) pathotypes can lead to the identification of possible risks of their

dissemination and the development of enteric disease. Identification of ETEC as one of major diarrheagenic agent is important. Children are sensitive to the loss of water and electrolytes caused by diarrheal disease. Accurate identification and the amount of bacteria have an important role in their treatment, because they reflect the degree of infection, so that a higher number of bacteria indicates a greater volume of bacteria inside the body. The number of bacteria in the dilution between 10-1 to a 10-9 is well distinguished in the present study. This method can reduce the time of detection from days to many hours. In addition, this method can only detect viable cell which can multiply and cause disease."

2. Methods:

Please correct the spelling of McConkey agar in the text and figure.

Please replace CT with Ct in all cases.

3. Results:

3.1. Please rewrite the following sentence. It is confusing:

"The result shows that was in the dilution that not treated with PMA, from 10-1 to 10-8 the CT 194 numbers were 11.5, 13.5, 15, 19, 21.5, 24, 27, and 30 respectively. In the dilution 10-9 CT, the increased to 33.5, and finally in the dilution 10-10 the CT threshold was undetectable. These result are almost the same as the results of the standard curve."

3.2. Please use the past tense in the results section.

3.3. Please do not compare your results with results of other studies in the results section. Such comparisons should be transferred to the discussion section.

Example: "In previous studies the different dilutions of 10, 20, 40 and 100 µM of PMA were investigated, which showed good efficiency against free DNA and the lowest effect on the live cell. In this study we used a 50 mM dilution as the standard dilution (21-23). However, to treat a clinical sample, 50 mM of PMA should be used for optimal inhibitory effects.When a diluted sample was treated with PMA, the CT value increased due to the removal of DNA released by dead bacteria."

Discussion:

1. A comparison between the studied method and other methods that are available for the detection of viable pathogens should be presented in the discussion section. The LOD, sensitivity, and specificity of the methods and their importance in clinical settings should also be described.

2. Vegetative is a term that is generally used for spore-forming bacteria. Please correct.

Figure:

While stool was described as the test sample in your study, milk was presented in the description line of Figure 1.

Associate Editor

MAJOR REVISION 20 Mar 2023

Dear author

1- "To quantify the quantity of live bacteria …" is not the aim of a research study. Please clarify the main purpose of the researchers in conducting this research.

2- It should be specified whether this study is an exploratory, descriptive, or explanatory study. If the study is descriptive, the factors that are to be described should be clearly stated in the objective section of the study. If the study is exploratory, it is necessary for the "discussion" section to have a specific procedure for interpreting and summarizing the results, in order to determine the value of the obtained results from a research or clinical point of view, and it is also necessary to provide hypotheses for further studies. In an explanatory study, there must already be a hypothesis that the study can test.

Regards

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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.

Rebuttal letter

Dear Editor

As requested, the manuscript has been heavily proofread and edited. In other words, for the purpose of greater clarity and professional readability, we have made extensive modification to the manuscript in terms of linguistic and overall language structure. Please Note that due to extensive change made, we did not make highlight, but instead used track change tool in Microsoft word.

Please find both the current revised file (with track change) and the final clean file attached.

We hope the current revised form of submitted manuscript would meet the high standard of the journal.

All the best.

> Reviewers' Comments:

Reviewer 1:

Dear Editor in Chief of APID,

The manuscript presented a comparative results for the assessment of the application of Propidium monoazide (A viable dye) for detection of viable ETEC by real time PCR in stool samples. The results were compared with conventional culture and qRT-PCR. The study is mainly methodological and laboratory based.

General comment:

1. The manuscript should be edited thoroughly to correct its syntax and grammar mistakes.

Response: The manuscript was edited by a professional editor.

Specific comments:

Please correct your manuscript according to the following comments:

1. ABSTRACT:

The conclusion should presents overview and statements about your findings. Please deleted general points (as depicted below) that are unrelated to your results:

“Monitoring of Escherichia coli (E. coli) pathotypes can lead to the identification of possible risks of their dissemination and the development of enteric disease. Identification of ETEC as one of major diarrheagenic agent is important. Children are sensitive to the loss of water and electrolytes caused by diarrheal disease. Accurate identification and the amount of bacteria have an important role in their treatment, because they reflect the degree of infection, so that a higher number of bacteria indicates a greater volume of bacteria inside the body. The number of bacteria in the dilution between 10-1 to a 10-9 is well distinguished in the present study. This method can reduce the time of detection from days to many hours. In addition, this method can only detect viable cell which can multiply and cause disease.”

Response: Dear reviewer, thank you for your helpful comment. Based on your comment, we rewrite the conclusion section.

2. Methods:

Please correct spell of McConkey agar in the text and figure.

Response: Done and acknowledged.

Please replace Ct instead of CT in all the cases.

Response: Done and acknowledged.

3. Results:

3.1. Please rewrite the following sentence. It is confusing:

“The result shows that was in the dilution that not treated with PMA, from 10-1 to 10-8 the CT

numbers were 11.5, 13.5, 15, 19, 21.5, 24, 27, and 30 respectively. In the dilution 10-9 CT, the increased to 33.5, and finally in the dilution 10-10 the CT threshold was undetectable. These result are almost the same as the results of the standard curve.”

Response: Done and acknowledged.

3.2. Please use past sentences in the results section

Response: Done and acknowledged.

3.3. Please do not compare your results with results of other studies in the results section. Such comparisons should be transfer to discussion section

Example: “In previous studies the different dilutions of 10, 20, 40 and 100 µM of PMA were investigated, which showed good efficiency against free DNA and the lowest effect on the live cell. In this study we used a 50 mM dilution as the standard dilution (21-23). However, to treat a clinical sample, 50 mM of PMA should be used for optimal inhibitory effects.When a diluted sample was treated with PMA, the CT value increased due to the removal of DNA released by dead bacteria.”

Response: Dear reviewer, thank you for your helpful comment. Based on your comment, we rewrite the result and discussion sections.

Discussion:

1. A comparison between the studied method with other methods that are available for detection of viable pathogens should be presented in discussion section. LOD, sensitivity, specificity of the methods and their importance at clinical settings also should be described.

Response: Done and acknowledged.

2. Vegetative is a terminology that generally for spore-forming bacteria. Please correct.

Response: Done and acknowledged.

Figure:

While stool was described as the test sample in your study, milk was presented in description line of Figure 1.

Response: Done and acknowledged.

Reviewer 2:

Dear Editor

Thank you so much for considering me as a reviewer in your journal. This manuscript is about “Effect of propodium monoazide in the detection of enterotoxigenic Escherichia coli in the pediatric”. This manuscript needs major revision.

Comments

- The English of the manuscripts needs improvement.

Response: The manuscript was edited by a professional editor.

- The figures were not observed in my reviewer profiles.

- The advantage and disadvantages of this method in contrast to conventional or usual Real-Time PCR should be discussed.

Response: Dear reviewer, thank you for your helpful comment. Based on your comment, we discussed the advantage and disadvantage of each method.

- The author should use up-date references.

Response: Done and acknowledged.

Associate Editor 2:

Dear author

1- “To quantify the quantity of live bacteria …” is not the aim of a research study. Please clarify the main purpose of the researchers to conduct this research.

2- It should be specified whether this study is an exploratory or descriptive or explanatory study. If the study is descriptive, the factors that are to be described should be clearly stated in the objective section of the study. If the study is exploratory, it is necessary for the "discussion" section to have a specific procedure for interpreting and summarizing the results, in order to determine the value of the obtained results from a research or clinical point of view, and it is also necessary to provide hypotheses for further studies. In an explanatory study, there must already be a hypothesis that the study can test.

Regards

Response: since conventional method in distinguishing between live and dead bacteria have limitation and are not very effective, a hypothesis was put forward as to whether it is possible to distinguish between live and dead bacteria using a new method. In this study, we introduced a new method and examined its sensitivity and specificity compared to conventional methods.

2 Revision 1

1 comment · 31 Mar 2023

Associate Editor

ACCEPT 31 Mar 2023

Dear Editor-in-Chief of APID,
The authors responded to all the comments and revised the manuscript accordingly. The manuscript could be accepted at this step.

Regards