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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Virology

ESPS manuscript NO: 20174

Title: Rapid genotyping of human rotavirus using SYBR green real-time reverse transcription-polymerase chain reaction with melting curve analysis

Reviewer's code: 00484099

Reviewer's country: Chile

Science editor: Yue-Li Tian

Date sent for review: 2015-06-02 08:59

Date reviewed: 2015-06-02 22:52

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input checked="" type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input checked="" type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Duplicate publication	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade D: Rejected	<input checked="" type="checkbox"/> No	<input checked="" type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor		BPG Search:	<input type="checkbox"/> Major revision
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input checked="" type="checkbox"/> No	

COMMENTS TO AUTHORS

This article provides new an very useful tool for genotyping rotavirus. I recomend to show the results of sequencing, since they are demosntration that the technique works as it should.



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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Virology

ESPS manuscript NO: 20174

Title: Rapid genotyping of human rotavirus using SYBR green real-time reverse transcription-polymerase chain reaction with melting curve analysis

Reviewer's code: 00053556

Reviewer's country: Egypt

Science editor: Yue-Li Tian

Date sent for review: 2015-06-02 08:59

Date reviewed: 2015-06-15 04:22

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input checked="" type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input checked="" type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input checked="" type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Duplicate publication	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade D: Rejected	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor		BPG Search:	<input type="checkbox"/> Major revision
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input checked="" type="checkbox"/> No	

COMMENTS TO AUTHORS

Comments to the Editor: Thanks for inviting me to review the article entitled "Rapid genotyping of human rotavirus using SYBR Green real-time RT-PCR with melting curve analysis". Comments to the authors: Minor Comment: o Minor editing polishing is needed o Language evaluation: "A". Comments to Authors: 1. TITLE Reflect the major content of the article 2. ABSTRACT It gives a clear delineation of the research background, including important data and conclusions; however, the aim of the study is not clearly identified. Also, the type of the samples was missing. 3. INTRODUCTION Provides sufficient background regarding the studied topic and the aim of the study is clearly identified 4. MATERIALS AND METHODS: Full description is provided for this section; however and in order to satisfy the reader, some important issues are better to be elaborated. o Subheadings for clinical specimens viral RNA extraction, R-T reaction and Real time PCR,.... were missing and are better to be maintained. o Preparation of stool suspension for RNA extraction is better to be included. o The detection limit and the detection range of the developed assay are better to be mentioned. o The source of positive controls of specific G and P genotypes has to be mentioned.



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o Statistical methods are missing and have to be mentioned. 5. RESULTS: ? An overall theoretical analysis of the study results is well covered. ? Provide sufficient experimental data, however, Paragraph 3 (lines 3-6): it is a discussion rather than results. This has to be revised ? Figures & tables are well presented 6. Discussion: The section is almost well organized; an overall theoretical analysis concerning the provided data is well covered, however, the results of the first paragraph were not clearly presented in the results section; specificity, sensitivity, correlation. 7-REFERENCES: Relevant and sufficient references were adequately cited and PMID/DOI is well maintained for all the cited references.



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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Virology

ESPS manuscript NO: 20174

Title: Rapid genotyping of human rotavirus using SYBR green real-time reverse transcription-polymerase chain reaction with melting curve analysis

Reviewer's code: 00070481

Reviewer's country: China

Science editor: Yue-Li Tian

Date sent for review: 2015-06-02 08:59

Date reviewed: 2015-06-02 16:00

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input checked="" type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input checked="" type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input checked="" type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Duplicate publication	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade D: Rejected	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor		BPG Search:	<input type="checkbox"/> Major revision
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input checked="" type="checkbox"/> No	

COMMENTS TO AUTHORS

The method described by the authors had been conducted by others to genotype other viruses or mRNA. But the work should be meaningful for the rotavirus genotyping.

ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Virology

ESPS manuscript NO: 20174

Title: Rapid genotyping of human rotavirus using SYBR green real-time reverse transcription-polymerase chain reaction with melting curve analysis

Reviewer's code: 00504174

Reviewer's country: Italy

Science editor: Yue-Li Tian

Date sent for review: 2015-06-02 08:59

Date reviewed: 2015-06-09 16:26

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good		<input type="checkbox"/> Duplicate publication	
<input checked="" type="checkbox"/> Grade D: Fair	<input checked="" type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Plagiarism	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade E: Poor	<input type="checkbox"/> Grade D: Rejected	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Minor revision
		BPG Search:	<input checked="" type="checkbox"/> Major revision
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input checked="" type="checkbox"/> No	

COMMENTS TO AUTHORS

Tong and colleagues developed and validated a different approach to perform rotavirus G and P genotyping using a two-step SYBR Green real-time RT-PCR (rt-gPCR). In this effort the authors selected published cnRT-PCR genotype specific primers and optimized the amplification conditions using melting temperature to genotype the rotaviruses in the different samples. The sensitivity of the rt-gPCR, evaluated by using 16 samples of G and P genotypes, was the same of the conventional nested RT-PCR without cross-reactions with other gastroenteritis viruses. Using this techniques the authors genotyped 121 rotaviruses samples previously identified by electron microscopy. In this analysis the authors reports different genotypes G1P[8] (42.6%), G2P[4] (4.9%), G3P[8] (10.7%), G9P[8] (10.7%), G9P[4] (6.6%), G12P[8] (23.0%), and unknown GP[8] (0.8%). Collectively this study remarks the importance of developing new sensitive and rapid molecular techniques to monitor of rotavirus genotypes is important. However few points are not clear and need to be improved. Main point 1- In the first part of the results section, lines 9-11, the authors mentioned sequence data of the amplicons but they are not reported any methods used for sequencing. Why it is not reported the sequence data



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and specificities of the methods ? Are the sequences obtained previously ? 2- In the first part of the results section, lines 14-15, the authors should specify the type of the viruses. 3- In the first part of the results section, lines 18-19, the issue is not clear. 4- In the first part of the discussion, lines 6-14, what is the meaning of carry out a specific genotype examination before other genotype. This strategy has a longer turn-around-time. Why the authors have not considered to amplify shorter genomic regions using primers different from those published but able to genotype the different rotaviruses. Is it not possible because the regions are conserved ? the use of short regions (100-150 bp or lower) should improve the sensitivity and specificity of the assay. Minor points 1- Introduction, first page line 23. Conventional nested RT-PCR should be changed in "Conventional Reverse transcriptase nested PCR" 2- Introduction, second page line 9. Real time RT-PCR should be changed in "Reverse transcriptase (RT) Real time PCR" as in Materials and methods. 3- In materials and methods, EM should be written completely. 4- All typos in the text should be corrected.



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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Virology

ESPS manuscript NO: 20174

Title: Rapid genotyping of human rotavirus using SYBR green real-time reverse transcription-polymerase chain reaction with melting curve analysis

Reviewer's code: 00503952

Reviewer's country: Canada

Science editor: Yue-Li Tian

Date sent for review: 2015-06-02 08:59

Date reviewed: 2015-06-10 04:57

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good		<input type="checkbox"/> Duplicate publication	
<input checked="" type="checkbox"/> Grade D: Fair	<input checked="" type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Plagiarism	<input checked="" type="checkbox"/> Rejection
<input type="checkbox"/> Grade E: Poor	<input type="checkbox"/> Grade D: Rejected	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Minor revision
		BPG Search:	<input type="checkbox"/> Major revision
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input checked="" type="checkbox"/> No	

COMMENTS TO AUTHORS

The authors reported that "The mean T_m ($^{\circ}C \pm SD$) for each of the genotypes were: G1 at $80.0^{\circ}C \pm 0.20$, G2 at $80.9^{\circ}C \pm 0.49$, G3 at $81.7^{\circ}C \pm 0.22$, G4 at $80.7^{\circ}C \pm 0.20$, G9 at $80.9^{\circ}C \pm 0.45$, G12 at $80.6^{\circ}C \pm 0.37$, P[4] at $80.7^{\circ}C \pm 0.50$, and P[8] at $80.0^{\circ}C \pm 0.34$." However, I noticed that G1=P[8]; and G2=G9; and from figure 1 and figure 2, I was not convinced that the mean T_m can be used to separate different genotypes of rotaviruses. Minor mistakes: On Page 4, "which encodes six structure proteins (VP14, VP6 and VP7)" should be "which encodes six structure proteins (VP1-4, VP6 and VP7). There are some grammatical errors.



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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Virology

ESPS manuscript NO: 20174

Title: Rapid genotyping of human rotavirus using SYBR green real-time reverse transcription-polymerase chain reaction with melting curve analysis

Reviewer's code: 00504271

Reviewer's country: Japan

Science editor: Yue-Li Tian

Date sent for review: 2015-06-02 08:59

Date reviewed: 2015-06-10 12:02

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Duplicate publication	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade D: Rejected	<input type="checkbox"/> Plagiarism	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor		[Y] No	<input type="checkbox"/> Major revision
		BPG Search:	
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

The manuscript by Tong et al. introduced the new genotyping method of human Rotaviruses. This manuscript should be published because this method is worthwhile. This method can be applied for clade-analysis of closely related viruses, which the multiplex PCR method is not available as indicated in the manuscript (p. 8, l. 15-21). The figure legend is insufficient and should be revised properly.



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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Virology

ESPS manuscript NO: 20174

Title: Rapid genotyping of human rotavirus using SYBR green real-time reverse transcription-polymerase chain reaction with melting curve analysis

Reviewer's code: 00504881

Reviewer's country: United States

Science editor: Yue-Li Tian

Date sent for review: 2015-06-02 08:59

Date reviewed: 2015-06-15 23:28

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Duplicate publication	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade D: Rejected	<input type="checkbox"/> Plagiarism	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor		<input type="checkbox"/> No	<input type="checkbox"/> Major revision
		BPG Search:	
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input type="checkbox"/> No	

COMMENTS TO AUTHORS

The article describes a new assay for genotyping the human rotavirus. The results presented seem pretty convincing and conclusions are valid. Even though the clinical implications might be limited as the simultaneous assay for multiple genotypes cannot be performed by this assay (due to narrow range of Tm among the genotypes). Authors should describe the basic principle (mechanism) of using melting temp "Tm" as distinguishing feature for genotyping. Difference in "Tm" has been extensively used in bacterial and other microbial phylogenetics to identify a new species. In introduction- second paragraph- first sentence- VP14 should be VP1-4.