March 23, 2015

Dear Editor,

Please find enclosed the revised manuscript in Word format (file name: ***16610-review.doc***). Below, we address all comments by the reviewers, and discuss them point-by-point.

Title: **Next-Generation Sequencing in Clinical Virology: Discovery of New Viruses**

Authors: Sibnarayan Datta, Raghvendra Budhauliya, Bidisha Das, Soumya Chatterjee, Vanlalhmuaka, Vijay Veer

Name of Journal: ***World Journal of Virology***

ESPS Manuscript NO: 16610

The manuscript has been improved according to the suggestions of reviewers.

1. Format has been updated

2. Revision has been made according to the suggestions of the entire three reviewers. All suggested changes to wording and punctuation have been incorporated.

Comments by Reviewer 1 (504045): This manuscript reviewed the evolution of virus discovery and sequencing methods, the current next-generation sequencing (NGS) methodologies and virus enrichment technologies and their applications in the clinical virus discovery. The manuscript was well organized and written. The authors are suggested to include illustrations to demonstrate different NGS virus enrichment technologies. The manuscript should be improved in grammar. Detailed correction can be found in uploaded revised manuscripts.

Our response: We sincerely thank the reviewer for his appreciation of our manuscript. We have now included additional figures to describe the virus enrichment technologies such as SISPA, VIDISCA, RCA etc., as suggested by the reviewer. We have also revised the manuscript thoroughly, and also incorporated all the corrections suggested by the reviewer in the revised manuscript.

Comments by Reviewer 2 (504019): Some errors need correct:

i) Severe Acute Respiratory Syndrome (SARS), should be abbreviated at the first time;

ii) Change "and having significant experimental basis" to "and need significant experimental basis";

iii) Change "Additionally, repeated passaging of the virus to obtain high titer is suggested to change the population of virus being sought" to "Additionally, repeated passaging of the virus to obtain high titer is believed could change the population of virus being sought";

iv) Change "The limitations of sequence dependent techniques encouraged the investigators to resort to 'metagenomics'," to "The limitations of sequence dependent techniques prompted the investigators to resort to 'metagenomics'";

v) Change "Alternatively, in an another study" to "Alternatively, in another study";

vi) Change "here are numerous application pf NGS" to "here are numerous application of NGS".

Our response: We are extremely thankful to the reviewer for his suggestions and corrections. We have now incorporated all the suggested changes in the revised manuscript.

Comments by Reviewer 3 (00646291)

Major comments :

i) In the section entitled EVOLUTION OF VIRUS DISCOVERY TECHNIQUES insert a Table indicating similarities and differences, and advantages and disadvantages between the different virus discovery techniques?

ii) In the section entitled EVOLUTION OF SEQUENCING TECHNOLOGIES insert a diagram indicating the added features at each stage of the different generations of sequence technologies?

iii) In the section entitled SAMPLE PREPARATION FOR VIRAL METAGENOMICS & DISCOVERY a brief description of SISPA, VIDISCA, RCA, TUViD-VM instead of merely listing the viruses discovered using each one of these methodological approaches would benefit the review?

iv) In the section entitled "BIOINFORMATICS CHALLENGES ASSOCIATED WITH NGS add a Table indicating the bioinformatics challenges and the action that have been taken or have been proposed to overcome them.

Our response to Major comments:

i) We have now incorporated Table 1 showing a comparison of different Virus discovery techniques, as suggested by the reviewer.

ii) We completely agree with the reviewer that diagrams indicating the evolution of different generations of sequencing technologies would surely have added more edge to the present manuscript. Initially, we also planned to include the same, but taking into consideration the complex chemical and physical processes involved in the latest NGS systems, we observed that their inclusion made the manuscript complex and lengthy at the cost of loss of primary focus of the manuscript i.e. reviewing the application of Next Generation sequencing technologies in virus discovery. So, to keep the emphasis on the application of these technologies, within considerable manuscript size, we did not include them. Nevertheless, a good number of excellent reviews (referred to in the text) describing in-depth details of the different NGS technologies along with diagrammatic representations have been published, most of which are freely accessibly.

iii) We have now included detailed figures, with legends briefly describing the SISPA, VIDISCA and RCA techniques. Since TUViD-VM is just a modification of SISPA for tissue samples, it was not included. The details of the TUViD-VM can be had from the original article by *Kohl et al., 2015*, which is freely accessible.

iv) We have now incorporated Table 2 showing the bioinformatic challenges and their available/proposed solutions, as suggested by the reviewer.

Minor comments :

i) Page 4: "and also in their prevention" replace with "in prevention of viral infections"

ii) Page 4: By the late-1950s, it was generally believed that most of the human pathogenic viruses "have" replace with "had"

iii) Page 4: Delete (;) after rapid

iv) Page 6: replace "virus(s)" with "virus(es)"
v) Page 6: Rephrase the sentence "Virus isolate(s) were then purified from the cells or cell supernatant using density gradient and other high speed centrifugal techniques, followed by structural characterization of viral particles, antigens, nucleic acids, through different biophysical and biochemical methods"
vi) Page 7: replace "a technique that do not" with "a technique that does not"
vii) Page 7: The sentence "Metagenomic involve direct unbiased amplification of total genetic material present in a given sample, without culturing the organisms, that are otherwise genetically too diverse to be detected by degenerate or consensus PCR, their cloning and sequencing" does not make sense. ?
viii) Page 12: replace "the scientific literature is very less as compared to Roche 454" with "the scientific literature is limited compared to Roche 454"
ix) Page 16: replace "a novel viruses" with "a novel virus"
x) Page 16: rephrase the sentence: "that were designed not anneal to ribosomal RNA"
xi) Page 17: replace "numerous application pf NGS" with "numerous applications of NGS"
xii) Page 19: the sentence at the top of the page is not complete
xiii) The references 13, 79 and 94 should be formatted in a similar way as the rest of the references.

Our response to Minor comments: We have now made all the suggested changes in the revised manuscript.

3 References and typesetting were corrected

Thank you again for considering our manuscript for publishing in the ***World Journal of Virology***.

Sincerely yours,

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