Reviewer's report

Title: A novel pancoronavirus RT-PCR assay: frequent detection of human coronavirus NL63 in children hospitalized with respiratory tract infections in Belgium

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Reviewer: Eric C.J. CJ Claas

Reviewer's report:

General

This manuscript describes the detection of 7 cases of the recently discovered human coronavirus NL63 in hospitalized children in Belgium using a pancoronavirus PCR. In addition, HCoV-229E and OC43 are detected in these patients.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

- The manuscript lacks information on the analytical and clinical sensitivity and the specificity of the pancoronavirus PCR. These issues are of particular importance when using highly degenerate primers.
- The manuscript would be improved by adding clinical data of the patients infected with NL63. The description URTI and LRTI in table 1 is too general. For the patient group that has been tested, terms as "serious respiratory symptoms" (page 5) and "relatively severe respiratory diseases" (page 9) is not very informative either.
- Page 8, last line: "These results.." needs to be put in perspective as these results are biased by the fact that sampling is only performed from January to May. This is also the case for the coinciding epidemic seasons for OC43 and NL63. In addition, the numbers are too small for a conclusion like that.
- Some discussion should be added on the fact that based on the discordant phylogeny when comparing different genes. Based on the ORF1a sequences there appear to be four clades of viruses. However, within these clades different subtypes based on the spike region can be observed (specifically NL-p223 and HCOV-NL). Basically this means that the true phylogeny can only be established by analyzing full-length sequences.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

- The experimental procedure for detection of HCoV-NL63 is not clear. Using the pancoronavirus primers, every coronavirus results in a 251 bp fragment (figure 2). How are the viruses differentiated? The text reads that HCoV-NL63 positives are confirmed by amplification and sequence analysis of four other regions, but how the primary identification of NL63 (and also for the 229E and OC43) is made remains elusive.
- Page 5: Discard the use of the "detection frequency" as the numbers are low: 7.7% when 1 out of 13 samples is positive is misleading. Figure 3A and B can be easily combined to one figure. This
should also be adjusted in the discussion on page 8.  
- Page 5 and figure 2: It is not clear what has been tested in figure 2: cell lysates, purified virus, something else? Do these degenerate primers generate specific PCR products on clinical specimens? 
- Page 11: If the samples are exclusively tested for RSV, indicate that no other pathogens were tested.

Discretionary Revisions (which the author can choose to ignore)

- Page 2, line 8 and page 10, line 4: samples do not get infected. 
- Figure 1: it seems more logic to combine the sequences by phylogeny of the viruses. Now the primer sequence looks more heterologous by the scattered appearance of NL63, PEDV, TGEV and 229E in the alignment. 
- Page 8, line 15: Provide some information on VATER as well (analogous to SLI). 
- Table 1: Remove RTI abbreviation in footnote and do not abbreviate it in the title.

What next? : Accept after minor essential revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests: 
I declare that I have no competing interests