Reviewer's report

Title: Detection of Norovirus genogroup I and II by multiplex real-time 3'-minor groove binder TaqMan assay

Version: 1 Date: 24 November 2005

Reviewer: Xiaoli Pang

Reviewer's report:

General
This is scientifically sound research with good rationale for applying one-tube multiplex real-time RT-PCR for detection and quantitation of Norovirus GI and GII. Although there were several publications of multiplex real time RT-PCR for detection of Norovirus in stool samples from outbreaks and sporadic of acute gastroenteritis, authors applied 3'- MGB labeled ‘shorter’ probe to increase broadly detecting in diversity norovirus genotypes. Authors have used a new development of the probe and primers in the high conserved sequence region for detection of norovirus GI and combined two monoplex PCR together for a multiplex PCR. The sensitivity of monoplex and multiplex PCR has been compared for detection of norovirus GI and GII. The results and analysis were clear and reasonable even though there was little redundant description in the results. A broad dynamic range allowed accurately quantification of norovirus in stool sample and provided a useful tool for further understanding viral shed of norovirus and clinical relevance of infection. Discussion was sound and conclusion was concise and appropriate.

Reviewer noticed that the study compared retrospectively the samples previously assayed using nest-RT PCR and monoplex real-time PCR. Time differences between two assays carried out might introduce variations and mis-interpret the sensitivity of newly developed assay. It is acceptable during the assay development phase. It would be good to see the data from further validation study in which the same samples are run in parallel using two mentioned assays.

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

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

1. Page of Abstract: last sentence in the conclusion: does the ‘single cases’ mean ‘sporadic cases’?

2. Page of results:
   a. There were a lot of redundancies in the first paragraph that had been described in the materials and methods. Suggest revising.
   b. Paragraph 3 (starting from overall...)
      i. Pair t-test should express as ‘p>0.05’
      ii. Last sentence ‘by factor’: What does the factor mean here? It should express the variation as coefficient variation (CV) (also refer to figure 4). It should be indicated clearly.
   c. Paragraph 4 (also refer to Table 3): Results showed in the table that there was reasonable correlation between expected and detected quantity when the copy numbers of plasmid DNAs were higher. However, the correlation was not good when the copy numbers were lower (for instance GI and GII at 10^2). The low copy number of known viral RNA mixture should be included for evaluating the efficiency of the assay.
3. Page of discussion:
   a. Paragraph 1 line 11: ‘lower the cost’ is better replaced by ‘reduce’
   b. 97% detection rate was obtained by multiplex assay compared to previous PCR assay but there was no further discussion why 3% discordance was obtained in the multiplex assay. It would be interested to find out.

4. Page of materials and methods:
   a. Paragraph One tube multiplex real-time RT-PCR, line 10; consider revising ‘96 tube plates’.

Discretionary Revisions (which the author can choose to ignore)

What next?: Accept after minor essential revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

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