Reviewer's report

Title: Improved primers for the detection and identification of human enterovirus 71 by RT PCR

Version: 1 Date: 6 March 2004

Reviewer: Hiroyuki Shimizu

Reviewer's report:

General

The paper describes a new diagnostic RT-PCR system of enterovirus 71 (EV71) using an improved primer pair, MAS01S/MAS02A. As the authors mentioned, development of rapid and more reliable laboratory diagnosis of EV71 for hand, foot and mouth disease is still needed because EV71 infection can be associated with severe neurological manifestations including fatal cases. The object of the study is clear and the methods used are appropriate, however, more detailed comparative evaluation and validation should be conducted to clarify the advantage of this methodology in comparison with the EV71-specific RT-PCR systems previously reported by other groups.

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

As mentioned by the authors in the section of Discussion (page 14), several previous reports already described specific detection of EV71 by RT-PCR including the design of the primer pairs. The authors provided several possible advantages of this MAS01S/MAS02A primer pair in the Discussion (page 14, line 15 - page 15, line 2). However, the discussion seems to be too speculative and the experimental data is insufficient to support the discussion. Comparative analysis of several RT-PCR systems using some representative virus isolates and primary clinical samples needs to be provided to evaluate the reliability and sensitivity of each system.

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. The specimen numbers, corresponding to those in Table 2 (list of clinical specimens), should be indicated on Figure 4 (RT-PCR on the clinical specimens).

2. The development and validation of a new diagnostic RT-PCR of EV71 is the major objective of this study. Therefore, the phylogenetic analysis of CA16 (Figure 6 and page 12) and related discussions can be deleted although the data itself would be valuable.

3. On page 18, line 18, MSA01S should be MAS01S.

4. On page 18, line 23, MAS02/A should be MAS02A.

Discretionary Revisions (which the author can choose to ignore)
What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory 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:

We are working in the same field.