Reviewer’s report

Title: Development of real-time NASBA assays with molecular beacon detection to quantify mRNA coding for HHV-8 lytic and latent genes

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Reviewer: Dr HGM Niesters

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Accept after discretionary revisions

The paper by Polstra et al describes the development and limited use of a real time based NASBA assay to detect gene expression of genes of HHV-8. The paper is written very clearly and shows that this NASBA technology is an alternative for real time based PCR assay. The use of the RNA detection is applied only in a limited number of patients (2). The paper should be published after some additional comments are clarified or addressed.

1. Page 3, last paragraph. Both NASBA and PPC|R based technologies are developing rapidly into real time based assays, making them both robust and less prone to contamination. A remark could be made indicating that NASBA based primers have different restrictions in their design.

2. Page 5. The method describing the NASBA technology in detail should be omitted from the paper. The journal is not a textbook.

3. Page 6. Give more details on the origin of the plasmids (company name, place, country)

4. Page 6. Top paragraph. I can imagine that it is not clear for the reader that the beacon is designed to be open at a relative low temperature (41 C) i.e. the NASBA operating temperature.

5. Page 7, line 18. How is the threshold easily designed?

6. Page 8, bottom paragraph. Can the authors give more details on the actual influence of this background RNA that disturbs quantification (is there a reference?).

7. Page 9, 3rd paragraph. I conclude from figure 2, that there are differences in quantification among the RNAs detected. Imagine that the ttp should be identical for the different targets (equal efficiency of amplification), I notice that the ttp for 3 log ORF73 RNA is 25, while for vBCL2 this is 35. Is my observation and interpretation correct? Of course the most difficult part is the accurate quantification of the in vitro synthesized RNA.

8. Page 11. Why are the data presented in Table 3 not quantified? The methodology is presented in the paper, but omitted in this table (to my surprise).

I feel that the technology has enormous potential to analyze in more detail expression levels for a number of transcripts of HHV8 and the paper is worthwhile publishing.

Competing interests:
None declared.