Reviewer's report

Title: Clinical Utility of a Nested Nucleic Acid Amplification Format in Comparison to Viral Culture for the Diagnosis of Mucosal Herpes Simplex Infection

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Reviewer: Dr Marek Slomka

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

Advice on publication: Accept after revision, which I do not need to see

Overall a well-conducted scientific study, though there are a few points:

Major point
Beginning with the "Conclusions", here the authors present many valid issues concerning the use of PCR for the diagnosis of genital herpes. The point about clinical utility is particularly important. However, I feel that here there is one other area which merits some attention in the Discussion, namely the observation that the authors have used a relatively simple & straightforward means of sample preparation (ie simple vortexing, p5). Judging by the high sensitivity of the HSV PCR on these samples, it strikes me that the use of a nested HSV PCR has overcome any inhibitor problems. This is worth stressing, because other published studies on genital herpes ["single round"] PCR tend to use more involved extraction procedures to achieve comparable levels of sensitivity. Inhibitors of HSV PCR from genital specimens have been reported / alluded, hence the need for sample preparation protocols prior to ["single round"] HSV PCR. Coyle et al appear to have successfully sidestepped the need for such a pre-PCR stage, ie a nested PCR probably compensates for any inhibition which would compromise a "single round" HSV PCR. Long / labour intensive sample preparation protocols is one reason why nucleic acid amplification methods in general have not yet received universal acceptance in many routine diagnostic settings, so I would encourage the authors to point out this achievement in the Discussion.

The matter of specificity concerns for nested PCRs in diagnostic use (ie false positives) was also important - that's covered perfectly well.

Minor point
On a separate issue, it would be clearer if the authors distinguished where they tested anogenital as opposed to oral swabs. I have assumed that these are all "lumped together" throughout the manuscript eg those with "herpetic" infection ("Patients" paragraph in "Methods"), but some distinction / clarification would help. Staying in this paragraph, how did the authors know they had "(ii) first clinical episode of
non-primary HSV”? Please explain.

**Competing interests:**

None declared.