Reviewer's report

Title: Development of a multiplex methylation-specific PCR as candidate triage test for women with an HPV-positive cervical scrape

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Reviewer: Matthias Lechner

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In their study Snellenberg et al. describe the consecutive experimental steps to set up a multiplex qMSP for CADM1, MAL and has-miR-124-2 and the reference gene beta-actin. The optimization of the method and the validation of these results look sound overall. The question is well-defined.

The methods are described in detail. However, when looking at the result section, one feels that a large proportion of the text may actually be moved to M&Ms. A list of the primer sequences used may be helpful.

One concern I would raise here is that the correlations that are presented between singleplex and multiplex msQPCR reactions (although good) might lead to misinterpretation of the absolute value of methylation.

Overall, I feel that the study would be far more powerful if the authors assessed the diagnostic and predictive validity of this method by testing them thoroughly on cervical cancer samples. Sensitivity and specificity may be measured and ROC curves obtained. This should then be compared with the sens/spec of cytology testing alone. It will be interesting to see whether their proposed method may be more powerful than the commonly used cytology approach (or whether it has any advantage if used as an adjunct to cytology testing).

In conclusion, looking at the scope of this journal, I think that it would make the described work far more interesting if the multiplex reaction is applied to the testing of cervical samples. Currently, it reads as one large optimisation experiment (which is fine) but this is not novel as lots of people do this.

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

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests.