Reviewer's report

Title: Evaluation of a single round polymerase chain reaction assay using dried blood spots for diagnosis of HIV-1 infection in infants in an African setting

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Reviewer: GERARDO GARCIA-LERMA

Reviewer's report:

This is a nice and well designed study that evaluates the performance characteristics of a single round PCR assay for early infant diagnoses on DBS specimens. The authors use a comprehensive approach by first determining the sensitivity of the method using serial dilutions of known HIV copy numbers, followed by a detailed evaluation of assay performance using both well characterized DBS specimens and freshly prepared samples. The results are clearly presented and support the conclusions. The main findings are:

1-. The single round PCR assay is highly sensitive and specific suggesting that this method may be a good alternative to more expensive methods. The use of a one-step PCR protocol is also advantageous since it reduces costs and minimizes risks of contamination.

2-. Amplification of small pol fragments from DBS is possible even after 3 to 7 years of storage at room temperature. These findings are reassuring since DBS are considered an attractive alternative to plasma for molecular diagnostics.

Minor comments:

1. Page 7. Please specify the subtype of the archived samples and the freshly-collected specimens. Also, please elaborate on the performance of the assay on non-B subtypes (page 9) since this method might be especially useful in resource-limited settings.

2. Page 7. Please specify if the archived DBS were stored in ziplock bags and if desiccant was used. Also, please define “room temperature” in the storage location.

3. Page 7. Please specify if the clinical specimens were also prepared on 903 paper. Why was 903 paper selected for the initial studies with ACH2 cells? Some reports have suggested that FTA might be a better option for DNA amplifications.

4. Page 9 (last paragraph). Please elaborate on the statement regarding the use of the gag PCR assay to control for DNA integrity. Degradation may only be evident if the assay amplifies a large fragment. Was the fragment size similar for the gag and pol assays?

5. Page 10 line 3..."compromised"

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

Statistical review: No, the manuscript does not need to be seen by a statistician.

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