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: Sharon Cassol

Reviewer's report:

This is a well-written manuscript that deals with an issue that is of critical importance for the developing world, i.e. the development and evaluation of a simple and affordable assay for the diagnosis of pediatric HIV-1. Elimination of the need for nested PCR would represent an important advance with respect to cost and quality assurance. The work is of particular important given recent international guidelines recommending universal screening and early treatment of all pediatric HIV-1 infections. If untreated, approximately 40% of HIV-1-infected neonates die during the first year of life.

Major Compulsory Revisions:

1. Further clarification of how the spiked standards were made would be useful. Did all the spots contain the same amount of cellular DNA? Were the HIV-1-infected ACH2 cells serially diluted into an uninfected cell line and then mixed with HIV-1 seronegative whole blood, prior to spotting on filter paper.

2. The paper is based on the amplification and detection of a 166 bp fragment of the HIV-1 pol gene. The data would be strengthened, at least in the initial phase of assay development and evaluation, by sequence analysis showing that the 166 bp band detected in ethidium stained agarose gels was indeed the expected HIV-1 product.

3. The paper should also contain a photo of the gel showing the intensity of the bands.

Discretionary Revisions:

4. There is no description of the demographic and clinical data of the infants. I would suggest including a Table providing information on the infant’s age at the time of diagnosis, epidemiology of HIV-1 transmission and duration of infection (if available). Diagnosis of neonatal infection is dependent on these variables, especially during the first few weeks of infection.

5. The authors say that their “in-house” FP-DBS assay works across different subtypes but they do not show any data

6. Is it possible to make the assay more robust by including an additional confirmatory step, i.e. does the PCR product contain a conserved enzyme restriction site that could be used to cleave and further confirm the nature of the
product? To use labeled primers and hybridize the PCR product to a probe bound to a microtiter plate? Many diagnostic laboratories in the developing world have ELISA equipment. Are there any plans for further development?

Level of interest: An article of importance in its field

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