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: Davey M Smith

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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

Summary: This paper is a very well-written study in the validation of dried blood spots on filter paper for use for identifying newborn HIV infection.

- Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)

1. All of the tables are visually nice, but Table 1 can be a supplementary table and Tables 2 and 3 are not necessary given the data presented in the Results.
2. It would be nice for the authors to discuss the issue surrounding how a newborn HIV detection assay should function. Like, how many copies of HIV DNA are routinely needed to detect in a newborn, and how false positives may be more clinically tolerable than false negatives. This would help support the authors’ argument about needing a “reliable” diagnostic test.
3. Define the abbreviation “RT” where it concerns “room temperature” and KEMRI.

- Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. The goal is stated that the study was to develop an economical method for identifying newborn HIV infection, but no cost-analyses were performed. The authors should at least acknowledge this limitation if formal cost-analysis cannot be done retrospectively. The authors should be able provide an estimate in comparison to what approved assays, like Roche, or in-house double step PCR methods would require to perform the same number of assays.
2. It is somewhat confusing how come the authors’ performed the tests in quadruplicate, as discussed in the second paragraph of the Discussion, and how would this increase the cost of the assay. My confusion may stem from the last paragraph of the Results, which is difficult to follow. I would recommend re-wording both of these sections and describing better how and why and the cost of performing the test in quadruplicate (versus singly via Roche?). Additionally, the Methods should state clearer how the assays were compared
(statistics and study design).

3. Need to describe limitation of performing a retrospective study, since the authors’ cannot truly provide the test characteristics of the diagnostic assay within the population, i.e. sensitivity and specificity for the assay in Kenya, since in the current study the number of HIV infected samples tested were preset and not based on the prevalence of HIV infected newborns tested in the study population, i.e. the pretest probability influences the test characteristics. This should be acknowledged, and the authors should state how the assay should next be evaluated prospectively in a clinical setting.

- Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

None

**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