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

Version: 2 Date: 22 November 2010

Reviewer: Sharon Cassol

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Second review following revision:
The manuscript has been improved by addressing the specific concerns raised in the initial review and by including information on the age and subtype of the infants tested and the sequence of the PCR product, as well as a photograph showing the quality of the PCR bands as detected by ethidium bromide staining of agarose gels.

Below, I have summarized additional specific improvements and raise a couple of additional points that may require further clarification.

Possible Compulsory Revisions

1) I am a bit confused by some of the calculations. These should be re-analyzed and expressed more clearly. In the second paragraph of the results, it states that the number of ACH2 cells added to the spiked FP-DBS was calculated so that 2 µl of the final lysate would be expected to contain 10, 5, 2 and 1 copy of HIV-1 proviral DNA. I assume this is correct.

However, in the third paragraph of the methods, it says the ACH2 standards were made to contain 10, 5, 2 and 1 copy of HIV-1 DNA per 10 µl (ie. the volume of ACH2 cells spotted onto the filter paper). This was then eluted in 100 µl of lysate buffer and 2 µl of the eluate was carried over into each PCR reaction (ie. the input into the PCR reaction was 1/50th of a blood spot). To get a PCR input of 10, 5, 2 and 1 HIV-1 DNA copies, the standards applied to the filter must have contained 500, 250, 100 and 50 copies of DNA/10 µl?). Please check and clarify so that the methods and results add up.

2) Similar, in the third paragraph of the discussion, it states that the Roche assay typically tests for the amount of HIV-1 DNA contained in ~10 µL of blood (ie. 50/200 x 50 = 12.5). Is the size of blood spot not the same for the HIV-1 pol FP-DBS assay? (ie. 50/100 x 2 = 1 µl)(ie. the input volume used for the Amplicor is 12.5 times that of the pol assay?). If correct, for clinical specimens this corresponds to 5,000 PBMC or 5 X 75.4 ± 104.3 of HIV-1 DNA). Please check and clarify in case I am not correct.

3) In the 5th paragraph of the discussion, it states that the initial Roche Amplicor test gave false positive results (n=8). Since repeat testing of a second independent specimen from these same children gave negative results, by both
the Roche and pol assays, these initial false-positive results may have been due to specimen mix-up rather than assay error. This possibility can be excluded only by HLA typing of the specimens in question.

Discretionary Revisions

4) I assume that the archived FP-DBS (n=115) of known infection status were collected from children in the United States who were mostly infected with HIV-1 subtype B. Also, that these DBS specimens were stored at ambient room temperatures in a laboratory in the United States in the absence of dessicant. This should be clarified since conditions of temperature and humidity in the USA may be vastly different from those in Kenya and other tropical countries where DBS technology is most needed.

Minor Revisions

5). Figure 1 is missing information on the size of the gag PCR product

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