Reviewer’s report

Title: Assessment of Feto-maternal Hemorrhage among Rhesus D negative pregnant Mothers using the Kleihauer-Betke test (KBT) and Flowcytometry (FCM) in Addis Ababa, Ethiopia

Version: 1
Date: 6 May 2014
Reviewer: Cynthie Anderson

Reviewer’s report:

- Major Compulsory Revisions

1. The Methods section should state the names of all participating institutions, define the beginning and ending dates for enrollment, and outline the inclusion criteria and recruitment methods that were employed.

2. In addition to the statement at the end of the paper, the Methods section should state that the current study was reviewed and approved by the ethics boards of all participating institutions and that all participants gave written or oral informed consent (based on participant literacy) prior to enrollment.

3. Materials and Methods first paragraph, sentence four, should be deleted since it is a summary of demographic data. This information is appropriately presented in the Results section and should not be repeated here.

4. Discussion section tenth paragraph, sentence one, states, “…gestational age 20-28 weeks was significantly different from other gestational ages by one-way ANOVA…” Similarly, Discussion section paragraph eleven, sentence one, states, “…there appears to be no difference in the incidence of large fetomaternal hemorrhage between cesarean and vaginal deliveries…” None of this data is presented in the Results section. As a rule, new data should not be introduced in the Discussion. Authors should present all relevant data with summary statistics in the Results section and limit the Discussion to an interpretation of how that data fits into current understanding of the literature. I would suggest including relevant summary data on gestational age of participants, quantity of fetomaternal hemorrhage by gestational number, and any other risk factors examined (Table format may be appropriate) in the Results section and deleting this data from the Discussion.

5. In the Results section second paragraph, sentence three, authors state that three samples were excluded from statistical analysis due to “excess FMH…” An operational definition of “excess FMH” should be provided. Investigators should describe the rationale for exclusion of these cases and provide appropriate supporting references, if applicable.

6. Limitations of the current investigation relative to existing literature should be presented in the discussion section.

7. The discussion section is too long and repeats presentation of much of the
data from the Results section. Repeated data should be deleted and the discussion should focus on key findings that support the authors’ conclusions and recommendations.

- Minor Essential Revisions

1. In keeping with convention in the current medical literature, flow cytometry should be written as two words in the title and throughout the manuscript.

2. In the abstract, FMH as an abbreviation does not need to be written in full text more than once.

3. Throughout the manuscript, there are too many abbreviations (FMH, KBT, FCM, HDF/N, HDFN, RHIG, RhIG, ANC, Rh, RhD, Rh D, EDTA, PBS, RBC). Excessive and/or inconsistent use of abbreviations detracts from clarity of the writing. For example, Results section sixth paragraph, sentence three, “FCM has detected FMH greater than KBT…” Authors should choose three (or four at most) abbreviations, write them in full text at the time they are used for the first time, and the remainder of the paper should be written in full text with care taken to avoid sentence structures that rely on multiple abbreviations.

4. Background section sixth paragraph, sentence three, is incomplete and should be edited for accuracy and meaning.

5. Background section seventh paragraph, sentence two, after “universal access to…” I suggest authors insert the words, “appropriate doses of prophylactic anti-D…” which would correct the grammatical error currently present in that sentence location.

6. Background section seventh paragraph, sentence two, “Africa” should conclude the sentence. “There is also a need…” should be the beginning of a third sentence in that paragraph.

7. The background section is too long. Readers of this journal should be sufficiently familiar with hemolytic disease of the fetus and newborn so that background paragraphs one and two could be deleted. Background paragraph seven is compelling and provides an excellent rationale for the current investigation. I suggest paragraph seven be moved to the top of the background section. Paragraphs three, four, and five could be combined and edited into one shorter paragraph. Paragraph eight is also key to the rationale for this investigation and should be emphasized.

8. Description of the methods for Kleihauer-Betke testing and flow cytometry should be condensed given that appropriate references are provided. The Mollison 1972 study, if relevant, should be included in standard fashion with the other references or deleted if an appropriate alternate reference has been provided.

9. Results first paragraph, sentence two, “multiple delivery pregnancy” should be clarified. It is unclear whether this refers to twins, more than one prior pregnancy, or some other meaning.

10. Results first paragraph, sentence three, “ANC” should be written in full text since this is the first (? only) appearance of this abbreviation.
11. Results first paragraph, sentence five, “normal delivery” should be defined. It is unclear whether this means spontaneous vaginal versus induced, spontaneous vaginal versus operative vaginal, vaginal versus cesarean, or some other meaning.

12. Upon completion of Major Compulsory Revision #1 above, headings of Tables I, II, and III, can be shortened by eliminating the institutional names and dates of study enrollment since this information would have been appropriately presented in the Methods section.

13. Results section fourth paragraph, sentence three, begins, “As it is shown in Table II…” However, it appears that the data being referenced is actually in Table I. This should be corrected.

14. Reference #22 contains an abbreviation that does not appear in the referenced manuscript’s title as published. This should be corrected and other references reviewed for accuracy.

- Discretionary Revisions

1. Abstract’s background could more clearly state that the goal of the current investigation was to quantify and compare the volume of fetomaternal hemorrhage using two techniques, Kleihauer-Betke test and flow cytometry, to determine if patient-specific doses of prophylactic anti-D warrant further investigation in the Ethiopian and wider African context.

2. Abstract’s conclusion could more clearly state that, given the current investigation, further investigation into the cost-effectiveness and scalability of patient-specific dosing of prophylactic anti-D appears warranted.

3. At the end of the Discussion section, authors may consider condensing recommendations to one or two more general statements that might be relevant to a wider audience of readers, for example, “Based on the current study, further investigation into the cost-effectiveness and scalability of patient-specific dosing of prophylactic anti-D is warranted. Generation of data to inform the development of optimized testing and dosing protocols is needed.”

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests