Reviewer's report

Title: Seasonal variation in haematological and biochemical reference values for healthy young children in The Gambia

Version: 3 Date: 11 August 2015

Reviewer: Seth Owusu-Agyei

Reviewer's report:

- Major Compulsory Revisions

1. From the results, table 1, there were marked differences between the reference value quoted based on the Mean±2SD and that of the 2.5th and 97.5th percentiles. For example, total white cell counts 4.7 - 15.2 versus 5.7 - 15.8; Neutrophils 0.05 – 6.7 versus 1.24 - 7.49; Platelets 181.4 – 730.4 versus 446 - 749.9. This could be due to the fact that these parameters were not normally distributed. For parameters which are not normally distributed, using the Mean ±2SD does not give a reliable value. In this case the data will have to be transformed first OR the 2.5th and 97.5th percentiles (non-parametric method) used to define the reference range.

2. As the study was aimed at establishing reference values for healthy children, it would be necessary to know how many children were excluded because they were not ‘healthy’ (i.e. did not meet the inclusion criteria during screening/physical examination by the study clinician).

- Minor Essential Revisions

3. Please provide a justification for the sample size used.

4. Method (line 78) : It is not clear if the same participants were used for the two cross-sectional studies or different sample was selected for the two cross sectional survey in the two seasons. This could equally account for a variation that might not exclusively be limited to season.

5. Since the haematological and biochemical parameters in this study were quantitative (not qualitative), the use of Positive and Negative Controls will not be appropriate in this case. What should be used in this case is Normal and Abnormal Controls. Also, standards are not used for daily checks. Standards (or calibrators) are used periodically (according to standard operating procedure) to calibrate the analyser. These should be clearly stated in the paper.

6. Please include the assay precision (coefficient of variation) for each biochemical and haematological parameter during the study.

7. Include the analytical method for the biochemical tests. E.g. Sodium – ion selective electrodes, etc.

8. You indicated the reference intervals were determined by age <24 months and
#24 months. However, in the methods (line 88) and Results (line 172), #30 months and >30 months, is used. Please clarify.

9. You state under Discussion (second paragraph) that ‘values for haemoglobin and WBC were similar to earlier reports in the western part of the country [15] and in Africa [7] which suggest a role for more stable genetic factors such as haemoglobin polymorphisms including sickle cell anaemia’

a) It is not clear how this statement is supported by your results

b) Reference [7] which is the paper of Horn & Pesce was not related to reference values for Africans. More appropriate papers relating to reference values in Africans should be cited. There are a number of such papers in your Reference section.

10 Line 47: Please include the mean values of the parameters in the results sections of abstract and main manuscript.

11 Please include biochemical parameters in the aim of the study.

12 You state in abstract that ‘most biochemical parameters did not vary between seasons or age but creatinine and alanine aminotransferase values were lower’. You, however, did not indicate which values you were comparing your values to

13 Reference intervals across seasons, age and gender (Line 167): It is not clear which of the seasons the statement “Children >30 months had lower mean values for WBCT, lymphocytes, monocytes and neutrophils” on line 172-173 refer to. It will be appropriate to make that addition to aid readers to put the values in context. From the preceeding sentence, one may infer the statement is about the wet season but this needs to be stated.

14 Please provide a detailed background on the seasonality of malaria transmission and other conditions that affect haematological indices if available in the study area. This will enable readers to justapose the effect of such factors on the reference values obtained for the population. This has been done better in the discussions compared to the background.

15 Can you please explain why higher WBC (mostly due to neutrophils) would suggest low level of infections? (Discussion Line 212)

- Discretionary Revisions

These are recommendations for improvement which the author can choose to ignore. For example clarifications, data that would be useful but not essential.

None

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.
Declaration of competing interests:

'I declare that I have no competing interests'