Reviewer's report

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

Version: 3
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Reviewer: Bertrand Dr Lell

Reviewer's report:

This manuscript describes laboratory reference intervals in children from The Gambia and looks at the effect of seasonality.

Two other studies have recently published reference intervals in infants and children from The Gambia (one only for hematology), so the importance of the present study must be seen in this context.

A very large number of children, many times the recommended number of subjects to determine reference intervals were sampled, making the results robust.

Major Compulsory Revisions

My major issue concerns the methods used to calculate reference intervals. The CLSI has published a comprehensive guideline on how to perform these calculations. It is not clear if the authors have tried to follow them. They are not referenced in the method section, but an older version is referenced in the background section. The choice of defining reference intervals as mean ± 2 * SD is unusual and there is no reference to support this. The CLSI guideline recommend 2.5 and 97.5 centiles and it is not clear why these are shown in the results but are not used as reference intervals.

Minor Essential Revisions

A Gaussian distribution is assumed for each parameter, and the reader needs at least assurance that the Kolmogorov-Smirnow Test supports this.

The choice of using Horn/Tukey's method for outlier detection (rather than Dixon's Test typically used for reference limit calculations) is also based on the assumption of Gaussian distribution. The reference Nr 14 for this seems wrong.

If the reference population is supposed healthy, more information has to be given on how this was assured. The authors mention eligibility criteria for this, but the information is very vague. For example, no temperature limits to determine fever are mentioned, nor are references to a standard health questionnaire given. Z-scores were calculated but it seems that undernourished children were eligible?

The number of outliers per parameter do not add relevant information in my opinion. In contrast, results of regression analysis mentioned in the methods
The units of the parameters in the tables should be checked (μmol/L for creatinine, U/L for AST and ALT).

**Level of interest:** An article of limited interest

**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