Author's response to reviews

Title: Method Validation of Circulating Tumour Cell Enumeration at Low Cell Counts

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Author's response to reviews: see over
Ms Cherry Battad c/o
Professor Evi Lianidou
Journal Editorial Office
BMC Cancer
BioMed Central

RE: MS 1146830020100769
Dear Professor Lianidou

Thank you very much for reviewing our article as a Technical Advance in BMC cancer entitled: Method Validation of Circulating Tumour Cell Enumeration at Low Cell Counts

We are particularly grateful to the two reviewers for their helpful and constructive comments.

The manuscript has now been revised taking account of all the comments of the two referees, as detailed point by point below.

**The first referee (Eric Rozet) requested 3 revisions.**

1. **Major Compulsory revision:**

   While the tolerance interval methodology is an excellent methodology and perfectly recognized approach to globally assess analytical results’ reliability, other classical approaches could be usefully used to make some diagnostics assessment in the results obtained. This should be done at least in section “Analytical accuracy of the CellSearch system characterized by beta-expectation tolerance intervals” on page 9. Indeed on the second paragraph of page 9, formal statistical analysis could be performed to measure the statistical significance of the effects, using ANOVA F-tests and students t-tests when appropriate, and/or provide confidence intervals of the estimates.

Authors Response:
As a major compulsory revision we have conducted the additional statistical tests required by the referee in section “Analytical accuracy of the CellSearch system characterized by beta-expectation tolerance intervals”. This analysis involved both ANOVA and F-test with Newman-Keuls Multiple Comparison post-test. These new data are incorporated into pages 9 and 10 of revised manuscript.

2. Minor essential revision

   On the last line of page 10: “The average recovery...”: please also provide BETI values for this analysis.

   The BETI values associated with this result have been calculate and are now included in page 10 of the revised manuscript.

Authors Response:

3. Discretionary Revision

   Define N in the equations used on page 8: section “Mathematical calculation and statistical analysis”.

Authors Response:

The term N refers to the number of patient samples analysed in this evaluation and the definition of this term has been included in page 8 of the revised manuscript.

The second referee (Luc Dirix) requested 2 minor essential revisions

1. In all figures the lines should be changed into actual observational points, there is no valid reason to "connect" this separate observations, this obvious for all figures, but most obvious so for fig 2D were separate analyst pairs are shown. They have no relationship whatsoever that could justify the connection line.

Authors Response:

The referee makes a valid observation in this comment. Nonetheless, it is the convention in the field of bioanalytical science to present accuracy profiles based on β-expectation tolerance intervals (BETI) with lines connecting the points, even if the profile consists of only 2 or 3 QC samples extending over a large range of concentrations. There are numerous publications including many seminal papers from
Dr Rozet’s laboratory to support this convention. In our paper, Figure 1 is a classic example of an accuracy profile, hence the reason we have followed the normal convention to present these plots with lines.

In Figure 2 we present a modified version of an accuracy profile and incurred sample reproducibility (ISR). Here, there are few in any precedents as how to present these profiles. In response to the referee’s comments, we looked at a number of different options – observational points (as recommended by the reviewer), bar charts, etc and concluded that the best way to convey the information contained in this figure is as we originally presented it, with lines connecting the different analysts.

2. On p. 5 '27 over a 3 month period' and on p. 9 '21 over a 3 month period' Which is correct?

Authors Response:

The correct value is 27 and this typographical error has been correct on page 9 of the revised manuscript.

I hope these revisions are acceptable.

Yours Faithfully
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