

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Methodology**ESPS manuscript NO:** 7840**Title:** Methodical and preanalytical characteristics of a Multiplex Cancer Biomarker Immunoassay**Reviewer code:** 01992073**Science editor:** Huan-Huan Zhai**Date sent for review:** 2013-12-04 14:53**Date reviewed:** 2014-02-07 20:00

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> Existing	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade D: Rejected	BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor		<input type="checkbox"/> Existing	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

The paper submitted by Hermann and colleagues reports the analysis of the methodological performance of MILLIPLEX[®] MAP Human Circulating Cancer Biomarker Magnetic Bead Panel 1. Overall, the major drawback of the paper resides in its presentation, as the English form needs extensive revision. In fact, the impaired syntactic structure makes difficult for the reader a thorough understanding of the logical steps that led the authors to their conclusions, especially for a non-expert reader. As regards the methodological approach, the study design is well defined and the methods are appropriate, although unnecessarily over-detailed. However, in my opinion, the study aims are not fully achieved, in particular for the issue regarding the clinical applicability of the assay. The assumption of the Authors that the use of extrapolated results, which they "...assume to be more realistic regarding the use of the assay in clinical routine measurements" is highly speculative, indeed, and not supported by the results obtained, at least in the way they are presented. Concerning the results obtained, it is unclear to me why Authors decided to exclude the second set from the analysis, leaving its presentation to the supplementary material section. The fact that there were differences between plates tested six months apart is of utmost importance in the definition of the inter-assay variability and all sets of experiments should be represented together. Finally, it would be valuable to include subsets of different patients' sera in order to provide a provisional estimate of the clinical performance of the assay.

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Methodology

ESPS manuscript NO: 7840

Title: Methodical and preanalytical characteristics of a Multiplex Cancer Biomarker Immunoassay

Reviewer code: 02506941

Science editor: Huan-Huan Zhai

Date sent for review: 2013-12-04 14:53

Date reviewed: 2014-02-07 20:23

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input checked="" type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> Existing	<input type="checkbox"/> High priority for publication
<input checked="" type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade D: Rejected	BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor		<input type="checkbox"/> Existing	<input checked="" type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

The manuscript treats a problem of major importance and has general merits, however it has to be improved for publication. The language needs serious improvements. The description of work and selection of data has some flows. The selected panel: it is true that Merck-Millipore offers this panel with 24 markers (with the possibility to select between total PSA and free PSA). However, it is very unlikely to have all these markers at the same time. It's understood that the purpose was a general evaluation of the components, which is a bit different than a real validation of a clearly focused biomarker panel. The samples were run on a pool of sera; it is not clear how many sera were pooled? Also, it seems that the pool involved sera from different pathologies, so as to create a mixture of markers. The selection of data to calculate the CV and recovery based on ST7 is atypical, since usually these concentration is most often outside the linearity range. I suggest to use for this purpose another concentration (S6, S5). Some pre-analytical condition were thoroughly investigated, establishing the optimal conditions for best results for most analytes. Some variants would be useful in the assay, for instance, setting higher the number of events; 50 is low and can be a source of the problems. Examination of the pre-analytical conditions was made thoroughly; thus, contribution in the analytical process reproducibility and a maximization of the reliability for most samples was achieved. However, it looks like the only parameter to estimate intra- and inter-assay imprecision was Mean FI, while other elements (for instance, number of events) was not considered. I don't



BAISHIDENG PUBLISHING GROUP INC

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com

<http://www.wjgnet.com>

understand how the recovery% was calculated for VEGF and sFASL; the data presented report concentrations below the limits of detection for both, in all experiments. Design of the tests: some imprecision in explaining when and what: - Two sample pools, seeming to be the same, but diluted; no info on the number of sera in the pool - Two sera from patients for testing pre-analytical conditions (they seem to be distinct from the previous) - The test for plasma run comparatively with serum - one case? The effective concentrations in serum and plasma would be of more use.

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Methodology

ESPS manuscript NO: 7840

Title: Methodical and preanalytical characteristics of a Multiplex Cancer Biomarker Immunoassay

Reviewer code: 02445950

Science editor: Huan-Huan Zhai

Date sent for review: 2013-12-04 14:53

Date reviewed: 2014-02-07 21:01

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> Existing	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade D: Rejected	BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor		<input type="checkbox"/> Existing	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

The manuscript is very well presented and highlights factors influencing the measurements of the key performance indicators of the multiplex cancer biomarker panel, which constitutes a highly interesting study. The findings from the comparison of the critical measurement parameters between physiological sera in parallel with synthetic internal controls will be of particular interest to a wide audience. The technical details are clearly defined and the interpretations are thorough with robust scientific conclusions. I recommend the publication of this manuscript. The only very minor editing detail I have spotted was a missing full stop at the end of the fourth paragraph of page 9 of the manuscript: "Observed concentration-based CVs ranged from 1.68% (MIF) to 36.09% (b-HCG) with 12 biomarkers measured with a CV below 5% and 4biomarkers exceeding the 10% mark (table 1)." Evidently, there has been thorough attention to detail during the preparation of this manuscript for review, which was a pleasure to read.

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Methodology

ESPS manuscript NO: 7840

Title: Methodical and preanalytical characteristics of a Multiplex Cancer Biomarker Immunoassay

Reviewer code: 02511983

Science editor: Huan-Huan Zhai

Date sent for review: 2013-12-04 14:53

Date reviewed: 2014-02-07 21:47

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> Existing	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair		BPG Search:	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade E: Poor	<input type="checkbox"/> Grade D: Rejected	<input type="checkbox"/> Existing	<input type="checkbox"/> Minor revision
		<input type="checkbox"/> No records	<input type="checkbox"/> Major revision

COMMENTS TO AUTHORS

The manuscript "Methodical and pre-analytical characteristics of a Multiplex Cancer Biomarker Immunoassay" approaches an issue of major importance, yet it needs to be improved for publication. The article is well written and we appreciate the good quality of their work, however further studies are needed to show the clinical applicability of the analysed kit, as the authors themselves have mentioned. Regarding the article content, we suggest the authors to give more details about the two serum pools (pool 1 and pool 2) used for the physiological external control. Validation for clinical diagnostic use (IVD) requires a large cohort of patients and controls for specificity and sensitivity evaluation of each analyte (false positive, false negative results, etc). The selected biomarkers panel is too large to be suitable in clinical routine for one single patient, in order to have a cost-effective analysis. We are also suggesting a few minor revisions in terms of spelling check and phrase topics. Considering these suggestions, we recommend the publication of the article after minor revisions are made.

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Methodology

ESPS manuscript NO: 7840

Title: Methodical and preanalytical characteristics of a Multiplex Cancer Biomarker Immunoassay

Reviewer code: 02520754

Science editor: Huan-Huan Zhai

Date sent for review: 2013-12-04 14:53

Date reviewed: 2014-02-08 14:48

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input checked="" type="checkbox"/> Grade A: Priority publishing	Google Search:	<input checked="" type="checkbox"/> Accept
<input checked="" type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> Existing	<input type="checkbox"/> High priority for publication
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COMMENTS TO AUTHORS

In this manuscript you tested the methodical and pre-analytical performance of a new multiplex cancer biomarker Magnetic Bead Panel Kit, This kit includes reagents for the detection of 24 biomarkers. You showed that the Human Cancer Biomarker Magnetic Bead Panel 1 assay is a stable and precise method for detection of most into the kit included biomarkers although single markers have to be interpreted with care. The experiment are carefully done and the interpretations are likely correct. Therefore, I believe that this manuscript is worth publication in World Journal of Methodology as it is.

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Methodology

ESPS manuscript NO: 7840

Title: Methodical and preanalytical characteristics of a Multiplex Cancer Biomarker Immunoassay

Reviewer code: 00009616

Science editor: Huan-Huan Zhai

Date sent for review: 2013-12-04 14:53

Date reviewed: 2014-01-22 12:07

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input checked="" type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input checked="" type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> Existing	<input checked="" type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
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<input type="checkbox"/> Grade E: Poor		<input type="checkbox"/> Existing	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

Good study. Accept as it is.