Author's response to reviews

Title: Decentral gene expression analysis: analytical validation of the Endopredict genomic multianalyte breast cancer prognosis test

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Version: 2 Date: 27 September 2012

Author's response to reviews: see over
Dear Dr. Lee,

Thank you very much for the review of our manuscript and the opportunity to submit a revised version. We have revised the manuscript according to each of the editorial and the reviewers’ comments. Please find below a point-by-point description of the changes made.

Comments of editorial office:

The editorial office asked for a statement regarding the ethical approval of the study.

It was an omission on our part not to refer to the ethical approval of the study in the first version of the manuscript. In the revised version we included the following sentence at the end of the first paragraph of the methods section (“Reference and testing Materials”, page 6):

This study was carried out in compliance with the Helsinki Declaration and was approved by the Ethics Committee of the Charité Hospital (Ref. No. EA1/139/05, Amendment 2008).

Comments of Referee 1:

There were no comments to address.

Comments of Referee 2:

Minor Essential Revisions:

“The authors mention that the total variation of EP in different laboratories is in the range of 1%. It would be interesting if this number was compared to other diagnostic tests like antibody-based assays which are regularly used in pathologies.”

“Although instruments and reagents are standardized for EP, the results of this study very likely depend on the experience of each laboratory and the personnel performing the assay. For sure the Institute of Pathology in Berlin is more experienced with RNA work than many other pathology. Eventually, this aspect should be considered, especially for laboratories with less experienced operators.”

We are grateful for these two important points. The regularly used diagnostic test system in pathologies is antibody-based immunohistochemistry. Recently, an interesting study was published from the Swiss Working Group of Gyneco- and Breast Pathologists evaluating the variability of Ki-67 immunohistochemistry in breast cancer and showing standard deviations ranging from 21.7% to 24.1% (Varga et al., 2012). A comparison of the reproducibility of EndoPredict with especially Ki-67 is interesting since both tests are used for the clinical decision whether a patient with luminal (ER pos., Her2 neg.) breast cancer should be treated with cytotoxic chemotherapy.
We also agree that the reproducibility of the multigene assay might depend on the experience of the laboratory.

To address both points we included the following two paragraphs in the discussion section of the revised version of the manuscript (top of page 15):

A recent study assessed the variability of Ki-67 immunochemistry, which is a standard antibody-based diagnostic test in pathology used for treatment decision making in luminal breast cancer [27]. The authors found standard deviations of Ki-67 results obtained by 15 pathologists on centrally stained slides of three breast carcinomas ranging from 21.7% to 24.1%. Interestingly, even clear guidelines how to assess Ki-67 could not improve variability. Although our results might suggest a higher reproducibility of the PCR-based test using standardized instruments and reagents, it is important to know that the pathological laboratories involved in this technical verification study as well in the proficiency testing of EndoPredict were highly experienced in molecular work. Therefore, the results might be different in laboratories with less molecular diagnostic experience and ongoing quality control by periodical round robin tests might be reasonable.

Discretionary Revisions

“page 14: please delete "can" (… which can must be performed in laboratories, …)"

We deleted “can” in the revised version.

The authors would like to thank the editor and the reviewers once again for their insightful comments for improving the manuscript.

We are looking forward to hearing from you.

Sincerely yours,

Dr. Ralf Kronenwett
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