Author's response to reviews

Title: Real-time PCR complements immunohistochemistry in the determination of HER-2/neu status in breast cancer

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Author's response to reviews: see over
Dear Editors:

**Re:Manuscript # 1299282527749247**

Thank you for reviewing our manuscript entitled “Real-time complements immunohistochemistry in the determination of HER-2/neu status in breast cancer”. We have read the reviewers’ comments and have found them very useful. We have now addressed each reviewer’s comments to the best of our ability and have incorporated the necessary changes in the text of the manuscript. As a result, we believe that the quality of the manuscript is much improved.

Please find attached the revised manuscript and our itemized responses to the two reviewers. We hope you find these changes acceptable.

Sincerely,

Yvonne Myal PhD
Responses to Reviewer #1 (Douglas Demetrick)

1. We have now clearly stated in the “Abstract” that we are evaluating a commercially available quantitative real time PCR LightCycler assay.

2. We have further extended a review of the HER2 literature, as suggested by this reviewer, to include more references pertaining to existing quantitative real time PCR assays. These changes have been incorporated in both the “Introduction” and “Discussion” sections.

3. Further discussion on the sensitivity and specificity, as well as false positive and/or negative rates in comparison to FISH, has now been incorporated into the “Discussion” section. Comparison to other recently published LightCycler assays of Her-2 copy number has also been included in the Discussion.

4. On the last page of the “Discussion” section we have included comments regarding the impact of the prevalence of cancer cells in the specimen chosen for DNA extraction vs. FISH, as well as included comments on the impact of microdissection on the real time PCR assay, as suggested by this reviewer.
Response to Reviewer #2 (Chris De Wolf-Peeters)

**Major Revisions:**

1. In this study we are evaluating a commercially available kit in which the gastrin gene is used as the control gene. That being said, we agree with the reviewer that as the gastrin gene being situated on 17q21, a subregion that is frequently amplified in breast carcinoma, could impact to some degree on the interpretation of the data, especially in the case of polysomy 17. We recognize that polysomy 17 can occur in a detectable frequency and therefore the use of an additional control gene in the future could help alleviate this problem. Also, in retrospect, we agree that our explanation regarding the phenomenon of polysomy 17 and the 2 FISH negative cases in the Discussion was not very clear. We have tried our best to clarify our statement.

2. We have expanded the “Introduction” section to include more discussion regarding the technical advantages and/or shortcomings of the methodologies used, as suggested by the reviewer. We have also included some comments about the CISH technique.

3. The discussion section has been revised to include the four publications as suggested by this reviewer.

**Minor Revisions:**

1. We have changed the sentence to now read, “suggests that real-time PCR is more accurate in determining the patients who are true candidates for trastuzumab therapy”.

2. A cut-off value of 2.0 for the real time PCR is the value suggested by the manufacturers of the LightCycler quantification kit. We used a cut-off value of 2.2 for FISH analysis as we observed that a range of values (2.0-2.4) have been used in the literature, and we therefore chose a value that was more often used.