Reviewer’s report

**Title:** Human Papillomavirus (HPV) in breast tumors: prevalence in a group of Mexican patients.

**Version:** 2  **Date:** 30 May 2008

**Reviewer:** Evi S Lianidou

**Reviewer’s report:**

In this manuscript by Cantu de Leon et al, present the detection of HPV in breast cancer patients. However there are some serious concerns about publishing this manuscript in its present form.

**Major Compulsory Revisions**

1) HPV detection: On page 6, the authors use 5uL of isolated DNA for their PCR reactions. They use the same volume of DNA in all their samples. However, they don’t quantify their DNA, and its well known that DNA quantity between FFPE samples can dramatically vary, thus all samples are not comparable concerning the reliability of PCR results.

2) HPV PCR systems: It is really unbelievable to describe an experimental approach where some clinical samples are analyzed by nested PCR (when the control gene shows that DNA fragments more than 400bp can be amplified) and some by single PCR (when the control gene shows that DNA fragments more than 400bp cannot be amplified). The sensitivity of these PCR approaches is by no means comparable, and taken into account the previous note that DNA quantity is not the same in all samples, one can easily conclude that some samples can be found positive or negative just because of the DNA quantity and not for other reasons.

3) DNA sequencing: Authors describe on pg 6 that they also performed DNA sequencing in their samples. They don’t explain why and they don’t give any comment on their results in the Discussion session.

4) RESULTS: On pg 7 the authors claim that the risk of developing a malignancy
in the presence of HPV in breast tissue was OR 43.7 (CI 95%: 2.53 – 754.6). This is a very important statement that could have an amazing impact on breast cancer prevention worldwide. However the number of samples analyzed is small, and the quality of experimental design is poor. I think its very risky to make such a profound statement.

5) Development of the analytical method: High Resolution Melting Curve Analysis (HRMA) is a very effective and promising new technique for DNA Analysis. The authors use a very well established and very efficient instrument for this purpose, which is especially designed for HRMA. The idea of using HRMA for free DNA quantitative measurements, without performing real time PCR is interesting. However, the data presented are not sufficient to claim that a new analytical method for fDNA has been developed.

MINOR Comments:
- The gel shown on Fig 1 is of very bad quality to be published.
- The quality of written English is bad, and in some paragraphs English is difficult to understand

What next: Unable to accept the manuscript in its present form. The authors have to improve their manuscript by responding to the major compulsory revisions.

Level of interest: As stated clearly by the authors this is an article whose findings are important mainly for Mexican population

Quality of written English: Not acceptable

Statistical review: Is it essential that this manuscript should be seen by an expert statistician

Declaration of competing interests: 'I declare that I have no competing interests'

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