Author's response to reviews

Title: Touchdown General Primer (GP5+/GP6+) PCR and optimized sample DNA concentration support the sensitive detection of human papillomavirus

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Author's response to reviews: see over
1. I still fundamentally disagree with the title. Strike the verbiage beyond the colon. The point of the paper is the method, not the tissues. The relationship between breast cancer and HPV is quite uncertain. Even though you argue (with reviewer 1) that the rates are comparable (somewhat less) than those found by de Villier et al. However, this paper is detecting an entirely different set of types, types found in the cervix, whereas the types detected by de Villier et al were primarily skin types. This makes me suspicious of contamination. And, given the differences in the cell types, I am more inclined to believe the de Villier paper but I have no evidence that what was done in this paper was wrong. However, I disagree with the use of "Breast" in the title, which emphasizes a role of HPV in breast cancer. I cannot relent on this issue.

Response:
The title has been changed to:
‘Touchdown General Primer (GP5+/GP6+) PCR and optimized sample DNA concentration support the sensitive detection of human papillomavirus.’

‘GP5+/GP6+’ has been added to make it clear which general primers have been examined.

2. The authors did not evaluate reliability, despite their claims. There are two measures of reliability, intra- and inter-laboratory reliability. That is, do they get the same answer on repeat assays and will another lab also get the same answer. It is a limitation that just need to acknowledge...i.e., they have not assessed it.

Response:
The following subsection has been added to the Methods:

**Protocol Reliability**
Touchdown protocols were assessed on two or more occasions on SiHa cell/C-33A DNA dilutions, and the TDP3 was also tested several times on recombinant HPV plasmid samples to determine data reproducibility. Clinical samples (cytology, FFPE VAIN and breast tumors) were tested once only with PCR protocols. Inter-laboratory tests of different PCR assays were not performed.

Confirmation of the reproducibility of cell line and HPV/plasmid experimental data is included in the 'Results' section.

3. Another limitation that I would like the authors to acknowledge the absence of a negative tissue control.

Response:
The negative tissue control used in the assessment of cytology samples was C-33A cell line DNA as already indicated. Negative control tissues were not used in the study of FFPE samples.

The following has been added in the **Discussion** at the end of the sub-section on **Detection of HPV in FFPE Samples**:
Additionally, given the disparate estimates of HPV prevalence in breast tumors, future studies might incorporate more rigorous control measures such as cutting sections of HPV negative
tissues for DNA extraction and PCR in between successive breast tumor specimens to test for cross-contamination with HPV positive samples.

**Other Changes**
- An additional reference has been added, reporting high-risk anogenital HPV types in 48% of breast carcinomas (*Br J Cancer* 2005, 93:946-948).
- Several minor textual changes have been made to improve manuscript readability.
- Table 1 has been simplified to fit portrait format.
- Table 2 has been rearranged to fit portrait format.