Author's response to reviews

Title: Prevalence and type distribution of human papillomavirus among adult women diagnosed with invasive cervical cancer (stage 1b or higher) in New Zealand

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Author's response to reviews: see over
Dear Editor,

Please find enclosed a manuscript entitled “Prevalence and type distribution of human papillomavirus among adult women diagnosed with invasive cervical cancer (stage 1b or higher) in New Zealand” which we are resubmitting for consideration by BMC Infectious Diseases.

Please also find attached itemized responses to reviewers’ comments. The authors appreciate the constructive comments and have attempted to modify the manuscript as requested within the limits of the study objectives. Changes from the original submitted manuscript are highlighted in track changes.

We hope the revised manuscript is now acceptable for publication in BMC Infectious Diseases.

On behalf of all authors,

Yours sincerely,

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Reviewer's report 1:

Minor Essential Revisions

1. Please give more detail about the DNA extraction method used.
Response: The procedure for DNA extraction are described in detail on Lines 134–141, and two testing methodology papers are cited should the readers require further details. The following sentence was added to clarify the controls for DNA testing: “Positive and negative controls were used for each run to monitor DNA isolation, PCR amplification, HPV detection and genotyping. Re-testing of HPV negative samples was conducted after diluting DNA tenfold.” (Lines 141–143)

2. Please describe the internal control used to determine which HPV-negative samples were true negatives (undetectable) versus samples which simply had poor overall DNA quantity or quality. Generally beta-globin detection is used for this purpose.
Response: Samples which tested negative for HPV had DNA diluted tenfold before being re-tested. This was the only additional step to remove potential inhibition and has been added to the Methods section (lines 141–143). We did not perform any additional assays (e.g. a housekeeping gene) to test for amplifiable human DNA, as the aim of this epidemiology study was to assess the HPV type-distribution rather than positivity rates.

Please include the numbers of "true negatives" versus "unsatisfactory" samples in your results and Figure 1.
Response: We would like to clarify that we did not test the extent to which HPV negative samples were indeed true negatives (other than as described above). All the samples taken from women enrolled in the study were tested and histological diagnosis was performed.
Figure 1 was modified to reflect the reason for excluding 15 women as histologically not confirmed as ICC.

3. Was any attempt made to overcome the limitations of the SPF10 assay in detecting integrated DNA - if so please describe.
Response: We did not perform any additional HPV tests which targeted other HPV genomic areas such as E6/E7. It is worth noting that rare cases with L1 loss could be misclassified as HPV negative. However as the aim of our study was to assess the HPV type-distribution, this was not further researched.
Reviewer's report 2:

Minor Essential Revisions:

Materials and Methods:
It should be specified what “gene” was amplified along with the HPV DNA to serve as control?
Response: We would like to clarify that “true negatives” were not tested in this study as our main aim was to assess the HPV-type distribution and not the positivity rates of HPV in New Zealand.

No additional assays with any housekeeping gene to test for amplifiable human DNA were run. In lines 141–143 we have added the following statements to clarify the control process: “Positive and negative controls were used for each run to monitor DNA isolation, PCR amplification, HPV detection and genotyping. Re-testing of HPV negative samples was conducted after diluting DNA tenfold.”

General comments:
The authors excluded Stage 1a patients from study on the ground that the volume of cancer in the paraffin block may not be adequate. This is not a valid concern as this is a PCR based study with DNA amplification. I think this is a flaw of the study. The stage 1a cancers should have been included. Having said that I think this manuscript should be accepted for publication. It will, as the authors claimed, fill up epidemiologic data gap in New Zealand.
Response: We note the reviewer’s concern regarding this. One major reason for not including patients with Stage 1a was that stage 1a tumors are not necessarily managed in cancer centers in New Zealand. Therefore national coverage would be poor. In addition, based on our experience from other studies, the tumor is frequently cut through following the primary histological evaluation and there is no residual invasive tumor on the blocks. Therefore a significant number of micro invasive tumors would not be available for further testing (as per our study protocol), thereby limiting the representative nature of the study. It is for these reasons combined that we excluded Stage 1a tumours. The lack of representativeness for Stage 1a patients is stated as a limitation of the study (lines 333–336).

The authors may consider changing the title to specify “1b and higher stage cervical cancer”.
Response: The title has been modified as “Prevalence and type distribution of human papillomavirus among adult women diagnosed with invasive cervical cancer (stage 1b or higher) in New Zealand.”

Some recently published literature including the one in Human Pathology 2014; 45, 303-309 may be discussed and added for completeness.
Response: Two recent papers, Quddus et al, (Hum Pathol 2014, 45: 303-309) and Pirog et al, (Mod Pathol 2014. doi: 10.1038/modpathol.2014.55. [Epub ahead of print]) providing the most recent data on the HPV type distribution in ADC cases were added to the Discussion. (Lines 256–257)