

Author's response to reviews

Title: Rapid, Sensitive Type Specific PCR Detection of the E6 and E7 regions of Human Papillomavirus Type 16 and 18 from Paraffin Embedded Sections of Cervical Carcinoma

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Author's response to reviews: see over

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Rapid, Sensitive Type Specific PCR Detection of the E6 and E7 regions of Human Papillomavirus Type 16 and 18 from Paraffin Embedded Sections of Cervical Carcinoma
Iana Lesnikova, Marianne Lidang, Stephen Hamilton-Dutoit, and Jørn Koch

Dear Editor,

Thank you for your email of 19.08.2009 - which we for a variety of reasons have not replied to before now.

Thanking the reviewers for their comments we attach a new version of the manuscript which has been adjusted according to their instructions, and in this letter we list the changes made as part of a point-by-point response to the concerns raised.

We hope you find the edited manuscript suitable for publication.

Yours sincerely

Iana Lesnikova

Referee 1 had not replied.

Referee 2 (Phaik-Leng Cheah):

1. The article is divided into sections (title page, abstract, findings, list of abbreviations used, competing interests, authors' contributions, acknowledgements, references, figure legends, and tables) following the Instructions for Infectious Agents and Cancer authors for short report articles. (http://www.infectagentscancer.com/info/instructions/?txt_jou_id=10116&txt_ms_t_id=73798)
2. There were two points of criticism regarding the title. The first was about the use of the word “rapid” in the title, as we do not document that the method is more rapid than something else, and the other was about the mentioning of both E6 and E7, though we only analyze for E7.
 - a. The use of the word “rapid” in the title of the paper refers to the fact that our method - like other PCR methods - is fast and simple. It should *not* be taken to imply, that our method is more rapid than other PCR methods, and we never state anything to that effect. So in this wording we make no special claims about our method in particular that would need verification in the paper, and we have maintained the word in the title.
 - b. It is very much true that we only detect the E7 and not E6, as might be believed from the original title of the paper. Consequently, we have removed the word “E6” from the title and from the manuscript as a whole.
3. We should refer to previous studies of type specific detection of HPV (both in unfixed and FFPE tissues). We actually do this (page 3), but later studies have reported difficulties in reproducing the specificity of the methods in those studies, which was why we set out to create our own method for the purpose.

4. We are also requested to describe the method for the DNA extraction. However, we have simply bought a DNA extraction kit from QIAmp and followed the instructions in the kit, and the contents of the reagents included are a trade secret of the manufacturer, so it would not be possible to give more than a reference to the kit.
5. The referee wonders why we use one PCR protocol for the detection of β -actin and another for the detection of the HPV E6 gene. There is nothing strange in that, since the two PCRs for obvious reasons employ different primer sets, each operating under its own optimal conditions. In practice we paired our new protocol for HPV detection with an established protocol for the detection of a housekeeping gene.
6. We should shorten the text, and we have done that as requested.
7. The primers designs are specified in Table 2 (page 6)

Referee 3 (Peter Weismann):

Major Compulsory Revisions

1. We should expand the “methods”-section, and we have done that as requested.
2. The referee wonders why we work from FFPE material and not from fresh material. It is true that we have developed the method to work on the most difficult starting material and not the easiest. However there are good practical reasons for this seemingly stupid choice, as fresh unfixed tissue, which is an optimal source for molecular diagnostics, is almost never available for diagnostic purposes. Shortly after admissions all surgical samples are fixed, cut and paraffin

embedded and only afterwards are they available for diagnostics. For the same reasons, most other routine diagnostic procedures in pathology (such as immunohistochemistry and in situ hybridization method) are also performed in FFPE sections. Thus, in creating a method that would work on this inferior source we generated a method that could be applied under standard conditions as they exist today - and if it works on this kind of material, it should work on *anything*. Indeed we also show results with the same methods on unfixed material (the cell lines)

3. The referee disagrees on the copy numbers that we give for the HPV 16 and 18 genes in the cell lines. However, the copy numbers we give are those provided by the supplier of the SiHa and HeLa cell lines [American Type Culture Collection (Manassas, VA 20108 USA)], and we would find it odd to give copy numbers that differ from this, but we have now further specified the source of the information.
4. We have changed the text according to the comment (changed “quality of the DNA extraction” to the “purity of extracted DNA”).
5. We have “improve the scientific expressions” as requested.

Minor Essential Revisions

1. The concentrations of the reagents in the master mix (μM , nM, mM) are given as - well - concentrations. Changing that into molecular counts (μmol , nmol, mmol) would not be meaningful.
2. We have changed the label on Figure 1 as requested.
3. We have made the changes in this sentence (changed “carcinoma” to “squamous cell carcinoma”).
4. We have made the changes in this phrase (changed “experienced gynaecological pathologist” to “pathologist experienced in evaluation of gynecologic pathology”).

5. Tables, figures, and references have been checked an extra time.