Author’s response to reviews

Title: Bioelectronic DNA Detection of Human Papillomaviruses Using eSensor: A Model System for Detection of Multiple Pathogens

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To The Editor:

Thank you for considering our manuscript entitled “Bioelectronic DNA Detection of Human Papillomaviruses Using eSensor: A Model System for Detection of Multiple Pathogens” for on-line publication in BMC Infectious Diseases. We have addressed all compulsory and discretionary revisions either directly in the manuscript and/or below in the response to reviewer’s comments. We have also followed the authors’ checklist (medicine) for formatting of this revised manuscript. Thank you once again for considering our revised manuscript for publication in BMC Infectious Diseases.

Sincerely,

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For Reviewer Wim Quint:

Compulsory revisions:
1. We respectfully disagree that our statement in the introduction that “The ideal detection…. Integrated detection device” is not relevant and should be omitted. Bioelectronic detection devices can be, and currently are being integrated with the multiple steps required for rapid detection of pathogens in field and clinical settings. We have kept this statement in the introduction as it does no harm and is meant to stimulate new thoughts and ideas.
2. The reviewer is directed to Table 4 where multiple pathogens (in this case the model system HPV) were present in a single clinical sample and detected by the eSensor. This statement in the Introduction is addressed and presented in the results and left in the closing paragraph of the Introduction.
3. This has been clarified in the text to read “To allow for strand separation, only the downstream primers designated PGM11 and one β-globin primer were biotinylated (Table 1).” The primers that are biotinylated are also noted in Table 1.
4. The sentence has been changed to: “Avidin-coated beads were used to harvest the biotin-labeled DNA strand from 50 uL of product. The biotin-free DNA strand was then used in the assay.”
5. The reason we say 2-8 hrs is due to target concentrations in the clinical specimens. Samples with low target concentrations required up to 8 hrs of incubation, while those with higher concentration required only 2 hrs.
6. Figure mis-numbering has been corrected.
7. We used an inappropriate word (“substantially”). We did not mean to imply that this strategy (of introducing mismatched bases in relevant probes) "more or less" took care of the problem of non-specificity. What we meant to say is that this strategy effectively eliminated non-specific signal without compromising detection of specific hybridization. We have therefore changed the sentence as follows to more accurately describe what we mean: "Applying this strategy, we were able to effectively reduce the cross-reactivity among the signal and capture probes for those HPV types where problems of non-specific signal had initially been encountered without compromising their hybridization strengths.”
8. We have done extensive discrepant testing for the reverse line blot assay as described in Vernon SD, Unger ER, Williams D: Comparison of human papillomavirus detection and typing by cycle sequencing, line blot and hybrid capture. J Clin Microbiol 2000, 38: 651-5, and are very confident in using reverse line probe results as our reference for this demonstration project described here. In addition, as part of our HPV typing quality control, we sequence 10% of samples typed with the reverse line probe assay on an ongoing basis. This is a very important aspect of our program since we type over 10,000 samples a year for National surveillance projects.
9. The Conclusions section is part of the required format for BMC medicine journal research articles.
For Reviewer Jeanne Carr:
Discretionary revisions:
1. Changed heading in 2<sup>nd</sup> column Table 4 to “by Reverse Line Probe” as suggested.
2. Changed as suggested
3. Added x-axis title as suggested
4. HPV capture probe sequences are provided in Table 1.
5. Changed Qiagen to all capital letters in text
Compulsory revisions:
1. Corrected figure numbering and citation in text and corrected figure legend numbering.
2. Corrected figure 3 numbering in text.

For Reviewer Michael J Heller
Discretionary Revisions:
1. This is a fair question by the reviewer. The points we make in the paragraph in question relate to the fact that there were no plans to commercialize an HPV assay on the eSensor platform. This work was taken on as a model for pathogen detection on the platform. Given that excellent, if not perfect (concordance), results were obtained we felt we had made the point. Clearly, if commercialization or clinical test development had been the goal, significantly more work would have been invested in many variables of the system (on PCR optimization, hybridization condition optimization, probe length optimization, etc.) to generate the kind of nearly perfect to perfect results obtained with this platform in so many other feasibility studies (including agricultural, pharmacogenetic and genetic disease targets).