Author’s response to reviews

Title: Low concordance of oral and genital HPV infection among male patients with sexually transmitted infections in Vietnam

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Author’s response to reviews:

Technical Comments

1. Improvements to the English language within your manuscript have been requested, and so you should have your manuscript reviewed by someone who is fluent in English.

Response:

In accordance with the comment, our manuscript has been reviewed by the San Francisco Edit, which provides a Scientific Medical, and General Proofreading and Editing service.

2. In Funding, please state whether or not the funding body played any roles in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Response:

In accordance with the comment, we have added the following sentence in the "Funding" section (Page 15, lines 13-15): “The funding body did not play any role in the design of the study, sample collection, analysis and interpretation of data, and in writing the manuscript.”
3. The Authors Contributions section should be specifically mentioned by their unique initials rather than their full name.

Response:

In accordance with the comment, we have changed the authors’ names from their full names to their unique initials in the "Authors Contributions" section (Page 15 line 22- Page 16 line 1).

4. In the Ethics approval and consent to participate please clarify that consent was informed.

Response:

In accordance with the comment, we have revised the sentence as follows: "The written informed consent was obtained from all participants." (Page16, line 7).

5. We note that the current submission contains some textual overlap with other previously published works, in particular:


This overlap mainly exists in the Methods section. While we understand that you may wish to express some of the same ideas contained in these publications, please be aware that we cannot condone the use of text from previously published work. Please rephrase these sections to minimise overlap.

Response:

In accordance with the comment, we have modified the method section as follows:

(1) In the "Sample collection" paragraph:

The paragraph (Page 6, lines 5-18, 18-02310R1 version) “Penile, urethral, urinary, and oral samples were collected from the 210 patients as described previously except oral samples [14]. Firstly, penile swabs were obtained by rubbing swabs along penile surfaces (including the glans, shaft, and scrotum) with nylon tips soaked in saline solution (FLOQSwab R100; Copan Diagnostics Inc., Murrieta, CA). Secondly, urethral swabs were obtained by inserting saline-soaked nylon tips (FLOQSwab U80; Copan Diagnostics Inc.) approximately 3 cm in the urethral meatus and rubbing against the inner walls. Each collected nylon tip was placed in an Eppendorf tube containing 1.5 ml LiquiPrep preservation solution (LGM International Inc., Fort Lauderdale, FL), then mixed gently to release cells from the tip in the solution. The cell suspension was stored at 4oC until use. Thirdly, a volume of 30 ml of midstream urine was collected to avoid
contamination by urethra. Finally, oral samples were collected by gargling with 15 ml of phosphate buffered saline (PBS) with facing up for about 10 seconds. Each urinary or oral sample was centrifuged at 1,500g for 10 min, and the supernatant was discarded. The urinary and oral cell pellets were resuspended in 3 ml and 2.5 ml of LiquiPrep solution, respectively, and stored at 4oC until use [18].” has been changed to:

“Penile, urethral, and urinary samples were collected from the 210 patients, as described previously [14]. Briefly, penile and urethral swabs were obtained with nylon tips soaked in saline solution (FLOQSwab R100 and FLOQSwab U80, respectively; Copan Diagnostics Inc., Murrieta, CA). The swabs were soaked in 1.5 ml LiquiPrep preservation solution (LGM International Inc., Fort Lauderdale, FL), and the released-cell suspension was stored at 4 °C until use. We also collected 30 ml of midstream urine, to avoid contamination by urethral cells. Oral samples were collected by asking patients to gargle with 15 ml of phosphate buffered saline with the face pointing upward for about 10 s, then spitting the solution into a receptacle. Urinary and oral samples were centrifuged at 1500 g for 10 min, and the cell pellets were resuspended in 3 ml and 2.5 ml of LiquiPrep solution, respectively. These samples were stored at 4 °C until use [14, 18].” (Page 6, lines 7-16, present version)

(2) In the "DNA extraction" paragraph:

The paragraph (Page 6, line 21 –page 7 line 4, 18-02310R1 version) “Aliquots of the cell suspensions (1.3 ml of the urinary and oral sample and 0.7 ml of the penile and urethral samples) were centrifuged at 2,000g for 10 min. After discarding the supernatant, the cell pellet was washed twice with 1.0 ml of E-MEM (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The cell pellet was resuspended in 50 µl of E-MEM. Next, DNA was extracted from the cells with a DNA extraction kit (SMI Test; Genome Science Laboratories, Fukushima, Japan) according to the manufacturer's instructions. The extracted DNA was qualified by PCR amplification of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene [14].” has been changed to:

“DNA was extracted from the cell suspensions as described previously [14]. Briefly, after the cell suspension was centrifuged, the cell pellet was washed twice with E-MEM (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and resuspended in 50 µl of E-MEM. DNA was extracted from the cells with a DNA extraction kit (SMI Test; Genome Science Laboratories, Fukushima, Japan). The quality of the extracted DNA was confirmed, based on PCR amplification of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene [14].” (Page 6, line 19- page 7 line 2, present version)

(3) In the "HPV genotyping" paragraph:

The sentences (page 7, lines 14–22, 18-02310R1 version) “The HPV DNA-positive samples were genotyped with the 21 HPV GenoArray Diagnostic Kit (Hybribio, Chaozhou, China), with which HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 6, 11, 42, 43, 44 and 81 can be genotyped, according to the manufacturer's instructions. HPV DNA-positive samples that were not genotyped with the GenoArray Diagnostic Kit were sequenced as described previously
The obtained sequences were compared with reference sequences of various HPV genotypes in GenBank using the BLAST sequence alignment program for HPV genotyping. The detected HPV genotypes were classified into high-risk (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), probably high-risk (HPV68), possibly high-risk (HPV30, 53, and 66), or low-risk (HPV6, 11, 40, 42, 43, 44, 74, 76, 81, 90, and 114) [19].

“HPV genotypes were determined with the 21 HPV GenoArray Diagnostic Kit (Hybribio, Chaozhou, China), which could genotype HPV16, 18, 31, 33, 35, 39, 45, 51-53, 56, 58, 59, 66, 68, 6, 11, 42-44, and 81, according to the manufacturer's instructions. HPV DNA-positive samples that could not be genotyped with this kit were sequenced and genotyped with the BLAST program (U.S. National Library of Medicine, Bethesda, MD), as described previously [14]. The detected HPV genotypes were classified into high-risk (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), probably high-risk (HPV68), possibly high-risk (HPV30, 53, and 66), or low-risk (HPV6, 11, 40, 42, 43, 44, 74, 76, 81, 90, and 114) [19] groups.” (Page 7, lines 11-18, present version)

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Response:

In accordance with the comment, we have removed any tracked changes or highlighting and included only a single clean copy of the manuscript.

DR. David Mesher (Reviewer 1):

The authors have address previous concerns about the statistical analysis and used an appropriate approach to consider infections from different samples collected from the same individual.

I have one minor comment that authors should take care not to put too much emphasis on arbitrary cut-offs (e.g. to say there was poor concordance with kappa 0.20 but fair concordance with kappa 0.21)

Response:

Thank you for your comments. In accordance with your comment, we have changed the sentence (Page 10, lines 14–16, 18-02310R1 version) “Among the genital sites, the concordance of HPV infection was poor between the penis and the urethra (kappa = 0.20), and fair between the penis and the urine (kappa = 0.21), while the concordance was good between the urethra and the urine...
Among the genital sites, HPV infections were slightly concordant between the penis and the urethra (kappa = 0.20) and between the penis and the urine (kappa = 0.21), but the concordance was good between the urethra and the urine (kappa = 0.62) (Table 1).
- Authors' Contributions
- Acknowledgements

Response:

Thank you for your advice. We have checked the Editorial Policies and reviewed our manuscript. We believe that we have adhered to your editorial requirement when we revised our manuscript.