PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Rationale and design of The Self-TI Study protocol: a cross-sectional human papillomavirus self-testing pilot study among transgender adults in England
AUTHORS	Jackson, Sarah; O'Callaghan, Stewart; Ward, Elanore; Orkin, Chloe; Clarke, Megan; Berner, Alison

VERSION 1 – REVIEW

REVIEWER	Nyitray, Alan Medical College of Wisconsin
REVIEW RETURNED	11-Apr-2024

GENERAL COMMENTS	The authors present a protocol for collection of exfoliated cells form 4 different anatomical sites in trans men and transmasculine non-binary individuals and trans women and transfeminine non-binary individuals. The protocol is strengthened through creation of a Patient and Public Involvement Group, including gender minorities, that helped design the protocol. It's a strong protocol although I have a few comments. MAJOR Feasibility is assessed by the return of swabs. But it seems to me that the swabs must also be adequate specimens. If not enough appropriate material/tissue is collected to make the specimen adequate, then the returned swab is no better than a non-returned swab. MINOR Strengths and Limitations Page 4/30 – No limitations are listed although study generalizability is limited by the convenience sampling. Page 5/30 line 12 – I think it's appropriate to add that widespread implementation only occurred in areas with the resources to do it (e.g., not sub-Saharan Africa), and that methods and strategies used often did not address barriers encountered by many individuals in disenfranchised communities, e.g., (access to culturally-appropriate screening). Page 6/30 line 15 – "bivadults"? P 8/30 line 40+ - knowing how individuals learned about the study, e.g., after a cervical cancer screening, or from Instagram, should be recorded. P 9/30 L 26 – Please explain how testosterone exposure might affect accuracy (sensitivity and specificity) and acceptability by

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	different sampling modes. P 12/30 L 38 – Please explain why dry-collected specimens were chosen for each of these sites. P 13/30 L 6 – Comparing home vs in-clinic sampling with regard to comfort, safety, security, and confidence in collection (not to mention feasibility) is an important aspect. P13/30 L6 – Is there a validated scale for acceptability of self-sampling that can be used?

REVIEWER	Tiyayon, Jitima Rajavithi Hospital
REVIEW RETURNED	03-May-2024

GENERAL COMMENTS	I am not sure that 'bivadults' is generally recognised worldwide. Is it 'bivalent' HPV vaccine. According to www.nhs.uk, there is a type of HPV vaccine given in the UK which is 9-valent HPV vaccine. The limitation is not clearly inform in this document, for example the difficulty of an enrolment that can be included in the study period.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Alan Nyitray, Medical College of Wisconsin
Comments to the Author:
Review of BMJ Open protocol for Self-TI

The authors present a protocol for collection of exfoliated cells form 4 different anatomical sites in trans men and transmasculine non-binary individuals and trans women and transfeminine non-binary individuals. The protocol is strengthened through creation of a Patient and Public Involvement Group, including gender minorities, that helped design the protocol. It's a strong protocol although I have a few comments.

MAJOR
Feasibility is assessed by the return of swabs. But it seems to me that the swabs must also be adequate specimens. If not enough appropriate material/tissue is collected to make the specimen adequate, then the returned swab is no better than a non-returned swab.

- **When we designed Self-TI, we were concerned about barriers to HPV screening in the trans community and felt that the first steps were to assess acceptability (do we have buy-in from the participants?) and logistical feasibility (are the procedures appropriate for the patient population and not too onerous?) in the community.**
- **We did look at the literature comparing dry vs wet swab for self-sampling and found that accuracy was similar between the two methods.**
    - **Eperon et al., found the good overall agreement between wet and dry vaginal self-samples taken in the clinic (85.7%; 95% CI 77.8-91.6) with a κ of 0.70 (95% CI 0.57-0.70).**
    - **Wolfram et al, found almost perfect agreement was observed between paired samples for detecting any HPV (91.9%, κ: 0.85) or type-specific HPV (90.1%, κ: 0.90) in dry vs. wet collection methods from vaginal samples collected by participants at home.**

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- **We did not find literature that compared dry vs wet swabs for HPV self-sampling of the anus. However, we have no reason to assume dry swabs will not work as well for detecting anal HPV as for vaginal.**
- **Our methods are in line with what other similar studies, namely Welsh et al., have used for vaginal and anal collection in trans populations.**
- **We take the reviewer's point seriously and will monitor the adequacy of the specimens. This is a pilot study that is designed to identify issues like this before we expand to a larger sample of participants.**

MINOR
Strengths and Limitations
Page 4/30 – No limitations are listed although study generalizability is limited by the convenience sampling.

- **We have added this to the limitations section, which now reads in part: "Potential study limitations include the inability to generalize our results to the wider transgender population in England because that we used sexual health clinics in two major cities to recruit our participants. Compared to the general population of transgender individuals, our study participants may be more engaged in care, have greater access to care, and have higher health seeking behaviors."**

Page 5/30 line 12 – I think it's appropriate to add that widespread implementation only occurred in areas with the resources to do it (e.g., not sub-Saharan Africa), and that methods and strategies used often did not address barriers encountered by many individuals in disenfranchised communities, e.g., (access to culturally-appropriate screening).

- **We have updated lines 47-48 to read as follows (highlighting indicates added text): "Prior research conducted in cisgender women (largely from high-income countries) has shown that self-sampling for HPV with PCR-based assays has comparable performance to clinician-collected samples for the detection of cervical HPV."**
- **We have added to the discussion the following sentence: "The implementation of HPV self-sampling methods and strategies may reduce barriers for transgender and non-binary people in high-resourced areas, but barriers will remain for individuals who live in areas where there is widespread discrimination resulting in a lack of access to culturally appropriate screening."**

Page 6/30 line 15 – "bivadults"?

- **Apologies this is a typo. It has been corrected to "bivalent."**

P 8/30 line 40+ - knowing how individuals learned about the study, e.g., after a cervical cancer screening, or from Instagram, should be recorded.

- **We are limited to the amount of information we record from participants for research purposes. Collection of this data will need to go through a protocol amendment to the regulatory bodies in England (REC-4). We will consider adding this question to our screening form for future amendments.**

P 9/30 L 26 – Please explain how testosterone exposure might affect accuracy (sensitivity and specificity) and acceptability by different sampling modes.

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- **Previous research by Reisner et al. has shown good concordance of self-sampling compared to clinician collected samples. However, this study enrolled TMNB participants with a variety of testosterone use histories. As the majority (>90%) of patient population the study sites serve are currently using testosterone (as well as the majority [>80%] of TMNB patients overall) we wanted our results to be applicable to this population. Further, as testosterone use has been associated with an increase in inadequate cervical samples, we felt self-sampling options are most needed for this population.**
- **We have added the following text to lines 101-103 (highlighting indicates new text): "Testosterone exposure is a requirement of study participation as it is associated with vaginal atrophy such that speculum and swab insertion to the recommended depth could be painful, unpleasant, or necessitate additional lubricant affecting the accuracy and acceptability of clinician- and self-collected HPV testing.[9, 39]**

P 12/30 L 38 – Please explain why dry-collected specimens were chosen for each of these sites.

- **Please see our response above to this question.**
- **We have added the following sentence to lines 142-144: "The choice of sampling methods was based on a review of previous studies that examined the same body sites and concordance between in-clinic and at-home sampling methods." [42,43]**

P 13/30 L 6 – Comparing home vs in-clinic sampling with regard to comfort, safety, security, and confidence in collection (not to mention feasibility) is an important aspect.

- **We agree! We have provided more detail on the contents of the acceptability questionnaire on lines 166-169: "The survey includes questions on based on previously validated surveys that capture sensitive demographic characteristics, acceptability of self-sampling (e.g., physical and emotional comfort, confidence in collection),[38]…"**

P13/30 L6 – Is there a validated scale for acceptability of self-sampling that can be used?

- **We adapted the acceptability scale for self-sampling from Reisner et al. We have added space for participants to provide comments so that we can update or revise the survey in response to participant feedback if needed.**
- **We have noted on line 164 that this aspect of the survey was adapted from Reisner et al.**

Reviewer: 2
Dr. Jitima Tiyayon, Rajavithi Hospital
Comments to the Author:
I am not sure that 'bivadults' is generally recognised worldwide. Is it 'bivalent' HPV vaccine. According to www.nhs.uk, there is a type of HPV vaccine given in the UK which is 9-valent HPV vaccine.

- **Apologies this is a typo. It has been corrected to "bivalent."**
- **You are correct, we have updated lines 27-30 as follows (highlighting indicates new text): "England began a national HPV immunisation programme for adolescent girls with the bivalent HPV vaccine in 2008, switching to the quadrivalent in 2012 and the nonavalent in 2021.[17 18]**

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The limitation is not clearly inform in this document, for example the difficulty of an enrolment that can be included in the study period.

- **We have added a limitations section to the document. We address your concern of enrolling an appropriate sample size in limited time on lines 291-294 as follows: "This recruitment strategy was chosen to maximize the proportion of positive HPV tests, to enable recruitment of a reasonable sample size in a short amount of time."**

References cited in this response in alphabetical order
[Please note that numbered references used in the text are located in the manuscript.]

Eperon I, Vassilakos P, Navarria I, et al. Randomized comparison of vaginal self-sampling by standard vs. dry swabs for human papillomavirus testing. *BMC Cancer* 2013;13:353.

Reisner SL, Deutsch MB, Peitzmeier SM, et al. Comparing self- and provider-collected swabbing for HPV DNA testing in female-to-male transgender adult patients: a mixed-methods biobehavioral study protocol. *BMC Infect Dis* 2017;17(1):444.

Reisner SL, Deutsch MB, Peitzmeier SM, et al. Test performance and acceptability of self- versus provider-collected swabs for high-risk HPV DNA testing in female-to-male trans masculine patients. *PLoS One* 2018;13(3):e0190172.

Welsh EF, Andrus EC, Sandler CB, et al. Cervicovaginal and anal self-sampling for HPV testing in a transgender and gender diverse population assigned female at birth: comfort, difficulty, and willingness to use. *medRxiv* 2023

Wolfrum SG, Koutsky LA, Hughes JP, et al. Evaluation of dry and wet transport of at-home self-collected vaginal swabs for human papillomavirus testing. *Journal of Medical Microbiology* 2012;61(11):1538-45.

VERSION 2 – REVIEW

REVIEWER	Nyitray, Alan Medical College of Wisconsin
REVIEW RETURNED	10-Jun-2024

GENERAL COMMENTS	Thanks for responding to my previous review. The answers are clear and help to allay any concerns I had. I only have 2 more comments with both on page 58/60 of the response from the authors. line 31: Note that feces carries microorganisms which may inhibit PCR assays. Those microorganisms are lysed by STM, but may continue inhibitory activities in a swab transported dry (although, TypeSeq may support better adequacy than, say, SPF10 assays). There is some literature on this, including Nitkowski J, Giuliano A, Ridolfi T, Chiao E, Fernandez ME, Schick V, Swartz MD, Smith JS, Schneider EA, Brzezinski B, Nyitray AG. Effect of the environment on home-based self-sampling kits for anal cancer screening. J Virol Methods. 2022;310:114616. Epub 20220909. doi: 10.1016/j.jviromet.2022.114616. PubMed PMID: 36096333; PMCID: PMC9645463. line 33: Welsh et al. methods (borrowed from Eisenberg et al. and Patel et al. methods) put the anal swab into STM for transport which

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	I believe is not the same as the current protocol in which the anal swab is mailed in a dry state. A slight change in the wording may be needed in the manuscript (page 42/60 line 144 in the marked-up manuscript). Note that the current discussion is related to the transport of specimens rather than whether the swab is inserted into the anus in a dry state or it is wetted before insertion. Dry swabs may be more painful which could affect acceptability: Weidlich S, Schellberg S, Scholten S, Schneider J, Lee M, Rothe K, Wantia N, Spinner CD, Noe S. Evaluation of self-collected versus health care professional (HCP)-performed sampling and the potential impact on the diagnostic results of asymptomatic sexually transmitted infections (STIs) in high-risk individuals. Infect Dis Rep. 2023;15(5):470-7. Epub 20230825. doi: 10.3390/idr15050047. PubMed PMID: 37736994; PMCID: PMC10514875.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Thanks for responding to my previous review. The answers are clear and help to allay any concerns I had. I only have 2 more comments with both on page 58/60 of the response from the authors.

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Note that the current discussion is related to the transport of specimens rather than whether the swab is inserted into the anus in a dry state or it is wetted before insertion. Dry swabs may be more painful which could affect acceptability: Weidlich S, Schellberg S, Scholten S, Schneider J, Lee M, Rothe K, Wantia N, Spinner CD, Noe S. Evaluation of self-collected versus health care professional (HCP)-performed sampling and the potential impact on the diagnostic results of asymptomatic sexually transmitted infections (STIs) in high-risk individuals. Infect Dis Rep. 2023;15(5):470 PubMed -7. Epub 20230825. doi: 10.3390/idr15050047. PubMed PMID: 37736994; PMCID: PMC10514875

- **We have made the following changes to the Methods on lines 145-146 (underlining indicates new text): The choice of sampling methods was based on a review of previous studies that examined the same body sites and concordance between in-clinic and at-home sampling methods.[43 44], though the mailing of dry swabs without transport media may affect anal specimen adequacy if fecal matter is present.[45]**
- **We have made the following changes to the Discussion on lines 304-306 (underlining indicates new text): Our decision to use dry swabs for anal sampling may affect acceptability as dry swabs were reported to cause pain in 19% of users[49] as well as adequacy as the presence of feces was shown to inhibit HPV assays.[45]**

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