Self-sampled specimens demonstrate comparable accuracy and consistency to clinician-sampled specimens for HPV detection among men who have sex with men in China

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ABSTRACT

Objectives Despite a high risk of human papillomavirus (HPV) infection among men who have sex with men (MSM), few have ever tested. This study aimed to evaluate the feasibility and accuracy of HPV self-sampling among Chinese MSM, with the purpose of measuring the feasibility of self-sampling as an alternative in HPV testing scenarios.

Methods Eligible participants were those who were assigned male at birth, aged 18 or above, had sex with men in the past year and had never gotten HPV vaccine. Participants followed the instructions to self-sample and were also clinician-sampled from the same anatomical sites (oral fluid, penis and rectum) in both approaches. All specimens were processed using multiple PCR assay. The reference standard of an individual with a true positive for HPV is determined via PCR test, regardless of sampling methods. Sensitivity and specificity were calculated for each approach independently and kappa test was used to assess the consistency between the two approaches.

Results Overall, 211 MSM were recruited at the local clinic from April to October 2020 in Zhuhai, China. The mean age was 31 years old. Only 3% of the participants sought help from healthcare providers during self-sampling. The prevalence of HPV was 49% (103 of 211). Clinician sampling detected 91 of 103 MSM infected with HPV, with a sensitivity of 88.3% (95% CI 80.2 to 93.6) and a specificity of 100.0% (95% CI 95.7 to 100.0). Self-sampling detected 81 of 103 MSM infected with HPV, with a sensitivity of 78.6% (95% CI 69.2 to 85.9) and a specificity of 100.0% (95% CI 95.7 to 100.0). The level of agreement was moderate between clinician sampling and self-sampling (κ=0.67).

Conclusions Self-sampled HPV testing demonstrated comparable accuracy and consistency to clinician sampling among MSM in China. It holds the potential to complement sexual health services especially among key populations.

INTRODUCTION

The absence of human papillomavirus (HPV) and anal cancer screening guidelines among men generates obstacles for men who have sex with men (MSM) and perpetuates inequities and preventable health disparities. HPV infection is one of the most prevalent STIs worldwide. In addition to the well-established association between high-risk HPV infection and cervical cancer, persistent high-risk HPV infection has been linked to the development of other malignancies, including oropharyngeal, oesophageal and anal cancers. Although anal cancer is relatively rare in the general male population, the incidence rate for HIV-negative MSM and MSM living with HIV is 20-fold and 30-fold higher, respectively, compared with the general male population. Among MSM living with HIV, the estimated incidence of high-risk HPV infection is 40%–88% and for HIV-negative MSM it is 22%–34%. Furthermore, HPV and HPV-related abnormalities are also more prevalent in MSM living with HIV, with a concerning high incidence of anal cancer. These significant associations between HPV infection and HIV acquisition among MSM have been discussed in other studies.

In China, a rising trend in the prevalence of HIV/AIDS and other STIs has received attention. For instance, a cross-sectional study illustrated that anal HPV infection is high among MSM in three Chinese cities; which in general was estimated to be 66.3%, suggesting a need to improve behaviour interventions and inclusive healthcare among at-risk populations. Other studies have also suggested HPV testing strategies to increase HPV screening for high-risk populations, especially MSM, to detect and manage the infection as secondary prevention approach. Additionally, accessibility constraints such as hours of operation, travel distance, and lack of access to primary care providers and regular screening methods conducted in clinical settings making it difficult for MSM to get tested. Alternate routes of effective HPV screening methods, such as self-testing, must be explored to facilitate testing.

Self-sampled HPV testing is an empowering method that can reduce testing barriers to allow rapid, confidential and cost-effective sampling. In HIV testing, self-testing has also been recommended by the WHO as an effective approach to engage marginalised populations. This strategy might also be applied to HPV screening. We define self-sampled HPV testing as individuals who want to know their HPV infection status by self-sampling and sending them to the laboratory
for interpretation of a test report. Self-sampled HPV testing has been widely piloted among women to screen for cervical cancer, to potentially increase cervical cancer screening for women who were reluctant or not yet tested. Previous meta-analyses have demonstrated that delivering home-based self-collected kits to women leads to higher participation rates than facility-based testing. Considering the decentralised nature of self-sampled HPV testing, it may also hold the potential to be applied among MSM due to the confidentiality it provides. This can contribute to improved capacity and access of sexual health services among MSM. This testing method has been approved in some developed regions, including the USA, Canada and Hong Kong, as acceptable and feasible among MSM. However, in mainland China, similar programmes for MSM have not been provided or evaluated. Therefore, this study aimed to evaluate the consistency between self-sampling and clinician sampling for HPV testing.

METHODS

Study design
This study was conducted in Zhuhai City, Guangdong Province, between 28 April 2020 and 28 October 2020. We aimed to enrol 200 eligible MSM to conduct this study. Laboratory analyses were conducted in BGI in Shenzhen City, Guangdong Province. We collaborated with the following: Zhuhai Xutong Volunteer Services Center, a local gay-led community-based organisation (CBO); Lingnan Clinic, a community health clinic providing routine HIV testing services; and the BGI Group, a Chinese life science and genomics organisation. We posted study information on the CBO’s official account on WeChat, the most popular social networking site in China. Study introduction, clinic hours and directions to the clinic were all provided in the posts. Besides, MSM who visited the Lingnan Clinic for HPV testing were also informed about the study by the CBO staff or clinicians.

Criteria
Participants who (1) were assigned as male at birth, (2) were 18 years of age or older, (3) had sex with another man in the past year and (4) had never received HPV vaccine were recruited by the CBO staff or the clinician at the Lingnan Clinic and provided their consent.

Survey
Participants were requested to complete a baseline survey through the CBO online platform before self-sampling. Survey questions include sociodemographic characteristics, sexual history and previous HIV and HPV testing experience. After completing the baseline survey, participants needed to sign two consent forms for permission to collect specimens.

All participants were requested to conduct self-sampling first in the clinic’s bathroom by themselves. The study participants were only provided with written instruction (without clinicians’ supervision) and used swabs to self-collect specimens from the oral, penile and rectal orifices. Participants then had to place the swab tips first into the provided tube, break the swab handles at the marked line and screw the cap back on tightly. Then, the CBO staff took the tube and led participants to a private area to conduct clinician sampling. For clinician sampling, a clinician or a trained CBO staff followed the same instructions, collected participants’ specimens from the same anatomical sites and put swabs in the tube accordingly. The CBO staff shipped the specimen sampling tubes to the laboratory approximately twice a week.

Sampling method
Each testing package contained six sterile swabs (three swabs for self-sampling and three for clinician sampling), two tubes with RNA storage media (supplied by the BGI Group), and written instructions for both self-sampling and clinician sampling. We assigned a unique number to each tube and divided it into two groups for the participants: odd numbers for self-sampling and even numbers for clinician sampling.

Results notification
We received laboratory reports 7 business days after delivering the tubes. Participants received HPV test results through a text message from the CBO staff. For participants who tested HPV-positive, the CBO staff called them and provided consultation and linkage to care. The CBO staff contacted the participants whose obtained specimens (either clinician sampling method or self-sampling method) were not sufficient for PCR analyses and asked them to return to the clinic to get retested.

Data collection, assurance and privacy
In the baseline survey, we asked the study participants to provide their cellphone numbers as the only identifiable data for receiving reports. A verified code was sent to participants to ensure the provided cellphone number was correct. Participants were required to disclose aliases when signing consent forms. All other data from the baseline survey were de-identified.

Specimen extraction and testing
We used a multiplex HPV genotyping assay to identify 14 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and 2 low-risk HPV genotypes (6 and 11) based on the amplification of a fragment of the L1 gene region using GP5+/GP6+ consensus primers and the subsequent use of a type-specific extension primer to distinguish the 16 HPVs in one reaction. Second, we used the MassARRAY (Agena Bioscience) technique based on the matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry platform to analyse the extension products, which allows detection and genotyping of all 16 HPV types simultaneously in one reaction with high throughput. Third, multiplex amplification of the GP5+/GP6+ PCR products with modified primary primers was performed to obtain suitable HPV genotyping primary templates. PCR was performed in a solution with a final volume of 5 µL. The PCR cocktail consisted of 1 µL of DNA template (10–25 ng/µL), 1× PCR buffer (including 2 mmol/L magnesium chloride), 2 mmol/L magnesium chloride, 500 pmol/L deoxynucleoside triphosphate (dNTP) mix, 0.1 pmol/µL of each preprimer and 0.5 U of HotstartTaq (Roche). PCR conditions were as follows: denaturation at 94°C for 15 min, followed by 45 cycles of 20 s at 94°C, 30 s at 56°C, 1 min at 72°C and a final extension of 3 min at 72°C. The final primary PCR reaction mix was treated with shrimp alkaline phosphatase to dephosphorylate unincorporated dNTP. The iPLEX primer extension reaction was performed to identify the HPV genotypes; procedures followed the iPLEX kit standard protocol (Agena). To desalt the iPLEX extension products before mass spectrometric analysis, 6 mg clean resin were added into the 384-well PCR plate. We dispensed 3–10 nL products onto a 384-element SpectroCHIP bioarray (Agena). TYPE V.4.0 software
(Agena) was used to process and analyse the iPLEX SpectroCHIP bioarrays.

Statistical analysis
We calculated a sample size of 200 enrolled study participants, considering an α of 5%, power of 80%, HPV prevalence of 0.4, expected sensitivity of 75%, specificity of 100%, desired precision of 0.10 and a loss to follow-up rate of 10%. We used descriptive statistics to present the baseline characteristics of the study participants and collected reports from lay health providers regarding sampling feasibility. Regardless of sampling methods, we defined overall PCR test results as the gold standard, and estimated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and respective 95% CI to measure the diagnostic performance of the self-sampling method and the clinician sampling method in detection of HPV. McNemar’s χ² test was used to estimate the consistency of sensitivity values between clinician sampling and self-sampling methods (PCR test being the reference).26 We also evaluated the performance of different sampling tests in study participants infected with high-risk HPV or low-risk HPV. Then we considered the results of the clinician-sampled test as the reference to measure the performance of the self-sampled test. In addition, we calculated the kappa statistics with respective 95%CI to evaluate the agreement between the two sampling methods. Levels of agreement were judged using the following scale: >0.90 (almost perfect), 0.80–0.90 (strong), 0.60–0.79 (moderate), 0.40–0.59 (weak), 0.21–0.39 (minimal) and 0.0–0.20 (none).27 We also estimated the accuracy of both clinician sampling and self-sampled tests in specific HPV genotypes (HPV16 and HPV11) according to the frequency distribution of HPV subtypes. There were no missing data. All data were presented in the following formats as appropriate: number, percentage, mean and SD. Data were analysed using R V.3.6.2.

RESULTS
We enrolled 211 study participants during the study period, with a total of 426 collected specimens. Of these specimens, 4 (1%; 1 from self-sampling and 3 from clinician sampling) were insufficient to detect DNA, resulting in 422 specimen results being included in the final analyses. Only 7 of 211 study participants (3%) sought assistance from clinicians or trained CBO staff during self-sampling because they did not understand some of the steps of the instruction.

All study participants’ baseline characteristics are summarised in table 1. The mean age overall was 31 years. Of the participants, 69% earned a college degree or above, 61.1% self-identified as gay, 82.0% were single, 87.2% had previous experience testing for HIV and 8.5% ever tested for HPV. Four (2.4%) study participants self-reported living with HIV and three of them tested HPV-positive.

Table 2 shows the performance of different sampling tests, using the PCR test as the reference. Overall, 103 participants tested HPV-positive from PCR test results. The clinician-sampled test gave higher sensitivity (88.3%, 95%CI 80.2 to 93.6) and NPV (90.0%, 95%CI 82.8 to 94.3) than the self-sampled test (sensitivity: 78.6%, 95%CI 69.2 to 85.9; NPV: 83.1%, 95%CI 75.3 to 88.9). However, there were no significant differences between these two sensitivities (p=0.1227). The clinician-sampled test and the self-sampled test had similar specificity values and PPVs.

We assessed the performance of different sampling tests in detecting high-risk and low-risk HPV infections, as shown in table 3. The sensitivity for detection of high-risk HPV was 77.2% (95% CI 66.1 to 85.6) in the self-sampled test and 88.6% (95% CI 79.0, 94.3) in the clinician-sampled test; for detection of low-risk HPV, the sensitivity was 87.5% (95% CI 72.4 to 95.3) in the self-sampled test and 90.0% (95% CI 75.4 to 96.7) in the clinician-sampled test. Both sensitivity comparisons were not significantly different between the self-sampled test and the clinician-sampled test in the detection of high-risk HPV (p=0.1237) and low-risk HPV (p=1.0000) (table 3).
test and the clinician-sampled test was moderate (Cohen’s kappa coefficient = 0.67) (table 4).

The frequency of detected HPV genotypes is presented in online supplemental figure 1. In summary, HPV16 (21.4%, 22 of 103) was the most common high-risk HPV infection genotype among the 103 participants, while HPV11 (23.3%, 24 of 103) was the most common low-risk HPV infection genotype.

Online supplemental tables 1 and 2 show the sensitivity and specificity of self-sampling and clinician-sampled tests for participants tested with HPV16 and HPV11. The sensitivity of the clinician-sampled method was 81.8% (95% CI 59.0 to 94.0) for HPV16 detection and 75.0% (95% CI 52.9 to 89.4) for HPV11 detection and the corresponding specificities were both 100.0% (95% CI 97.5 to 100.0). The sensitivity of the self-sampled method was 63.6% (95% CI 40.8 to 82.0) for HPV16 detection and 87.5% (95% CI 66.5 to 96.7) for HPV11 detection and the corresponding specificities were both 100.0% (95% CI 97.5 to 100.0). No adverse event related to the study was reported. De-identified data can be found in supplementary files (online supplemental files 2 and 3).

**DISCUSSION**

Despite MSM bearing a high burden of HPV infection, few have ever been tested due to the absence of HPV and anal cancer screening guidelines among MSM and other constraints that hinder MSM from attending clinics for HPV screening. Adding self-sampled HPV testing as an MSM-focused approach can be effective and empowering to improve sexual health services among them. Our study was designed to evaluate the accuracy and feasibility of self-sampled HPV testing among MSM, which might provide evidence and opportunity for integration of HPV screening guidelines for MSM in China. We found that this decentralised approach demonstrated comparable accuracy and feasibility among MSM compared with clinician-sampled HPV testing.

Our findings demonstrate self-sampling had a moderate performance in HPV detection compared with clinician sampling. The specificity of self-sampling in detection of HPV was high and equivalent to the specificity of clinician sampling. Regarding sensitivity, clinician sampling had a higher sensitivity than self-sampling, which may indicate that it is critical to provide easy and comprehensive instructions for the participants to follow the sampling mechanisms. Moreover, study results showed that a minority of HPV-positive results were exclusively detected by self-sampling. Although we have adopted PCR results as the gold standard to avoid bias, it is also worth noting that this was consistent with a prior study: that is, the lack of a criteria standard detection technique for HPV detection is highlighted by the fact that some of the clinician-sampled swabs were insufficient for processing. Therefore, we also adopted both PCR results and clinician sampling results as references to assess the accuracy of the sensitivity and specificity. The test results revealed that the prevalence of HPV among MSM is high, which is consistent with a prior meta-analysis on HPV prevalence in China. Providing preventive measures for MSM, a high-risk group for HPV infection, especially those living with HIV, is warranted. In addition to primary prevention including HPV vaccine, and prevention and control of HIV infection, secondary prevention measures such as HPV testing can also play an essential role for further monitoring and treatments.

As demonstrated by the data from the clinicians and CBO staff, self-sampled HPV testing could be feasible among MSM. First, in terms of sampling, there was only one specimen that was insufficient to detect DNA, which indicated that most of the participants performed the sampling accurately. Second, only 7 of 211 participants (3.3%) sought assistance from clinicians.

### Table 2 Sensitivity and specificity analyses of different sampling HPV tests, using PCR test as the reference (N=211)

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
<th>Total (%)</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>PPV, % (95% CI)</th>
<th>NPV, % (95% CI)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinician-sampled test</td>
<td>Negative: 108/108 (51.2)</td>
<td>Positive: 0/0 (0.0)</td>
<td>Total: 108 (51.2)</td>
<td>88.3 (80.2 to 93.6)</td>
<td>100.0 (95.7 to 100.0)</td>
<td>100.0 (95.0 to 100.0)</td>
<td>90.0 (82.8 to 94.5)</td>
<td>0.1227</td>
</tr>
<tr>
<td>Self-sampled test</td>
<td>Negative: 108/108 (51.2)</td>
<td>Positive: 0/0 (0.0)</td>
<td>Total: 108 (51.2)</td>
<td>78.6 (69.2 to 85.9)</td>
<td>100.0 (95.7 to 100.0)</td>
<td>100.0 (94.4 to 100.0)</td>
<td>83.1 (75.3 to 88.9)</td>
<td>0.1237</td>
</tr>
</tbody>
</table>

*McNemar’s χ² test was done to compare sensitivity values between clinician-sampled and self-sampled tests.

### Table 3 Performance of different sampling tests among study participants with different risks of HPV infections, using PCR test as the reference

<table>
<thead>
<tr>
<th>Test Type</th>
<th>High-risk HPV</th>
<th>Low-risk HPV</th>
<th>High-risk HPV</th>
<th>Low-risk HPV</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity, % (n/N)</td>
<td>88.6 (70/79) (79.0 to 94.3)</td>
<td>90.0 (36/40) (75.4 to 96.7)</td>
<td>0.1237</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>Specificity, % (n/N)</td>
<td>87.4 (72.4 to 95.3)</td>
<td>97.7 (95.7 to 100.0)</td>
<td>0.0100</td>
<td>0.6250</td>
<td></td>
</tr>
<tr>
<td>PPV, % (n/N)</td>
<td>100.0 (108/108) (95.7 to 100.0)</td>
<td>100.0 (108/108) (95.7 to 100.0)</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPV, % (n/N)</td>
<td>97.3 (108/111) (91.7 to 99.3)</td>
<td>93.1 (108/116) (86.4 to 96.8)</td>
<td>0.0089</td>
<td>0.6250</td>
<td></td>
</tr>
</tbody>
</table>

*McNemar’s χ² test was done to compare sensitivity values between clinician-sampled and self-sampled tests.

HPV, human papillomavirus; NPV, negative predictive value; PPV, positive predictive value.
before or during self-sampling, mainly for not understanding some of the operational procedures. This reveals that most of the participants were able to understand the instructions; the lack of understanding on operational procedures can be resolved by providing a clearer instruction. Finally, clinicians mentioned that some participants felt embarrassed about clinician sampling because it involved sampling of their private areas. Self-sampling can overcome these highlighted concerns for MSM by ensuring confidentiality.

Given that our study was organised by a local gay-friendly CBO and since self-sampled HPV tests presented considerable accuracy and feasibility, this approach could be considered for future policymaking to be incorporated into the health services of local CBOs or sexual health clinics. For example, it could be combined with the HIV self-testing currently being implemented in China to provide digital network-based HIV/STI testing.30 For MSM who are sexually active or living with HIV, self-sampled HPV tests could be a testing strategy that is beneficial for infection detection, monitoring and clinic attendance. Additionally, self-sampled HPV testing may benefit future HPV vaccination programmes among MSM by providing a novel and convenient method to surveil vaccination-related outcomes. Future studies are required to assess the willingness of MSM to perform the self-sampled HPV tests, including facilitators and barriers. It is also essential to pay attention to the promotion of this service among MSM.

This study has two limitations. First, since our study combined swabs from all three sites in one tube, some of the HPV-positive participants who were not yet showing symptoms were not able to know their specific site of infection. However, we believe that this sampling method can capture a more complete picture of participants’ infection status. Second, the subtype analyses of prevalence had a small sample, which might impact the results; however, since this study was not designed for this aspect, the focus was instead on the accuracy and feasibility of the self-sampled HPV testing.

To conclude, self-sampled HPV testing is feasible among MSM in China and holds the potential to complement current sexual health services and facilitate future HPV vaccination programmes among marginalised populations.

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Contributors YN, YiL and SG drafted the manuscript. YN, YL, XH, YuL, NL and WT contributed to study design. YN, YL, XH, YuL, Yol., SG, CX, XW, NL and WT contributed to the development and implementation of study procedures. YN, YL, XH, YuL, JQD, XY and WT participated in the statistical analysis and interpretation of the findings. WT is responsible for the overall content as the guarantor. All authors critically reviewed and edited the manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study involves human participants and was approved by the Dermatology Hospital of Southern Medical University in Guangzhou, China (ID: 2020019). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

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REFERENCES

Table 4 Sensitivity, specificity and correlation analyses of different sampling HPV tests, using clinician-sampled test as the reference (N=211)

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
<th>Total (%)</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>PPV, % (95% CI)</th>
<th>NPV, % (95% CI)</th>
<th>Kappa (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-sampled</td>
<td>108 (51.2)</td>
<td>22 (10.4)</td>
<td>130 (61.6)</td>
<td>75.8 (65.5 to 83.9)</td>
<td>90.0 (82.8 to 94.5)</td>
<td>85.2 (75.2 to 91.8)</td>
<td>83.1 (75.3 to 88.9)</td>
<td>0.67 (0.56 to 0.77)</td>
</tr>
<tr>
<td>Self-sampled</td>
<td>12 (5.7)</td>
<td>69 (32.7)</td>
<td>81 (38.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>120 (56.9)</td>
<td>91 (43.1)</td>
<td>211 (100.0)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

HPV, human papillomavirus; NPV, negative predictive value; PPV, positive predictive value.

Key messages
⇒ As demonstrated by the data from the clinicians and community-based organisation staff, self-sampled human papillomavirus (HPV) testing could be feasible among men who have sex with men (MSM).
⇒ Self-sampling shows a moderate agreement with clinician sampling for HPV detection among Chinese MSM.
⇒ HPV self-sampling could be an alternative HPV testing method for MSM.
⇒ HPV prevalence remains relatively high among MSM in Guangdong, China.

Feasibility of incorporating self-collected rectal swabs into a community venue-based survey to measure the prevalence of HPV infection in men who have sex with men. Sex Transm Infect 2011;38:964–9.


