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# Diagnostic Performance Evaluation of Urine HIV-1 Antibody Rapid Test Kits in Screening Diverse Populations: A Real-World Study



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# Diagnostic Performance Evaluation of Urine HIV-1 Antibody Rapid Test Kits in Screening Diverse Populations: A Real-World Study


## Abstract

**Objective:** To evaluate the diagnostic performance of urine human immunodeficiency virus (HIV) antibody rapid test kits in screening diverse populations and to analyse subjects' willingness regarding reagent types, purchase channels, acceptable prices, and self-testing.

**Design:** Screening study

**Participants:** A total of 2606 valid and eligible samples were collected in the study, including samples from female sex workers (FSWs), persons with injection drug use (IDU), pregnant women (PW), subjects undergoing voluntary HIV counselling and testing (VCT), and students in higher education (STUs). The receiver operator characteristic (ROC) curve was drawn to evaluate the diagnostic performance of urine HIV-1 antibody rapid test kits in on-site screening, and the cluster analysis model was applied to analyse the subjects' intentionality regarding HIV antibody testing options.

**Results:** The sensitivity, specificity, and area under the curve (AUC) of the urine HIV-1 antibody rapid test kits were 92.16%, 99.92%, and 0.960 (95% confidence interval (CI): 0.952-0.968, $p<0.001$), respectively, among 2606 samples collected during on-site screenings. The kits showed good diagnostic performance in persons with IDU (*AUC*: 1.000, 95% CI: 1.000-1.000, $p<0.001$), PW (*AUC*: 0.999, 95% CI: 0.999-1.000, $p<0.001$), and FSWs (*AUC*: 1.000, 95% CI: 1.000-1.000, $p<0.001$). The AUC of the urine reagent kits in subjects undergoing VCT was 0.941 (95% CI: 0.876-0.978, $p<0.001$). The "acceptable price" had the greatest influence on STUs (Predictor importance, $Pi=1.000$) and PW ($Pi=1.000$), the "purchase channel" had the greatest influence on subjects undergoing VCT ($Pi=1.000$) and persons with IDU ($Pi=1.000$), and the “reagent types” had the greatest influence on FSWs ($Pi=1.000$).

**Conclusions:** The urine HIV-1 rapid test kit has reliable diagnostic performance in screening the general population and high-risk populations for HIV, and its use can be further promoted to generate sufficient data and experience. Physicians of subjects undergoing VCT should prudently select HIV antibody testing reagents based on the subjects' actual conditions.

**Ethics statement:** This study was approved by the Ethics Committee of the Guangxi Zhuang Autonomous Region Center for Disease Control and Prevention (approval number GXIRB2019-0047).


**Keywords:** HIV, urine, rapid test kits, ROC

**Strengths and limitations of this study:**

1. Few studies have evaluated the diagnostic performance of urine HIV-1 rapid test kits in screening both the general population and high-risk populations.
2. This manuscript provides a preliminary evaluation of the acceptability of urine HIV-1 rapid test kits in high-risk HIV populations and the general population.
3. No positive samples were found among the students, and therefore, ROC curves could not be plotted for this subgroup.

## 1. Introduction

The prevalence of HIV/AIDS varies widely across China[1, 2]. Guangxi Zhuang Autonomous Region, the only minority region in southern China, is a serious HIV/AIDS hotspot; in the past decade, this region had a much higher HIV/AIDS prevalence than any other Chinese coastal or inland province[3, 4]. Therefore, the public health administration in Guangxi is attempting to expand the scale of HIV screening to diagnose HIV-infected patients at an early stage and provide highly active antiretroviral therapy (HAART) in a timely manner to reduce HIV/AIDS mortality and transmission[5, 6], especially in high-risk populations[7].

In recent years, extensive HIV/AIDS publicity and education have increased the Chinese population's awareness and willingness to get tested[8]. With the reduction in cost, urine HIV antibody testing is gradually gaining attention and acceptance by public health institutions and the general public due to its advantages of being convenient, noninvasive and safe[9, 10]. The satisfactory sensitivity and specificity of the urine reagent kits have been described in many previous studies and have shown good performance under controlled laboratory conditions[11-13].

Their noninvasiveness has made urine reagent strips for HIV antibody testing more popular among target populations and has led to public health policymakers being willing to choose urine reagent strips for population screening in areas with HIV epidemics, such as Guangxi[14], increasing acceptance among target populations, especially with the availability of urine rapid test kits that can be used for direct on-site screening. In contrast, previous urine HIV antibody reagents required that urine samples be transported to the laboratory for centralized testing because of methodological limitations.

It is worth noting that although some studies have evaluated the diagnostic performance of urine HIV-1 antibody rapid test kits using standard samples under controlled laboratory conditions, no studies have yet reported on their diagnostic performance in practical screening applications in different populations; therefore, an adequate scientific basis for the application of urine rapid test kits for HIV screening has not been provided for public health authorities in high-prevalence areas.

This study, based on a special study of the Chinese National Science and Technology Major Project (NSTMP) for infectious diseases, aimed to evaluate the diagnostic performance of urine HIV-1 antibody rapid test kits in a practical screening setting and to preliminarily analyse the willingness of subjects regarding the types of reagents, purchase channels, and acceptable prices to provide a valuable scientific basis for the application of urine HIV antibody rapid test reagents for screening.

## 2. Materials and methods

### *2.1 Samples and Sources*

Subjects were recruited from the most commonly screened populations for HIV antibodies in the real world, including high-risk populations, individuals identified through sentinel surveillance, and the general population, and divided into the following five categories: Female sex workers (FSWs), persons with injection drug use (IDU), pregnant women (PW), subjects undergoing voluntary HIV counselling and testing (VCT), and students at colleges and universities (STUs).

FSWs and persons with IDU are high-risk populations for HIV infection, and both groups were recruited by sentinel surveillance in this study. PW are routinely screened for HIV, and women receiving care during pregnancy were recruited from women and children's hospitals. Subjects undergoing VCT were consulted or referred to provincial CDC VCT clinics. The STUs were enrolled in higher education schools or colleges. This study was conducted from August 1, 2020, to September 31, 2020.

To improve the external validity and to match the characteristics of the real-world HIV screening population, no strict inclusion or exclusion criteria were set for this study. Researchers informed subjects of the purpose, methods, potential harms, and personal privacy issues of this study in detail before informed consent forms were signed.

### *2.2 Urine and blood sample testing methods*

Three HIV antibody test reagents were used in the study: (1) Reagent A, named the Urine HIV-1 Antibody Rapid Test Kit (colloidal gold), was packaged as a rapid test kit and manufactured by Wantai (20193400550); (2) Reagent B, named Determine™ HIV1/2 (colloidal selenium), was packaged as a rapid test kit and manufactured by Abbott (20163400427); and (3) Reagent C, named GENscreen™ ULTRA HIV Ag-Ab (Enzyme-Linked Immunosorbent Assay, ELISA), which was manufactured by Bio-Rad (72388C).

HIV antibody tests were divided into on-site tests (for Reagents A and B) and laboratory tests (for Reagent C only). Reagents A and B were used to test for HIV-1 antibodies in urine samples and peripheral blood samples taken from fingertips, respectively. Reagent B is the most common testing method for HIV-1 antibodies in VCT clinics. Urine and venous blood samples were collected from the study subjects using a 100 ml urine cup and a 4 ml EDTA vacuum blood collection tube for Reagents A and C, respectively.

Reagent A and B results were simultaneously identified and recorded by two trained practitioners, and the results were classified as negative, positive, or invalid according to the reagent instructions. If the two

practitioners disagreed on the identification of the same reagent, they uploaded an electronic photo of the reagent, and the result was judged by the quality control team. The anticoagulated blood samples were transferred to the local CDC HIV confirmation laboratory and tested for HIV-1 antibodies under controlled conditions by Reagent C, which was used as the reference method in the study.

All reagents were used in strict accordance with the manufacturer's instructions, and samples with positive results were tested again in the HIV confirmation laboratory and confirmed by both ELISA and Western blotting.

*2.3 Data management and statistical analysis*

The subjects' information, including basic information such as their name, sex, date of birth, occupation type, education level, and ethnicity, as well as their willingness regarding HIV-1 antibody testing methods, purchase channels, acceptable prices, and self-tests, was collected through questionnaires.

The main data management and statistical software used in this study included EPIDATA v3.1, Microsoft Excel 2019, R v4.1.0, RStudio v1.4. 1103, and IBM SPSS v26.0. The sensitivity, specificity, receiver operator characteristic (ROC) curve, and area under the curve (AUC) were used to assess the diagnostic performance of the urine HIV-1 antibody reagents in the on-site screening of different populations. The two-step cluster analysis method was used to evaluate the intentionality and user characteristics of the study subjects regarding HIV antibody reagent types, acceptable prices, purchase channels, and self-tests. The level of statistical significance was set at $\alpha$=0.05.

The information recorded in the paper questionnaire was entered in pairs using EPI DATE V3.1 and compared for consistency, with key information (age, sex, population category, education level, willingness to use reagents, etc.), HIV antibody test results, and other auxiliary information, with consistency levels of 100%, 100%, and 99.5%, respectively.

## 3. Results

### *3.1 Basic information of the subjects*

A total of 2606 valid and eligible samples were collected from the FSWs, persons with IDU, PW, STUs, and subjects undergoing VCT included in this study, with 202 (7.7%), 304 (11.7%), 1000 (38.4%), 1000 (38.4%), and 100 (3.8%) collected samples, respectively. The basic information of each population subgroup is shown in **Table 1**.

### *3.2 Consistency of the results of the 3 reagents*

Reagents A and B both showed quality control bands in the 2606 samples tested, and no reagent invalidation occurred. The results of the three reagents are shown in **Supplemental Table S1**.

The number of probable HIV-positive individuals detected by Reagents A, B, and C was 49, 51, and 51, respectively. Of these, 51 individuals with HIV-positive samples detected by Reagents B and C were confirmed to show HIV positivity by both ELISA and WB tests. Of the 49 HIV-positive samples detected by Reagent A, 47 were eventually confirmed to show HIV positivity. Of the 3 PW diagnosed with HIV by Reagent A, 2 were misdiagnosed.

The results of Reagent A were fully consistent with those of the reference method for the FSWs (*Kappa*=1.000, $p<0.001$) and persons with IDU (*Kappa*=1.000, $p<0.001$), with *kappa* values of 0.499 ($p<0.001$) and 0.908 ($p<0.001$) in the PW and subjects undergoing VCT, respectively. The results of Reagent B were fully consistent with those of the reference method, and there were no missed or misdiagnosed cases, as shown in **Supplemental Table S2**.

### *3.3 Diagnostic performance*

The overall sensitivity of Reagent A was 92.16%, the specificity was 99.92%, and the AUC was 0.960 (95% CI: 0.952-0.968, $p<0.001$) for the 2606 on-site tests. Reagent B showed identical results to the reference method in the 2606 on-site assays (*AUC*: 1.000, 95% CI: 0.999-1.000, $p<0.001$), and the overall performance of Reagent A was slightly lower than that of Reagent B ($z=2.083$, $p<0.05$), as presented in **Table 2**. The ROC curves of the 2 reagents are shown in **Figure 1**.

Reagent A showed good performance in the on-site application for persons with IDU (*AUC*: 1.000, *95% CI*: 1.000-1.000, $p<0.001$), FSWs (*AUC*: 1.000, *95% CI*: 1.000-1.000, $p<0.001$), and PW (*AUC*: 0.999, *95% CI*: 0.997-1.000, $p<0.001$), but the performance differences in in each application setting were significant

($z$=2.908, $p$<0.005), as shown in **Supplemental Table S3**. The ROC curves of the different application settings are shown in **Supplemental Figure 1**. In this study, the false negative rate (FNR) of Reagent A in the subjects undergoing VCT was 6.25% (2/32), and the false positive rate (FPR) in the PW was 0.20% (2/999).

The AUC of Reagent A in the on-site application for subjects undergoing VCT was 0.941 (95% CI: 0.876-0.978, $p$<0.001). We further dissected and reviewed the causes of this problem: Of the four subjects undergoing VCT with inconsistent results between Reagent A and the reference method, two were men who have sex with men (MSM) who are regularly tested at Non-governmental organizations and were recently determined to have HIV-1 antibody positivity, which we speculate may have been due to recent infection. The other two subjects were HIV-infected individuals receiving HAART who requested recertification reports from the VCT for referral to hospitals in other provinces for treatment.

*3.4 Willingness regarding and cluster analysis of HIV-1 antibody reagents, prices, and channels among different populations*

The willingness regarding HIV-1 antibody test reagent types ($\chi^2$=430.498, $p$<0.001), purchase channels ($\chi^2$=494.970, $p$<0.001), acceptable prices ($\chi^2$=152.710, $p$<0.001), and self-tests ($\chi^2$=245.966, $p$<0.001) were significant among the different subgroups, as presented in **Table 3**.

The two-step cluster analysis models showed that the "acceptable price" had the greatest influence on STUs (*Pi*=1.000) and PW (*Pi*=1.000), the "purchase channel" had the greatest influence on subjects undergoing VCT (*Pi*=1.000) and persons with IDU (*Pi*=1.000), and the "reagent types" had the greatest influence on FSWs (*Pi*=1.000), as presented in **Supplemental Table S4**.

The user profiles of STUs, PW, subjects undergoing VCT, persons with IDU, and FSWs were classified into 7, 8, 5, 3, and 3 patterns, respectively. The main patterns of the five populations were as follows and are presented in **Figure 2**: “priced less than $4.35, purchased at a pharmacy, blood reagents, and willing to self-test” for STUs; “priced below $4.35, purchased at a medical institution, urine reagents, and nonself-testing” for PW; “purchased at a medical institution, willing to self-test, priced between $4.35 and $8.69 or more than $17.40, and blood reagents” for subjects undergoing VCT; “purchased at a medical institution, willing to self-test, and blood reagents” for persons with IDU; and "blood reagents, priced at $4.35–$8.69, willing to self-test, and purchased at medical facilities" for FSWs.

## 4. Discussion

Due to obvious advantages such as noninvasiveness and convenience[15], urine testing for HIV antibodies began in the 1990s, and their diagnostic performance has been confirmed in many studies[16-18]. Urine HIV antibody tests have been used in practice for more than a decade[19], and their convenience has been further promoted in recent years with the advent of colloidal gold rapid test kits[12, 20]. These rapid test kits further enhance the convenience of HIV antibody testing by eliminating the requirement for centralized testing in specialized infectious disease laboratories. However, few studies have reported on the diagnostic performance of rapid urine HIV antibody test kits for practical application in large, complex populations in the real world.

The NSTMP is considered to be the most important scientific and research project in China. Its infectious disease prevention and control projects have been carried out in Guangxi for decades to assess the key issues in the HIV epidemic[21, 22], including the low willingness of the population to be screened and the high mortality rate in rural areas due to late HIV detection and diagnosis[23, 24]. To explore solutions to these problems, we conducted a special study to estimate the diagnostic performance and acceptance of a rapid urine HIV antibody test kit in different populations.

In this study, based on real-world samples, we found that urine HIV antibody rapid test kits showed satisfactory sensitivity, specificity, and ROC curves, especially in high-risk populations such as persons with IDU and FSWs. Commercial heterosexual infections are the main transmission route of HIV in Guangxi, and as a high-risk population, FSWs are a key node in this transmission route[25, 26]. Both persons with IDU and FSWs are high-risk groups for HIV, and currently, sentinel surveillance and special investigations are the primary public health strategies for identifying HIV-positive patients in high-risk populations. ELISA is the major approach to test for the HIV antibody, which requires the collection of venous whole blood samples from study subjects and transportation to a dedicated HIV laboratory at the CDC for cryopreservation and testing.

In contrast, urine testing offers greater advantages in terms of convenience, acceptability, and timeliness. The administration of injection drugs requires regular urine sample collection for recent opioid, methamphetamine, and ketamine abuse, and efficiency and subject acceptance can be improved if urine HIV antibody testing is also conducted instead of blood testing. However, the sentinel surveillance and special investigation of some high-risk groups for HIV infection also require testing for HCV and syphilis[27, 28], and the single function of the current urine HIV rapid reagent test limits its applicability.

In areas with high HIV prevalence, maternal HIV screening helps to identify HIV-infected PW at an early stage and provide timely drug interventions to interrupt mother-to-child transmission[29], which has a positive effect on reducing vertical transmission[30, 31]. Urine reagent strips showed satisfactory ROC curves in maternal HIV-1 antibody screening, but there were two false positive tests out of 1000 tests. The reasons for occasional false-positive HIV antibody tests in PW need to be further investigated, and similar occasional occurrences have previously been reported in ELISA screening tests[32].

In practice, physicians treating subjects undergoing VCT are dealing with a very complex population, which is even more complex than the high-risk population. In this study, we routinely tested subjects for blood HIV antibodies and additionally used urine reagent strips to evaluate their performance under complex practice conditions. The urine rapid test kit showed four false-negative cases among 100 subjects undergoing VCT; 2 were MSM with new infections detected by regular testing at NGOs, and two were patients receiving in-treatment HAART. In the present study, the ROC curve of the urine rapid test kit could have been affected by these false negative cases if the routine VCT consultation procedure had been followed, and similar false-negative results have been found in some previous studies[14, 33]. It should be added that the urine reagent's instructions stated that samples from HIV-infected individuals in the window period or those receiving treatment may yield false-negative results. However, if the instructions were followed, these four subjects would not have been able to use the urine rapid test kits to complete the VCT subsequent and confirmation procedures.

Considering the complexities and psychologically protective behaviours of some subjects undergoing VCT, it may be more appropriate to choose an antigen-antibody combined reagent with higher sensitivity and specificity to reduce the possibility of false negatives in some cases where it is difficult for physicians treating these subjects to obtain true and accurate information[34, 35]. Some subjects with significant psychological fear of HIV but no high-risk exposure may consider using noninvasive urine reagent strips to reduce trauma and receive psychological counselling.

Despite some limitations, urine rapid test kits can be offered as an option for HIV self-testing in high-risk populations such as MSM, FSWs, and persons with IDU who require regular testing due to their operability, noninvasiveness, and safety; these test kits can have a positive effect on increasing subjects' willingness to accept and participate in screening[13, 36].

Previous studies have evaluated urine HIV antibody reagents for general population screening, but this approach required centralized testing by qualified laboratories[20, 37]. Combined with the internet platform

and logistics industry, rapid test kits with urine reagent strips can improve operability through anonymous testing, which may be able to further expand the coverage of general population screening.

This study initially assessed the willingness of different populations regarding the type of HIV reagents, purchase channels, acceptable prices, and self-tests and further classified and analysed the different user profiles of each subgroup. We found that STUs and PW preferred reagent prices below $4.35, which may be related to the lack of financial income for STUs and the higher cost of childbirth, resulting in price sensitivity for these two groups. We also observed a higher willingness to self-test among the student population, which may be related to the extensive HIV propaganda work carried out in colleges and universities in the past decade[38, 39].

The low willingness to self-test among persons with IDU and FSWs may be related to the fact that local CDCs conduct free HIV, HCV, and syphilis testing for such high-risk populations several times per year. At the same time, persons with IDU and FSWs enrolled in long-term health interventions develop trusting relationships with the CDC, so they are more inclined to choose the medical institution channel and blood reagents. In this study, FSWs preferred urine HIV reagents, which may be related to the noninvasive operation of the rapid test kits. Although the diagnostic performance has been proven in some studies [40], a low percentage of subjects in this study chose the oral secretion HIV antibody test kit, probably due to its expensive price and complicated operation.

People undergoing VCT were more likely to have their HIV antibodies tested in medical institutions, had the highest willingness to undergo self-testing and were also willing to accept more expensive reagents. However, for subjects undergoing VCT, we speculated that their acceptance of HIV-1 antibody testing options, particularly regarding price, may be influenced by factors such as the reason for seeking medical services and psychological status, as all HIV antibody tests conducted in the VCT centres were free of charge.

There were limitations in this study. First, no positive samples were identified in the STUs, and therefore, ROC curves could not be drawn for this subgroup. Second, patients receiving HAART treatment and MSM in the window period were included in the VCT subgroups, which is not consistent with the recommended suggestions for the use of urine HIV reagents; however, this is a complexity that doctors treating subjects undergoing VCT face every day. Despite these limitations, this study evaluated the diagnostic performance of HIV urine rapid test kits in a complex real-world setting and provided a valuable scientific basis for the practical application of urine reagent strips.

## 5. Conclusions

The urine rapid test kits showed good diagnostic performance in the practical application of screening tests in different populations. However, physicians treating subjects undergoing VCTs should carefully select HIV-1 antibody testing reagents based on each subject's situation.

## 6. Author contributions

HX Lu, HH Chen, SJ Liang, YH Ruan, QY Zhu, GH Lan, and M Lin contributed to conception and design of the study. HX Lu, GJ Tan, WL Cai, and YJ Zhou organized the database. HX Lu and YH Ruan performed the statistical analysis. HX Lu, HH Chen, and SJ Liang wrote the first draft of the manuscript. XW Pang, JJ Li, XM Ge, wrote sections of the manuscript. HX Lu, HH Chen, and SJ Liang contributed equally to the current work. All authors contributed to manuscript revision and read and approved the submitted version.

## 7. Data sharing statement

The original database for this study contains private information about the study participants. For non-commercial use and reasonable purposes, anonymised data of the current work can be obtained from the corresponding author.

## 8. Findings

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**Table 1** The basic information of the 2606 FSWs, persons with IDU, PW, STUs, and subjects undergoing VCT in the sample

| Variables | Subgroups | The sample sizes of each population groups [n (%)] | | | | | Total |
|---|---|---|---|---|---|---|---|
| | | FSWs | Persons with IDU | PW | STUs | Subjects undergoing VCT | |
| Sex | Male | 0(0) | 256(84.2) | 0(0) | 255(25.5) | 48(48.0) | 559 |
| | Female | 202(100) | 48(15.8) | 1000(100) | 745(74.5) | 52(52.0) | 2047 |
| Age | <20 | 1(0.5) | 2(0.7) | 38(3.8) | 846(84.6) | 2(2.0) | 889 |
| | 20-29 | 12(5.9) | 16(5.3) | 524(52.4) | 113(11.3) | 57(57.0) | 722 |
| | 30-39 | 68(33.7) | 126(41.4) | 417(41.7) | 41(4.1) | 18(18.0) | 670 |
| | ≥40 | 121(59.9) | 160(52.6) | 21(2.1) | 0(0) | 23(23.0) | 325 |
| Ethnicity | Han | 120(59.4) | 279(91.8) | 692(69.2) | 526(52.6) | 56(56.0) | 1673 |
| | Zhuang | 58(28.7) | 20(6.6) | 281(28.1) | 402(40.2) | 40(40.0) | 801 |
| | Other | 24(11.9) | 5(1.6) | 27(2.7) | 72(7.2) | 4(4.0) | 132 |
| Education level | Illiterate | 33(16.3) | 5(1.6) | 1(0.1) | 0(0) | 1(1.0) | 40 |
| | Primary school | 94(46.5) | 54(17.8) | 40(4) | 0(0) | 8(8.0) | 196 |
| | Junior middle school | 69(34.2) | 217(71.4) | 471(47.1) | 0(0) | 18(18.0) | 775 |
| | Senior high school | 6(3) | 28(9.2) | 193(19.3) | 472(47.2) | 19(19.0) | 718 |
| | Junior college | 0(0) | 0(0) | 292(29.2) | 527(52.7) | 54(54.0) | 873 |
| | Bachelor's degree or above | 0(0) | 0(0) | 3(0.3) | 1(0.1) | 0(0) | 4 |
| Total | | 202 | 304 | 1000 | 1000 | 100 | 2606 |

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**Table 2** The receiver operator characteristic curves for Reagents A and B in the 2606 subjects

| Reagents | Results | Results | | Statistical parameters of ROC curves | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | - | + | *AUC* | 95% *CI* | Sensitivity | Specificity | Youden index | *p* |
| A | - | 2553 | 2 | 0.960 | 0.952-0.968 | 92.16 | 99.92 | 0.921 | <0.001 |
| | + | 4 | 47 | | | | | | |
| B | - | 2555 | 0 | 1.000 | 0.999-1.000 | 100.00 | 100.00 | 1.000 | <0.001 |
| | + | 0 | 51 | | | | | | |

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**Table 3** Acceptance of HIV-1 antibody testing methods, access and prices in different populations

| Questions | Classification | Population [n (%)] | | | | | $\chi^2$ | |
|---|---|---|---|---|---|---|---|---|
| | | STUs | PW | Subjects undergoing VCT | Persons with IDU | FSWs | | |
| Reagent types | Blood | 781(78.1) | 599(59.9) | 85(85.0) | 74(24.3) | 88(43.6) | 430.498 | <0.001 |
| | Saliva | 72(7.2) | 45(4.5) | 6(6.0) | 13(4.3) | 6(3.0) | | |
| | Urine | 147(14.7) | 356(35.6) | 9(9.0) | 217(71.4) | 108(53.5) | | |
| Purchase channels | Pharmacy | 382(38.2) | 202(20.2) | 26(26.0) | 176(57.9) | 107(53) | 494.970 | <0.001 |
| | Online shopping | 38(3.8) | 42(4.2) | 24(24.0) | 66(21.7) | 9(4.5) | | |
| | Medical institution | 565(56.5) | 725(72.5) | 45(45.0) | 39(12.8) | 85(42.1) | | |
| | Vending machine | 15(1.5) | 31(3.1) | 5(5.0) | 23(7.6) | 1(0.5) | | |
| Acceptable price (USD$) | <4.35 | 537(53.7) | 575(57.5) | 20(20.0) | 222(73.0) | 99(49.0) | 152.710 | <0.001 |
| | 4.35-8.69 | 285(28.5) | 252(25.2) | 39(39.0) | 63(20.7) | 86(42.6) | | |
| | 8.70-17.39 | 117(11.7) | 128(12.8) | 23(23.0) | 17(5.6) | 16(7.9) | | |
| | ≥17.40 | 61(6.1) | 45(4.5) | 18(18.0) | 2(0.7) | 1(0.5) | | |
| Willingness to self-test | Yes | 762(76.2) | 451(45.1) | 83(83.0) | 143(47.0) | 106(52.5) | 245.966 | <0.001 |
| | No | 238(23.8) | 549(54.9) | 17(17.0) | 161(53.0) | 96(47.5) | | |

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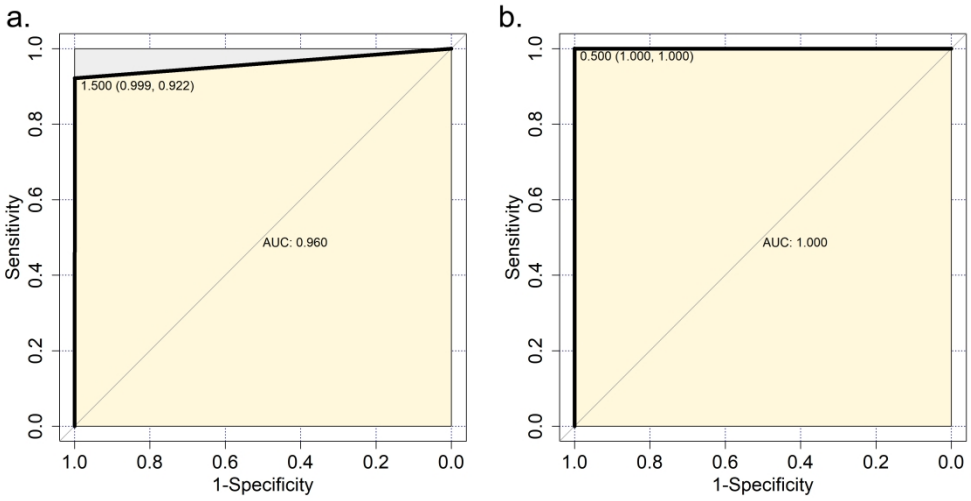



Figure 1 The receiver operator characteristic curves of Reagents A and B for the 2606 samples

338x169mm (300 x 300 DPI)

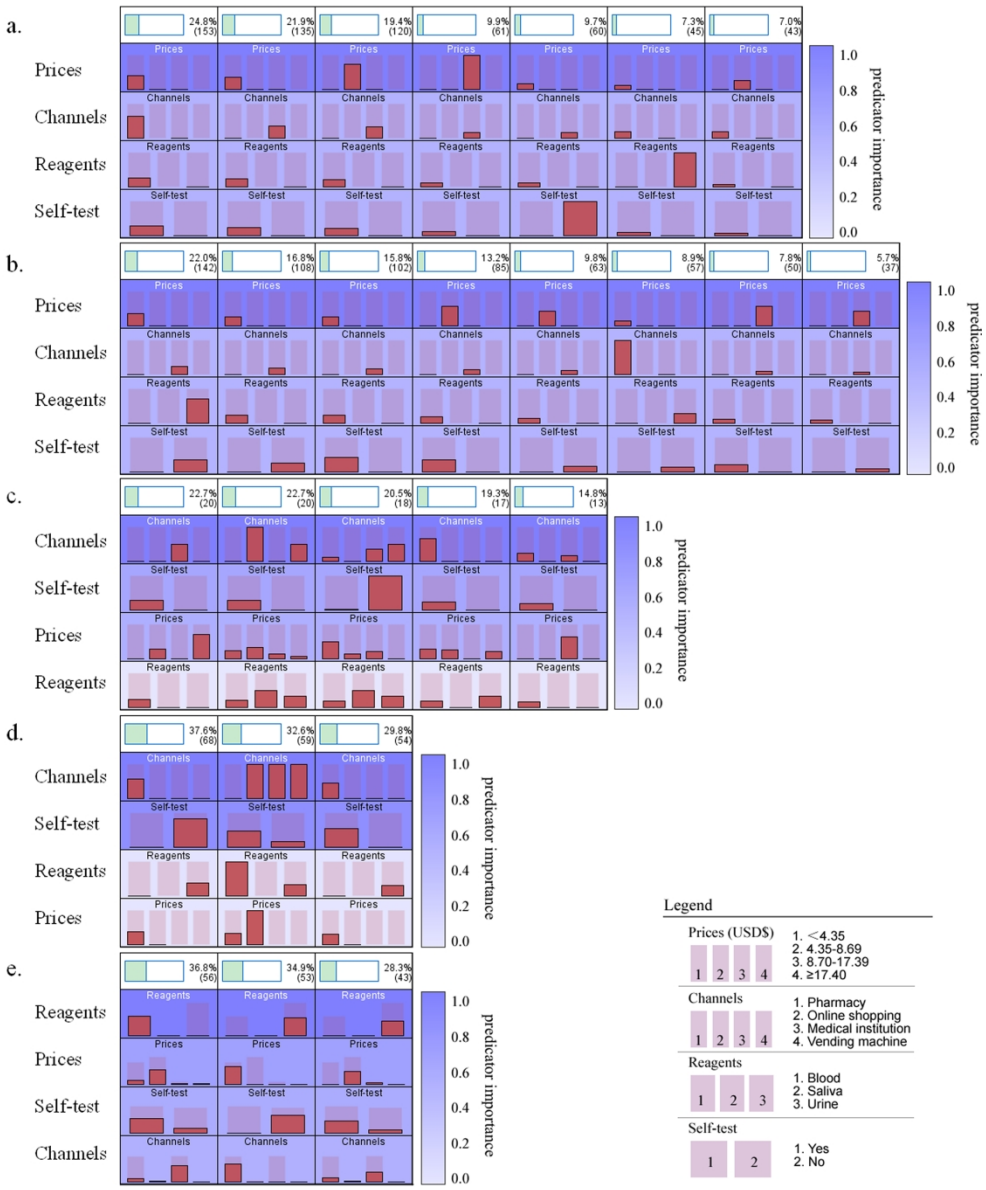



Figure 2 The user profile patterns of subjects in the two-step cluster analyses and the patterns of STUs, PW, subjects undergoing VCT, persons with IDU, and FSWs are illustrated in a, b, c, d, and e, respectively.

869x1027mm (72 x 72 DPI)

**Supplemental Table S1** The performance of two HIV-1 antibody reagents in field testing [n (%)]

| Groups | Reagent A | | Reagent B | | Reagent C[a] | | total |
|---|---|---|---|---|---|---|---|
| | - | + | - | + | - | + | |
| FSWs | 201(99.5) | 1(0.5) | 201(99.5) | 1(0.5) | 201(99.5) | 1(0.5) | 202 |
| Persons with IDU | 289(95.1) | 15(4.9) | 289(95.1) | 15(4.9) | 289(95.1) | 15(4.9) | 304 |
| PW | 997(99.7) | 3(0.3) | 999(99.9) | 1(0.1) | 999(99.9) | 1(0.1) | 1000 |
| STUs | 1000(100.0) | 0(0) | 1000(100.0) | 0(0) | 1000(100.0) | 0(0) | 1000 |
| Subjects undergoing VCT | 70(70.0) | 30(30.0) | 66(66.0) | 34(34.0) | 66(66.0) | 34(34.0) | 100 |
| Total | 2557(98.1) | 49(1.9) | 2555(98.0) | 51(2.0) | 2555(98.0) | 51(2.0) | 2606 |

a. Reagent C was set as the reference method in this study

**Supplemental Table S2** Consistency check of two HIV-1 antibody reagents in diverse populations

| Group | Reference | Result | Reagent A | | | | Reagent B | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | - | + | *kappa* | *p* | - | + | *kappa* | *p* |
| FSWs | Reagent C | - | 201 | 0 | 1.000 | <0.001 | 201 | 0 | 1.000 | <0.001 |
| | | + | 0 | 1 | | | 0 | 1 | | |
| IDU | Reagent C | - | 289 | 0 | 1.000 | <0.001 | 289 | 0 | 1.000 | <0.001 |
| | | + | 0 | 15 | | | 0 | 15 | | |
| PW | Reagent C | - | 997 | 2 | 0.499 | <0.001 | 999 | 0 | 1.000 | <0.001 |
| | | + | 0 | 1 | | | 0 | 1 | | |
| STUs | Reagent C | - | 1000 | 0 | - | - | 1000 | 0 | - | - |
| | | + | 0 | 0 | | | 0 | 0 | | |
| Subjects undergoing VCT | Reagent C | - | 66 | 0 | 0.908 | <0.001 | 66 | 0 | 1.000 | <0.001 |
| | | + | 4 | 30 | | | 0 | 34 | | |
| Total | Reagent C | - | 2553 | 2 | 0.939 | <0.001 | 2555 | 0 | 1.000 | <0.001 |
| | | + | 4 | 47 | | | 0 | 51 | | |

**Supplemental Table S3** The receiver operator characteristic curves for Reagent A in each group

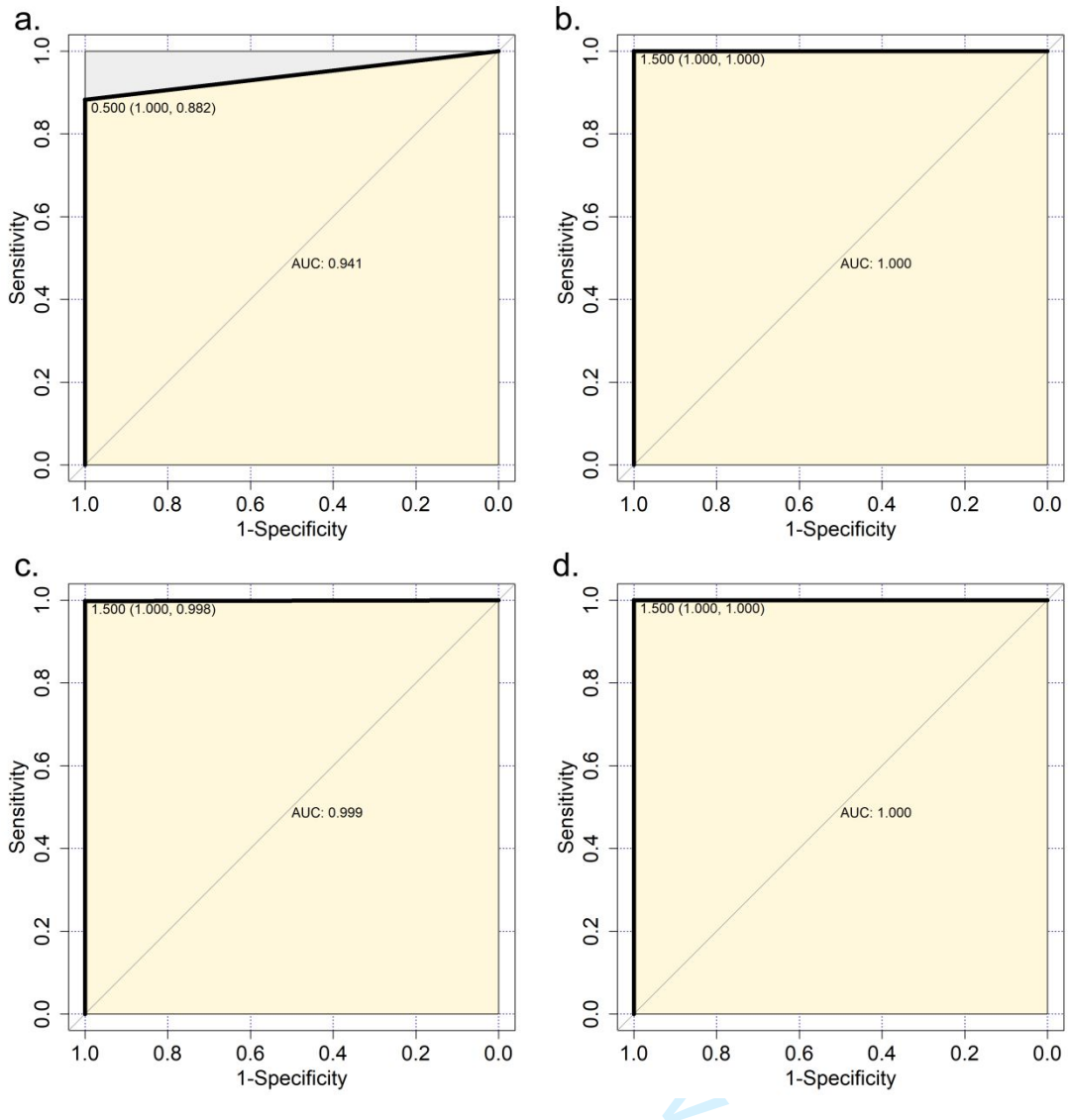
| Groups | Reference | Reagent A | | Statistical parameters of ROC curves | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | - | + | *AUC* | 95% CI | Sensitivity | Specificity | Youden index | *P* |
| Subjects undergoing VCT | - | 66 | 0 | 0.941 | 0.876-0.978 | 88.24 | 100.00 | 0.882 | <0.001 |
| | + | 4 | 30 | | | | | | |
| Persons with IDU | - | 289 | 0 | 1.000 | 0.999-1.000 | 100.00 | 100.00 | 1.000 | <0.001 |
| | + | 0 | 15 | | | | | | |
| PW | - | 997 | 2 | 0.999 | 0.997-1.000 | 99.80 | 100.00 | 0.998 | <0.001 |
| | + | 0 | 1 | | | | | | |
| FSWs | - | 201 | 0 | 1.000 | 0.999-1.000 | 1.000 | 1.000 | 1.000 | <0.001 |
| | + | 0 | 1 | | | | | | |
| STUs | - | 1000 | 0 | - | - | - | - | - | |
| | + | 0 | 0 | | | | | | |

**Supplemental Table S4** The user profiles of different populations regarding HIV-1 antibody testing methods, channels, and prices

| Population | Clustering model parameters | | | Predictor importance (*Pi*) [b] | | | |
|---|---|---|---|---|---|---|---|
| | clusters | Fit quality [a] | *AIC* | reagent types | channels | prices | self-s |
| STUs | 7 | 1.00 | 126.00 | 0.50 | 0.50 | 1.00 | 0.5 |
| PW | 8 | 1.00 | 144.00 | 0.50 | 0.50 | 1.00 | 0.5 |
| Subjects undergoing VCT | 5 | 0.50 | 197.88 | <0.01 | 1.00 | 0.54 | 0.6 |
| Persons with IDU | 3 | 0.80 | 54.00 | 0.03 | 1.00 | 0.01 | 0.8 |
| FSWs | 3 | 0.70 | 54.00 | 1.00 | 0.53 | 0.69 | 0.5 |

a: Clustering Fit quality ranged from -1 to 1, where 0.5-1 is good and ≥0.51 is excellent

b: Variable importance scores ranged from 0 to 1, with 0 being the lowest and 1 being the highest




**Supplemental Figure 1** The ROCs of urine HIV-1 antibody reagent in VCTs(a)、IDUs(b)、PWs(c), and FSWs(d) Groups

BMJ Open

# Diagnostic Performance Evaluation of Urine HIV-1 Antibody Rapid Test Kits in A Real-life Routine Care Setting in China

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| | |

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# Diagnostic Performance Evaluation of Urine HIV-1 Antibody Rapid Test Kits in A Real-life Routine Care Setting in China


## Abstract

**Objectives:** To evaluate the diagnostic performance of urine human immunodeficiency virus (HIV) antibody rapid test kits in screening diverse populations and to analyse subjects' willingness regarding reagent types, purchase channels, acceptable prices, and self-testing.

**Designs:** Diagnostic accuracy studies

**Participants:** A total of 2606 valid and eligible samples were collected in the study, including 202 samples from female sex workers (FSWs), 304 persons with injection drug use (IDU), 1000 pregnant women (PW), 100 subjects undergoing voluntary HIV counselling and testing (VCT), and 1000 students in higher education schools or colleges (STUs). Subjects should simultaneously meet the following inclusion criteria: (1) being at least 18 years old and in full civil capacity; (2) signing an informed consent form; and (3) providing truthful identifying information to ensure the subjects and their samples are unique.

**Results:** The sensitivity, specificity, and area under the curve (AUC) of the urine HIV-1 antibody rapid test kits were 92.16%, 99.92%, and 0.960 (95% confidence interval (CI): 0.952-0.968, $p<0.001$), respectively, among 2606 samples collected during on-site screenings. The kits showed good diagnostic performance in persons with IDU (*AUC*: 1.000, 95% CI: 1.000-1.000, $p<0.001$), PW (*AUC*: 0.999, 95% CI: 0.999-1.000, $p<0.001$), and FSWs (*AUC*: 1.000, 95% CI: 1.000-1.000, $p<0.001$). The AUC of the urine reagent kits in subjects undergoing VCT was 0.941 (95% CI: 0.876-0.978, $p<0.001$). The "acceptable price" had the greatest influence on STUs (*Pi*=1.000) and PW (*Pi*=1.000), the "purchase channel" had the greatest influence on subjects undergoing VCT (*Pi*=1.000) and persons with IDU (*Pi*=1.000), and the “reagent types” had the greatest influence on FSWs (*Pi*=1.000).

**Conclusions**: The rapid urine test kits showed a good diagnostic validity in practical applications, despite a few cases involving misdiagnosis and underdiagnosis.


**Keywords:** HIV, urine, rapid test kits, ROC

**Strengths and limitations of this study:**

1. This study has evaluated the diagnostic validity of urine HIV-1 rapid test kits in screening both the general population and high-risk populations.
2. Cluster analysis provides a clear profile of the main concerns and selection preferences of the different populations when they choose HIV test reagents.
3. No positive samples were found among the students, and therefore, ROC curves could not be plotted for this subgroup.

## 1. Introduction

The prevalence of HIV/AIDS varies widely across China[1, 2]. Guangxi Zhuang Autonomous Region, the only minority region in southern China, is a serious HIV/AIDS hotspot; in the past decade, this region had a much higher HIV/AIDS prevalence than any other Chinese coastal or inland province[3, 4]. Therefore, the public health administration in Guangxi is attempting to expand the scale of HIV screening to diagnose HIV-infected patients at an early stage and provide highly active antiretroviral therapy (HAART) promptly to reduce HIV/AIDS mortality and transmission[5, 6], especially in high-risk populations[7].

With the cost reduction, urine HIV antibody testing is gradually gaining attention and acceptance by public health policymakers, health institutions, and the general public due to its advantages of being convenient, noninvasive, safe[8-10], and reliable [11-14]. However, these urine HIV antibody reagents required that urine samples be transported to the laboratory for centralized testing because of methodological limitations, which limits their convenience of application.

A urine HIV-1 antibody rapid test reagent with colloidal gold method has been granted marketing approval by the China Food and Drug Administration in 2019. This reagent can present the results within 15 minutes, and all operations can be completed on-site. Due to the advantages of noninvasive, convenient, and rapid, the Guangxi health department is very interested in this reagent and believes that adopting it may help to further increase the acceptance of the population to HIV screening. It is worth noting that although some studies have evaluated the diagnostic performance of urine HIV-1 antibody rapid test kits using standard samples under controlled laboratory conditions, no studies have yet reported on their diagnostic performance in practical applications and the acceptance of different populations; therefore, an adequate scientific basis for the application of urine rapid test kits for HIV screening has not been provided for public health authorities in high-prevalence areas.

This study, based on a special study of the Chinese National Science and Technology Major Project (NSTMP) for infectious diseases, aimed to evaluate the diagnostic performance of urine HIV-1 antibody rapid test reagents in a practical screening setting and to preliminarily analyse the willingness of subjects regarding the types of reagents, purchase channels, and acceptable prices to provide a valuable scientific basis for the application of urine HIV antibody rapid test reagents for screening.

## 2. Materials and methods

### *2.1 Samples and Sources*

Subjects were recruited from the most commonly screened populations for HIV antibodies in the real world. The subjects of this study were categorized into four groups based on HIV-related risk behaviours as follows: (1) the key population, including Female sex workers (FSWs) and persons with injection drug use (IDU); (2) the vulnerable population, in this study, were pregnant women (PW) who received regular pregnancy check-up's; (3) general population, which in this study were students at colleges or universities (STUs); and (4) subjects undergoing voluntary HIV counselling and testing (VCT).

FSWs and persons with IDU are high-risk populations for HIV infection, and both groups were recruited by sentinel surveillance in this study by the CDC. PW are routinely screened for HIV, and women receiving care during pregnancy were recruited from women and children's hospitals. Subjects undergoing VCT were consulted or referred to provincial CDC VCT clinics. The STUs were enrolled in higher education schools or colleges. This study was conducted from August 1, 2020, to September 31, 2020. No researcher knows whether the subjects were infected with HIV before testing because of previously reported cases that were excluded through the ID card system.

To improve the external validity and to match the characteristics of the real-world HIV screening population, no strict inclusion or exclusion criteria were set for this study, only the following requirements need to be met concurrently: (1) the subject should be at least 18 years of age and in full civil capacity; (2) the subject should have signed the informed consent form and volunteered to participate in the study as a subject; (3) the subject should provide truthful identifying information, such as a driver's license or identification card, to ensure the subject and the sample are unique, and to exclude previously reported HIV cases. Researchers informed subjects of the purpose, methods, potential harms, and personal privacy issues of this study in detail before informed consent forms were signed. Following the signing of the informed consent form, each subject was required to be taken three samples, a whole blood sample, a fingertip peripheral blood sample, and a urine sample, and to complete the questionnaire after sampling.

The urine rapid test reagent AUC area was predicted to be between 0.85 and 0.98, and the confidence level (1-alpha), confidence interval width, sample dropout rate, and screening sample size were set to 0.95, 0.10, 5%, and 2,500 cases, respectively, requiring a positive sample size of 5-34 cases as estimated by the PASS 2015 software package.

*2.2 Urine and blood sample testing methods*

Three HIV antibody test reagents were used in the study: (1) Reagent A, named the Urine HIV-1 Antibody Rapid Test Kit (colloidal gold), was packaged as a rapid test kit and manufactured by Wantai (20193400550); (2) Reagent B, named Determine™ HIV1/2 (colloidal selenium), was packaged as a rapid test kit and manufactured by Abbott (20163400427); and (3) Reagent C, named GENscreen™ ULTRA HIV Ag-Ab (Enzyme-Linked Immunosorbent Assay, ELISA), which was manufactured by Bio-Rad (72388C).

HIV antibody tests were divided into on-site tests (for Reagents A and B) and laboratory tests (for Reagent C only). Reagents A and B were used to test for HIV-1 antibodies in urine samples and peripheral blood samples taken from fingertips, respectively. Reagent B is the most common testing method for HIV-1 antibodies in VCT clinics. Urine and venous blood samples were collected from the study subjects using a 100 ml urine cup and a 4 ml EDTA vacuum blood collection tube for Reagents A and C, respectively.

Reagent A and B results were simultaneously identified and recorded by two trained practitioners, and the results were classified as negative, positive, or invalid according to the reagent instructions. If the two practitioners disagreed on the identification of the same reagent, they uploaded an electronic photo of the reagent, and the result was judged by the quality control team. The anticoagulated blood samples were transferred to the local CDC HIV confirmation laboratory and tested for HIV-1 antibodies under controlled conditions by Reagent C immediately, which was used as the reference method in the study.

All reagents were used in strict accordance with the manufacturer's instructions, and samples with positive results were tested again in the HIV confirmation laboratory and confirmed by both ELISA and Western blotting, according to the diagnostic criteria of the Chinese Guidelines for Diagnosis and Treatment of Human Immunodeficiency Virus Infection/Acquired Immunodeficiency Syndrome (2020 edition). Three laboratories with HIV-confirmation qualifications participated in the study, including the HIV-confirmation laboratories of Guangxi Provincial CDC, Guigang CDC, and Liuzhou CDC.

*2.3 Data management and statistical analysis*

The subjects' information, including basic information such as their name, sex, date of birth, occupation type, education level, and ethnicity, as well as their willingness regarding HIV-1 antibody testing methods, purchase channels, acceptable prices, and self-tests, was collected through questionnaires.

The main data management and statistical software used in this study included EPIDATA v3.1, Microsoft Excel 2019, R v4.1.0, RStudio v1.4. 1103, and IBM SPSS v26.0. The sensitivity, specificity, receiver

operator characteristic (ROC) curve, and area under the curve (AUC) were used to assess the diagnostic validity of the urine HIV-1 antibody reagents in the on-site screening of different populations, these processes are synchronized in the ROC analysis module of SPSS and the PROC package of the R language. The two-step cluster analysis method in SPSS was used to evaluate the intentionality and user profiles of the study subjects regarding HIV antibody reagent types, acceptable prices, purchase channels, and self-tests. The level of statistical significance was set at $\alpha$=0.05.

The information recorded in the paper questionnaire was entered in pairs using EPI DATE V3.1 and compared for consistency, with key information (ID information, age, sex, population category, education level, willingness to use reagents, etc.), HIV antibody test results, and other auxiliary information, with consistency levels of 100%, 100%, and 99.5%, respectively.

### *2.4 Patient and Public Involvement*

This study was mainly completed by Guangxi CDC, with Guigang CDC, Luzhai CDC, and Binyang CDC as the specific implementors of the study. The public and patients (mainly potential patients in this study) were not directly involved in the design and implementation of this study. However, the findings of this study may have some influence on local HIV-related public health strategies in Guangxi, such as promoting noninvasive urine testing reagents for HIV screening in the general population to increase its acceptability and adopting more sensitive and specific methods for screening high-risk populations to find HIV-infected individuals at the early stage.

## 3. Results

### *3.1 Basic information about the subjects*

A total of 2606 valid and eligible samples were collected from the FSWs, persons with IDU, PW, STUs, and subjects undergoing VCT included in this study, with 202 (7.7%), 304 (11.7%), 1000 (38.4%), 1000 (38.4%), and 100 (3.8%) collected samples, respectively. No adverse events were reported. The flowchart is presented in **Figure 1**. The basic information of each population subgroup is shown in **Table 1**.

### *3.2 Consistency of the results of the 3 reagents*

Reagents A and B both showed quality control bands in the 2606 samples tested, and no reagent invalidation occurred. The results of the three reagents are shown in **Table 2**.

The number of probable HIV-positive individuals detected by Reagents A, B, and C was 49, 51, and 51,

respectively. Of these, 51 individuals with HIV-positive samples detected by Reagents B and C were confirmed to show HIV positivity by both ELISA and WB tests. Of the 49 HIV-positive samples detected by Reagent A, 47 were eventually confirmed to show HIV positivity. Of the 3 PW diagnosed with HIV by Reagent A, 2 were misdiagnosed.

The results of Reagent A were fully consistent with those of the reference method for the FSWs (*Kappa*=1.000, $p<0.001$) and persons with IDU (*Kappa*=1.000, $p<0.001$), with *kappa* values of 0.499 ($p<0.001$) and 0.908 ($p<0.001$) in the PW and subjects undergoing VCT, respectively. The results of Reagent B were fully consistent with those of the reference method, and there were no missed or misdiagnosed cases, as shown in **Table 3**.

*3.3 Diagnostic performance*

The overall sensitivity of Reagent A was 92.16%, the specificity was 99.92%, and the AUC was 0.960 (95% CI: 0.952-0.968, $p<0.001$) for the 2606 on-site tests. Reagent B showed identical results to the reference method in the 2606 on-site assays (*AUC*: 1.000, 95% CI: 0.999-1.000, $p<0.001$), and the overall performance of Reagent A was slightly lower than that of Reagent B ($z=2.083$, $p<0.05$), as presented in **Table 4**. The ROC curves of the 2 reagents are shown in **Figure 2**.

Reagent A showed good performance in the on-site application for persons with IDU (*AUC*: 1.000, *95% CI*: 1.000-1.000, $p<0.001$), FSWs (*AUC*: 1.000, *95% CI*: 1.000-1.000, $p<0.001$), and PW (*AUC*: 0.999, *95% CI*: 0.997-1.000, $p<0.001$), but the performance differences in in each application setting were significant ($z=2.908$, $p<0.005$), as shown in **Table 5**. The ROC curves of the different application settings are shown in **Figure 3**. In this study, the false negative rate (FNR) of Reagent A in the subjects undergoing VCT was 6.25% (2/32), and the false positive rate (FPR) in the PW was 0.20% (2/999).

The AUC of Reagent A in the on-site application for subjects undergoing VCT was 0.941 (95% CI: 0.876-0.978, $p<0.001$). We further dissected and reviewed the causes of this problem: Of the four subjects undergoing VCT with inconsistent results between Reagent A and the reference method, two were men who have sex with men (MSM) who are regularly tested at Non-governmental organizations and were recently determined to have HIV-1 antibody positivity, which we speculate may have been due to recent infection. The other two subjects were HIV-infected individuals receiving HAART who requested recertification reports from the VCT for referral to hospitals in other provinces for treatment.

*3.4 Willingness regarding and cluster analysis of HIV-1 antibody reagents, prices, and channels among*

*different populations*

The willingness regarding HIV-1 antibody test reagent types ($\chi^2$=430.498, $p$<0.001), purchase channels ($\chi^2$=494.970, $p$<0.001), acceptable prices ($\chi^2$=152.710, $p$<0.001), and self-tests ($\chi^2$=245.966, $p$<0.001) were significant among the different subgroups, as presented in **Table 6**.

The two-step cluster analysis models showed that the "acceptable price" had the greatest influence on STUs (*Pi*=1.000) and PW (*Pi*=1.000), the "purchase channel" had the greatest influence on subjects undergoing VCT (*Pi*=1.000) and persons with IDU (*Pi*=1.000), and the "reagent types" had the greatest influence on FSWs (*Pi*=1.000), as presented in **Table 7**.

The user profiles of STUs, PW, subjects undergoing VCT, persons with IDU, and FSWs were classified into 7, 8, 5, 3, and 3 patterns, respectively. The main patterns of the five populations were as follows and are presented in **Figure 4**: “priced less than $4.35, purchased at a pharmacy, blood reagents, and willing to self-test” for STUs; “priced below $4.35, purchased at a medical institution, urine reagents, and nonself-testing” for PW; “purchased at a medical institution, willing to self-test, priced between $4.35 and $8.69 or more than $17.40, and blood reagents” for subjects undergoing VCT; “purchased at a medical institution, willing to self-test, and blood reagents” for persons with IDU; and "blood reagents, priced at $4.35–$8.69, willing to self-test, and purchased at medical facilities" for FSWs.

## 4. Discussion

Due to obvious advantages such as noninvasiveness and convenience[15], urine testing for HIV antibodies began in the 1990s, and their diagnostic performance has been confirmed in many studies[16-18]. Urine HIV antibody tests have been used in practice for more than a decade[19], and their convenience has been further promoted in recent years with the advent of colloidal gold rapid test kits[12, 20]. These rapid test kits further enhance the convenience of HIV antibody testing by eliminating the requirement for centralized testing in specialized infectious disease laboratories. However, few studies have reported on the diagnostic performance of rapid urine HIV antibody test kits for practical application in large, complex populations in the real world.

The NSTMP is considered to be the most important scientific and research project in China. Its infectious disease prevention and control projects have been carried out in Guangxi for decades to assess the key issues in the HIV epidemic[21, 22], including the low willingness of the population to be screened and the high mortality rate in rural areas due to late HIV detection and diagnosis[23, 24]. We conducted the study to estimate the diagnostic validity and acceptance of a rapid urine HIV antibody test kit in different populations. As far as we know, such studies are rarely reported.

In this study, based on real-world samples, we found that urine HIV antibody rapid test kits showed satisfactory sensitivity, specificity, and ROC curves, especially in high-risk populations such as persons with IDU and FSWs. Commercial heterosexual infections are the main transmission route of HIV in Guangxi, and as a high-risk population, FSWs are a key node in this transmission route[25, 26]. Both persons with IDU and FSWs are high-risk groups for HIV, and currently, sentinel surveillance and special investigations are the primary public health strategies for identifying HIV-positive patients in high-risk populations. ELISA is the major approach to test for HIV antibodies, which requires the collection of venous whole blood samples from study subjects and transportation to a dedicated HIV laboratory at the CDC for cryopreservation and testing.

In contrast, urine testing offers greater advantages in terms of convenience and timeliness. The administration of injection drugs requires regular urine sample collection for recent opioid, methamphetamine, and ketamine abuse, and efficiency and subject acceptance can be improved if urine HIV antibody testing is also conducted instead of blood testing. However, the sentinel surveillance and special investigation of some high-risk groups for HIV infection also require testing for HCV and syphilis[27, 28], and the single function of the current urine HIV rapid reagent test limits its applicability.

In practice, physicians treating subjects undergoing VCT are dealing with a very complex population, which is even more complex than the high-risk population. In this study, we routinely tested subjects for blood HIV antibodies and additionally used urine reagent strips to evaluate their performance under complex practice conditions. The urine rapid test kit showed four false-negative cases among 100 subjects undergoing VCT; two were MSM with new infections detected by regular testing at NGOs, and two were patients receiving in-treatment HAART. In the present study, the ROC curve of the urine rapid test kit could have been affected by these false-negative cases if the routine VCT consultation procedure had been followed, and similar false-negative results had been found in some previous studies[14, 33]. It should be added that the urine reagent's instructions stated that samples from HIV-infected individuals in the window period or those receiving treatment may yield false-negative results.

Considering the complexities and psychologically protective behaviours of some subjects undergoing VCT, it may be more appropriate to choose an antigen-antibody combined reagent with higher sensitivity and specificity to reduce the possibility of false negatives in some cases where it is difficult for physicians treating these subjects to obtain true and accurate information[34, 35]. Some subjects with significant psychological fear of HIV but no high-risk exposure may consider using noninvasive urine reagent strips to reduce trauma and receive psychological counselling.

Despite some limitations, urine rapid test kits can be offered as an option for HIV self-testing in high-risk populations such as MSM, FSWs, and persons with IDU who require regular testing due to their operability, noninvasiveness, and safety; these test kits can have a positive effect on increasing subjects' willingness to accept and participate in screening[13, 36].

Previous studies have evaluated urine HIV antibody reagents for general population screening, but this approach required centralized testing by qualified laboratories[20, 37]. Combined with the internet platform and logistics industry, rapid test kits with urine reagent strips can improve operability through anonymous testing, which may be able to further expand the coverage of general population screening.

In areas with high HIV prevalence, maternal HIV screening helps to identify HIV-infected PW at an early stage and provides timely drug interventions to interrupt mother-to-child transmission[29], which has a positive effect on reducing vertical transmission[30, 31]. Urine reagent strips showed satisfactory ROC curves in maternal HIV-1 antibody screening, but there were two false positive tests out of 1000 tests. The reasons for occasional false-positive HIV antibody tests in PW need to be further investigated, and similar occasional occurrences have previously been reported in ELISA screening tests[32]. Overall, the false

positive rate of urine rapid test reagents in the PW population is acceptable given the considerable advantages of the noninvasive operation. No positive case was found in the STUs, which we believe is related to the very low prevalence of HIV infection in this population. Thus, the validity of the urine rapid reagent in STUs requires a larger sample size in future studies.

User profiles are the behavioural characteristics of a customer group in selecting or using a product, which is one of the hot analytical approaches in e-business. The current study innovatively applied user profiles to assess the characteristics and tendencies of different population subgroups when choosing reagents for HIV testing. We found that STUs and PW preferred reagent prices below $4.35, which may be related to the lack of financial income for STUs and the higher cost of childbirth, resulting in price sensitivity for these two groups. We also observed a higher willingness to self-test among the student population, which may be related to the extensive HIV propaganda work carried out in colleges and universities in the past decade[38, 39].

The low willingness to self-test among persons with IDU and FSWs may be related to the fact that local CDCs conduct free HIV, HCV, and syphilis testing for such high-risk populations several times per year. At the same time, persons with IDU and FSWs enrolled in long-term health interventions develop trusting relationships with the CDC, so they are more inclined to choose the medical institution channel and blood reagents. In this study, FSWs preferred urine HIV reagents, which may be related to the noninvasive operation of the rapid test kits. Although the diagnostic performance has been proven in some studies [40], a low percentage of subjects in this study chose the oral secretion HIV antibody test kit, probably due to its expensive price and complicated operation.

People undergoing VCT were more likely to have their HIV antibodies tested in medical institutions, had the highest willingness to undergo self-testing, and were also willing to accept more expensive reagents. However, for subjects undergoing VCT, we speculated that their acceptance of HIV-1 antibody testing options, particularly regarding price, may be influenced by factors such as the reason for seeking medical services and psychological status, as all HIV antibody tests conducted in the VCT centres were free of charge.

There were limitations in this study. First, no positive samples were identified in the STUs, and therefore, ROC curves could not be drawn for this subgroup. Second, patients receiving HAART treatment and MSM in the window period were included in the VCT subgroups, which is not consistent with the recommended suggestions for the use of urine HIV reagents; however, this is a complexity that doctors treating subjects

undergoing VCT face every day. Despite these limitations, this study evaluated the diagnostic validity of HIV urine rapid test kits in a complex real-world setting and provided some valuable scientific cues for the practical application of urine reagent strips.

## 5. Conclusions

Overall, the rapid urine test kits showed a good diagnostic validity in practical applications, despite a few cases involving misdiagnosis and underdiagnosis. We recommend that physicians providing testing services to subjects undergoing VCTs should carefully select HIV testing reagents based on each subject's situation.

## 6. Author contributions

HX Lu, HH Chen, SJ Liang, YH Ruan, QY Zhu, GH Lan, and M Lin contributed to the conception and design of the study. HX Lu, GJ Tan, WL Cai, and YJ Zhou organized the database. HX Lu and YH Ruan performed the statistical analysis. HX Lu, HH Chen, and SJ Liang wrote the first draft of the manuscript. XW Pang, JJ Li, XM Ge, wrote sections of the manuscript. HX Lu, HH Chen, and SJ Liang contributed equally to the current work. All authors contributed to the manuscript revision and read and approved the submitted version.

## 7. Data sharing statement

The original database for this study contains private information about the study participants. For non-commercial use and reasonable purposes, anonymised data of the current work can be obtained from the corresponding author.

## 8. Findings

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## 9. Ethics statement

This study was approved by the Ethics Committee of the Guangxi Zhuang Autonomous Region Center for Disease Control and Prevention (approval number GXIRB2019-0047).

**Table 1** The basic information of the 2606 FSWs, persons with IDU, PW, STUs, and subjects undergoing VCT in the sample

| Variables | Subgroups | The sample sizes of each population group [n (%)] | | | | | Total |
|---|---|---|---|---|---|---|---|
| | | FSWs | Persons with IDU | PW | STUs | Subjects undergoing VCT | |
| Sex | Male | 0(0) | 256(84.2) | 0(0) | 255(25.5) | 48(48.0) | 559 |
| | Female | 202(100) | 48(15.8) | 1000(100) | 745(74.5) | 52(52.0) | 2047 |
| Age | <20 | 1(0.5) | 2(0.7) | 38(3.8) | 846(84.6) | 2(2.0) | 889 |
| | 20-29 | 12(5.9) | 16(5.3) | 524(52.4) | 113(11.3) | 57(57.0) | 722 |
| | 30-39 | 68(33.7) | 126(41.4) | 417(41.7) | 41(4.1) | 18(18.0) | 670 |
| | ≥40 | 121(59.9) | 160(52.6) | 21(2.1) | 0(0) | 23(23.0) | 325 |
| Ethnicity | Han | 120(59.4) | 279(91.8) | 692(69.2) | 526(52.6) | 56(56.0) | 1673 |
| | Zhuang | 58(28.7) | 20(6.6) | 281(28.1) | 402(40.2) | 40(40.0) | 801 |
| | Other | 24(11.9) | 5(1.6) | 27(2.7) | 72(7.2) | 4(4.0) | 132 |
| Education level | Illiterate | 33(16.3) | 5(1.6) | 1(0.1) | 0(0) | 1(1.0) | 40 |
| | Primary school | 94(46.5) | 54(17.8) | 40(4) | 0(0) | 8(8.0) | 196 |
| | Junior middle school | 69(34.2) | 217(71.4) | 471(47.1) | 0(0) | 18(18.0) | 775 |
| | Senior high school | 6(3) | 28(9.2) | 193(19.3) | 472(47.2) | 19(19.0) | 718 |
| | Junior college | 0(0) | 0(0) | 292(29.2) | 527(52.7) | 54(54.0) | 873 |
| | Bachelor's degree or above | 0(0) | 0(0) | 3(0.3) | 1(0.1) | 0(0) | 4 |
| Total | | 202 | 304 | 1000 | 1000 | 100 | 2606 |

**Table 2** The performance of two HIV-1 antibody reagents in field testing [n (%)]

| Groups | Reagent A | | Reagent B | | Reagent C[a] | | total |
|---|---|---|---|---|---|---|---|
| | - | + | - | + | - | + | |
| FSWs | 201(99.5) | 1(0.5) | 201(99.5) | 1(0.5) | 201(99.5) | 1(0.5) | 202 |
| Persons with IDU | 289(95.1) | 15(4.9) | 289(95.1) | 15(4.9) | 289(95.1) | 15(4.9) | 304 |
| PW | 997(99.7) | 3(0.3) | 999(99.9) | 1(0.1) | 999(99.9) | 1(0.1) | 1000 |
| STUs | 1000(100.0) | 0(0) | 1000(100.0) | 0(0) | 1000(100.0) | 0(0) | 1000 |
| Subjects undergoing VCT | 70(70.0) | 30(30.0) | 66(66.0) | 34(34.0) | 66(66.0) | 34(34.0) | 100 |
| Total | 2557(98.1) | 49(1.9) | 2555(98.0) | 51(2.0) | 2555(98.0) | 51(2.0) | 2606 |

a. Reagent C was set as the reference method in this study

**Table 3** Consistency check of two HIV-1 antibody reagents in diverse populations [a]

| Group | Reference Reagent | Reagent A | | Reagent A | |
|---|---|---|---|---|---|
| | | *kappa* | *p* | *kappa* | *p* |
| FSWs | C | 1.000 | <0.001 | 1.000 | <0.001 |
| IDU | C | 1.000 | <0.001 | 1.000 | <0.001 |
| PW | C | 0.499 | <0.001 | 1.000 | <0.001 |
| STUs | C | - | - | - | - |
| Subjects undergoing VCT | C | 0.908 | <0.001 | 1.000 | <0.001 |
| Total | C | 0.939 | <0.001 | 1.000 | <0.001 |

a. Table 3 is a summary table and detailed results have been presented in Table 3(detail) of the supplementary material.

**Table 4** The receiver operator characteristic curves for Reagents A and B in the 2606 subjects [a]

| Reagents [b] | Statistical parameters of ROC curves | | | | | |
|---|---|---|---|---|---|---|
| | *AUC* | 95% *CI* | Sensitivity | Specificity | Youden index | *p* |
| A | 0.96 | 0.952-0.968 | 92.16 | 99.92 | 0.921 | <0.001 |
| B | 1 | 0.999-1.000 | 100 | 100 | 1 | <0.001 |

a: Table 4 is a summary table and detailed results have been presented in Table 4(detail) of the supplementary material.

b: The reference standard is Reagent C (ELISA)

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**Table 5** The receiver operator characteristic curves for Reagent A in each group [a]

| Groups | Statistical parameters of ROC curves [b] | | | | | |
|---|---|---|---|---|---|---|
| | *AUC* | 95% CI | Sensitivity | Specificity | Youden index | *p* |
| Subjects undergoing VCT | 0.941 | 0.876-0.978 | 88.240 | 100.000 | 0.882 | <0.001 |
| Persons with IDU | 1.000 | 0.999-1.000 | 100.000 | 100.000 | 1.000 | <0.001 |
| PW | 0.999 | 0.997-1.000 | 99.800 | 100.000 | 0.998 | <0.001 |
| FSWs | 1.000 | 0.999-1.000 | 1.000 | 1.000 | 1.000 | <0.001 |
| STUs | - | - | - | - | - | - |

a: Table 5 is a summary table and detailed results have been presented in Table 5(detail) of the supplementary material.

b: The reference standard is Reagent C (ELISA)

**Table 6** Acceptance of HIV-1 antibody testing methods, access, and prices in different populations

| Questions | Classification | Population [n (%)] | | | | | $\chi^2$ | |
|---|---|---|---|---|---|---|---|---|
| | | STUs | PW | Subjects undergoing VCT | Persons with IDU | FSWs | | |
| Reagent types | Blood | 781(78.1) | 599(59.9) | 85(85.0) | 74(24.3) | 88(43.6) | 430.498 | <0.001 |
| | Saliva | 72(7.2) | 45(4.5) | 6(6.0) | 13(4.3) | 6(3.0) | | |
| | Urine | 147(14.7) | 356(35.6) | 9(9.0) | 217(71.4) | 108(53.5) | | |
| Purchase channels | Pharmacy | 382(38.2) | 202(20.2) | 26(26.0) | 176(57.9) | 107(53) | 494.970 | <0.001 |
| | Online shopping | 38(3.8) | 42(4.2) | 24(24.0) | 66(21.7) | 9(4.5) | | |
| | Medical institution | 565(56.5) | 725(72.5) | 45(45.0) | 39(12.8) | 85(42.1) | | |
| | Vending machine | 15(1.5) | 31(3.1) | 5(5.0) | 23(7.6) | 1(0.5) | | |
| Acceptable price (USD$) | <4.35 | 537(53.7) | 575(57.5) | 20(20.0) | 222(73.0) | 99(49.0) | 152.710 | <0.001 |
| | 4.35-8.69 | 285(28.5) | 252(25.2) | 39(39.0) | 63(20.7) | 86(42.6) | | |
| | 8.70-17.39 | 117(11.7) | 128(12.8) | 23(23.0) | 17(5.6) | 16(7.9) | | |
| | ≥17.40 | 61(6.1) | 45(4.5) | 18(18.0) | 2(0.7) | 1(0.5) | | |
| Willingness to self-test | Yes | 762(76.2) | 451(45.1) | 83(83.0) | 143(47.0) | 106(52.5) | 245.966 | <0.001 |
| | No | 238(23.8) | 549(54.9) | 17(17.0) | 161(53.0) | 96(47.5) | | |

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**Table 7** The user profiles of different populations regarding HIV-1 antibody testing methods, channels, and prices

| Population | Clustering model parameters | | | Predictor importance [b] | | | |
|---|---|---|---|---|---|---|---|
| | clusters | Fit quality [a] | *AIC* | reagent types | channels | prices | self-tests |
| STUs | 7 | 1.00 | 126.00 | 0.50 | 0.50 | 1.00 | 0.5 |
| PW | 8 | 1.00 | 144.00 | 0.50 | 0.50 | 1.00 | 0.5 |
| Subjects undergoing VCT | 5 | 0.50 | 197.88 | <0.01 | 1.00 | 0.54 | 0.5 |
| Persons with IDU | 3 | 0.80 | 54.00 | 0.03 | 1.00 | 0.01 | 0.5 |
| FSWs | 3 | 0.70 | 54.00 | 1.00 | 0.53 | 0.69 | 0.5 |

a: Clustering Fit quality ranged from -1 to 1, where 0.5-1 is good and ≥0.51 is excellent

b: Variable importance scores ranged from 0 to 1, with 0 being the lowest and 1.00 being the highes

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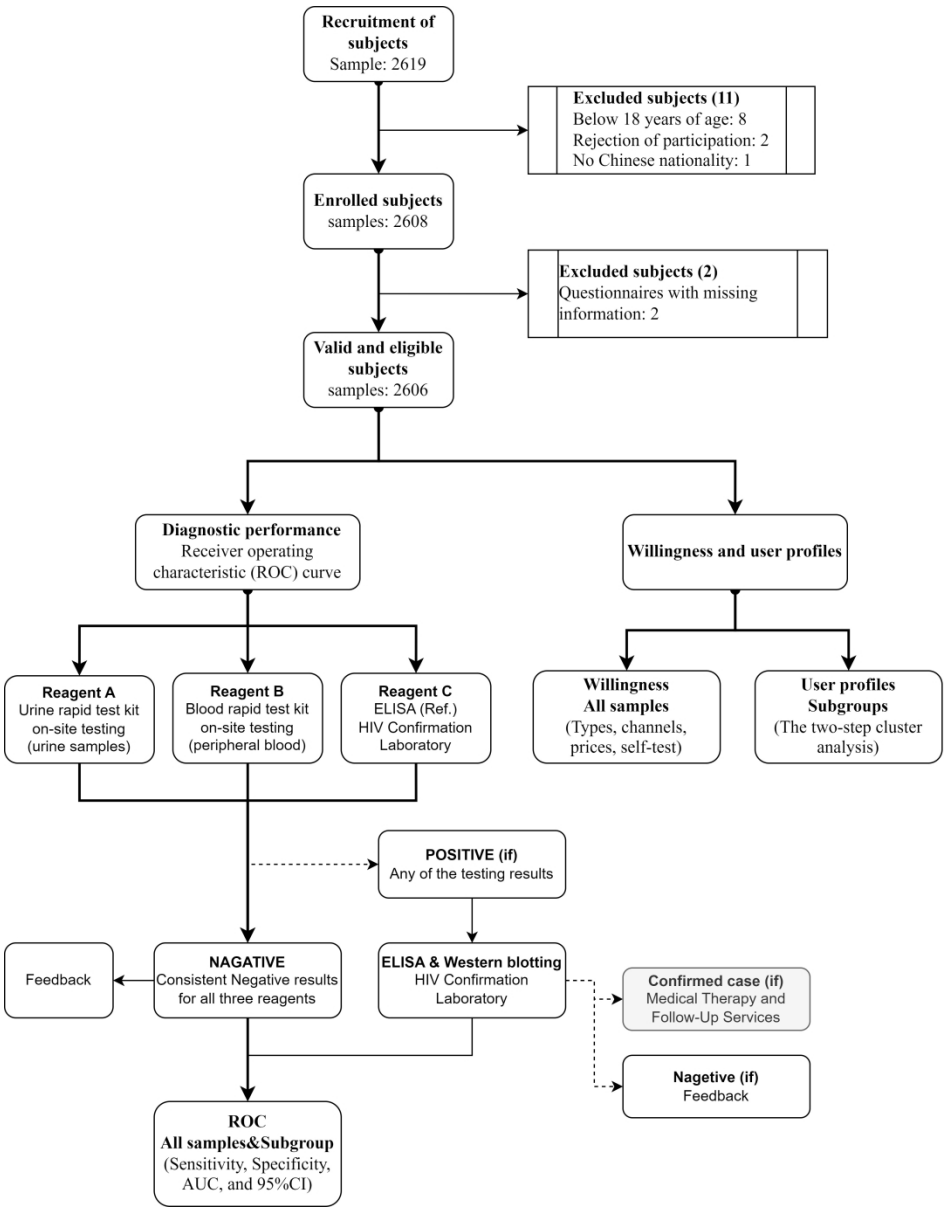


Figure 1. The flowchart

1329x1696mm (72 x 72 DPI)

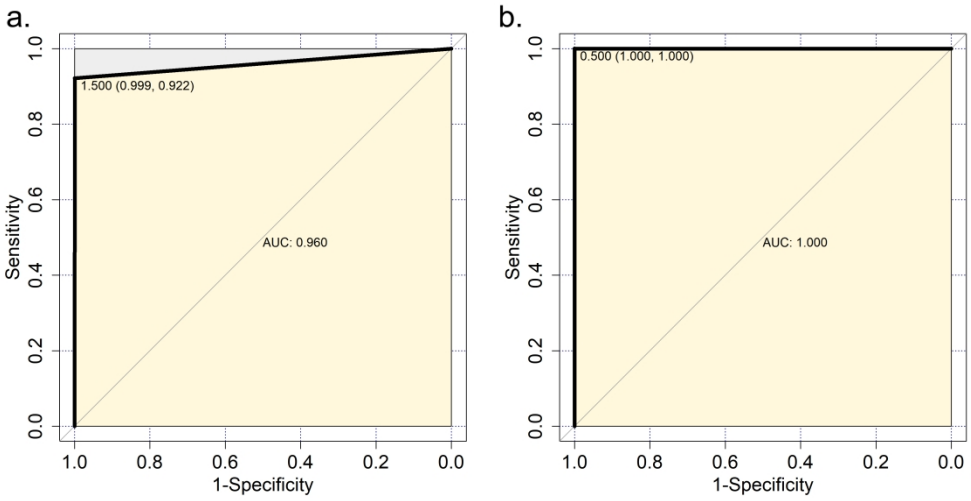


Figure 2. The receiver operator characteristic curves of reagents A and B in 2606 samples

338x169mm (300 x 300 DPI)

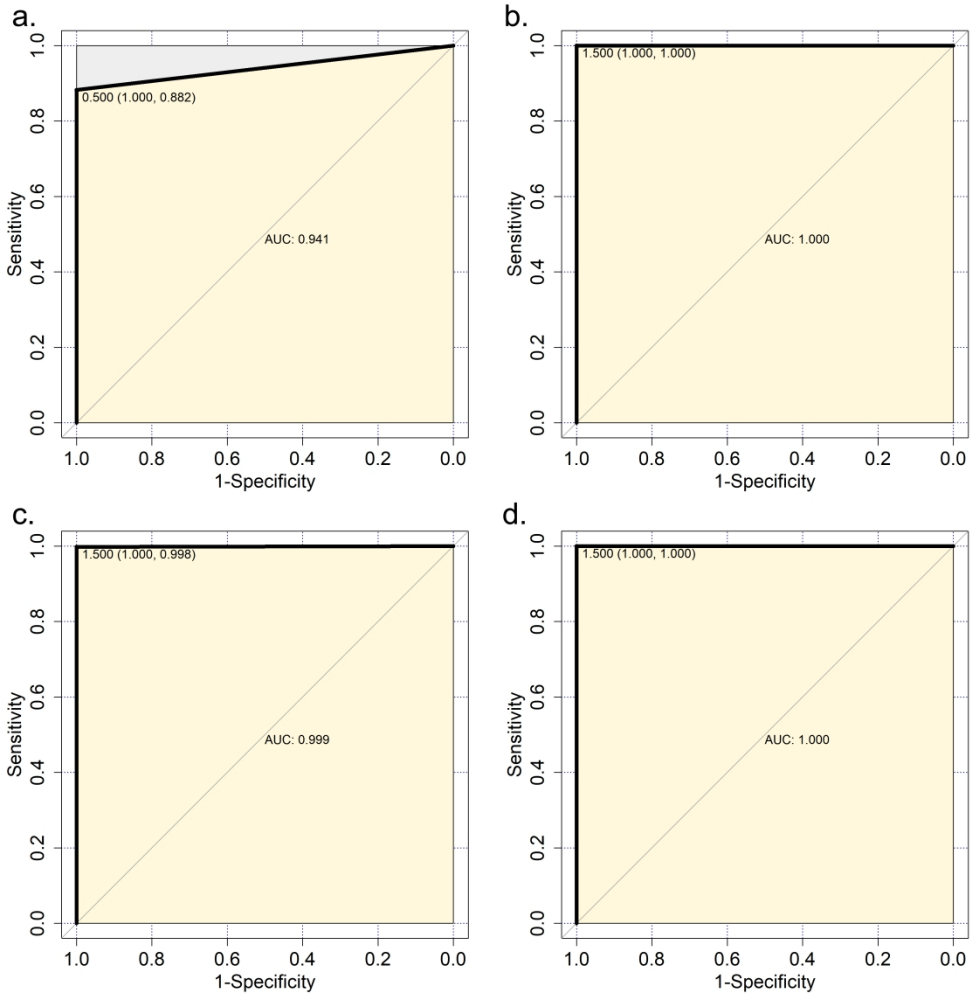


Figure 3. The ROCs of urine HIV-1 antibody reagent in VCTs(a)、IDUs(b)、PWs(c), and FSWs(d) Groups

338x338mm (300 x 300 DPI)

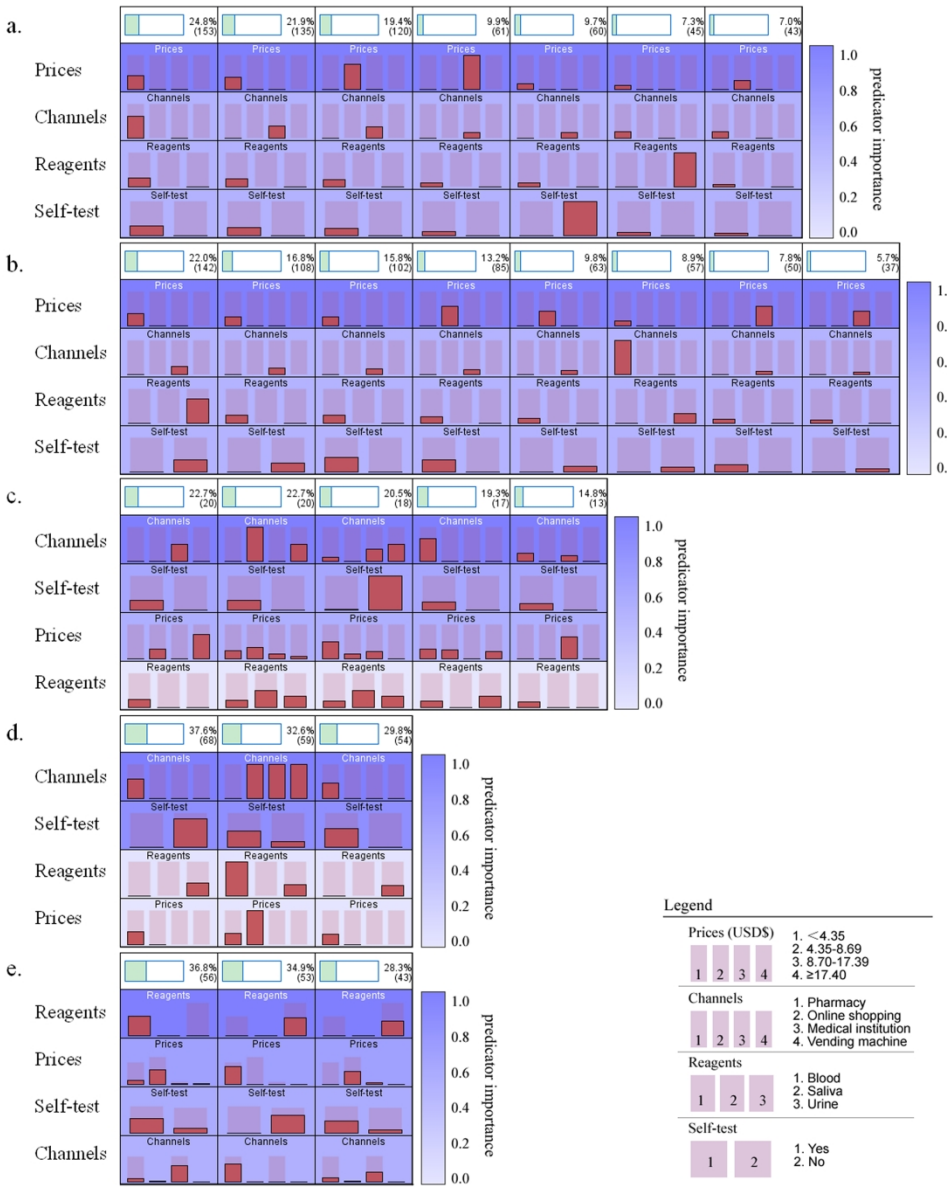


Figure 4. The user profiles patterns of subjects in the two-steps cluster analyses, the patterns of STUs, PWs, VCTs, IDUs, and FSWs illustrated in a, b, c, d, and e, respectively.

869x1027mm (72 x 72 DPI)

**Table 3** Consistency check of two HIV-1 antibody reagents in diverse populations **(detail)**

| Group | Reference | Result [a] | Reagent A | | | | Reagent B | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | - | + | *kappa* | *p* | - | + | *kappa* | *p* |
| FSWs | Reagent C | - | 201 | 0 | 1.000 | <0.001 | 201 | 0 | 1.000 | <0.001 |
| | | + | 0 | 1 | | | 0 | 1 | | |
| IDU | Reagent C | - | 289 | 0 | 1.000 | <0.001 | 289 | 0 | 1.000 | <0.001 |
| | | + | 0 | 15 | | | 0 | 15 | | |
| PW | Reagent C | - | 997 | 2 | 0.499 | <0.001 | 999 | 0 | 1.000 | <0.001 |
| | | + | 0 | 1 | | | 0 | 1 | | |
| STUs | Reagent C | - | 1000 | 0 | - | - | 1000 | 0 | - | |
| | | + | 0 | 0 | | | 0 | 0 | | |
| Subjects undergoing VCT | Reagent C | - | 66 | 0 | 0.908 | <0.001 | 66 | 0 | 1.000 | <0.001 |
| | | + | 4 | 30 | | | 0 | 34 | | |
| Total | Reagent C | - | 2553 | 2 | 0.939 | <0.001 | 2555 | 0 | 1.000 | <0.001 |
| | | + | 4 | 47 | | | 0 | 51 | | |

a: Table 3 (detail) presents the detailed diagnostic results for Reagent A and Reagent B.

**Table 4** The receiver operator characteristic curves for Reagents A and B in the 2606 subjects **(detail)**

| Reagents | Results [a] | Results | | Statistical parameters of ROC curves | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | - | + | *AUC* | 95% *CI* | Sensitivity | Specificity | Youden index | *p* |
| A | - | 2553 | 2 | 0.960 | 0.952-0.968 | 92.16 | 99.92 | 0.921 | <0.001 |
| | + | 4 | 47 | | | | | | |
| B | - | 2555 | 0 | 1.000 | 0.999-1.000 | 100.00 | 100.00 | 1.000 | <0.001 |
| | + | 0 | 51 | | | | | | |

a: Table 4 (detail) presents the detailed diagnostic results for Reagent A and Reagent B.

**Table 5** The receiver operator characteristic curves for Reagent A in each group **(detail)**

| Groups | Reference | Reagent A | | Statistical parameters of ROC curves | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | - | + | *AUC* | 95% CI | Sensitivity | Specificity | Youden index | |
| Subjects undergoing VCT | - | 66 | 0 | 0.941 | 0.876-0.978 | 88.24 | 100.00 | 0.882 | <0.001 |
| | + | 4 | 30 | | | | | | |
| Persons with IDU | - | 289 | 0 | 1.000 | 0.999-1.000 | 100.00 | 100.00 | 1.000 | <0.001 |
| | + | 0 | 15 | | | | | | |
| PW | - | 997 | 2 | 0.999 | 0.997-1.000 | 99.80 | 100.00 | 0.998 | <0.001 |
| | + | 0 | 1 | | | | | | |
| FSWs | - | 201 | 0 | 1.000 | 0.999-1.000 | 1.000 | 1.000 | 1.000 | <0.001 |
| | + | 0 | 1 | | | | | | |
| STUs | - | 1000 | 0 | - | - | - | - | - | - |
| | + | 0 | 0 | | | | | | |

a: Table 5 (detail) presents the detailed diagnostic results for Reagent A in each subgroup.

| Section & Topic | No | Item | Reported on page # |
|---|---|---|---|
| **TITLE OR ABSTRACT** | | | |
| | **1** | Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC) | Lines 1-2, Page 1 |
| **ABSTRACT** | | | |
| | **2** | Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts) | Lines 3-34, Page 1 |
| **INTRODUCTION** | | | |
| | **3** | Scientific and clinical background, including the intended use and clinical role of the index test | Lines 41-55, Page 2 |
| | **4** | Study objectives and hypotheses | Lines 56-60, Page 2 |
| **METHODS** | | | |
| *Study design* | **5** | Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study) | Lines 70-74, Page 3 |
| *Participants* | **6** | Eligibility criteria | Lines 102-107, Page 4 |
| | **7** | On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry) | Lines 64-69, Page 3 |
| | **8** | Where and when potentially eligible participants were identified (setting, location and dates) | Lines 70-74, Page 3 |
| | **9** | Whether participants formed a consecutive, random or convenience series | Lines 77-78, Page 3 |
| *Test methods* | **10a** | Index test, in sufficient detail to allow replication | Lines 62-129, Pages 3-5 |
| | **10b** | Reference standard, in sufficient detail to allow replication | Lines 92-111, Page 4. |
| | **11** | Rationale for choosing the reference standard (if alternatives exist) | Lines 108-113, Page 4 |
| | **12a** | Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory | Lines 102-107, Page 4 |
| | **12b** | Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory | Lines 108-111, Page 4 |
| | **13a** | Whether clinical information and reference standard results were available to the performers/readers of the index test | Lines 108-111, Page 4 |
| | **13b** | Whether clinical information and index test results were available to the assessors of the reference standard | Lines 108-111, Page 4 |
| *Analysis* | **14** | Methods for estimating or comparing measures of diagnostic accuracy | Lines 118-122, Page 5 |
| | **15** | How indeterminate index test or reference standard results were handled | Lines 102-105, Page 4 |
| | **16** | How missing data on the index test and reference standard were handled | Lines 126-129, Page 5 |
| | **17** | Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory | Lines 102-107, Page 4 |
| | **18** | Intended sample size and how it was determined | Lines 87-90, Page 3 |
| **RESULTS** | | | |
| *Participants* | **19** | Flow of participants, using a diagram | Line 143, Page 5 |
| | **20** | Baseline demographic and clinical characteristics of participants | Lines 139-143, Page 5 |
| | **21a** | Distribution of severity of disease in those with the target condition | Lines 139-143, Page 5 |
| | **21b** | Distribution of alternative diagnoses in those without the target condition | Not applicable |
| | **22** | Time interval and any clinical interventions between index test and reference standard | Lines 105-107, Page 4 |
| *Test results* | **23** | Cross tabulation of the index test results (or their distribution) by the results of the reference standard | Table 3, Page 15 |
| | **24** | Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals) | Lines 167-175, Page 6 |
| | **25** | Any adverse events from performing the index test or the reference standard | Line 112, Page 5 |
| **DISCUSSION** | | | |
| | **26** | Study limitations, including sources of potential bias, statistical uncertainty, and generalisability | Lines 280-286, Pages 10-11 |
| | **27** | Implications for practice, including the intended use and clinical role of the index test | Lines 288-290, Page 11 |
| **OTHER INFORMATION** | | | |
| | **28** | Registration number and name of registry | Lines 309-311, Page 11 |
| | **29** | Where the full study protocol can be accessed | Lines 299-301, Page 11 |
| | **30** | Sources of funding and other support; role of funders | Lines 303-308, Page 11 |

# STARD 2015

## AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

## EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

## DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

BMJ Open

# Diagnostic Performance Evaluation of Urine HIV-1 Antibody Rapid Test Kits in A Real-life Routine Care Setting in China

| | |
|---|---|
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| Primary Subject Heading: | HIV/AIDS |
| Secondary Subject Heading: | Epidemiology, Diagnostics |
| Keywords: | HIV & AIDS < INFECTIOUS DISEASES, Public health < INFECTIOUS DISEASES, Sensitivity and Specificity |
| | |

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# Diagnostic Performance Evaluation of Urine HIV-1 Antibody Rapid Test Kits in A Real-life Routine Care Setting in China


## Abstract

**Objectives:** To evaluate the diagnostic performance of urine human immunodeficiency virus (HIV) antibody rapid test kits in screening diverse populations and to analyse subjects' willingness regarding reagent types, purchase channels, acceptable prices, and self-testing.

**Designs:** Diagnostic accuracy studies

**Participants:** A total of 2606 valid and eligible samples were collected in the study, including 202 samples from female sex workers (FSWs), 304 persons with injection drug use (IDU), 1000 pregnant women (PW), 100 subjects undergoing voluntary HIV counselling and testing (VCT), and 1000 students in higher education schools or colleges (STUs). Subjects should simultaneously meet the following inclusion criteria: (1) being at least 18 years old and in full civil capacity; (2) signing an informed consent form; and (3) providing truthful identifying information to ensure the subjects and their samples are unique.

**Results:** The sensitivity, specificity, and area under the curve (AUC) of the urine HIV-1 antibody rapid test kits were 92.16%, 99.92%, and 0.960 (95% confidence interval (CI): 0.952-0.968, $p$<0.001), respectively, among 2606 samples collected during on-site screenings. The kits showed good diagnostic performance in persons with IDU (*AUC*: 1.000, 95% CI: 1.000-1.000, $p$<0.001), PW (*AUC*: 0.999, 95% CI: 0.999-1.000, $p$<0.001), and FSWs (*AUC*: 1.000, 95% CI: 1.000-1.000, $p$<0.001). The AUC of the urine reagent kits in subjects undergoing VCT was 0.941 (95% CI: 0.876-0.978, $p$<0.001). The "acceptable price" had the greatest influence on STUs (*Pi*=1.000) and PW (*Pi*=1.000), the "purchase channel" had the greatest influence on subjects undergoing VCT (*Pi*=1.000) and persons with IDU (*Pi*=1.000), and the “reagent types” had the greatest influence on FSWs (*Pi*=1.000).

**Conclusions**: The rapid urine test kits showed a good diagnostic validity in practical applications, despite a few cases involving misdiagnosis and underdiagnosis.


**Keywords:** HIV, urine, rapid test kits, ROC

**Strengths and limitations of this study:**

1. This study has evaluated the diagnostic validity of urine HIV-1 rapid test kits in screening both the general population and high-risk populations.
2. Cluster analysis provides a clear profile of the main concerns and selection preferences of the different populations when they choose HIV test reagents.
3. No positive samples were found among the students, and therefore, ROC curves could not be plotted for this subgroup.

## 1. Introduction

The prevalence of HIV/AIDS varies widely across China[1, 2]. Guangxi Zhuang Autonomous Region, the only minority region in southern China, is a serious HIV/AIDS hotspot; in the past decade, this region had a much higher HIV/AIDS prevalence than any other Chinese coastal or inland province[3, 4]. Therefore, the public health administration in Guangxi is attempting to expand the scale of HIV screening to diagnose HIV-infected patients at an early stage and provide highly active antiretroviral therapy (HAART) promptly to reduce HIV/AIDS mortality and transmission[5, 6], especially in high-risk populations[7].

With the cost reduction, urine HIV antibody testing is gradually gaining attention and acceptance by public health policymakers, health institutions, and the general public due to its advantages of being convenient, noninvasive, safe[8-10], and reliable [11-14]. However, these urine HIV antibody reagents required that urine samples be transported to the laboratory for centralized testing because of methodological limitations, which limits their convenience of application.

A urine HIV-1 antibody rapid test reagent with colloidal gold method has been granted marketing approval by the China Food and Drug Administration in 2019. This reagent can present the results within 15 minutes, and all operations can be completed on-site. Due to the advantages of noninvasive, convenient, and rapid, the Guangxi health department is very interested in this reagent and believes that adopting it may help to further increase the acceptance of the population to HIV screening. It is worth noting that although some studies have evaluated the diagnostic performance of urine HIV-1 antibody rapid test kits using standard samples under controlled laboratory conditions, no studies have yet reported on their diagnostic performance in practical applications and the acceptance of different populations; therefore, an adequate scientific basis for the application of urine rapid test kits for HIV screening has not been provided for public health authorities in high-prevalence areas.

This study, based on a special study of the Chinese National Science and Technology Major Project (NSTMP) for infectious diseases, aimed to evaluate the diagnostic performance of urine HIV-1 antibody rapid test reagents in a practical screening setting and to preliminarily analyse the willingness of subjects regarding the types of reagents, purchase channels, and acceptable prices to provide a valuable scientific basis for the application of urine HIV antibody rapid test reagents for screening.

## 2. Materials and methods

### *2.1 Samples and Sources*

Subjects were recruited from the most commonly screened populations for HIV antibodies in the real world. According to the CDC HIV Sentinel Surveillance Implementation Plan, the subjects of this study were categorized into four groups based on HIV-related risk behaviours as follows: (1) The key population, including Female sex workers (FSWs) and persons with injection drug use (IDU). FSWSs, defined as women currently involved in the commercial sex trade. IDU, defined as a person who injects opioids (mainly heroin) and has had a positive urine test for morphine in the last month. FSWs and IDU were sampled and surveyed at the place of sex trade and methadone clinics, respectively. (2) The vulnerable population, in this study, were pregnant women (PW), defined as women undergoing maternal health care in preparation for childbirth, were sampled and surveyed at maternity units in general hospitals or women's and children's hospitals. (3) In this study, the general population was students enrolled in tertiary institutions (STUs) who were sampled and surveyed at the school dispensary. (4) The subjects undergoing voluntary HIV counselling and testing (VCT), were sampled and surveyed at the CDC's HIV testing clinic.

PW are routinely screened for HIV, and women receiving care during pregnancy were recruited from women and children's hospitals. Subjects undergoing VCT were consulted or referred to provincial CDC VCT clinics. This study was conducted from August 1, 2020, to September 31, 2020. No researcher knows whether the subjects were infected with HIV before testing because of previously reported cases that were excluded through the ID card system.

To improve the external validity and to match the characteristics of the real-world HIV screening population, no strict inclusion or exclusion criteria were set for this study, only the following requirements need to be met concurrently: (1) the subject should be at least 18 years of age and in full civil capacity; (2) the subject should have signed the informed consent form and volunteered to participate in the study as a subject; (3) the subject should provide truthful identifying information, such as a driver's license or identification card, to ensure the subject and the sample are unique, and to exclude previously reported HIV cases. Researchers informed subjects of the purpose, methods, potential harms, and personal privacy issues of this study in detail before informed consent forms were signed. Following the signing of the informed consent form, each subject was required to be taken three samples, a whole blood sample, a fingertip peripheral blood sample, and a urine sample, and to complete the questionnaire after sampling.

The urine rapid test reagent AUC area was predicted to be between 0.85 and 0.98, and the confidence level (1-alpha), confidence interval width, sample dropout rate, and screening sample size were set to 0.95, 0.10, 5%, and 2,500 cases, respectively, requiring a positive sample size of 5-34 cases as estimated by the PASS 2015 software package.

*2.2 Urine and blood sample testing methods*

Three HIV antibody test reagents were used in the study: (1) Reagent A, named the Urine HIV-1 Antibody Rapid Test Kit (colloidal gold), was packaged as a rapid test kit and manufactured by Wantai (20193400550); (2) Reagent B, named Determine™ HIV1/2 (colloidal selenium), was packaged as a rapid test kit and manufactured by Abbott (20163400427); and (3) Reagent C, named GENscreen™ ULTRA HIV Ag-Ab (Enzyme-Linked Immunosorbent Assay, ELISA), which was manufactured by Bio-Rad (72388C).

HIV antibody tests were divided into on-site tests (for Reagents A and B) and laboratory tests (for Reagent C only). Reagents A and B were used to test for HIV-1 antibodies in urine samples and peripheral blood samples taken from fingertips, respectively. Reagent B is the most common testing method for HIV-1 antibodies in VCT clinics. Urine and venous blood samples were collected from the study subjects using a 100 ml urine cup and a 4 ml EDTA vacuum blood collection tube for Reagents A and C, respectively.

Reagent A and B results were simultaneously identified and recorded by two trained practitioners, and the results were classified as negative, positive, or invalid within a specified time frame, according to the reagent instructions. If the two practitioners disagreed on the identification of the same reagent, they uploaded an electronic photo of the reagent, and the result was judged by the quality control team. The anticoagulated blood samples were transferred to the local CDC HIV confirmation laboratory and tested for HIV-1 antibodies under controlled conditions by Reagent C immediately, which was used as the reference method in the study.

All reagents were used in strict accordance with the manufacturer's instructions, and any samples from the same participant was positive, the whole blood sample was tested again in the HIV confirmation laboratory and confirmed by both ELISA and Western blotting, according to the diagnostic criteria of the Chinese Guidelines for Diagnosis and Treatment of Human Immunodeficiency Virus Infection/Acquired Immunodeficiency Syndrome (2020 edition). Three laboratories with HIV-confirmation qualifications participated in the study, including the HIV-confirmation laboratories of Guangxi Provincial CDC, Guigang CDC, and Liuzhou CDC.

*2.3 Data management and statistical analysis*

The subjects' information, including basic information such as their name, sex, date of birth, occupation type, education level, and ethnicity, as well as their willingness regarding HIV-1 antibody testing methods, purchase channels, acceptable prices, and self-tests, was collected through questionnaires.

The main data management and statistical software used in this study included EPIDATA v3.1, Microsoft Excel 2019, R v4.1.0, RStudio v1.4. 1103, and IBM SPSS v26.0. The sensitivity, specificity, receiver operator characteristic (ROC) curve, and area under the curve (AUC) were used to assess the diagnostic validity of the urine HIV-1 antibody reagents in the on-site screening of different populations, these processes are synchronized in the ROC analysis module of SPSS and the PROC package of the R language. The two-step cluster analysis method in SPSS was used to evaluate the intentionality and user profiles of the study subjects regarding HIV antibody reagent types, acceptable prices, purchase channels, and self-tests. The level of statistical significance was set at $\alpha$=0.05.

The information recorded in the paper questionnaire was entered in pairs using EPI DATE V3.1 and compared for consistency, with key information (ID information, age, sex, population category, education level, willingness to use reagents, etc.), HIV antibody test results, and other auxiliary information, with consistency levels of 100%, 100%, and 99.5%, respectively.

*2.4 Patient and Public Involvement*

This study was mainly completed by Guangxi CDC, with Guigang CDC, Luzhai CDC, and Binyang CDC as the specific implementors of the study. The public and patients (mainly potential patients in this study) were not directly involved in the design and implementation of this study. However, the findings of this study may have some influence on local HIV-related public health strategies in Guangxi, such as promoting noninvasive urine testing reagents for HIV screening in the general population to increase its acceptability and adopting more sensitive and specific methods for screening high-risk populations to find HIV-infected individuals at the early stage.

## 3. Results

*3.1 Basic information about the subjects*

A total of 2606 valid and eligible samples were collected from the FSWs, persons with IDU, PW, STUs, and subjects undergoing VCT included in this study, with 202 (7.7%), 304 (11.7%), 1000 (38.4%), 1000 (38.4%), and 100 (3.8%) collected samples, respectively. No adverse events were reported. The flowchart

is presented in **Figure 1**. The basic information of each population subgroup is shown in **Table 1**.

*3.2 Consistency of the results of the 3 reagents*

Reagents A and B both showed quality control bands in the 2606 samples tested, and no reagent invalidation occurred. The results of the three reagents are shown in **Table 2**.

The number of probable HIV-positive individuals detected by Reagents A, B, and C was 49, 51, and 51, respectively. Of these, 51 individuals with HIV-positive samples detected by Reagents B and C were confirmed to show HIV positivity by both ELISA and WB tests. Of the 49 HIV-positive samples detected by Reagent A, 47 were eventually confirmed to show HIV positivity. Of the 3 PW diagnosed with HIV by Reagent A, 2 were misdiagnosed.

The results of Reagent A were fully consistent with those of the reference method for the FSWs (*Kappa*=1.000, $p<0.001$) and persons with IDU (*Kappa*=1.000, $p<0.001$), with *kappa* values of 0.499 ($p<0.001$) and 0.908 ($p<0.001$) in the PW and subjects undergoing VCT, respectively. The results of Reagent B were fully consistent with those of the reference method, and there were no missed or misdiagnosed cases, as shown in **Table 3** and **supplementary Table 1**.

*3.3 Diagnostic performance*

The overall sensitivity of Reagent A was 92.16%, the specificity was 99.92%, and the AUC was 0.960 (95% CI: 0.952-0.968, $p<0.001$) for the 2606 on-site tests. Reagent B showed identical results to the reference method in the 2606 on-site assays (*AUC*: 1.000, 95% CI: 0.999-1.000, $p<0.001$), and the overall performance of Reagent A was slightly lower than that of Reagent B ($z=2.083$, $p<0.05$), as presented in **Table 4** and **supplementary Table 2**. The ROC curves of the 2 reagents are shown in **Figure 2**.

Reagent A showed good performance in the on-site application for persons with IDU (*AUC*: 1.000, *95% CI*: 1.000-1.000, $p<0.001$), FSWs (*AUC*: 1.000, *95% CI*: 1.000-1.000, $p<0.001$), and PW (*AUC*: 0.999, *95% CI*: 0.997-1.000, $p<0.001$), but the performance differences in in each application setting were significant ($z=2.908$, $p<0.005$), as shown in **Table 5** and **supplementary Table 3**. The ROC curves of the different application settings are shown in **Figure 3**. In this study, the false negative rate (FNR) of Reagent A in the subjects undergoing VCT was 6.25% (2/32), and the false positive rate (FPR) in the PW was 0.20% (2/999).

The AUC of Reagent A in the on-site application for subjects undergoing VCT was 0.941 (95% CI: 0.876-0.978, $p<0.001$). We further dissected and reviewed the causes of this problem: Of the four subjects undergoing VCT with inconsistent results between Reagent A and the reference method, two were men who

have sex with men (MSM) who are regularly tested at Non-governmental organizations and were recently determined to have HIV-1 antibody positivity, which we speculate may have been due to recent infection. The other two subjects were HIV-infected individuals receiving HAART who requested recertification reports from the VCT for referral to hospitals in other provinces for treatment.

*3.4 Willingness regarding and cluster analysis of HIV-1 antibody reagents, prices, and channels among different populations*

The willingness regarding HIV-1 antibody test reagent types ($\chi^2$=430.498, $p$<0.001), purchase channels ($\chi^2$=494.970, $p$<0.001), acceptable prices ($\chi^2$=152.710, $p$<0.001), and self-tests ($\chi^2$=245.966, $p$<0.001) were significant among the different subgroups, as presented in **Table 6**.

The two-step cluster analysis models showed that the "acceptable price" had the greatest influence on STUs (*Pi*=1.000) and PW (*Pi*=1.000), the "purchase channel" had the greatest influence on subjects undergoing VCT (*Pi*=1.000) and persons with IDU (*Pi*=1.000), and the "reagent types" had the greatest influence on FSWs (*Pi*=1.000), as presented in **Table 7**.

The user profiles of STUs, PW, subjects undergoing VCT, persons with IDU, and FSWs were classified into 7, 8, 5, 3, and 3 patterns, respectively. The main patterns of the five populations were as follows and are presented in **Figure 4**: “priced less than $4.35, purchased at a pharmacy, blood reagents, and willing to self-test” for STUs; “priced below $4.35, purchased at a medical institution, urine reagents, and nonself-testing” for PW; “purchased at a medical institution, willing to self-test, priced between $4.35 and $8.69 or more than $17.40, and blood reagents” for subjects undergoing VCT; “purchased at a medical institution, willing to self-test, and blood reagents” for persons with IDU; and "blood reagents, priced at $4.35–$8.69, willing to self-test, and purchased at medical facilities" for FSWs.

## 4. Discussion

Due to obvious advantages such as noninvasiveness and convenience[15], urine testing for HIV antibodies began in the 1990s, and their diagnostic performance has been confirmed in many studies[16-18]. Urine HIV antibody tests have been used in practice for more than a decade[19], and their convenience has been further promoted in recent years with the advent of colloidal gold rapid test kits[12, 20]. These rapid test kits further enhance the convenience of HIV antibody testing by eliminating the requirement for centralized testing in specialized infectious disease laboratories. However, few studies have reported on the diagnostic performance of rapid urine HIV antibody test kits for practical application in large, complex populations in the real world.

The NSTMP is considered to be the most important scientific and research project in China. Its infectious disease prevention and control projects have been carried out in Guangxi for decades to assess the key issues in the HIV epidemic[21, 22], including the low willingness of the population to be screened and the high mortality rate in rural areas due to late HIV detection and diagnosis[23, 24]. We conducted the study to estimate the diagnostic validity and acceptance of a rapid urine HIV antibody test kit in different populations. As far as we know, such studies are rarely reported.

In this study, based on real-world samples, we found that urine HIV antibody rapid test kits showed satisfactory sensitivity, specificity, and ROC curves, especially in high-risk populations such as persons with IDU and FSWs. Commercial heterosexual infections are the main transmission route of HIV in Guangxi, and as a high-risk population, FSWs are a key node in this transmission route[25, 26]. Both persons with IDU and FSWs are high-risk groups for HIV, and currently, sentinel surveillance and special investigations are the primary public health strategies for identifying HIV-positive patients in high-risk populations. ELISA is the major approach to test for HIV antibodies, which requires the collection of venous whole blood samples from study subjects and transportation to a dedicated HIV laboratory at the CDC for cryopreservation and testing.

In contrast, urine testing offers greater advantages in terms of convenience and timeliness. The administration of injection drugs requires regular urine sample collection for recent opioid, methamphetamine, and ketamine abuse, and efficiency and subject acceptance can be improved if urine HIV antibody testing is also conducted instead of blood testing. However, the sentinel surveillance and special investigation of some high-risk groups for HIV infection also require testing for HCV and syphilis[27, 28], and the single function of the current urine HIV rapid reagent test limits its applicability.

In practice, physicians treating subjects undergoing VCT are dealing with a very complex population, which is even more complex than the high-risk population. In this study, we routinely tested subjects for blood HIV antibodies and additionally used urine reagent strips to evaluate their performance under complex practice conditions. The urine rapid test kit showed four false-negative cases among 100 subjects undergoing VCT; two were MSM with new infections detected by regular testing at NGOs, and two were patients receiving in-treatment HAART. In the present study, the ROC curve of the urine rapid test kit could have been affected by these false-negative cases if the routine VCT consultation procedure had been followed, and similar false-negative results had been found in some previous studies[14, 29]. It should be added that the urine reagent's instructions stated that samples from HIV-infected individuals in the window period or those receiving treatment may yield false-negative results.

Considering the complexities and psychologically protective behaviours of some subjects undergoing VCT, it may be more appropriate to choose an antigen-antibody combined reagent with higher sensitivity and specificity to reduce the possibility of false negatives in some cases where it is difficult for physicians treating these subjects to obtain true and accurate information[30, 31]. Some subjects with significant psychological fear of HIV but no high-risk exposure may consider using noninvasive urine reagent strips to reduce trauma and receive psychological counselling.

Despite some limitations, urine rapid test kits can be offered as an option for HIV self-testing in high-risk populations such as MSM, FSWs, and persons with IDU who require regular testing due to their operability, noninvasiveness, and safety; these test kits can have a positive effect on increasing subjects' willingness to accept and participate in screening[13, 32].

Previous studies have evaluated urine HIV antibody reagents for general population screening, but this approach required centralized testing by qualified laboratories[20, 33]. Combined with the internet platform and logistics industry, rapid test kits with urine reagent strips can improve operability through anonymous testing, which may be able to further expand the coverage of general population screening.

In areas with high HIV prevalence, maternal HIV screening helps to identify HIV-infected PW at an early stage and provides timely drug interventions to interrupt mother-to-child transmission[34], which has a positive effect on reducing vertical transmission[35, 36]. Urine reagent strips showed satisfactory ROC curves in maternal HIV-1 antibody screening, but there were two false positive tests out of 1000 tests. The reasons for occasional false-positive HIV antibody tests in PW need to be further investigated, and similar occasional occurrences have previously been reported in ELISA screening tests[37]. Overall, the false

positive rate of urine rapid test reagents in the PW population is acceptable given the considerable advantages of the noninvasive operation. No positive case was found in the STUs, which we believe is related to the very low prevalence of HIV infection in this population. Thus, the validity of the urine rapid reagent in STUs requires a larger sample size in future studies.

User profiles are the behavioural characteristics of a customer group in selecting or using a product, which is one of the hot analytical approaches in e-business. The current study innovatively applied user profiles to assess the characteristics and tendencies of different population subgroups when choosing reagents for HIV testing. We found that STUs and PW preferred reagent prices below $4.35, which may be related to the lack of financial income for STUs and the higher cost of childbirth, resulting in price sensitivity for these two groups. We also observed a higher willingness to self-test among the student population, which may be related to the extensive HIV propaganda work carried out in colleges and universities in the past decade[38, 39].

The low willingness to self-test among persons with IDU and FSWs may be related to the fact that local CDCs conduct free HIV, HCV, and syphilis testing for such high-risk populations several times per year. At the same time, persons with IDU and FSWs enrolled in long-term health interventions develop trusting relationships with the CDC, so they are more inclined to choose the medical institution channel and blood reagents. In this study, FSWs preferred urine HIV reagents, which may be related to the noninvasive operation of the rapid test kits. Although the diagnostic performance has been proven in some studies [40], a low percentage of subjects in this study chose the oral secretion HIV antibody test kit, probably due to its expensive price and complicated operation.

People undergoing VCT were more likely to have their HIV antibodies tested in medical institutions, had the highest willingness to undergo self-testing, and were also willing to accept more expensive reagents. However, for subjects undergoing VCT, we speculated that their acceptance of HIV-1 antibody testing options, particularly regarding price, may be influenced by factors such as the reason for seeking medical services and psychological status, as all HIV antibody tests conducted in the VCT centres were free of charge.

There were limitations in this study. First, no positive samples were identified in the STUs, and therefore, ROC curves could not be drawn for this subgroup. Second, patients receiving HAART treatment and MSM in the window period were included in the VCT subgroups, which is not consistent with the recommended suggestions for the use of urine HIV reagents; however, this is a complexity that doctors treating subjects

undergoing VCT face every day. Despite these limitations, this study evaluated the diagnostic validity of HIV urine rapid test kits in a complex real-world setting and provided some valuable scientific cues for the practical application of urine reagent strips.

## 5. Conclusions

Overall, the rapid urine test kits showed a good diagnostic validity in practical applications, despite a few cases involving misdiagnosis and underdiagnosis. We recommend that physicians providing testing services to subjects undergoing VCTs should carefully select HIV testing reagents based on each subject's situation.

## 6. Author contributions

HX Lu, HH Chen, SJ Liang, YH Ruan, QY Zhu, GH Lan, and M Lin contributed to the conception and design of the study. HX Lu, GJ Tan, WL Cai, and YJ Zhou organized the database. HX Lu and YH Ruan performed the statistical analysis. HX Lu, HH Chen, and SJ Liang wrote the first draft of the manuscript. XW Pang, JJ Li, XM Ge, wrote sections of the manuscript. HX Lu, HH Chen, and SJ Liang contributed equally to the current work. All authors contributed to the manuscript revision and read and approved the submitted version.

## 7. Data sharing statement

The original database for this study contains private information about the study participants. For non-commercial use and reasonable purposes, anonymised data of the current work can be obtained from the corresponding author.

## 8. Findings

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## 9. Ethics statement

This study was approved by the Ethics Committee of the Guangxi Zhuang Autonomous Region Center for Disease Control and Prevention (approval number GXIRB2019-0047).

## 10. Competing Interest statement

No competing interest

**Table 1** The basic information of the 2606 FSWs, persons with IDU, PW, STUs, and subjects undergoing VCT in the sample

| Variables | Subgroups | The sample sizes of each population group [n (%)] | | | | | Total |
|---|---|---|---|---|---|---|---|
| | | FSWs | Persons with IDU | PW | STUs | Subjects undergoing VCT | |
| Sex | Male | 0(0) | 256(84.2) | 0(0) | 255(25.5) | 48(48.0) | 559 |
| | Female | 202(100) | 48(15.8) | 1000(100) | 745(74.5) | 52(52.0) | 2047 |
| Age | <20 | 1(0.5) | 2(0.7) | 38(3.8) | 846(84.6) | 2(2.0) | 889 |
| | 20-29 | 12(5.9) | 16(5.3) | 524(52.4) | 113(11.3) | 57(57.0) | 722 |
| | 30-39 | 68(33.7) | 126(41.4) | 417(41.7) | 41(4.1) | 18(18.0) | 670 |
| | ≥40 | 121(59.9) | 160(52.6) | 21(2.1) | 0(0) | 23(23.0) | 325 |
| Ethnicity | Han | 120(59.4) | 279(91.8) | 692(69.2) | 526(52.6) | 56(56.0) | 1673 |
| | Zhuang | 58(28.7) | 20(6.6) | 281(28.1) | 402(40.2) | 40(40.0) | 801 |
| | Other | 24(11.9) | 5(1.6) | 27(2.7) | 72(7.2) | 4(4.0) | 132 |
| Education level | Illiterate | 33(16.3) | 5(1.6) | 1(0.1) | 0(0) | 1(1.0) | 40 |
| | Primary school | 94(46.5) | 54(17.8) | 40(4) | 0(0) | 8(8.0) | 196 |
| | Junior middle school | 69(34.2) | 217(71.4) | 471(47.1) | 0(0) | 18(18.0) | 775 |
| | Senior high school | 6(3) | 28(9.2) | 193(19.3) | 472(47.2) | 19(19.0) | 718 |
| | Junior college | 0(0) | 0(0) | 292(29.2) | 527(52.7) | 54(54.0) | 873 |
| | Bachelor's degree or above | 0(0) | 0(0) | 3(0.3) | 1(0.1) | 0(0) | 4 |
| Total | | 202 | 304 | 1000 | 1000 | 100 | 2606 |

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**Table 2** The performance of two HIV-1 antibody reagents in field testing [n (%)]

| Groups | Reagent A | | Reagent B | | Reagent C[a] | | total |
|---|---|---|---|---|---|---|---|
| | - | + | - | + | - | + | |
| FSWs | 201(99.5) | 1(0.5) | 201(99.5) | 1(0.5) | 201(99.5) | 1(0.5) | 202 |
| Persons with IDU | 289(95.1) | 15(4.9) | 289(95.1) | 15(4.9) | 289(95.1) | 15(4.9) | 304 |
| PW | 997(99.7) | 3(0.3) | 999(99.9) | 1(0.1) | 999(99.9) | 1(0.1) | 1000 |
| STUs | 1000(100.0) | 0(0) | 1000(100.0) | 0(0) | 1000(100.0) | 0(0) | 1000 |
| Subjects undergoing VCT | 70(70.0) | 30(30.0) | 66(66.0) | 34(34.0) | 66(66.0) | 34(34.0) | 100 |
| Total | 2557(98.1) | 49(1.9) | 2555(98.0) | 51(2.0) | 2555(98.0) | 51(2.0) | 2606 |

a. Reagent C was set as the reference method in this study

**Table 3** Consistency check of two HIV-1 antibody reagents in diverse subgroups [a]

| Group | Reference Reagent | Reagent A | | Reagent A | |
|---|---|---|---|---|---|
| | | *kappa* | *p* | *kappa* | *p* |
| FSWs | C | 1.000 | <0.001 | 1.000 | <0.001 |
| IDU | C | 1.000 | <0.001 | 1.000 | <0.001 |
| PW | C | 0.499 | <0.001 | 1.000 | <0.001 |
| STUs | C | - | - | - | - |
| Subjects undergoing VCT | C | 0.908 | <0.001 | 1.000 | <0.001 |
| Total | C | 0.939 | <0.001 | 1.000 | <0.001 |

a. **Table 3** is a summary table and detailed results have been presented in **supplementary Table 1** of the supplementary material.

**Table 4** The receiver operator characteristic curves for Reagents A and B in the 2606 subjects [a]

| Reagents [b] | Statistical parameters of ROC curves | | | | | |
|---|---|---|---|---|---|---|
| | *AUC* | 95% *CI* | Sensitivity | Specificity | Youden index | *p* |
| A | 0.96 | 0.952-0.968 | 92.16 | 99.92 | 0.921 | <0.001 |
| B | 1 | 0.999-1.000 | 100 | 100 | 1 | <0.001 |

a: **Table 4** is a summary table and detailed results have been presented in **supplementary Table 2**.

b: The reference standard is Reagent C (ELISA)

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**Table 5** The receiver operator characteristic curves for Reagent A in each group [a]

| Groups | Statistical parameters of ROC curves [b] | | | | | |
|---|---|---|---|---|---|---|
| | *AUC* | 95% CI | Sensitivity | Specificity | Youden index | *p* |
| Subjects undergoing VCT | 0.941 | 0.876-0.978 | 88.240 | 100.000 | 0.882 | <0.001 |
| Persons with IDU | 1.000 | 0.999-1.000 | 100.000 | 100.000 | 1.000 | <0.001 |
| PW | 0.999 | 0.997-1.000 | 99.800 | 100.000 | 0.998 | <0.001 |
| FSWs | 1.000 | 0.999-1.000 | 1.000 | 1.000 | 1.000 | <0.001 |
| STUs | - | - | - | - | - | - |

a: **Table 5** is a summary table and detailed results have been presented in **supplementary Table 3**.

b: The reference standard is Reagent C (ELISA)

**Table 6** Acceptance of HIV-1 antibody testing methods, access, and prices in different populations

| Questions | Classification | Population [n (%)] | | | | | $\chi^2$ | |
|---|---|---|---|---|---|---|---|---|
| | | STUs | PW | Subjects undergoing VCT | Persons with IDU | FSWs | | |
| Reagent types | Blood | 781(78.1) | 599(59.9) | 85(85.0) | 74(24.3) | 88(43.6) | 430.498 | <0.001 |
| | Saliva | 72(7.2) | 45(4.5) | 6(6.0) | 13(4.3) | 6(3.0) | | |
| | Urine | 147(14.7) | 356(35.6) | 9(9.0) | 217(71.4) | 108(53.5) | | |
| Purchase channels | Pharmacy | 382(38.2) | 202(20.2) | 26(26.0) | 176(57.9) | 107(53) | 494.970 | <0.001 |
| | Online shopping | 38(3.8) | 42(4.2) | 24(24.0) | 66(21.7) | 9(4.5) | | |
| | Medical institution | 565(56.5) | 725(72.5) | 45(45.0) | 39(12.8) | 85(42.1) | | |
| | Vending machine | 15(1.5) | 31(3.1) | 5(5.0) | 23(7.6) | 1(0.5) | | |
| Acceptable price (USD$) | <4.35 | 537(53.7) | 575(57.5) | 20(20.0) | 222(73.0) | 99(49.0) | 152.710 | <0.001 |
| | 4.35-8.69 | 285(28.5) | 252(25.2) | 39(39.0) | 63(20.7) | 86(42.6) | | |
| | 8.70-17.39 | 117(11.7) | 128(12.8) | 23(23.0) | 17(5.6) | 16(7.9) | | |
| | ≥17.40 | 61(6.1) | 45(4.5) | 18(18.0) | 2(0.7) | 1(0.5) | | |
| Willingness to self-test | Yes | 762(76.2) | 451(45.1) | 83(83.0) | 143(47.0) | 106(52.5) | 245.966 | <0.001 |
| | No | 238(23.8) | 549(54.9) | 17(17.0) | 161(53.0) | 96(47.5) | | |

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**Table 7** The user profiles of different populations regarding HIV-1 antibody testing methods, channels, and prices

| Population | Clustering model parameters | | | Predictor importance [b] | | | |
|---|---|---|---|---|---|---|---|
| | clusters | Fit quality [a] | *AIC* | reagent types | channels | prices | self-tests |
| STUs | 7 | 1.00 | 126.00 | 0.50 | 0.50 | 1.00 | 0.5 |
| PW | 8 | 1.00 | 144.00 | 0.50 | 0.50 | 1.00 | 0.5 |
| Subjects undergoing VCT | 5 | 0.50 | 197.88 | <0.01 | 1.00 | 0.54 | 0.2 |
| Persons with IDU | 3 | 0.80 | 54.00 | 0.03 | 1.00 | 0.01 | 0.0 |
| FSWs | 3 | 0.70 | 54.00 | 1.00 | 0.53 | 0.69 | 0.2 |

a: Clustering Fit quality ranged from -1 to 1, where 0.5-1 is good and ≥0.51 is excellent

b: Variable importance scores ranged from 0 to 1, with 0 being the lowest and 1.00 being the highest

**Figure 1.** The flowchart of this study

Caption: The flowchart illustrated the line and methodology of this study

**Figure 2**. The receiver operator characteristic curves of reagents A and B in 2606 samples

Caption: Figure 2 presented the ROC curves for urine rapid test reagents and blood rapid test reagents

**Figure 3.** The ROCs of urine HIV-1 antibody reagent in VCTs(a), IDUs(b), PWs(c), and FSWs(d) Groups

Caption: Figure 3 presented the ROC curves of urine rapid test reagents in different population subgroups

**Figure 4.** The user profiles patterns of subjects in the two-steps cluster analyses, the patterns of STUs, PWs, VCTs, IDUs, and FSWs illustrated in a, b, c, d, and e, respectively.

Caption: Figure 4 presented the user profiles of different population subgroups by two-steps cluster analyses

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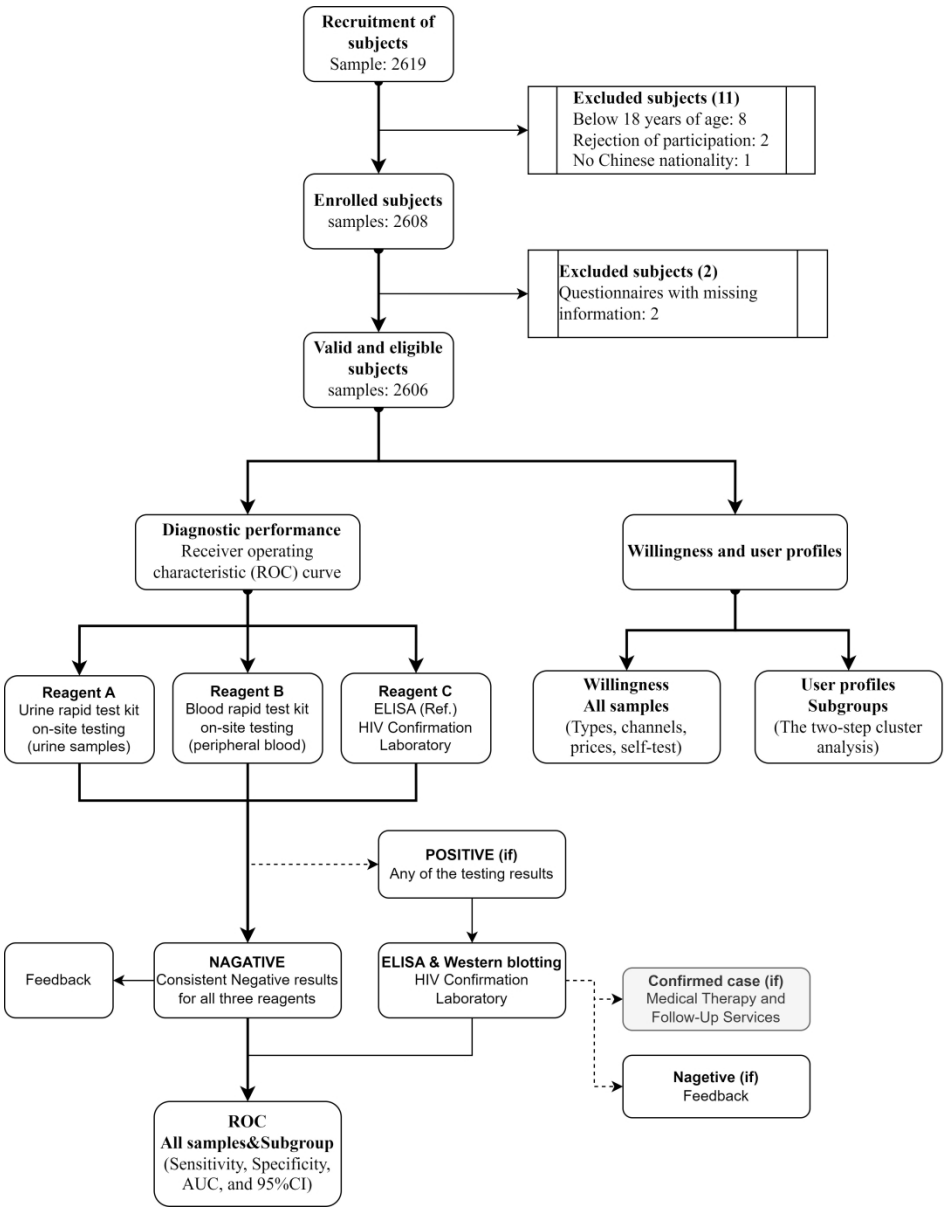


Figure 1. The flowchart of this study

1329x1696mm (72 x 72 DPI)

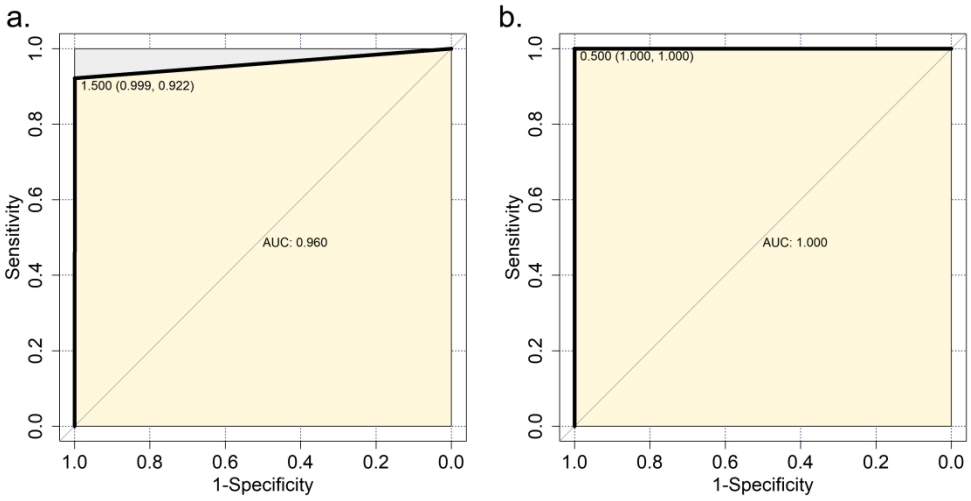


Figure 2. The receiver operator characteristic curves of reagents A and B in 2606 samples

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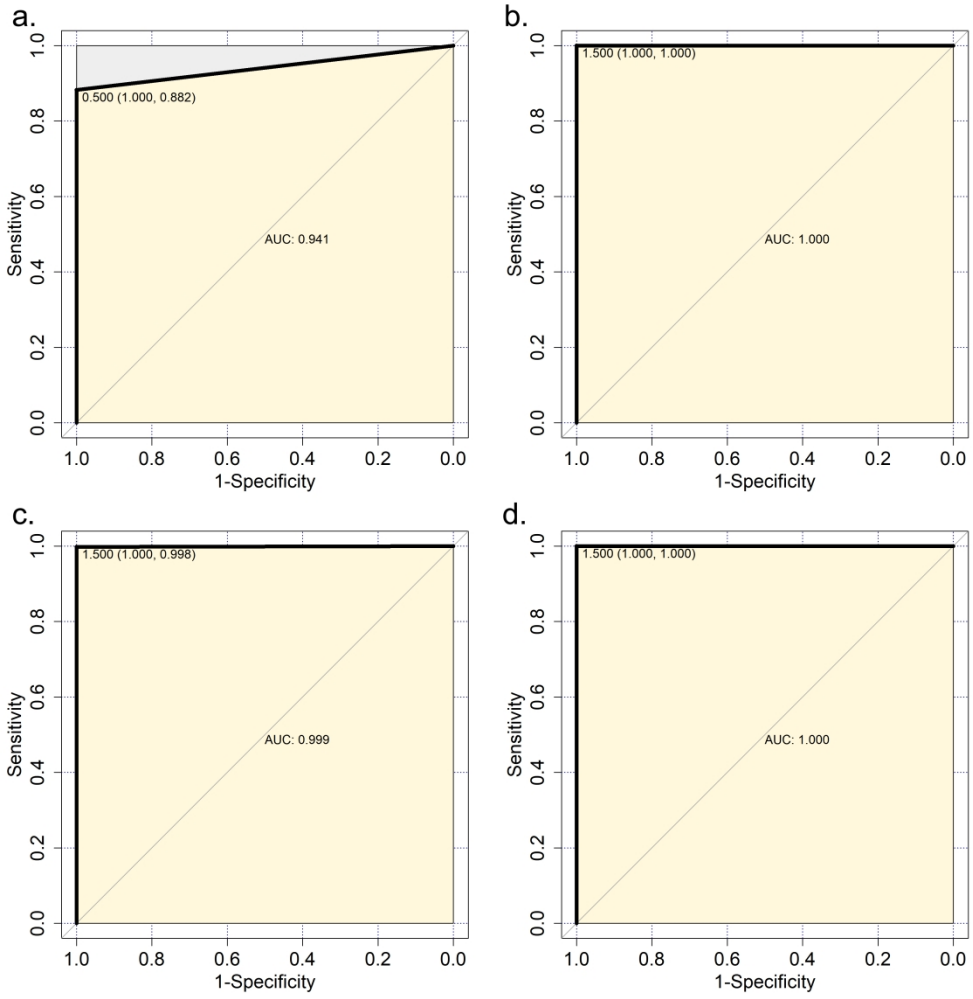


Figure 3. The ROCs of urine HIV-1 antibody reagent in VCTs(a), IDUs(b), PWs(c), and FSWs(d) Groups

338x338mm (300 x 300 DPI)

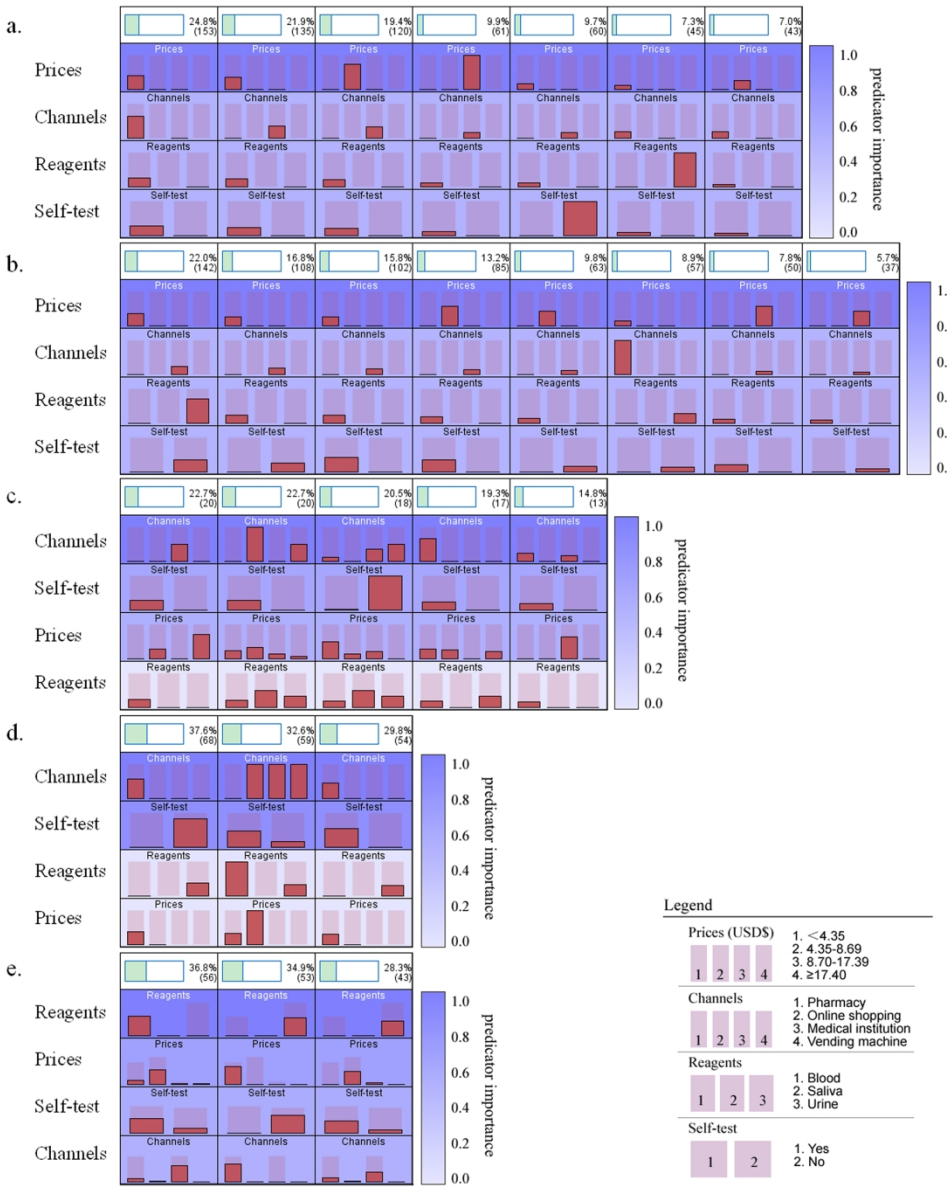


Figure 4. The user profiles patterns of subjects in the two-steps cluster analyses, the patterns of STUs, PWs, VCTs, IDUs, and FSWs illustrated in a, b, c, d, and e, respectively.

869x1027mm (72 x 72 DPI)

**supplementary Table 1** Consistency check of two HIV-1 antibody reagents in diverse subgroups

| Group | Reference | Result [a] | Reagent A | | | | Reagent B | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | - | + | *kappa* | *p* | - | + | *kappa* | *p* |
| FSWs | Reagent C | - | 201 | 0 | 1.000 | <0.001 | 201 | 0 | 1.000 | <0.001 |
| | | + | 0 | 1 | | | 0 | 1 | | |
| IDU | Reagent C | - | 289 | 0 | 1.000 | <0.001 | 289 | 0 | 1.000 | <0.001 |
| | | + | 0 | 15 | | | 0 | 15 | | |
| PW | Reagent C | - | 997 | 2 | 0.499 | <0.001 | 999 | 0 | 1.000 | <0.001 |
| | | + | 0 | 1 | | | 0 | 1 | | |
| STUs | Reagent C | - | 1000 | 0 | - | - | 1000 | 0 | - | |
| | | + | 0 | 0 | | | 0 | 0 | | |
| Subjects undergoing VCT | Reagent C | - | 66 | 0 | 0.908 | <0.001 | 66 | 0 | 1.000 | <0.001 |
| | | + | 4 | 30 | | | 0 | 34 | | |
| Total | Reagent C | - | 2553 | 2 | 0.939 | <0.001 | 2555 | 0 | 1.000 | <0.001 |
| | | + | 4 | 47 | | | 0 | 51 | | |

a: **supplementary Table 1** presents the detailed diagnostic results for Table 3.

**supplementary Table 2** The receiver operator characteristic curves for Reagents A and B in the 2606 subjects

| Reagents | Results [a] | Results | | Statistical parameters of ROC curves | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | - | + | *AUC* | 95% *CI* | Sensitivity | Specificity | Youden index | *p* |
| A | - | 2553 | 2 | 0.960 | 0.952-0.968 | 92.16 | 99.92 | 0.921 | <0.001 |
| | + | 4 | 47 | | | | | | |
| B | - | 2555 | 0 | 1.000 | 0.999-1.000 | 100.00 | 100.00 | 1.000 | <0.001 |
| | + | 0 | 51 | | | | | | |

a: **supplementary Table 2** presents the detailed diagnostic results for Table 4.

**supplementary Table 3** The receiver operator characteristic curves for Reagent A in each group

| Groups | Reference | Reagent A | | Statistical parameters of ROC curves | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | - | + | *AUC* | 95% CI | Sensitivity | Specificity | Youden index | |
| Subjects undergoing VCT | - | 66 | 0 | 0.941 | 0.876-0.978 | 88.24 | 100.00 | 0.882 | <0.001 |
| | + | 4 | 30 | | | | | | |
| Persons with IDU | - | 289 | 0 | 1.000 | 0.999-1.000 | 100.00 | 100.00 | 1.000 | <0.001 |
| | + | 0 | 15 | | | | | | |
| PW | - | 997 | 2 | 0.999 | 0.997-1.000 | 99.80 | 100.00 | 0.998 | <0.001 |
| | + | 0 | 1 | | | | | | |
| FSWs | - | 201 | 0 | 1.000 | 0.999-1.000 | 1.000 | 1.000 | 1.000 | <0.001 |
| | + | 0 | 1 | | | | | | |
| STUs | - | 1000 | 0 | - | - | - | - | - | - |
| | + | 0 | 0 | | | | | | |

a: **supplementary Table 3** presents the detailed diagnostic results for Table 5.

| Section & Topic | No | Item | Reported on page # |
|---|---|---|---|
| **TITLE OR ABSTRACT** | | | |
| | **1** | Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC) | Lines 1-2, Page 1 |
| **ABSTRACT** | | | |
| | **2** | Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts) | Lines 3-34, Page 1 |
| **INTRODUCTION** | | | |
| | **3** | Scientific and clinical background, including the intended use and clinical role of the index test | Lines 41-55, Page 2 |
| | **4** | Study objectives and hypotheses | Lines 56-60, Page 2 |
| **METHODS** | | | |
| *Study design* | **5** | Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study) | Lines 70-74, Page 3 |
| *Participants* | **6** | Eligibility criteria | Lines 102-107, Page 4 |
| | **7** | On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry) | Lines 64-69, Page 3 |
| | **8** | Where and when potentially eligible participants were identified (setting, location and dates) | Lines 70-74, Page 3 |
| | **9** | Whether participants formed a consecutive, random or convenience series | Lines 77-78, Page 3 |
| *Test methods* | **10a** | Index test, in sufficient detail to allow replication | Lines 62-129, Pages 3-5 |
| | **10b** | Reference standard, in sufficient detail to allow replication | Lines 92-111, Page 4. |
| | **11** | Rationale for choosing the reference standard (if alternatives exist) | Lines 108-113, Page 4 |
| | **12a** | Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory | Lines 102-107, Page 4 |
| | **12b** | Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory | Lines 108-111, Page 4 |
| | **13a** | Whether clinical information and reference standard results were available to the performers/readers of the index test | Lines 108-111, Page 4 |
| | **13b** | Whether clinical information and index test results were available to the assessors of the reference standard | Lines 108-111, Page 4 |
| *Analysis* | **14** | Methods for estimating or comparing measures of diagnostic accuracy | Lines 118-122, Page 5 |
| | **15** | How indeterminate index test or reference standard results were handled | Lines 102-105, Page 4 |
| | **16** | How missing data on the index test and reference standard were handled | Lines 126-129, Page 5 |
| | **17** | Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory | Lines 102-107, Page 4 |
| | **18** | Intended sample size and how it was determined | Lines 87-90, Page 3 |
| **RESULTS** | | | |
| *Participants* | **19** | Flow of participants, using a diagram | Line 143, Page 5 |
| | **20** | Baseline demographic and clinical characteristics of participants | Lines 139-143, Page 5 |
| | **21a** | Distribution of severity of disease in those with the target condition | Lines 139-143, Page 5 |
| | **21b** | Distribution of alternative diagnoses in those without the target condition | Not applicable |
| | **22** | Time interval and any clinical interventions between index test and reference standard | Lines 105-107, Page 4 |
| *Test results* | **23** | Cross tabulation of the index test results (or their distribution) by the results of the reference standard | Table 3, Page 15 |
| | **24** | Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals) | Lines 167-175, Page 6 |
| | **25** | Any adverse events from performing the index test or the reference standard | Line 112, Page 5 |
| **DISCUSSION** | | | |
| | **26** | Study limitations, including sources of potential bias, statistical uncertainty, and generalisability | Lines 280-286, Pages 10-11 |
| | **27** | Implications for practice, including the intended use and clinical role of the index test | Lines 288-290, Page 11 |
| **OTHER INFORMATION** | | | |
| | **28** | Registration number and name of registry | Lines 309-311, Page 11 |
| | **29** | Where the full study protocol can be accessed | Lines 299-301, Page 11 |
| | **30** | Sources of funding and other support; role of funders | Lines 303-308, Page 11 |

# STARD 2015

## AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

## EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

## DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.