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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

9 Participants: A total of 2606 valid and eligible samples were collected in the study, including samples from 10 female sex workers (FSWs), persons with injection drug use (IDU), pregnant women (PW), subjects 11 undergoing voluntary HIV counselling and testing (VCT), and students in higher education (STUs). The 12 receiver operator characteristic (ROC) curve was drawn to evaluate the diagnostic performance of urine 13 HIV-1 antibody rapid test kits in on-site screening, and the cluster analysis model was applied to analyse 14 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).

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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).
- 30 Keywords: HIV, urine, rapid test kits, ROC
 - 31 Strengths and limitations of this study:
 - 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
 37 for this subgroup.

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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.

68 2. Materials and methods

69 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.

80 To improve the external validity and to match the characteristics of the real-world HIV screening 81 population, no strict inclusion or exclusion criteria were set for this study. Researchers informed subjects of 82 the purpose, methods, potential harms, and personal privacy issues of this study in detail before informed 83 consent forms were signed.

84 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 DetermineTM HIV1/2 (colloidal selenium), was packaged as a rapid test kit and
manufactured by Abbott (20163400427); and (3) Reagent C, named GENscreenTM ULTRA HIV Ag-Ab
(Enzyme-Linked Immunosorbent Assay, ELISA), which was manufactured by Bio-Rad (72388C).

90 HIV antibody tests were divided into on-site tests (for Reagents A and B) and laboratory tests (for Reagent 91 C only). Reagents A and B were used to test for HIV-1 antibodies in urine samples and peripheral blood 92 samples taken from fingertips, respectively. Reagent B is the most common testing method for HIV-1 93 antibodies in VCT clinics. Urine and venous blood samples were collected from the study subjects using a 94 100 ml urine cup and a 4 ml EDTA vacuum blood collection tube for Reagents A and C, respectively.

95 Reagent A and B results were simultaneously identified and recorded by two trained practitioners, and

96 the results were classified as negative, positive, or invalid according to the reagent instructions. If the two 3/16

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97 practitioners disagreed on the identification of the same reagent, they uploaded an electronic photo of the 98 reagent, and the result was judged by the quality control team. The anticoagulated blood samples were 99 transferred to the local CDC HIV confirmation laboratory and tested for HIV-1 antibodies under controlled 100 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.

104 2.3 1

2.3 Data management and statistical analysis

105 The subjects' information, including basic information such as their name, sex, date of birth, occupation 106 type, education level, and ethnicity, as well as their willingness regarding HIV-1 antibody testing methods, 107 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 α =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

127 Reagents A and B both showed quality control bands in the 2606 samples tested, and no reagent 128 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**.

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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

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148 (z=2.908, p<0.005), as shown in **Supplemental Table S3**. The ROC curves of the different application 149 settings are shown in **Supplemental Figure 1**. In this study, the false negative rate (FNR) of Reagent A in 150 the subjects undergoing VCT was 6.25% (2/32), and the false positive rate (FPR) in the PW was 0.20% 151 (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

161 The willingness regarding HIV-1 antibody test reagent types (χ^2 =430.498, p<0.001), purchase channels 162 (χ^2 =494.970, p<0.001), acceptable prices (χ^2 =152.710, p<0.001), and self-tests (χ^2 =245.966, p<0.001) were 163 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. 7/16

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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.

225 Considering the complexities and psychologically protective behaviours of some subjects undergoing 226 VCT, it may be more appropriate to choose an antigen-antibody combined reagent with higher sensitivity 227 and specificity to reduce the possibility of false negatives in some cases where it is difficult for physicians 228 treating these subjects to obtain true and accurate information[34, 35]. Some subjects with significant 229 psychological fear of HIV but no high-risk exposure may consider using noninvasive urine reagent strips to 230 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 8/16

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and logistics industry, rapid test kits with urine reagent strips can improve operability through anonymoustesting, 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.

277 7. Data sharing statement

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

8. Findings

This work was supported by the National Natural Science Foundation of China (82160636 and 82260670),
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1 2 3		information of the 2606 FSWs, person						njopen-2023-078694 o d by copyright, inclugi	
4 5	Table 1 The basic	information of the 2606 FSWs, person	s with IDU,	PW, STUs, a	nd subjects ur	ndergoing V	CT in the samp		
б			The sample	e sizes of each	n population §	groups [n (%)]	24 F g fo	- -
7 8	Variables	Subgroups	FSWs	Persons with IDU	PW	STUs)] Subjects undergoing V	ebruar Enser ruses	Total
9 10	Sex	Male	0(0)	256(84.2)	0(0)	255(25.5)	48(48.0)	y 20 Pign rela	559
11		Female	202(100)	48(15.8)	1000(100)	745(74.5)	52(52.0)	eme	2047
12	Age	<20	1(0.5)	2(0.7)	38(3.8)	846(84.6)	2(2.0)	to to	889
3 4		20-29	12(5.9)	16(5.3)	524(52.4)	113(11.3)	57(57.0)	ry 2024. Downloaded from http://bmjopen.bmj.com/ on . eignement Superieur (ABES) . related to text and data mining, Al training, and similar	722
4 5		30-39	68(33.7)	126(41.4)	417(41.7)	41(4.1)	18(18.0)	erie and	670
6		≥ 40	121(59.9)	160(52.6)	21(2.1)	0(0)	23(23.0)	ed fi data	325
7	Ethnicity	Han	120(59.4)	279(91.8)	692(69.2)	526(52.6)	56(56.0)	ABE m	1673
8 9		Zhuang	58(28.7)	20(6.6)	281(28.1)	402(40.2)	40(40.0)	inin Sinin	801
0		Other	24(11.9)	5(1.6)	27(2.7)	72(7.2)	4(4.0)	g, A	132
1	Education level	Illiterate	33(16.3)	5(1.6)	1(0.1)	0(0)	1(1.0)	ul tra	40
2		Primary school	94(46.5)	54(17.8)	40(4)	0(0)	8(8.0)	aini pe	196
3 4		Junior middle school	69(34.2)	217(71.4)	471(47.1)	0(0)	18(18.0)	ng, a	775
5		Senior high school	6(3)	28(9.2)	193(19.3)	472(47.2)	19(19.0)	and	718
		Junior college	0(0)	0(0)	292(29.2)	527(52.7)	54(54.0)	sim	873
7 3		Bachelor's degree or above	0(0)	0(0)	3(0.3)	1(0.1)	0(0)	nilar	4
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Table 2 Th	he receive			aracteris	tic curves for I	-				
Reagents	Results	Rest					neters of ROC			
		-	+	AUC	95% CI	Sensitivity	Specificity	Youden index	<i>p</i>	
А	-	2553	2	0.960	0.952-0.968	92.16	99.92	0.921	< 0.001	
	+	4	47							
В	-	2555	0	1.000	0.999-1.000	100.00	100.00	1.000	< 0.001	
	+	0	51							
12/16								erie		

Table 3 Acceptance of HI	V-1 antibody testing m	ethods, acce					
Questions	Classification	STUs	PW	opulation [n (% Subjects undergoing VCT	Persons with IDU	FSWs	χ^2
Reagent types	Blood	781(78.1)	599(59.9)	85(85.0)	74(24.3)	88(43.6)	430.49
	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)	494.97
	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)	152.71
	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)	245.96
	No	238(23.8)	549(54.9)	17(17.0)	161(53.0)	96(47.5)	

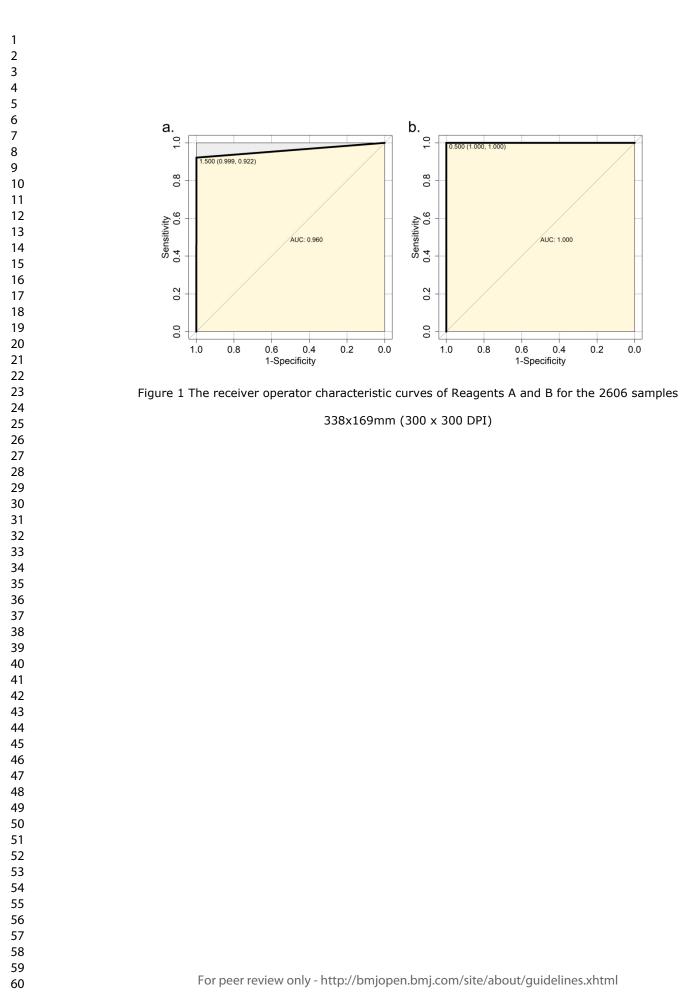
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40 41	321	10.	Lv Y, Zhu Q, Xu C, <i>et al.</i> Spatiotemporal Analysis of Online Purchase of HIV Self-testing Kits in
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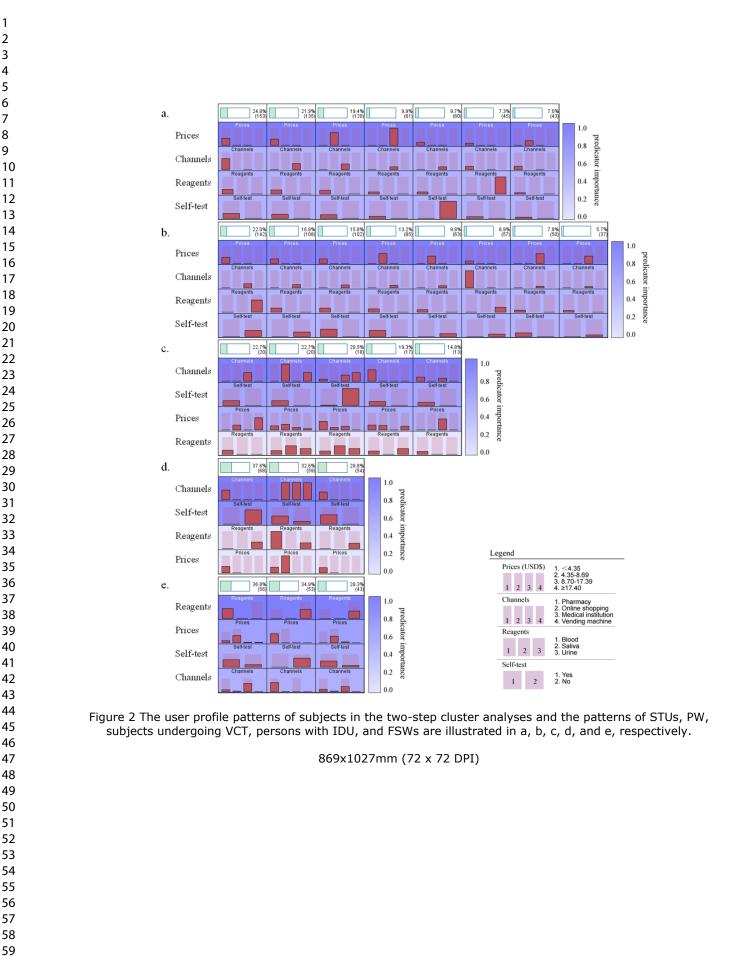
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Supplemental Table S1 Th				-		/ 2	
Groups	Reagent A		Reager		Reagen		total
	-	+	-	+	-	+	
FSWs	201(99.5)	1(0.5)	201(99.5)	1(0.5)	201(99.5)	1(0.5)	202
Persons with IDU		15(4.9)	289(95.1)	15(4.9)	289(95.1)	15(4.9)	304
PW	997(99.7)	3(0.3)	999(99.9)	1(0.1)	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		0(30.0)	66(66.0)	34(34.0)	66(66.0)	34(34.0)	100
Total	2557(98.1) 4	49(1.9)	2555(98.0)	51(2.0)	2555(98.0)	51(2.0)	2606
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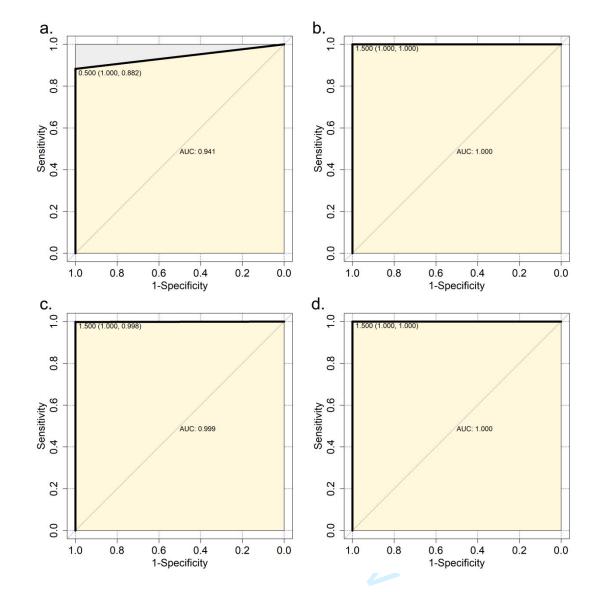
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<i>p</i> .001 .001 .001	.001	.001	.001	.001	<i>p</i> .001			
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				R	eagent A			Re	eagent B	
Group	Reference	Result	-	+	kappa	р	-	+	kappa	<i>p</i> <0.00 <0.00 <0.00
FSWs	Reagent C	-	201	0	1.000	< 0.001	201	0	1.000	< 0.00
		+	0	1			0	1		
IDU	Reagent C	-	289	0	1.000	< 0.001	289	0	1.000	<0.00
		+	0	15	0.400	-0.001	0	15	1 000	-0.0(
PW	Reagent C		997	2	0.499	< 0.001	999 0	0	1.000	<0.00
STUs	Reagent C	+	0 1000	1 0	_	_	1000	1 0	_	
5105	Reagent C	+	0	0			0	0		
Subjects undergoing VCT	Reagent C	-	66	0	0.908	< 0.001	66	0	1.000	<0.00
		+	4	30			0	34		
Total	Reagent C	-	2553	2	0.939	< 0.001	2555	0	1.000	<0.00
		+	4	47			0	51		

Preceiver operator characteristic curves for Reagent A in each group Reference Reagent A Statistical parameters of ROC curves - 66 0 0.941 0.876-0.978 88.24 100.00 0.882 + 4 30 - - - 66 0 0.941 0.876-0.978 88.24 100.00 0.882 + 4 30 -
Reagent A Statistical parameters of ROC of the statistica
Reagent A Statistical parame Reference Reagent A Statistical parame - 4 AUC 95% CI Sensitivity - 66 0 0.941 $0.876-0.978$ 88.24 + 4 30 - - - 289 0 1.000 $0.999-1.000$ 100.00 + 0 15 - - - 997 2 0.999 $0.997-1.000$ 99.80 + 0 1 - - - - 201 0 1.000 $0.999-1.000$ 1.000 + 0 1 - - - - 10000 0 - - -
Reference Reagent A Stat - + AUC 95% CI - 66 0 0.941 0.876-0.978 + 4 30 - - - 289 0 1.000 0.999-1.000 + 0 15 - - - 997 2 0.999 0.997-1.000 + 0 1 - - - 201 0 1.000 0.999-1.000 + 0 1 - - - 1000 0 - -
$\begin{array}{c c c c c c c } \hline Reagent A \\ \hline - & + & AUC \\ \hline - & 66 & 0 & 0.941 \\ + & 4 & 30 \\ - & 289 & 0 & 1.000 \\ + & 0 & 15 \\ - & 997 & 2 & 0.999 \\ + & 0 & 1 \\ - & 201 & 0 & 1.000 \\ + & 0 & 1 \\ - & 1000 & 0 & - \end{array}$
$\begin{tabular}{ c c c c } \hline Reagent A \\ \hline Reference & \hline - & + \\ \hline - & 66 & 0 \\ + & 4 & 30 \\ - & 289 & 0 \\ + & 0 & 15 \\ - & 997 & 2 \\ + & 0 & 1 \\ - & 201 & 0 \\ + & 0 & 1 \\ - & 1000 & 0 \\ \hline \end{tabular}$
Reference R - + - + - + - + - - - - - - - - - - -

FSWs 3 0.70 54.00 1.00 0.53 0.69 0.99 a: Clustering Fit quality ranged from -1 to 1, where 0.5-1 is good and ≥ 0.51 is excellent a a a a a b b a a a a b a a a a a a a a b a	Supplemental Table S4 T	The user pro	files of different	t populations	logarang m v i		.8	
clustersFit quality aAICreagent typeschannelspricesself &STUs71.00126.000.500.501.000.30PW81.00144.000.500.501.000.36Subjects undergoing VCT50.50197.88<0.011.000.540.66Persons with IDU30.8054.000.031.000.010.36FSWs30.7054.001.000.530.690.37At Clustering Fit quality ranged from -1 to 1, where 0.5-1 is good and ≥0.51 is excellent0.510.500.50b: Variable importance scores ranged from 0 to 1, with 0 being the lowest and 1 being the highest0.510.500.50At training Fit quality ranged from 0 to 1, with 0 being the lowest and 1 being the highest0.510.500.50		Clustering model parameters		Predictor importance (Pi) ^b			TOP	
PW 8 1.00 144.00 0.50 0.50 1.00 0.50 Subjects undergoing VCT 5 0.50 197.88 <0.01 1.00 0.54 0.69 Persons with IDU 3 0.80 54.00 0.03 1.00 0.01 0.56 FSWs 3 0.70 54.00 1.00 0.53 0.69 0.37 at: Clustering Fit quality ranged from -1 to 1, where 0.5-1 is good and ≥ 0.51 is excellent	ropulation	clusters	Fit quality ^a	AIC	reagent types	channels	prices	self-
Subjects undergoing VCT 5 0.50 197.88 <0.01 1.00 0.54 0.00 Persons with IDU 3 0.80 54.00 0.03 1.00 0.01 0.00 PSWs 3 0.70 54.00 1.00 0.53 0.69 0.00 the Clustering Fit quality ranged from -1 to 1, where 0.5-1 is good and ≥0.51 is excellent to: Variable importance scores ranged from 0 to 1, with 0 being the lowest and 1 being the highest of the transformer of the lowest and 1 being the highest of the highest of the lowest and 1 being the highest of t	STUs	7	1.00	126.00	0.50	0.50	1.00	0.5
Persons with IDU 3 0.80 54.00 0.03 1.00 0.01 0.05 FSWs 3 0.70 54.00 1.00 0.53 0.69 0.05 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 0.50 million of the lowest and 1 being the highest 0.50 million of the lowest and 1 being the highest 0.50 million of the lowest and 1 being the highest 0.50 million of the lowest and 1 being the highest 0.50 million of the lowest and 1 being the highest 0.50 million of the lowest and 1 being the highest 0.50 million of the lowest and 1 being the highest 0.50 million of the lowest and 1 being the highest 0.50 million of the lowest and 1 being the highest 0.50 million of the lowest 0.50 millio	PW	8	1.00	144.00	0.50	0.50	1.00	0.5
FSWs 3 0.70 54.00 1.00 0.53 0.69 0.87 In: Clustering Fit quality ranged from -1 to 1, where 0.5-1 is good and ≥0.51 is excellent 50<	Subjects undergoing VCT	5	0.50	197.88	< 0.01	1.00	0.54	0.6
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 At training, and similar technologi, and similar technologi, and similar technolo	Persons with IDU	3	0.80	54.00	0.03	1.00	0.01	0.8
b: Variable importance scores ranged from 0 to 1, with 0 being the lowest and 1 being the highest	FSWs	3	0.70	54.00	1.00	0.53	0.69	0.5
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Supplemental Figure 1 The ROCs of urine HIV-1 antibody reagent in VCTs(a), IDUs(b), PWs(c), and FSWs(d) Groups

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

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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: This study has evaluated the diagnostic validity of urine HIV-1 rapid test kits in screening both the 1. 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. No positive samples were found among the students, and therefore, ROC curves could not be plotted 3. for this subgroup. 1/21

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34 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.

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62 2. Materials and methods

63 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.

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91 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 DetermineTM HIV1/2 (colloidal selenium), was packaged as a rapid test kit and
manufactured by Abbott (20163400427); and (3) Reagent C, named GENscreenTM ULTRA HIV Ag-Ab
(Enzyme-Linked Immunosorbent Assay, ELISA), which was manufactured by Bio-Rad (72388C).

97 HIV antibody tests were divided into on-site tests (for Reagents A and B) and laboratory tests (for Reagent 98 C only). Reagents A and B were used to test for HIV-1 antibodies in urine samples and peripheral blood 99 samples taken from fingertips, respectively. Reagent B is the most common testing method for HIV-1 100 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.

114 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.

118 The main data management and statistical software used in this study included EPIDATA v3.1, Microsoft

119 Excel 2019, R v4.1.0, RStudio v1.4. 1103, and IBM SPSS v26.0. The sensitivity, specificity, receiver

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120 operator characteristic (ROC) curve, and area under the curve (AUC) were used to assess the diagnostic 121 validity of the urine HIV-1 antibody reagents in the on-site screening of different populations, these 122 processes are synchronized in the ROC analysis module of SPSS and the PROC package of the R language. 123 The two-step cluster analysis method in SPSS was used to evaluate the intentionality and user profiles of 124 the study subjects regarding HIV antibody reagent types, acceptable prices, purchase channels, and self-125 tests. The level of statistical significance was set at α =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.

130 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. Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

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**.

147 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 6/21

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different populations

178 The willingness regarding HIV-1 antibody test reagent types (χ^2 =430.498, p<0.001), purchase channels 179 (χ^2 =494.970, p<0.001), acceptable prices (χ^2 =152.710, p<0.001), and self-tests (χ^2 =245.966, p<0.001) were 180 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.

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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. 8/21

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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.

234 Considering the complexities and psychologically protective behaviours of some subjects undergoing 235 VCT, it may be more appropriate to choose an antigen-antibody combined reagent with higher sensitivity 236 and specificity to reduce the possibility of false negatives in some cases where it is difficult for physicians 237 treating these subjects to obtain true and accurate information[34, 35]. Some subjects with significant 238 psychological fear of HIV but no high-risk exposure may consider using noninvasive urine reagent strips to 239 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 9/21

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

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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.

291 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 noncommercial use and reasonable purposes, anonymised data of the current work can be obtained from the corresponding author.

302 8. Findings

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309 9. Ethics statement

310 This study was approved by the Ethics Committee of the Guangxi Zhuang Autonomous Region Center for

311 Disease Control and Prevention (approval number GXIRB2019-0047).

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	30-39	68(33.7)	126(41.4)	417(41.7)	41(4.1)	18(18.0)	oad and	670
	≥40	121(59.9)	160(52.6)	21(2.1)	0(0)	23(23.0)	ed f dat	325
nicity	Han	120(59.4)	279(91.8)	692(69.2)	526(52.6)	56(56.0)	ed from http://bmjopen.bmj.com/ on Jun ur (ABES) . data mining, Al training, and similar tec	1673
	Zhuang	58(28.7)	20(6.6)	281(28.1)	402(40.2)	40(40.0)	inin	801
	Other	24(11.9)	5(1.6)	27(2.7)	72(7.2)	4(4.0)	ġ, A	132
cation level	Illiterate	33(16.3)	5(1.6)	1(0.1)	0(0)	1(1.0)	omjo vl tra	40
	Primary school	94(46.5)	54(17.8)	40(4)	0(0)	8(8.0)	aini	196
	Junior middle school	69(34.2)	217(71.4)	471(47.1)	0(0)	18(18.0)	ng, i	775
	Senior high school	6(3)	28(9.2)	193(19.3)	472(47.2)	19(19.0)	nj.c	718
	Junior college	0(0)	0(0)	292(29.2)	527(52.7)	54(54.0)	sim	873
	Bachelor's degree or above	0(0)	0(0)	3(0.3)	1(0.1)	0(0)	ilar	4
ıl		202	304	1000	1000	100	Jun	2606
1	For peer re	view only - I	nttp://bmjope	en.bmj.com/	site/about/g	uidelines.x	13, 2025 at Agence Bibliographique nologies.	

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1 2 3 4		Table 2 The per
2 3 4 5 6 7		Groups
8		FSWs
9 10		Persons with ID
11 12		PW
13		STUs Subjects underg
14 15		Total
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36	313 314	a. Reagent C wa
 37 38 39 40 41 42 43 44 45 		13/21

rformance of two HIV-1 antibody reagents in field testing [n (%)]

C	Reagen	it A	Reager	nt B	Reagen	total	
Groups	-	+	-	+	-	+	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

Table 3 Consistency check of two HIV-1 antib	body reagents in diverse populations ^a
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Group	Reference	Reage	ent A	Reage	ent A	
Gloup	Reagent	kappa	р	kappa	р	
FSWs	С	1.000	< 0.001	1.000	< 0.001	
IDU	С	1.000	< 0.001	1.000	< 0.001	
PW	С	0.499	< 0.001	1.000	< 0.001	
STUs	С	-	-	-	-	
Subjects undergoing VCT	С	0.908	< 0.001	1.000	< 0.001	
Total	С	0.939	< 0.001	1.000	< 0.001	

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' of 30					BMJ Ope	'n	
	Table 4 The 1	receiver ope	rator characteristic cu		and B in the 2606 sub	jects ^a	
	Reagents ^b	AUC	95% CI	Sensitivity	Specificity	Youden index	р
	A	0.96	0.952-0.968	92.16	99.92	0.921	< 0.00
	В	1	0.999-1.000	100	100	1	< 0.00
					ted in Table 4(detail)		
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Table 5 The receiver	operator	characteristic	curves for	Reagent Δ	in each	oroun ^a
	operator	characteristic	cui ves 101	Reagent A	in cach	group

Table 5 The receiver operator	characteristic					
Groups	AUC	Statis 95% CI	tical parameter Sensitivity	rs of ROC curv Specificity	Ves ^b Youden index	(
Subjects undergoing VCT	0.941	0.876-0.978	88.240	100.000	0.882	
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	-	-	5	-	-	-
: The reference standard is Rea		-	resented in Tab		1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1	•
: The reference standard is Rea		-				-

			Ро	opulation [n (%	ó)]		
Questions	Classification	STUs	PW	Subjects undergoing VCT	Persons with IDU	FSWs	χ^2
Reagent types	Blood	781(78.1)	599(59.9)	85(85.0)	74(24.3)	88(43.6)	430.49
	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)	494.97
	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)	152.71
	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)	245.96
	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	f different po	opulations regard	ing HIV-1 aı	ntibody testing met	hods, channels,	and prices	
Population	Clust	ering model parai	neters		Predictor impo	ortance ^b	ng fo
Topulation	clusters	Fit quality ^a	AIC	reagent types	channels	prices	sel ^t uses
STUs	7	1.00	126.00	0.50	0.50	1.00	Sel Lusës rëlatëd të të text a
PW	8	1.00	144.00	0.50	0.50	1.00	0 <mark>6</mark> a
Subjects undergoing VCT	5	0.50	197.88	< 0.01	1.00	0.54	0
Persons with IDU	3	0.80	54.00	0.03	1.00	0.01	0
FSWs	3	0.70	54.00	1.00	0.53	0.69	ext and
							nining, Al train
				a ≥0.51 is excellent owest and 1.00 be			mining, Al training, and similar technologies.

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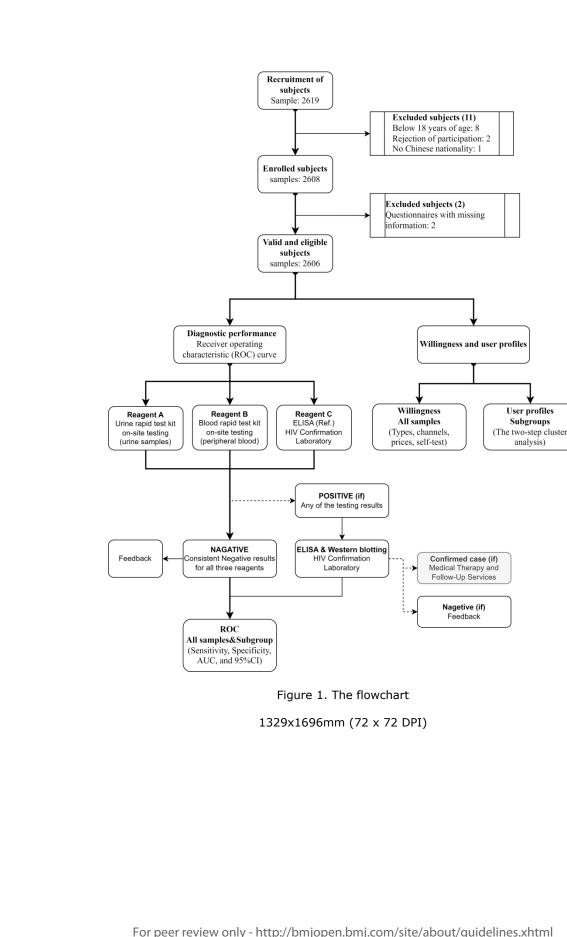
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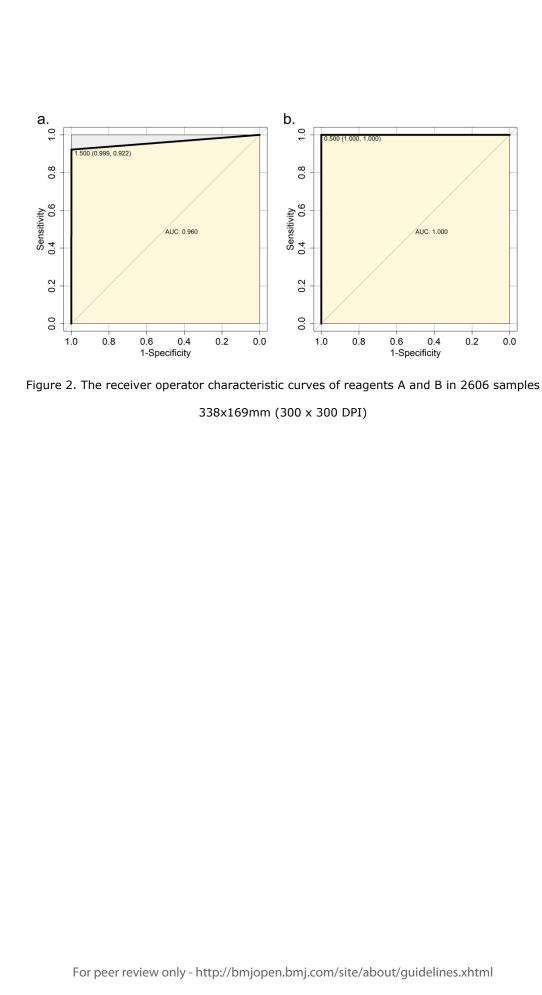
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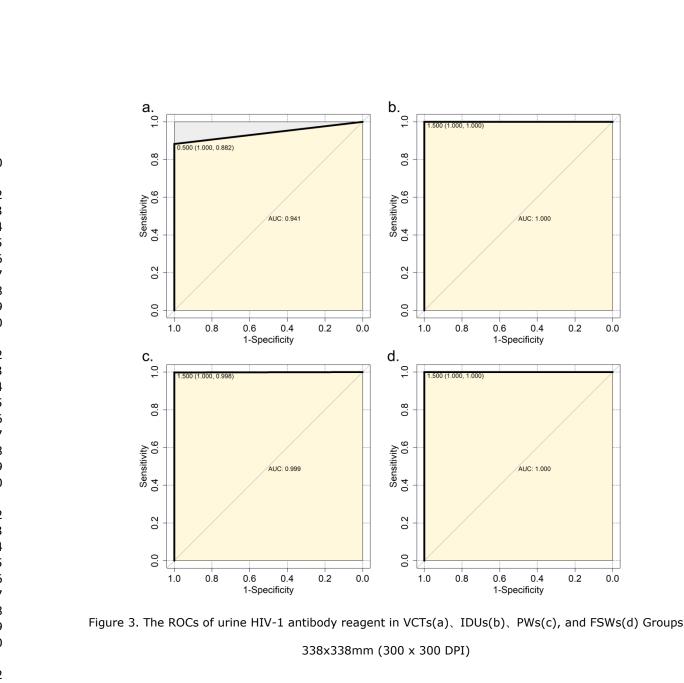
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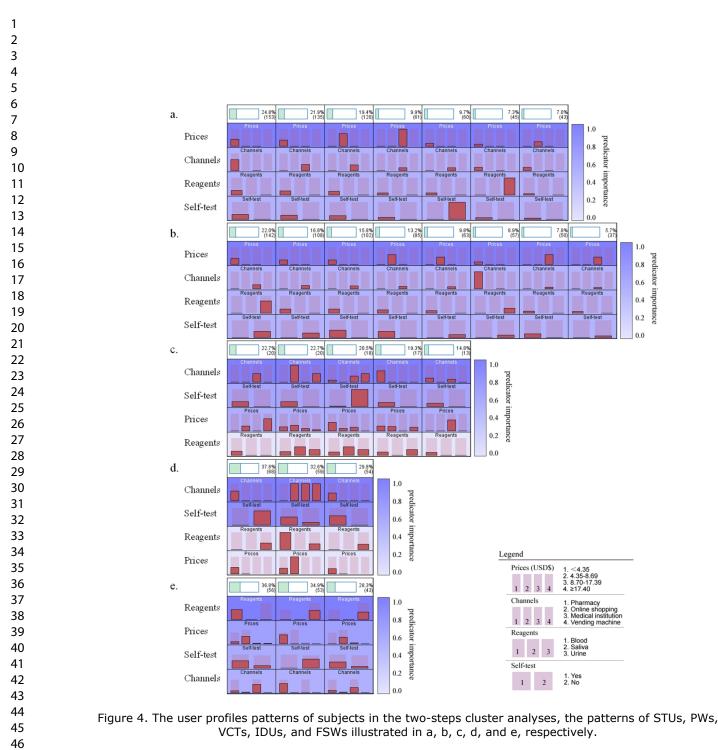
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Group	Reference	Result ^a	-	+	kappa	р	-	+	kappa	
FSWs	Reagent C	-	201	0	1.000	< 0.001	201	0	1.000	<i>p</i> uses <0.00
		+	0	1			0	1		late
IDU	Reagent C	~ -	289	0	1.000	< 0.001	289	0	1.000	<0.00
			0	15			0	15		<0.00
PW	Reagent C		997	2	0.499	< 0.001	999	0	1.000	<0.00 Ta nd
		+	0	1			0	1		a as
STUs	Reagent C	-	1000	0	-	-	1000 0	0	-	ata r
Subjects undergoing VCT	Reagent C	+	0 66	0 0	0.908	< 0.001	66	0 0	1.000	nin ===================================
Subjects undergoing VC1	Reagent C	+	4	30	0.900	<0.001	0	34	1.000	, 9
Total	Reagent C	-	2553	2	0.939	<0.001	2555	0	1.000	<u>₹</u> ±0.00
	0	+	4	47			0	51		<0.00#raini
a: Table 3 (detail) presents		,								ig, and similar technologies.

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Reagents Results a - + 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 - - 2555 0 1.000 0.999-1.000 100.00 100.00 1.000 <0.001 + 0 51 -	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Table 4 The receiver		Resu					eters of ROC		
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Table 5 The receiver operator characteristic curves for Reagent A in each group (det	ail)
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Page 31 of 30

Section & Topic	No	Item	Reported on pag
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy	Lines 1-2, Page 1
		(such as sensitivity, specificity, predictive values, or AUC)	
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions	Lines 3-34, Page
		(for specific guidance, see STARD for Abstracts)	
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	Lines 41-55, Page
	4	Study objectives and hypotheses	Lines 56-60, Page
METHODS			
Study design	5	Whether data collection was planned before the index test and reference standard	Lines 70-74, Page
	_	were performed (prospective study) or after (retrospective study)	
Participants	6	Eligibility criteria	Lines 102-107, Pa
	7	On what basis potentially eligible participants were identified	Lines 64-69, Page
	8	(such as symptoms, results from previous tests, inclusion in registry) Where and when potentially eligible participants were identified (setting, location and dates)	Lines 70-74, Page
	8 9	Whether participants formed a consecutive, random or convenience series	Lines 70-74, Page
Test methods	9 10a	Index test, in sufficient detail to allow replication	Lines 77-78, Page
	10a 10b	Reference standard, in sufficient detail to allow replication	Lines 92-111, Pag
		Rationale for choosing the reference standard (if alternatives exist)	
	11	Definition of and rationale for test positivity cut-offs or result categories	Lines 108-113, Pa Lines 102-107, Pa
	12a	of the index test, distinguishing pre-specified from exploratory	LINES 102-107, P
	12b	Definition of and rationale for test positivity cut-offs or result categories	Lines 108-111, P
	120	of the reference standard, distinguishing pre-specified from exploratory	
	13a	Whether clinical information and reference standard results were available	Lines 108-111, P
		to the performers/readers of the index test	
	13b	Whether clinical information and index test results were available	Lines 108-111, P
		to the assessors of the reference standard	
Analysis	14	Methods for estimating or comparing measures of diagnostic accuracy	Lines 118-122, P
	15	How indeterminate index test or reference standard results were handled	Lines 102-105, P
	16	How missing data on the index test and reference standard were handled	Lines 126-129, Pa
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	Lines 102-107, P
	18	Intended sample size and how it was determined	Lines 87-90, Page
RESULTS			
Participants	19	Flow of participants, using a diagram	Line 143, Page 5
	20	Baseline demographic and clinical characteristics of participants	Lines 139-143, P
	21a	Distribution of severity of disease in those with the target condition	Lines 139-143, P
	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, Pa
Test results	23	Cross tabulation of the index test results (or their distribution)	Table 3, Page 15
		by the results of the reference standard	
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	Lines 167-175, Pa
	25	Any adverse events from performing the index test or the reference standard	Line 112, Page 5
DISCUSSION	~~		Lines 200, 200, 7
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	Lines 280-286, Pa 10-11
	27	Implications for practice, including the intended use and clinical role of the index test	Lines 288-290, P
OTHER			
INFORMATION			
	28	Registration number and name of registry	Lines 309-311, P
	29	Where the full study protocol can be accessed	Lines 299-301, Pa
	30	Sources of funding and other support; role of funders	Lines 303-308, Pa
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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 <u>http://www.equator-network.org/reporting-guidelines/stard.</u>



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

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

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1 2	Diagnostic Performance Evaluation of Urine HIV-1 Antibody Rapid Test Kits in A Real-life Routine Care Setting in China
2	Abstract
4	Objectives: To evaluate the diagnostic performance of urine human immunodeficiency virus (HIV)
5	antibody rapid test kits in screening diverse populations and to analyse subjects' willingness regarding
6	reagent types, purchase channels, acceptable prices, and self-testing.
7	Designs: Diagnostic accuracy studies
8	Participants: A total of 2606 valid and eligible samples were collected in the study, including 202 samples
9	from female sex workers (FSWs), 304 persons with injection drug use (IDU), 1000 pregnant women (PW),
0	100 subjects undergoing voluntary HIV counselling and testing (VCT), and 1000 students in higher
1	education schools or colleges (STUs). Subjects should simultaneously meet the following inclusion criteria:
2	(1) being at least 18 years old and in full civil capacity; (2) signing an informed consent form; and (3)
3	providing truthful identifying information to ensure the subjects and their samples are unique.
4	Results: The sensitivity, specificity, and area under the curve (AUC) of the urine HIV-1 antibody rapid test
5	kits were 92.16%, 99.92%, and 0.960 (95% confidence interval (CI): 0.952-0.968, p<0.001), respectively,
6	among 2606 samples collected during on-site screenings. The kits showed good diagnostic performance in
7	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,
8	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
9	subjects undergoing VCT was 0.941 (95% CI: 0.876-0.978, p<0.001). The "acceptable price" had the
20	greatest influence on STUs (Pi=1.000) and PW (Pi=1.000), the "purchase channel" had the greatest
21	influence on subjects undergoing VCT (Pi=1.000) and persons with IDU (Pi=1.000), and the "reagent types
22	had the greatest influence on FSWs (Pi=1.000).
23	Conclusions: The rapid urine test kits showed a good diagnostic validity in practical applications, despite a
24	few cases involving misdiagnosis and underdiagnosis.
25	Keywords: HIV, urine, rapid test kits, ROC
26	Strengths and limitations of this study:
27	1. This study has evaluated the diagnostic validity of urine HIV-1 rapid test kits in screening both the
28	general population and high-risk populations.
29	2. Cluster analysis provides a clear profile of the main concerns and selection preferences of the different
30	populations when they choose HIV test reagents.
31	3. No positive samples were found among the students, and therefore, ROC curves could not be plotted
32	for this subgroup.
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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.

62 2. Materials and methods

63 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.

91 The urine rapid test reagent AUC area was predicted to be between 0.85 and 0.98, and the confidence
92 level (1-alpha), confidence interval width, sample dropout rate, and screening sample size were set to 0.95,
93 0.10, 5%, and 2,500 cases, respectively, requiring a positive sample size of 5-34 cases as estimated by the
94 PASS 2015 software package.

95 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 DetermineTM HIV1/2 (colloidal selenium), was packaged as a rapid test kit and
manufactured by Abbott (20163400427); and (3) Reagent C, named GENscreenTM 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

105 100 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.

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120 2.3 Data management and statistical analysis

121 The subjects' information, including basic information such as their name, sex, date of birth, occupation 122 type, education level, and ethnicity, as well as their willingness regarding HIV-1 antibody testing methods, 123 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 α =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.

136 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

146 A total of 2606 valid and eligible samples were collected from the FSWs, persons with IDU, PW, STUs,

147 and subjects undergoing VCT included in this study, with 202 (7.7%), 304 (11.7%), 1000 (38.4%), 1000

148 (38.4%), and 100 (3.8%) collected samples, respectively. No adverse events were reported. The flowchart 5/23

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

150 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**.

153 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

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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 (χ^2 =430.498, p<0.001), purchase channels (χ^2 =494.970, p<0.001), acceptable prices (χ^2 =152.710, p<0.001), and self-tests (χ^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 nonselftesting" 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.

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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. 8/23

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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.

240 Considering the complexities and psychologically protective behaviours of some subjects undergoing 241 VCT, it may be more appropriate to choose an antigen-antibody combined reagent with higher sensitivity 242 and specificity to reduce the possibility of false negatives in some cases where it is difficult for physicians 243 treating these subjects to obtain true and accurate information[30, 31]. Some subjects with significant 244 psychological fear of HIV but no high-risk exposure may consider using noninvasive urine reagent strips to 245 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].

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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 9/23

260 positive rate of urine rapid test reagents in the PW population is acceptable given the considerable 261 advantages of the noninvasive operation. No positive case was found in the STUs, which we believe is 262 related to the very low prevalence of HIV infection in this population. Thus, the validity of the urine rapid 263 reagent in STUs requires a larger sample size in future studies.

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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

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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.

297 **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.

304 7. Data sharing statement

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

308 **8. Findings**

This work was supported by the National Natural Science Foundation of China (82160636 and 82260670),
Guangxi Natural Science Foundation Project (2020GXNSFAA159020), Guangxi Key Laboratory of AIDS
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(AB19245044), Guangxi Bagui Honor Scholarship, Ministry of Science and Technology of China
(2022YFC2305200 and 2018ZX10715008), and Guangxi Medical and Health Key Discipline Construction
Project.

315 **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).

318 **10. Competing Interest statement**

9 319 No competing interest

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	information of the 2606 FSWs, pers		BN	/J Open			njopen-2023-078694 on d by copyright, inclugi 글	
Table 1 The basic	information of the 2606 FSWs, pers						incluging	
Variables	Subgroups	The sample FSWs	Persons with IDU	h population g	group [n (%)] STUs	Subjects undergoin	h 24 Februa Ensi ng for uses	To
Sex	Male	0(0)	256(84.2)	0(0)	255(25.5)	48(48.0)	ary 2 seigi srel;	55
	Female	202(100)	48(15.8)	1000(100)	745(74.5)	52(52.0)	ry 2024 eignem related	20
Age	<20	1(0.5)	2(0.7)	38(3.8)	846(84.6)	2(2.0)	се.	88
e	20-29	12(5.9)	16(5.3)	524(52.4)	113(11.3)	57(57.0)	Downloaded t int Superieur (to text and dated	72
	30-39	68(33.7)	126(41.4)	417(41.7)	41(4.1)	18(18.0)	loac and	67
	≥40	121(59.9)	160(52.6)	21(2.1)	0(0)	23(23.0)	d da	32
Ethnicity	Han	120(59.4)	279(91.8)	692(69.2)	526(52.6)	56(56.0)	from (ABE: ata mii	16
5	Zhuang	58(28.7)	20(6.6)	281(28.1)	402(40.2)	40(40.0)	from http://bmjopen.bmj.com/ (ABES) . ta mining, Al training, and sim	80
	Other	24(11.9)	5(1.6)	27(2.7)	72(7.2)	4(4.0)	ng, .	13
Education level	Illiterate	33(16.3)	5(1.6)	1(0.1)	0(0)	1(1.0)	Al tr	40
	Primary school	94(46.5)	54(17.8)	40(4)	0(0)	8(8.0)	aini'	19
	Junior middle school	69(34.2)	217(71.4)	471(47.1)	0(0)	18(18.0)	ing,	77
	Senior high school	6(3)	28(9.2)	193(19.3)	472(47.2)	19(19.0)	anc mj.o	71
	Junior college	0(0)	0(0)	292(29.2)	527(52.7)	54(54.0)	i sir	87
	Bachelor's degree or above	0(0)	0(0)	3(0.3)	1(0.1)	0(0)	ed from http://bmjopen.bmj.com/ on Jun ur (ABES) . data mining, Al training, and similar tec	4
Total	5	202	304	1000	1000	100	Jun r tec	26
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5 6 7		Groups
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7	Reager	nt A	Reagen	nt B	Reagen	it C ^a	40401
Groups	-	+	-	+	-	+	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

Table 3 Consistency check of two HIV-1 a	antibody reagents in diverse subgroups ^a
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Group	Reference	Reage	ent A	Reage	ent A	
Joup	Reagent	kappa	р	kappa	р	
FSWs	С	1.000	< 0.001	1.000	< 0.001	
IDU	С	1.000	< 0.001	1.000	< 0.001	
PW	С	0.499	< 0.001	1.000	< 0.001	
STUs	С	-	-	-	-	
Subjects undergoing VCT	С	0.908	< 0.001	1.000	< 0.001	
Total	С	0.939	< 0.001	1.000	< 0.001	

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	Table 4 The 1	receiver ope	rator characteristic co	urves for Reagents A	and B in the 2606 sub	ojects ^a	p
				Statistical param	eters of ROC curves		
	Reagents ^b	AUC	95% CI	Sensitivity	Specificity	Youden index	р
	А	0.96	0.952-0.968	92.16	99.92	0.921	<0.001
	В	1	0.999-1.000	100	100	1	< 0.001
	a: Table 4 is a	a summary t	table and detailed res	ults have been presen	ted in supplementary	y Table 2.	
	b: The referer	nce standard	is Reagent C (ELISA	A)			
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Table 5 The receiver operator	characterist			-	. h	
Groups	AUC	95% CI	tical parameter 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 a b: The reference standard is Rea		-	resented in sur			<u> </u>
: Table 5 is a summary table a		-	resented in sur		- Fable 3.	0



			Po	opulation [n (%	ó)]		-
Questions	Classification	STUs	PW	Subjects undergoing VCT	Persons with IDU	FSWs	χ^2
Reagent types	Blood	781(78.1)	599(59.9)	85(85.0)	74(24.3)	88(43.6)	430.49
	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)	494.97
	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)	152.71
	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)	245.96
	No	238(23.8)	549(54.9)	17(17.0)	161(53.0)	96(47.5)	

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BMJ Open Table 7 The user profiles of different populations regarding HIV-1 antibody testing methods, channels, and prices Population Clustering model parameters Predictor importance ^b STUs 7 1.00 126.00 0.50 0.50 1.00 PW 8 1.00 144.00 0.50 0.50 1.00 050 Stubes undergoing VCT 5 0.50 197.88 <0.01 1.00 0.54 066 Persons with IDU 3 0.80 54.00 1.00 0.53 0.69 066 StUstering Fit quality ranged from -1 to 1, where 0.5-1 is good and ≥0.51 is excellent					BMJ Op	en		by col
Population Clustering model parameters Predictor importance b clusters Fit quality a AIC reagent types channels prices selection								pyright, i
clusters Fit quality ^a AIC reagent types channels prices selEt	Table 7 The user profiles c	of different p	opulations regard	ing HIV-1 aı	ntibody testing met	hods, channels,	and prices	ncludi
clusters Fit quality ^a AIC reagent types channels prices sel E	Population	Clust	tering model parai	meters		Predictor impo	ortance ^b	ng fi
STUs 7 1.00 126.00 0.50 0.50 1.00 00 PW 8 1.00 144.00 0.50 0.50 1.00 00 Subjects undergoing VCT 5 0.50 197.88 <0.01 1.00 0.54 00 Persons with IDU 3 0.80 54.00 0.03 1.00 0.01 00 FSWs 3 0.70 54.00 1.00 0.53 0.69 00 Clustering Fit quality ranged from -1 to 1, where 0.5-1 is good and ≥0.51 is excellent secolent		clusters	Fit quality ^a	AIC	reagent types	channels	prices	sel
PW 8 1.00 144.00 0.50 0.50 1.00 Operation of the second standard	STUs	7	1.00	126.00	0.50	0.50	1.00	0 65
Persons with IDU3 0.80 54.00 0.03 1.00 0.01 0.08 FSWs3 0.70 54.00 1.00 0.53 0.69 0.69 Clustering Fit quality ranged from -1 to 1, where $0.5-1$ is good and ≥ 0.51 is excellent 0.53 0.69 0.69 Custoriable importance scores ranged from 0 to 1, with 0 being the lowest and 1.00 being the highest 0.69 0.69	PW	8	1.00	144.00	0.50	0.50	1.00	0ල්
Persons with IDU 3 0.80 54.00 0.03 1.00 0.01 05 FSWs 3 0.70 54.00 1.00 0.53 0.69 05 It Clustering Fit quality ranged from -1 to 1, where 0.5-1 is good and ≥0.51 is excellent It is excellent 0.01 05 0.69 05 0.69 05 06 05 06 05 06 05 06 <t< td=""><td>Subjects undergoing VCT</td><td>5</td><td>0.50</td><td>197.88</td><td>< 0.01</td><td>1.00</td><td>0.54</td><td>0</td></t<>	Subjects undergoing VCT	5	0.50	197.88	< 0.01	1.00	0.54	0
FSWs 3 0.70 54.00 1.00 0.53 0.69 000 t: Clustering Fit quality ranged from -1 to 1, where 0.5-1 is good and ≥0.51 is excellent to the lowest and 1.00 being the highest the	Persons with IDU	3	0.80	54.00	0.03	1.00	0.01	05
t: 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 and similar recomposition of the store of the	FSWs	3	0.70	54.00	1.00	0.53	0.69	0
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36 37 38 39	 333 334 335 336 337 338 339 340 341 342 343 344 	BMJ Open Portuge Propagation of this study Figure 1. The flowchart of this study Figure 2. The receiver operator characteristic curves of tragents A and B in 2606 samples Figure 2. The receiver operator characteristic curves of tragents A and B in 2606 samples Figure 2. The receiver operator characteristic curves of tragents and blood trapid test reagents Figure 2. The receiver operator characteristic curves of unine rapid test reagents and blood trapid test reagents Figure 2. The user profiles patterns of subjects in the two-steps cluster analyses, the patterns of STUS, PWs, VCT, Terper, and FWS, illustrated in a, b, c, d, and e, respectively. Figure 4. The user profiles patterns of subjects in the two-steps cluster analyses. Figure 4. The user profiles patterns of different population subgroups by two-steps cluster analyses. Figure 4. The user profiles patterns of different population subgroups by two-steps cluster analyses. Figure 4. The user profiles of different population subgroups by two-steps cluster analyses. Figure 4. The user profiles of different population subgroups by two-steps cluster analyses. Figure 4. The user profiles patterns of subjects in the two-steps cluster analyses. Figure 4. The user profiles of different population subgroups by two-steps cluster analyses. Figure 4. The user profile patterns of subjects in the two-steps cluster analyses. Figure 4. The user profile patterns of user profiles of different population subgroups. Figure 4. The user profile patterns of user patterns of user patterns of user patterns of user profiles of different population subgroups. Figure 4. The user profile patterns of user patterns of user patterns of u
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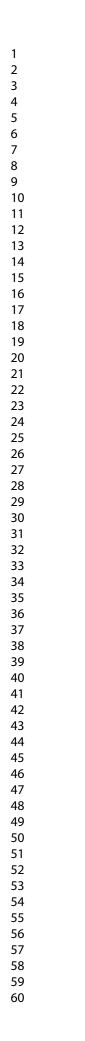
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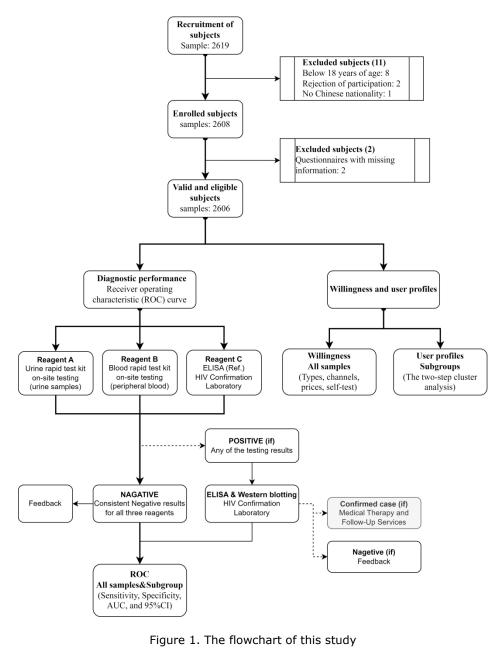
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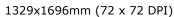
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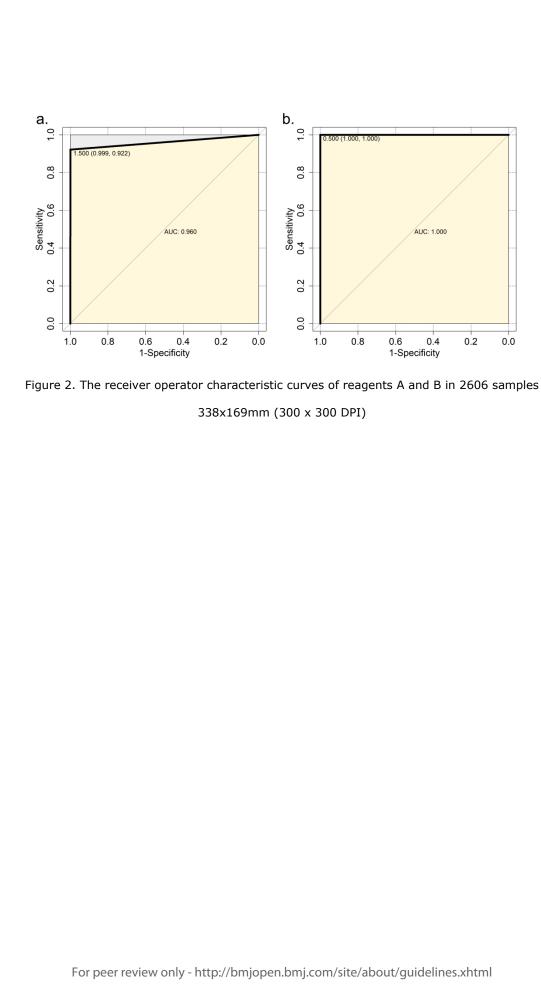
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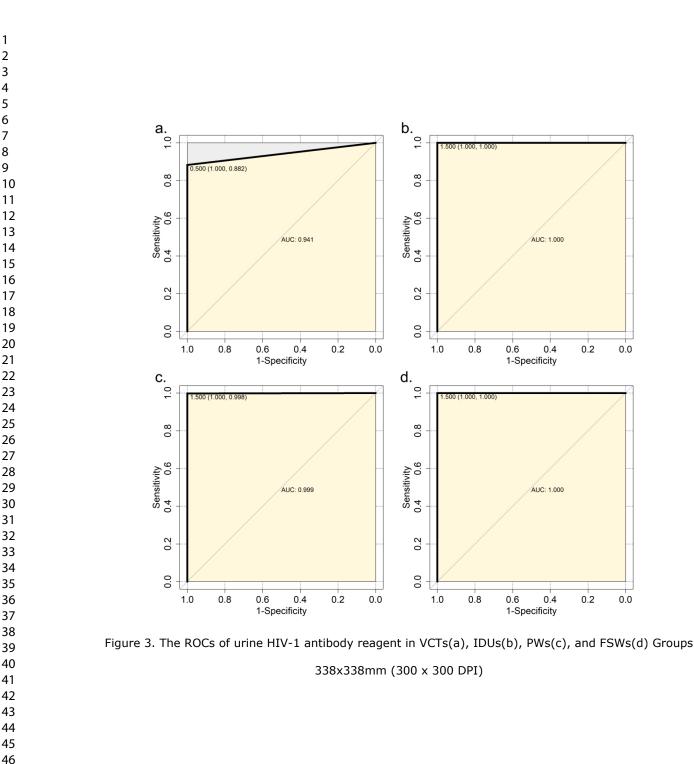




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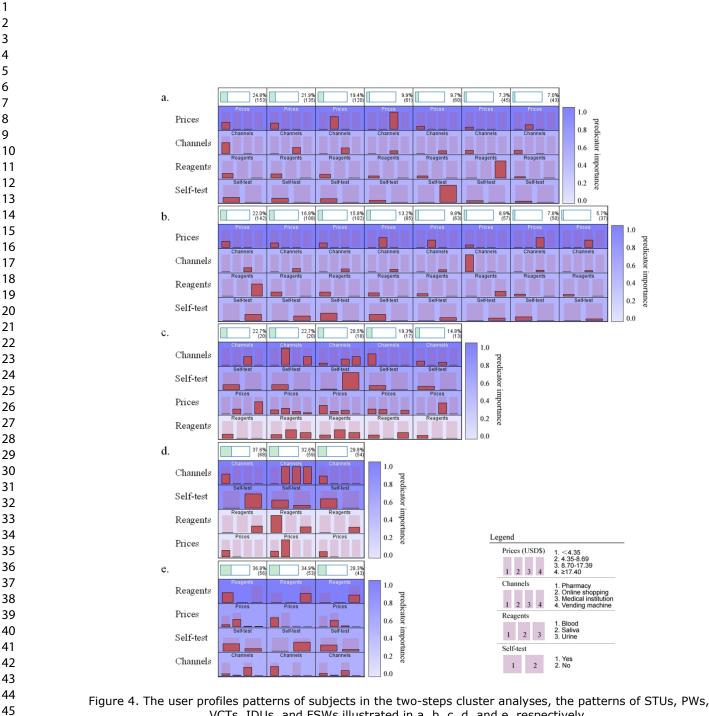
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VCTs, IDUs, and FSWs illustrated in a, b, c, d, and e, respectively.

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supplementary Table 1 Cons	sistency check of	of two HIV-1 a	antibody 1	reagen	ts in dive	rse subgrou	ps			by copyright, including
Group	Reference	Result ^a			eagent A				eagent B	tor u
_	Descent C		- 201	+ 0	<i>kappa</i>	<i>p</i>	- 201	+ 0	<i>kappa</i>	<i>p s</i> <0.00
FSWs	Reagent C	-	201	1	1.000	< 0.001	201	1	1.000	<0.00 1
DU	Reagent C		289	0	1.000	< 0.001	289	0	1.000	<0.00
		+	0	15			0	15		io te
PW	Reagent C	Qr	997	2	0.499	< 0.001	999	0	1.000	<0.00
		+	0	1			0	1		na a
STUs	Reagent C	-	1000	0	-	-	1000	0	-	Jata
		+	0	0			0	0		
Subjects undergoing VCT	Reagent C	-	66	0	0.908	< 0.001	66	0	1.000	<0.00
	D	+	4	30	0.020		0	34	1 000	, A
Fotal	Reagent C	- +	2553 4	2 47	0.939	< 0.001	2555 0	0 51	1.000	<0.004
a: supplementary Table 1 pr	esents the detai	led diagnostic	results fo	r Tabi	e 3.					ig, and similar technologies.

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supplementary Table 2	The receiver operator characte	eristic curves for Reagents A and B i	in the 2606 subjects
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Descents	Descrites a	Resu	ılts		Sta	tistical param	eters of ROC	curves	
Reagents	Results ^a	-	+	AUC	95% CI	Sensitivity	Specificity	Youden index	р
А	-	2553	2	0.960	0.952-0.968	92.16	99.92	0.921	< 0.001
	+	4	47						
В	-	2555	0	1.000	0.999-1.000	100.00	100.00	1.000	< 0.001
	+	0	51			Do)		

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

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supplementary Table 3 The	receiver oper	ator cha	ractei	ristic cur	ves for Reagen	t A in each gı	oup		uding
Groups	Reference	Reage	nt A		Star	tistical param	eters of ROC	curves	
-		-	+	AUC	95% CI	Sensitivity	Specificity	Youden index	ses
Subjects undergoing VCT	-	66	0	0.941	0.876-0.978	88.24	100.00	0.882	< 8
	+	4	30						lated
Persons with IDU	-	289	0	1.000	0.999-1.000	100.00	100.00	1.000	< 6
	+	0	15	6					text
PW	-	997	2	0.999	0.997-1.000	99.80	100.00	0.998	< ଥି ପ
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Page 33 of 32

Section & Topic	No	Item	Reported on pag
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy	Lines 1-2, Page 1
		(such as sensitivity, specificity, predictive values, or AUC)	
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions	Lines 3-34, Page
		(for specific guidance, see STARD for Abstracts)	
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	Lines 41-55, Page
	4	Study objectives and hypotheses	Lines 56-60, Page
METHODS			
Study design	5	Whether data collection was planned before the index test and reference standard	Lines 70-74, Page
		were performed (prospective study) or after (retrospective study)	
Participants	6	Eligibility criteria	Lines 102-107, Pa
	7	On what basis potentially eligible participants were identified	Lines 64-69, Page
	-	(such as symptoms, results from previous tests, inclusion in registry)	
	8	Where and when potentially eligible participants were identified (setting, location and dates)	Lines 70-74, Page
	9	Whether participants formed a consecutive, random or convenience series	Lines 77-78, Page
Test methods	10a	Index test, in sufficient detail to allow replication	Lines 62-129, Pag
	10b	Reference standard, in sufficient detail to allow replication	Lines 92-111, Pag
	11	Rationale for choosing the reference standard (if alternatives exist)	Lines 108-113, Pa
	12a	Definition of and rationale for test positivity cut-offs or result categories	Lines 102-107, Pa
		of the index test, distinguishing pre-specified from exploratory	
	12b	Definition of and rationale for test positivity cut-offs or result categories	Lines 108-111, Pa
		of the reference standard, distinguishing pre-specified from exploratory	
	13a	Whether clinical information and reference standard results were available	Lines 108-111, Pa
		to the performers/readers of the index test	
	13b	Whether clinical information and index test results were available	Lines 108-111, Pa
A		to the assessors of the reference standard	L'12
Analysis	14	Methods for estimating or comparing measures of diagnostic accuracy	Lines 118-122, Pa
	15	How indeterminate index test or reference standard results were handled	Lines 102-105, Pa
	16	How missing data on the index test and reference standard were handled Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	Lines 126-129, Pa
	17		Lines 102-107, Pa
	18	Intended sample size and how it was determined	Lines 87-90, Page
RESULTS	10	Flow of portigioants, using a diagram	
Participants	19	Flow of participants, using a diagram	Line 143, Page 5
	20 21-2	Baseline demographic and clinical characteristics of participants Distribution of severity of disease in those with the target condition	Lines 139-143, Pa Lines 139-143, Pa
	21a 21b	Distribution of severity of disease in those with the target condition	Lines 139-143, Pa
	210	Time interval and any clinical interventions between index test and reference standard	Lines 105-107, Pa
Test results	22	Cross tabulation of the index test results (or their distribution)	Table 3, Page 15
iest iesuils	23	by the results of the reference standard	rable 5, Fage 15
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	Lines 167-175, Pa
	24 25	Any adverse events from performing the index test or the reference standard	Line 112, Page 5
DISCUSSION		Any develop events nom performing the mack test of the reference standard	Ence IIZ, Fage J
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	Lines 280-286, Pa
		stary minuterior, mereaning sources of potential bias, statistical ancertainty, and generalisability	10-11
	27	Implications for practice, including the intended use and clinical role of the index test	Lines 288-290, Pa
OTHER			
INFORMATION			
	28	Registration number and name of registry	Lines 309-311, Pa
	29	Where the full study protocol can be accessed	Lines 299-301, Pa
	30	Sources of funding and other support; role of funders	Lines 303-308, Pa

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 <u>http://www.equator-network.org/reporting-guidelines/stard.</u>

