

## Original Article

# Detection of human immunodeficiency virus type-1 antibody from oral mucosal transudate using gelatin particles aggregation less-sensitive method

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**Abstract:** Health care workers in stomatology department are at a high risk of occupational HIV infection, and so a non-invasion detecting method with higher bio-safety is essential to be applied before therapy. The oral mucosal transudate (OMT) and venous blood specimens were collected in three groups, including HIV-1 antibody-positive group, high-risk group and general group. Simultaneously the OMT samples were detected by gelatin particles aggregation less-sensitive (PA-LS), and the serum samples were screened by ELISA and confirmed by Western blot (WB). According to the final results of ELISA/WB, it was to evaluate the sensitivity, specificity, omission diagnostic rate, mistake diagnostic rate, positive predictive value and negative predictive value of PA-LS method. For HIV-1 antibody-positive group, the sensitivity of PA-LS detecting HIV-1 antibody in OMT specimens was 100%, the omission diagnostic rate was 0. For high-risk group, the sensitivity of PA-LS detecting HIV-1 antibody on OMT samples was 100%, the specificity was 97.49%, the omission diagnostic rate was 0, the mistake diagnostic rate was 2.51%, the positive predictive value was 88.52% and the negative predictive value was 100%. Compared to ELISA detecting HIV-1 antibody in serum specimens, the consistency of PA-LS detecting HIV-1 antibody positive on OMT samples was excellent ( $Kappa > 0.8$ ), and the statistical difference of PA-LS detecting HIV-1 antibody in OMT specimens was significant ( $P < 0.05$ ). In conclusion, compared to HIV-1 antibody detection from serum specimens by ELISA/WB, PA-LS detecting HIV-1 antibody from OMT specimens was non-invasive and accurate.

**Keywords:** Human immunodeficiency virus type-1 antibody, gelatin particles aggregation less-sensitive method, oral mucosal transudate

## Introduction

The human immunodeficiency virus (HIV) is a lentivirus that causes HIV infection and over time acquired immunodeficiency syndrome (AIDS). AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Two types of HIV have been characterized according to serological reaction and nucleotide sequences: HIV-1 and HIV-2. HIV-1 is more virulent, more infective [1] and is also the cause of the majority of HIV infections globally, while HIV-2 is much less pathogenic than HIV-1 and is restricted in its worldwide distribution.

Since 1983 when the first incident of occupational HIV infection was reported in the United States, the rate for occupational transmission of HIV-1 in health setting had experienced an increasing tendency [2, 3]. Health care workers were defined as all paid and unpaid persons working in health care setting with the potential for exposure to infectious materials, such as blood, tissue, and specific body fluids, or contaminated medical supplies, equipment, or environmental surfaces. Since 1991, reports of occupationally acquired HIV in health care workers had been recorded by the National HIV Surveillance System following a standardized case investigation protocol [4]. And 51 documented cases and 108 possible cases of occu-

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tionally acquired HIV infection in healthcare workers in the United States had been reported to the Centers for Disease Control and Prevention until June 1996 [5]. Also, in Yunnan province China where is the area with higher incidence of HIV infection and AIDS, the healthcare workers were at adverse and higher risk of HIV infection in therapies [6]. Especially, medical and health care personnel in stomatology department sustained adverse exposure to patients, which induced to greater potential risk of accidental HIV infection. Hence, it is essential to screen HIV before the dental treatment. Chair-side diagnosis for HIV antibody could provide information whether the patient is infected with HIV. Then the healthcare workers could take targeted protection and timely intervene after exposure, which would reduce the risk of occupational HIV infection.

Recently, the detective markers of HIV include HIV antibody, specific protein (such as p24) [7, 8], RNA/DNA of viruses and count detection of CD+4 lymphocyte [9], but commonly HIV antibody is the clinical detective marker. In China, the detection program of HIV antibody is primary screening by ELISA and then confirmatory by Western blot (immunoblot analysis) for blood testing. However, it is difficult to acquire the same blood specimens repeatedly because of its trauma and invasion; meanwhile, clients and medical staffs might be at risk of cross-infection because of unintended exposures during sampling for ELISA testing. ELISA testing is not suitable for dental outpatient to test HIV antibody by chair-side diagnosis.

Yoshida et al. used gelatin particles aggregation method (PA) to test HIV, and the sensitivity of PA was similar to that of ELISA with 0% false positive proportion [10]. Since 1998, S/LS ELISA methods had been developed and applied widely, which depended on the concept that the antibody titers would rise in the first 6 months of infection. Li et al. applied gelatin particles aggregation less-sensitive method (PA-LS) based on PA and S/LS to estimate recent HIV incidence [11]. The sensitivity of PA-LS test was 100%, and the specificity was 99.8%, and even HIV serum positive specimen at 1:10000 dilution could be tested and identified by PA-LS. Besides, the attractive feature of PA-LS was its extremely low cost.

Hence, the aim of this study was to investigate whether PA-LS test could be used to screen

and detect HIV-1 antibody from oral mucosal transudate (OMT) specimens accurately, and to provide basis for further research on non-invasion chair-side HIV detection.

### Materials and methods

#### *Study participants*

All 1116 objects were Chinese citizens without regard to gender, age, nations, and virus infection, dental diseases and systemic diseases, but those who had no teeth, who had difficult to open mouth, and who were unwilling to cooperate with staffs were excluded. Objects could not brush their teeth in 1 hour before sampling. All participants were divided into three groups.

HIV-1 antibody-positive group: 50 research objects with HIV-1 antibodies-positive identification in serum specimens by Yunnan Centre for Disease Control and Prevention (YNCDC), but without considering HIV infection approach, symptoms and immunosuppressive therapy.

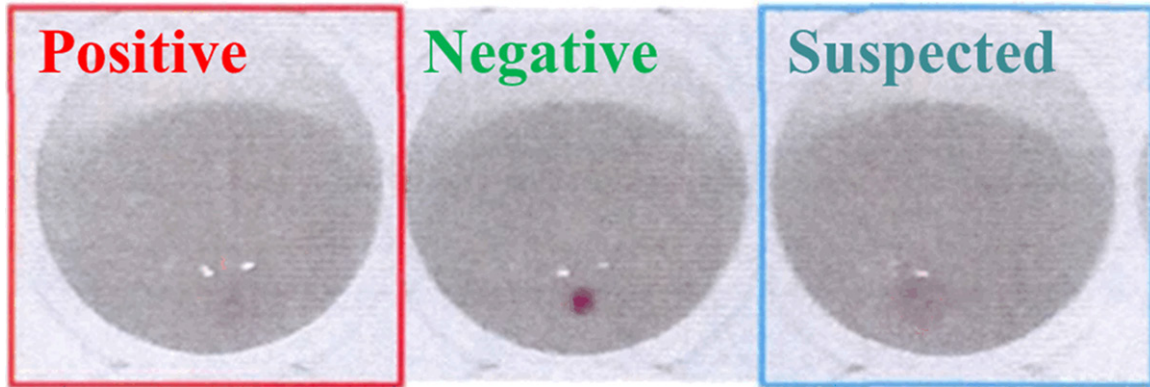
High-risk group: 666 drug addicts from Yunnan 3<sup>rd</sup> Quarantine Rehab in Jiuxi District and Compulsory Detoxification in Yingjiang County, Yunnan Province without considering duration of detoxification and form of drug administration.

General group: 400 objects were students from Kunming Medical University, medical staffs from Yunnan Province Dental Hospital, and patients in Yunnan Province Dental Hospital, who all denied any history of high-risk behaviors and transfusion associated with HIV infection.

#### *Study design*

1116 OMT specimens were collected from all 1116 objects to screen HIV-1 antibodies using PA-LS, while blood specimens were acquired from 666 objects of high-risk group, and were screened by ELISA. Besides, as for HIV-1 antibodies-negative or suspected cases in positive group, blood specimens were collected to recheck by ELISA and confirm by WB; as for HIV-1 antibodies-positive or suspected cases in high-risk group, it was essential to confirm HIV antibodies in blood specimens by WB; as for positive or suspected ones in general group, blood samples were collected to recheck and confirm HIV-1 antibodies by ELISA and WB respectively.

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**Figure 1.** PA-LS testing results of HIV-1 antibody. The positive one showed a large circle with uneven edge and agglutination (dispersed particule), the negative one showed a red particle with uniform and smooth edge in the bottom well, and the suspected one showed a small circle with uniform and smooth edge.

### *Sampling and preservation*

Applicator was rubbed repeatedly between buccal mucosa of vestibules and gum until that absorbent cotton on top of it was wet. And subsequently, the applicator with OMT specimen was put in sterile EP tube and stored hermetically at  $-70^{\circ}\text{C}$ . 3-5 ml venous blood samples of 666 drug addicts were collected, and were centrifuged to take the serum samples, respectively.

### *Detection*

OMT samples were thawed under room temperature, and mixed with 100  $\mu\text{l}$  M solution (YNCDC, China) uniformly to obtain specimens for testing. Sensitive gelatin particles (SP) solution was prepared as the instruction. 8  $\mu\text{l}$  of each specimen was added into plate with 38  $\mu\text{l}$  SP/L solution (SP:L=1:67, v/v), and the plate was shaken for 30 s. Then the plate was incubated in humidity chamber for overnight. The result was recorded as positive, suspected or negative (**Figure 1**). The positive one showed a large circle with uneven edge and agglutination (dispersed particle), the negative one showed a red particle with uniform and smooth edge in the bottom well, and the suspected one showed a small circle with uniform and smooth edge.

### *Evaluation indicators [12]*

Sensitivity/true positive rate: it reflected the capacity of a method to detect positive object as positive one.

Specificity/true negative rate: it reflected the capacity of a method to detect negative object as negative one.

Omission diagnostic rate/false negative rate: it reflected the capacity of a method to detect positive object as negative one.

Mistaken diagnostic rate/false positive rate: it reflected the capacity of a method to detect negative object as positive one. Positive predictive value: it was a probability of true positive object numbers in positive ones according to results of a testing method.

Negative predictive value: it was a probability of true negative object numbers in negative ones according to results of a testing method.

These indicators in each group were obtained by the following equations:

$$\text{Sensitivity} = a/(a+b)$$

$$\text{Specificity} = c/(c+d)$$

$$\text{Omission diagnostic rate} = b/(a+b)$$

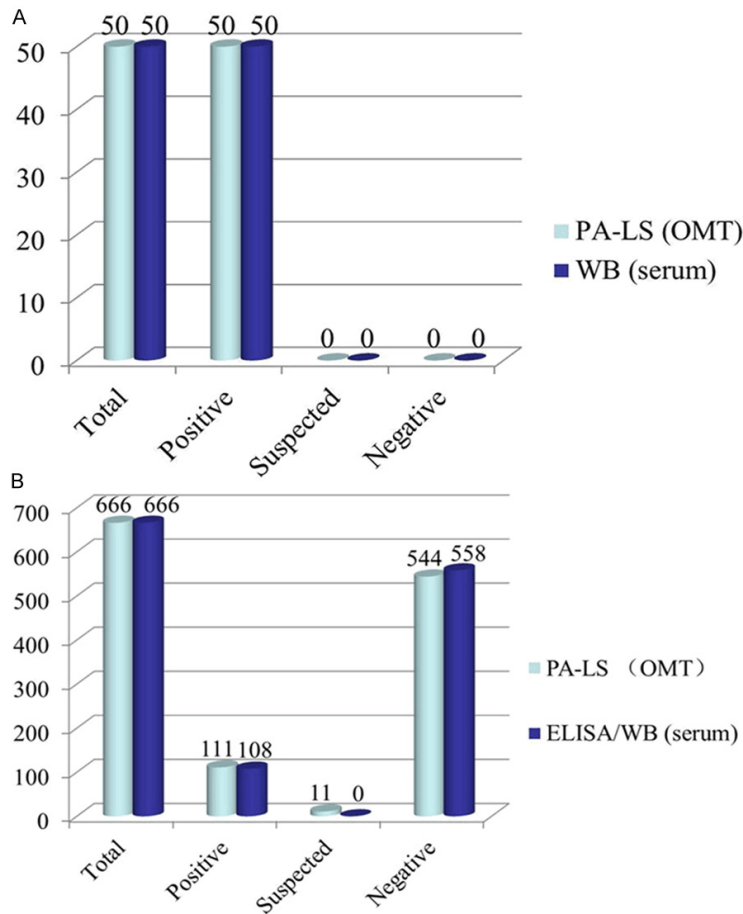
$$\text{Mistake diagnostic rate} = d/(c+d)$$

$$\text{Positive predictive value} = a/(a+d)$$

$$\text{Negative predictive value} = c/(c+b)$$

Where, letter a stood for true positive object numbers according to results of a testing method, letter b stood for false negative object numbers according to results of a testing method, letter c stood for true negative object numbers according to results of a testing method, and letter d stood for false positive object numbers according to results of a testing method.

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**Figure 2.** PA-LS and WB testing results of HIV-1 antibody-positive group and high-risk group. A. PA-LS and WB testing results of HIV-1 antibody-positive group. B. PA-LS and ELISA/WB testing results of high-risk group.

**Table 1.** Consistency analysis between PA-LS (OMT specimens) result and WB (serum specimens) result from positive and high-risk groups respectively

PA-LS (OMT)		WB (serum specimens)			Total
		Positive	Suspected	Negative	
Positive group	Positive	50	0	0	50
	Suspected	0	0	0	0
	Negative	0	0	0	0
	Total	50	0	0	50
High-risk group	Positive	108	0	3	111
	Suspected	0	0	11	11
	Negative	0	0	544	544
	Total	108	0	558	666

### Ethical considerations

We obtained the permissions from Student Affairs Office in Kunming Medical University, ethics committees in Yunnan Province Dental

Hospital, Yunnan Disease Prevention and Control Center, Yunnan 3<sup>rd</sup> Quarantine Rehab in Jiuxi District and Compulsory Detoxification in Yingjiang County, Yunnan Province. All participants consented to their clinical data being accessed and analyzed for research purposes. Medical staffs should fulfill informing obligation to ensure volunteers' confirmed consent right and to register personal information before sampling, and should offer feedback of testing results to objects in time.

### Statistical analysis

All data were analyzed with SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). Kappa value was used to check consistency of two testing methods. The significant difference of screening HIV-1 antibodies between PA-LS testing OMT specimens and ELISA testing serum specimens were assessed by the  $\chi^2$  test.  $P < 0.05$  was considered statistically significant.

## Results

### HIV-1 antibody-positive group

50 objects were diagnosed with positive HIV-1 antibodies by YNCDC, and their serum specimens had been also screened by ELISA and confirmed by WB with positive HIV-1 antibody. 38 out of 50 objects received Highly Active Antiretroviral Therapy (HAART) at the time of the sample collection. The result of PA-LS detecting HIV-1 antibody in OMT specimens was 50 positive cases, 0 suspected case, and 0 negative case (**Figure 2A**, [Supplementary Figure 1](#)).

According to HIV-1 antibody certification of YNCDC as diagnostic criteria, the sensitivity of PA-LA for HIV-1 antibody detection in OMT samples was 100% (50/50), omission diagnostic rate was 0% (**Table 1**).

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**Table 2.** Evaluation indicators of PA-LS testing results for OMT specimens

	Sensitivity	Specificity	Omission diagnostic rate	Mistake diagnostic rate	Positive predictive value	Negative predictive value
PA-LS (OMT)	100%	97.49%	0	2.51%	88.52%	100%

**Table 3.** Consistency analysis between PA-LS (OMT specimens) and ELISA/WB (serum specimens) using Kappa value

	Value	ASE <sup>a</sup>	T <sup>b</sup>	Sig.
K	0.926	0.019	23.975	0.000
n	666			

<sup>a</sup>, P<0.05; <sup>b</sup>, P<0.01.

### High-risk group

According to the HIV-1 antibody detection of serum specimens by ELISA, there were 108 positive cases, 0 suspected case, and 558 negative cases among 666 objects, particularly in which the 108 positive cases were confirmed by WB as true positive (positive detectable rate was 16.22%). However, 111 positive cases with HIV-1 antibodies in OMT specimens, 11 suspected ones, and 544 negative ones were identified using PA-LS (**Figure 2B**, [Supplementary Figure 2](#)).

Therefore, we re-screened serum specimens of above 111 positive objects, 11 suspected ones, and 544 negative ones using ELISA for HIV-1 antibodies and confirmed by WB respectively. It showed that there were 108 positive cases and 3 negative cases out of 111 positive ones, 11 negative cases out of 11 suspected ones, and 544 negative cases out of 544 negative ones. Namely, 14 PA-LS testing results (positive or suspected) were inconsistent with ELISA testing ones (negative). Among these 14 samples, there were 13 males whose routes of taking the drug were oral administration, intravenous injection or mixed routes, and 1 female object taking ephedrine orally.

According to ELISA screening and WB confirmation results as diagnostic criteria, the sensitivity of PA-LA detecting HIV-1 antibody in 666 OMT specimens of high-risk group was 100% (108/108), specificity 97.49%, omission diagnostic rate 0%, mistake diagnostic rate 2.51% (14/558), positive predictive value 88.52% (108/(111+11)), and negative predictive value 100% (553/553) (**Tables 1 and 2**). Under the same standard, statistical analysis was done

with PA-LS and ELISA/WB testing results using consistency check. It suggested that Kappa value > 0.8, which indicated that it was highly consistency between the two methods (**Table 3**). Similarly, statistical analysis of  $\chi^2$  test was done, and obtained P<0.05, which indicated there were significant differences between the two methods (**Table 4**).

### General group

OMT specimens from 400 objects were tested by PA-LS, and there were 0 positive case, 1 suspected case, and 399 negative cases ([Supplementary Figure 3](#)). The 1 suspected OMT specimen was re-tested by PA-LS to be negative one, but the blood specimen was not collected for HIV-1 antibody detection.

### Discussion

OMT specimens were collected from 1116 objects (divided into HIV-1 antibody-positive group, high-risk group and general group), and were tested using PA-LS for HIV-1 antibodies detection. However, the specificity value of PA-LS testing HIV-1 antibodies of OMT specimens in high-risk group (97.49%) was lower than that of serum specimens (98.8%) [11] reported by Li et al. It was due to that the HIV-1 antibody content in OMT was less than that in serum, and would be also influenced by repeated freeze-thaw cycles during transportation.

In this study, 50 objects from positive group were diagnosed with positive HIV-1 antibodies ranging from 3 months to 5 years, and varied with the clinical courses. The HIV-1 antibodies in 50 OMT specimens were accurately identified as positive ones and the clinical courses had no impact on the detection. One reason might be that the diseases of them had not reached an advanced stage, and higher HIV-1 antibody titer was easy to be detected. And this also suggested that PA-LS had a higher sensitivity to identify HIV-1 antibody in OMT. Besides, HAART treatment during sampling had no effect on PA-LS testing OMT specimens, which indicated that PA-LS was of high sensitivity and not

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**Table 4.** The significant difference of screening HIV-1 antibodies between PA-LS testing OMT specimens and ELISA testing serum specimens

Method	Negative	Positive	Suspected	Total	$\chi^2$	<i>P</i>
PA-LS (OMT)	544	111	11	666	16.881	0.001
ELISA (serum)	558	108	0	666		

affected by interference from HAART treatment. However, this might be owing to the limited objects, so more subjects should be included in the future experiment in order to study the effect of HARRT treatment on HIV-1 antibody detection deeply.

According to the results of PA-LS testing OMT specimens from 666 drug abusers, 14 cases (3 positive and 11 suspected) were inconsistent with results of serum specimens testing by ELISA (14 negative). One reason might be that HIV-1 antibodies in serum specimens of these HIV infected patients were not proofed using ELISA, and showed false negative. Type and level of antibodies in HIV-infected individual changed along with infection period. Antibodies against HIV antigens had not been completely generated at the initial stage of HIV infection, so antibody titers in the infectors were too low to detect and showed negative or suspected. But the level of antibody increased gradually with the development of disease, then led to a positive result [13]. Sandeep Ramalingam et al. detected HIV antibodies from 6654 serum specimens by PA and ELISA methods respectively [14]. They found that 63 negative cases by means of ELISA showed weak positive by means of PA, but they showed positive by means of ELISA after 3 years infection. This suggested that PA method was more sensitive to HIV-1 antibodies than ELISA under the condition of acute infection or low HIV antibodies level. So these 14 objects should be rechecked for HIV-1 antibodies in the case of omission [15], and Detoxification staffs should break them dependence of the drug and high risk behavior of HIV infection. However, the other reason of these inconsistent testing results might be that HIV-1 antibodies in OMT specimens from these 14 objects without HIV infection were identified as positive or suspected, namely, OMT specimens were false positive. Other retrovirus infection at the time of sampling would induce the false positive result. These retrovirus had homology with HIV, and

would generate antibodies to cross-react with HIV antigen, then led to abnormal cross-reactions of antibody-antibody. Besides, auto-antibodies in patients with autoimmune diseases (such as systematic lupus erythematosus), acute or chronic renal failure, and

malignant tumor would elicit abnormal immune response to HIV [16, 17], leading to the HIV-1 antibodies false positive or suspected. HIV-1 antibodies in blood specimens of patients with acute malaria were identified as positive, but they were negative after recovering [18]. Shi et al. [19] collected OMT and serum specimens from 15 HIV-infected cases, 15 HIV-uninfected cases, 30 periodontal cases and 570 general cases. 1 OMT specimen was tested as positive while the correspondent serum specimen was negative. Moreover, the results of BBI serum and OMT transition plate showed that HIV-1/2 antibodies could be detected from OMT specimens on the 28th day of clear exposure, but could be detected from serum specimen on the 33rd day of exposure. This suggested that antibodies from OMT specimens were sensitive to be detected than those from serum specimens. In this study, drug addicts from high-risk group had weak immune defense ability, and it was more likely for them to be infected with HIV. Besides, the complicated factors affected the HIV testing, so it was essential to test HIV-1 p24 antigen [20, 21] or carry out nucleic acid quantitative detection [22] when WB testing result was not sure or testing results varied with testing methods.

Besides, the possible reason of false positive result in MOT sample was due to that objects rinsed the mouth and discharged makeup products incompletely. This led to OMT samples contaminated with food residue in mouth or lipstick.

399 out of 400 general cases were negative by PA-LS testing OMT specimens, and 1 was suspected. The 1 suspected specimen was re-detected by PA-LS as negative. This object was one female patient at the age of 62 from Yunnan Dental Hospital, and without any high-risk HIV infection-related behavior and blood transfusion history, but this female suffered from systemic lupus erythematosus (SLE) for 15 years, diabetes for 2 years, and hyperten-

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sion for 7 years with various medicines. When sampling, this patient was subjected to acute exacerbation of left posterior teeth chronic apical periodontitis, and went to see oral medical doctor for treatment. Esteva et al. [23] tested HIV antibodies from blood specimens of 2 male patients with acute SLE for 3 times in 6 months, in which 2 times showed positive using ELISA test. But false positive result was occurred using WB test, which might be due to autoantibodies related with SLE. According to the previous medical history, this patient was affected with SLE, so it might be higher autoantibodies level that induced the antibody of OMT specimen tested as suspected. Higher autoantibodies bound to tagged specific antigens in gelatin granules, leading to agglutination phenomena, but the mechanism was unknown. That was to say that, diagnosis of HIV infection should be cautious in patients with SLE because of the presence of autoantibodies and cross-reactivity [24].

In general group, there was 1 suspected case in the initial screening. This object should be diagnosed again for HIV-1 antibody, and meanwhile supplementary identified detection for other diseases should be carried out according to individual health condition. Moreover, the impacts of diabetes, hypertension and other medications on HIV-1 antibodies detection were not clear, and importantly, these diseases were not excluded when recruiting volunteers for sampling. So as a medical staff and inspector, it was not reasonable to identify HIV-1 antibody positive based on one suspected or positive sample, lest patient's worried.

In conclusion, compared to serum, OMT was not one approach to transmit HIV [25, 26], so OMT specimen had higher bio-safety, and lowered the potential risk of cross-infection between participants and medical staffs effectively. The oral fluid collection was also non-invasive collection with painless, and relieved clients' miseries and fears. It was more convenient and acceptable for children, haemophilic, obese, the old, the weak, clients with inconspicuous superficial veins, and those needed repeated sampling to participate in the research for HIV detection [27-29]. Moreover, it was easy to sampling for professionals or non-professionals and there was no significant difference in the HIV antibodies detection rates between them [29]. Besides, PA-LS was based on the rapid testing kit without especial equip-

ment, and the testing results could be observed visually.

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### Disclosure of conflict of interest

None.

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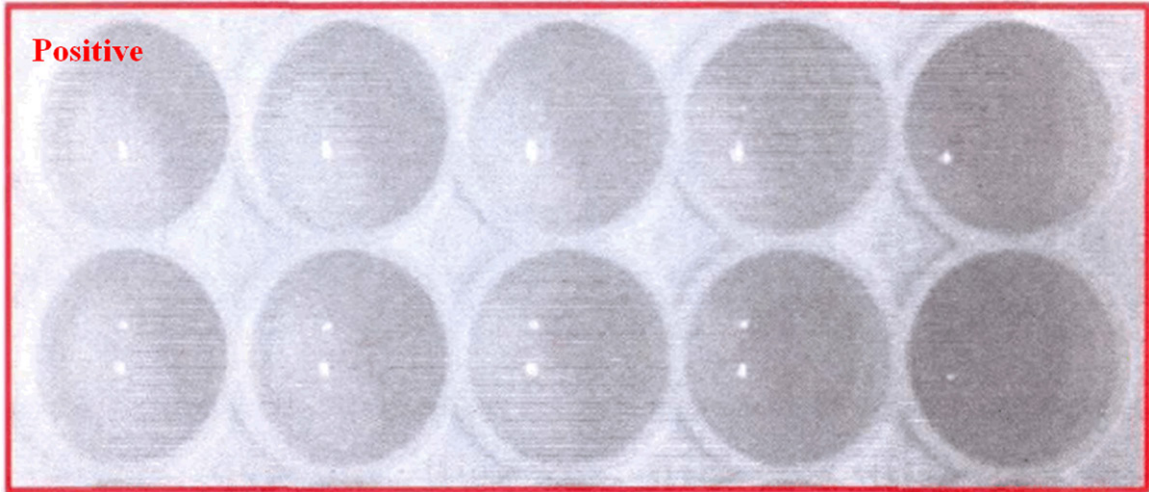
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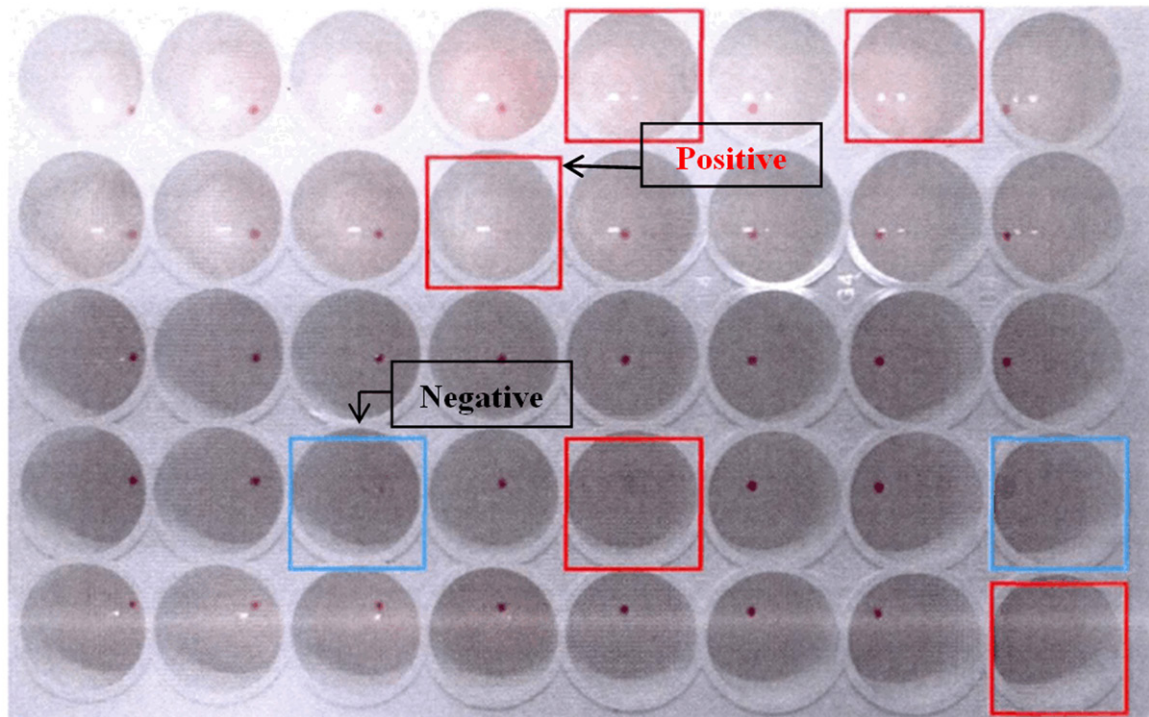
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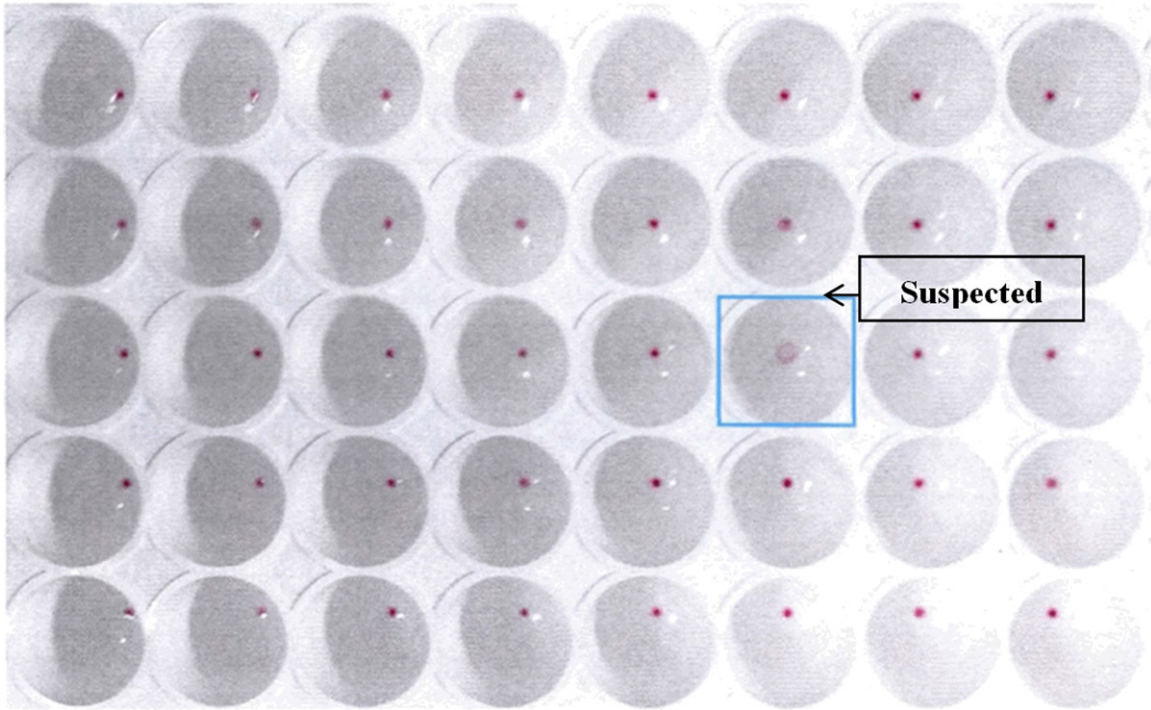


**Supplementary Figure 1.** Typical reactions of PA-LS testing in HIV-1 antibody-positive group. Agglutination (dispersed particles) represented a positive result.



**Supplementary Figure 2.** Typical reactions of PA-LS testing in high-risk group. A red button in the well of the plate represented a negative result, while agglutination (dispersed particles) represented a positive result.

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**Supplementary Figure 3.** Typical reactions of PA-LS testing in general group. A red button in the well of the plate represented a negative result, while agglutination (dispersed particles) represented a positive result, and a small circle with uniform and smooth edge represented a suspected result.