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Version No. 1.0

Anti HIV 1+2 Serum Rapid Test (Colloidal Gold)

Clinical Trial

Management of the study: R&D Department
Quality control department

Place of the study: Beijing China

Abstract

- **Background**

Antibody testing for human immunodeficiency virus (HIV) began with the introduction of enzyme-linked immunosorbent assays (ELISA) in the 1980s. Those tests, together with Western blotting, formed the cornerstone of HIV testing algorithms until 1992, when the Global Programme on AIDS of the World Health Organization (WHO) first recommended the use of simple rapid (SR) tests . Rapid tests have been recommended for use in blood safety screening, in surveillance, and in diagnosis in prenatal or voluntary counseling and testing (VCT) centers. Rapid tests have been widely used throughout Africa and the developing world.

- **Method**

A multi-center prospective study was conducted to evaluate the diagnostic sensitivity and specificity of HIV Rapid Test manufactured by Atlas Link (Beijing) Technology Co., Ltd in specimens from the patients clinically diagnosed as HIV infected.

- **Result**

The comparison study result confirmed relatively high diagnostic sensitivity and diagnostic specificity of HIV Test.

- **Conclusion**

The HIV test kit is a simple, rapid, easy-to-run and highly sensitive assay that can be used in both high- and low-risk populations for the diagnosis of HIV carriage. It appears to be a promising new tool for large-scale screening and diagnosis of HIV infection.

1. Clinical Trial Location:

Study site for Diagnostic Sensitivity:

Evaluation Site	Probands
PLA 302 Hospital	30 Commercial HIV seroconversion Panels(BBI and ZMC)
Sichuan Center for Disease Control and Prevention	482 HIV positive samples including 283 specimens known HIV genotype
Key Laboratory of Molecular Biology on Infectious Diseases, Chongqing University of Medical Sciences	157 HIV positive samples including 58 specimens known HIV genotype

Study site for Diagnostic Specificity:

Evaluation Site	Probands
PLA 302 Hospital	200 hospitalized patients
PLA 302 Hospital	200 pregnant women

Key Laboratory of Molecular **Biology** on Infectious Diseases, Chongqing University of Medical Science	151 specimens from patients of potential interference diseases
Sichuan Center for Disease Control and Prevention	90 specimens from patients of potential interference diseases
Key Laboratory of Molecular Biology on Infectious Diseases, Chongqing University of Medical Sciences	517 specimens from blood donors
Blood Center of Beijing Red Cross Society	593 specimens from blood donors

2. Diagnostic Sensitivity Study Summary

The sensitivity of HIV-1/2 Antibody Test for the detection of antibodies to HIV-1 Group M subtypes (A,B,C,D,E,F,G) from various geographic regions was assessed by testing a worldwide HIV performance panel.

10 seroconversion series from ZMC and 20 seroconversion series from BBI were tested on Atlas Link HIV1/2 Antibody Rapid Test. The assay results were compared with the data from the testing results of Abbot HIV1/2 Antibody EIA reported on the BBI and ZMC panel data sheets. Overall, Atlas Link HIV1/2 Antibody g Rapid Test demonstrated an ability to detect earlier in the conversion of a patient from seronegative to seropositive in comparison with Abbot HIV1/2 Antibody EIA products.

Study Method and Results

3 studies were carried out at different sites in China.

Total 30 commercial seroconversion panels including 10 from ZMC (ZeptoMetrix Corporation, ZMC, US) and 20 from BBI (SeraCare, US) were used to determine the relative diagnostic sensitivity of HIV1/2 Antibody Rapid Test. The relative diagnostic sensitivity was determined by comparing the earliest detection date of serum conversion of the patient to HIV seropositive with the HIV serological status of each series bleed with the data of CE-market HIV products- Abbott HIV1/2 EIA provided by ZMC or BBI. HIV1/2 Antibody Rapid Test showed earlier detection of seroconversion on panel PRB962, PRB964 , PRB971, PRB972 than Abbot's products, the value were -2, -1, -1and -1 respectively; later detection of seroconversion on panel HIV9089, PRB945 and PRB943, all the values were +1; the total value was -2. Overall, HIV1/2 Antibody g Rapid Test demonstrated an ability to detect earlier in the conversion of a patient from seronegative to seropositive in comparison with Abbot HIV1/2 Antibody EIA products. (Table blow)

Table Summary of Seroconversion Panels Detection at HIV1/2 Antibody Rapid Test

Panel Code	Period of Detection from the First Bleed Day		Atlas Link results value compared with Abbot
	Atlas Link HIV1/2 Antibody Rapid Test	Abbot HIV1/2 EIA	
HIV10234	35	35	0
HIV12007	87	87	0
HIV6248	27	27	0
HIV9012	22	22	0
HIV9014	10	10	0
HIV9075	31	31	0
HIV9076	69	69	0
HIV9081	23	23	0
HIV9089	24	20	+1
HIV9096	18	18	0
PRB914	NA	NA	0
PRB933	21	21	0
PRB945	15	13	+1
PRB943	19	14	+1
PRB946	NA	NA	0
PRB947	9	9	0
PRB948	NA	NA	0
PRB951	19	19	0
PRB955	12	12	0
PRB958	15	15	0
PRB962	14	NA	-2
PRB963	NA	NA	0
PRB964	22	NA	-1
PRB965	12	12	0
PRB967	17	17	0
PRB968	28	28	0
PRB970	10	10	0
PRB971	11	NA	-1
PRB972	21	NA	-1
PRB975	14	14	0

Total 639 positive serum samples including 507 HIV1 covering 13 HIV1 subtypes (including 129 non-B-subtypes), 104 HIV2 and 28 HIV1and HIV2 mixed infected (Among the 639 specimens, there were 482 from SCCDC and 157 from Chongqing Medical University) were selected to evaluate the diagnostic sensitivity of HIV1/2 Antibody Rapid Test and compare the results with CE marked Alere Determine HIV1/2 Test. From study results of SCCDC (Table 1), 480 of 482 positive samples were tested positive by HIV1/2 Antibody Rapid Test while 481 positive results by

Alere HIV1/2. The diagnostic sensitivity of HIV1/2 Antibody Rapid Test was 99.59% (480/482). From study results of Chongqing Medical University (Table 2), 157 of 157 HIV positive sera were tested positive by both HIV1/2 Antibody Rapid Test and Alere HIV1/2. The sensitivity of Atron HIV1/2 Antibody Rapid Test was 100% (157/157). All the samples with known genotype were tested as positive by both assays. Total 639 positive samples were tested 637 positive by HIV1/2 Test, while 638 positive by CE marked Alere HIV1/2; the one (No.335 from SCCDC)tested negative by both HIV1/2 Antibody Rapid Test and Alere HIV1/2, and the other one(No. 376 from SCCDC) tested negative only by were further confirmed positive by PCR. The genotype of the false negative samples were unknown. The diagnostic sensitivity of HIV1/2 Antibody Rapid Test was 99.69% (637/639) and could identify HIV1 subtypes and HIV2.

Table 1 Summary of HIV positive specimens' detection from SCCDC at HIV1/2 Antibody Rapid test

Genotype		Results of HIV1/2 Antibody Rapid Test		Results of CE Marked Test		Subtotal
		Positive	Negative	Positive	Negative	
HIV1	A	6	0	6	0	6
	B	19	0	19	0	19
	B'	26	0	26	0	26
	C	17	0	17	0	17
	CRF01-AE	18	0	18	0	18
	CRF02-AG	4	0	4	0	4
	CRF-BC	33	0	33	0	33
	D	7	0	7	0	7
	E	11	0	11	0	11
	F	4	0	4	0	4
	G	2	0	2	0	2
	H	3	0	3	0	3
	J	3	0	3	0	3
	Unknown Genotype	197	2	198	1	199
HIV2		102	0	102	0	102
HIV1&HIV2		28	0	28	0	28
Subtotal		480	2	481	1	482

Table 2 Summary of HIV Positive Specimens' Detection from Chongqing Medical University at HIV1/2 Antibody Rapid Test

Genotype		Results of HIV1/2 Antibody Rapid Test		Results of CE Marked Anti- HIV1/2 Tests		Subtotal
		Positive	Negative	Positive	Negative	
HIV-1	A	4	0	4	0	4
	B	33	0	33	0	4
	B'	2	0	2	0	2
	C	3	0	3	0	3
	CRF01-AE	2	0	2	0	2
	CRF02-AG	2	0	2	0	2
	D	2	0	2	0	2
	E	2	0	2	0	2
	F	2	0	2	0	2
	G	2	0	2	0	2
	H	1	0	1	0	1
	J	1	0	1	0	1
	unknown	99	0	99	0	99
HIV-2		2	0	2	0	2
Subtotal		157	0	157	0	157

Summary Result of HIV Positive Specimens' Detection at HIV1/2 Antibody Rapid Test

Genotype		Results of HIV1/2 Antibody Rapid Test		Results of Alere HIV1/2		Subtotal
		Positive	Negative	Positive	Negative	
HIV1	A	10	0	10	0	10
	B	52	0	52	0	52
	B'	28	0	28	0	28
	C	20	0	20	0	20
	CRF01-AE	20	0	20	0	20
	CRF02-AG	6	0	6	0	6
	CRF-BC	33	0	33	0	33
	D	9	0	9	0	9
	E	13	0	13	0	13
	F	6	0	6	0	6
	G	4	0	4	0	4
	H	4	0	4	0	4
	J	4	0	4	0	4

	Unknown genotype	296	2	297	1	298
HIV2		104	0	104	0	104
HIV1&HIV2		28	0	28	0	28
Subtotal		637	2	638	1	639

Table Comparison of the sensitivity of HIV1/2 Antibody Test with that of Alere HIV1/2

		Atlas Link HIV1/2Antibody Test		
		Positive	Negative	subtotal
Results of CE Marked HIV 1/2 Antibody Test	Positive	637	1	638
	Negative	0	1	1
Subtotal		637	2	639

Sensitivity of HIV1/2 Antibody Test: 99.69% (637/639)

Sensitivity of CE Marked HIV1/2 Antibody Test: 99.84% (638/639).

3. Diagnostic specificity Study Summary

The effect of seromarkers associated with unrelated medical conditions on the specificity of the Atlas Link HIV Antibody Test was assessed using a panel of specimens. The seromarkers studied were C-reactive protein (CRP), antistreptolysin O titre (ASOT), rheumatoid factor (RF), infectious mononucleosis, Helicobacter pylori IgG/IgM antibodies, hepatitis B virus seromarkers (HBsAg, anti-HBc IgG/IgM, and anti-HBs), hepatitis C virus (anti-HCV antibody), hepatitis A virus IgM (anti-HAV), parvovirus IgG/IgM, herpes simplex virus IgG (HSV), cytomegalovirus IgG/IgM, mycoplasma IgM, EBV, human T- Lymphotrophic virus, rubella IgM, syphilis reagin (RPR/TPPA),Dengue Virus IgG/IgM. The test panel was comprised of 195 HIV-1 antibody negative specimens. Of the 195 specimens, 95 contained one or more of the seromarkers while 100 were health negative specimens.

Study Method and Results

6 studies were carried out at different sites in China.

Total 200 specimens with known non-HIV positive from hospitalized patients in PLA 302 Hospital, PRC, were used to evaluate the diagnostic specificity among inpatients. 199 of 200 specimens were tested anti-HIV negative by both HIV1/2 Antibody Test and Alere HIV1/2. 1 specimen showed disagreement results between the product of and Alere. Result of was positive while that of Ortho was negative at sample No. 179. The reactive sample was tested repeatedly by these two Tests. Further Real-time PCR was carried out at the sample. PCR showed negative result. The diagnostic specificity of HIV1/2 Antibody Rapid Test in inpatients was 99.5% (199/200). (Table below)

Table Summary of Diagnostic Specificity of HIV1/2 Antibody Rapid Test among Inpatients

200 inpatients	Results of HIV1/2 Antibody Rapid Test		Results of Alere HIV1/2		Subtotal
	Negative	Positive	Negative	Positive	
True Negative	199	1	200	0	200

Among the 200 pregnant women, there were 23 multiparas. 200 of 200 specimens were tested anti-HIV1/2 negative by both HI1/2V Antibody Test and Alere HIV1/2. The diagnostic specificity of HIV1/2 Antibody Rapid Test in pregnant women was 100% (200/200). (Table below)

Summary of Diagnostic Specificity of HIV1/2 Antibody Rapid Test among Pregnant Women

200 Pregnant Women	Results of HIV1/2 Antibody Rapid Test		Results of Alere HIV1/2		Subtotal
	Negative	Positive	Negative	Positive	
True Negative	200	0	200	0	200

Total 241 specimens (151 from Chongqing Medical University and 90 from SCCDC) from patients with potential interference diseases were tested to evaluate the diagnostic specificity of HIV1/2 Antibody Rapid Test. The potential interference diseases status were HAV, HBV, HCV, Dengue, EBV, CMV, HTLV, Rubella and E.coli infections, CRF, ASOT, RF positive. There were at least 15 specimens for each interference status. 151 specimens from Chongqing Medical University included the interference status of HAV, HBV, HCV, EBV, RF, CMV, ASOT, CRF, Dengue, Rubella and HTLV positive. These 151 specimens had no interference with HIV1/2 Antibody Rapid Test (Table 3). 90 specimens from SCCDC included the interference status of HAV, HCV Dengue, HTLV and E.coli positive. 89 of the 90 specimens showed negative results by HIV1/2 Antibody Rapid Test. The one tested as false positive by is Anti-E.coli positive serum No. 89 (Table 4). Among the 241 interference specimens, 240 showed no interference with HIV1/2 Antibody Rapid Test. The diagnostic specificity among potential interference disease status was 99.59% (240/241)(Table 5)

Table 3 Summary of Diagnostic Specificity of HIV1/2 Antibody Rapid Test among Interference Disease Status (Chongqing Medical University)

Interference Diseases	Results of HIV1/2 Antibody Rapid Test		Results of Alere HIV1/2		Subtotal
	Positive	Negative	Positive	Negative	
HAV	0	12	0	12	12
HBV	0	20	0	20	20
HCV	0	20	0	20	20
Dengue	0	5	0	5	5
CMV	0	15	0	15	15
EBV	0	15	0	15	15

HTLV	0	4	0	4	4
Rubella	0	15	0	15	15
RF	0	15	0	15	15
CRF	0	15	0	15	15
ASOT	0	15	0	15	15
Subtotal	0	151	1	151	151

Table 4 Summary of Diagnostic Specificity of HIV1/2 Antibody Rapid Test among interference Disease Status (SCCDC)

Interference Diseases	Results of HIV1/2 Antibody Rapid Test		Results of Alere HIV1/2		Subtotal
	Positive	Negative	Positive	Negative	
HAV	0	20	0	20	20
HCV	0	20	0	20	20
Dengue	0	16	1	15	16
HTLV	0	13	0	13	13
Anti-E.coli	1	20	0	21	21
Subtotal	1	89	1	89	90

Table 5 Summary of Diagnostic Specificity of HIV1/2 Antibody Rapid Test among Interference Disease Status

Interference Diseases	Results of HIV1/2 Antibody Rapid Test		Results of Alere HIV1/2		Subtotal
	Positive	Negative	Positive	Negative	
HAV	0	32	0	32	32
HBV	0	20	0	20	20
HCV	0	40	0	40	40
Dengue	0	21	1	20	21
CMV	0	15	0	15	15
EBV	0	15	0	15	15
HTLV	0	17	0	17	17
Rubella	0	15	0	15	15
RF	0	15	0	15	15
CRF	0	15	0	15	15
ASOT	0	15	0	15	15
Anti-E.Coli	1	20	0	21	21
Subtotal	1	240	1	240	241

Total 1110 specimens from blood donors (593 from Blood Center of Beijing Red Cross Society and 517 from Blood Transfusion Department, the Secondary Affiliated Hospital of Chongqing Medical University) were used to evaluate the diagnostic specificity of HIV1/2 Antibody Rapid Test. The

1110 specimens consisted of 68 specimens from consecutive blood donations. Among the 593 specimens from Blood Center of Beijing Red Cross Society, 591 showed negative results by HIV1/2 Antibody Rapid Test, the diagnostic specificity was 99.66% (Table 6). Among the 517 specimens from Chongqing Medical University, 515 showed negative results by HIV1/2 Antibody Rapid Test, the diagnostic specificity was 99.61%(Table 7). Combined these two results together, total 1110 specimens from blood donors, 1106 showed negative results by HIV1/2 Antibody Rapid Test, 4 gave false positive; the diagnostic specificity among blood donors was 99.64%(Table 8)

Table 6 Summary of Diagnostic Specificity of HIV1/2 Antibody Rapid Test among Blood Donors (Blood Center of Beijing Red Cross Society)

Blood donors	Results of HIV1/2 Antibody Rapid Test		Results of Alere HIV1/2		Subtotal
	Negative	Positive	Negative	Positive	
True Negative	591	2	591	2	593

Table 7 Summary of Diagnostic Specificity of HIV1/2 Antibody Rapid Test among Blood Donors

Blood donors	Results of HIV1/2 Antibody Rapid Test		Results of Alere HIV1/2		Subtotal
	Negative	Positive	Negative	Positive	
True Negative	1106	4	1107	3	1110

Table 8 Summary of Diagnostic Specificity of HIV1/2 Antibody Rapid Test among Blood Donors

Blood donors	Results of HIV1/2 Antibody Rapid Test		Results of Alere HIV1/2		Subtotal
	Negative	Positive	Negative	Positive	
True Negative	1106	4	1107	3	1110

From the 6 diagnostic specificity studies among different populations, total 1727 negative samples including 1110 from blood donors, 200 from inpatients, 200 from pregnant women, 241 from potentially interfering diseases were selected to evaluate the specificity of HIV1/2 Antibody Rapid Test and Compare the results with CE marked Alere HIV1/2. Of the 1751 samples, 1745 were tested negative, 6 tested positive (4 from blood donors, 1 from potentially interfering diseases, 1 from inpatients) by HIV1/2 Antibody Rapid Test. All the results which showed inconsistent with CE marked Alere HIV1/2were further confirmed negative by PCR. The diagnostic sensitivity of HIV1/2 Antibody Rapid Test was 99.66% (1745/1751), false positive rate is 0.34% (Table below).

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Summary of Diagnostic Specificity of HIV1/2 Antibody Rapid Test

	Results of HIV1/2 Antibody Rapid Test		Results of CE Marked Alere HIV1/2		Subtotal
	Negative	Positive	Negative	Positive	
Blood Donors	1106	4	1107	3	1110
200 clinic al specimens	199	1	200	0	200
200 Pregnant Women	200	0	200	0	200
236 potentially interfering samples	240	1	240	1	241
Subtotal	1745	6	1747	4	1751

Comparison of the specificity of HIV1/2 Antibody Test with that of Alere

		Atlas Link HIV1/2Antibody Test		
		Positive	Negative	subtotal
Results of CE Marked HIV 1/2 Antibody Test	Positive	0	4	4
	Negative	6	1741	1747
Subtotal		637	1745	1751

Specificity of HIV1/2 Antibody Test: 99.66% (1745/1751)

Specificity of CE Marked HIV1/2 Antibody Test: 99.77% (1747/1751)

When evaluated the diagnostic sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and 95% Confidence limits (CL) of the calculated sensitivity and specificity of HIV1/2 Antibody Test with total 639 true positive and 1751 true negative HIV serum samples, the sensitivity was 99.69% (637/639), the specificity was 99.66% (1745/1751), PPV was 99.07% (637/643), NPV was 99.89% (1745/1747); 95% CL of sensitivity was 99.47%-99.91%, 95% CL of specificity was 99.43%-99.89%.

	Atlas Link HIV1/2Antibody Test		subtotal
	Positive	Negative	
True Positive	637	2	639
True Negative	6	1745	1751
Subtotal	643	1747	2390

Comparison of the sensitivity and specificity of HIV1/2 Antibody Test with those of CE Marked HIV 1/2 Test , the agreement of the two HIV1/2 Antibody test was 99.87%((641+1746)/2390), Statistical significance of sensitivity and specificity between HIV1/2 Antibody Test and CE Marked

HIV1/2 Antibody EIA was determined by x^2 test, and no significant difference between these two assays. (P>0.01)

	Atlas Link HIV1/2Antibody Test			
		Positive	Negative	subtotal
Results of CE Marked HIV 1/2 Antibody Test	Positive	641	1	642
	Negative	2	1746	1748
Subtotal		633	1747	2390

4. Conclusion

By analysis the data collected in these clinical investigations, it is concluded that HIV 1/2 AntibodyTest manufactured by Atlas Link (Beijing) Technology Co., Ltd has relatively high sensitivity, specificity and accuracy for a lateral flow One Step test. This test is convenient, fast and simple to operate. The test has been determined to be safe and effective. No special instrument is needed to read the test result. The background is clear and interpretation of the test result is very easy.