Performance Evaluation Report

One Step HIV Test

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1.0 Intended Clinical Use

One Step Anti-HIV 1+2 Test is a rapid direct binding test for the qualitative detection of antibodies to Human Immuno deficiency Virus Type 1 and Type 2 (HIV 1+2) in human blood as an aid in the clinical assessment of HIV infection.

PRECAUTIONS

1. Read directions for use carefully before performing this test. Pay attention to the position of the C and T line.
2. Do not use beyond the labeled expiration date.
3. Do not reuse the test devices.
4. Do not use if pouch is damaged or opened.
5. Do not touch the membrane on the strip.
6. Once open the pouch, the test device should be used immediately. Prolonged exposure to ambient humidity will cause product deterioration.
7. It is recommended that all specimens be used as if they are potentially infectious and handled in accordance with Biosafety Level 2 practices as described in the CDC NIH Publication: Biosafety in Microbiological and Biomedical laboratories [1], or other equivalent guidelines [2, 3].
8. Devices used for the assay should be sterilized before being disposed.
9. **The test can be used with serum/plasma specimen, carefully follow the procedure instruction.**

2.0 Principle of the Test

One Step Anti-HIV 1+2 Test is a tri-line rapid test. It uses “double antigen sandwich principle” for the detection of anti HIV 1 and anti HIV 2 virus antibodies in human blood specimen. For the detection of anti HIV 1 virus antibody, purified recombinant HIV 1 antigen (Ag 1, contains gp120 and gp41) was immobilized on the test band region, and polyclonal anti HIV 1 antibody was immobilized on the control band region of a nitrocellulose membrane. Another purified recombinant HIV 1 antigen (Ag 2, contains gp120 and gp41), coupled with colloidal gold particles, is dried on a conjugate pad. During the assay, specimen reacts with the colour conjugate to form the complex mixture; the mixture then migrates chromatographically along the membrane by capillary action. If the specimen contains anti-HIV 1 antibody, the purified recombinant HIV 1 antigen immobilized on the membrane will capture the antibody-colloidal gold antigen complex and form a red test band on the membrane, indicating a positive result. Absence of the test band suggests a negative result. The detection of anti HIV 2 virus antibody employs a similar principle to form a T2 region as indicator. The purified recombinant HIV 2 antigen contains gene gp36. Therefore, the appearance of T1 indicates anti HIV 1 virus antibody positive, and the appearance of T2 indicates anti HIV 2 virus antibody positive.
To serve as a procedural control, a color band at control region always appears, indicating the devices are functioning properly and sample volume is appropriate.

3.0 Purpose

This document was prepared mainly based on the requirement of Directive 98/79/EEC on in vitro diagnostic medical devices (IVD MDs) and standard EN13612:2002 for a description of performance evaluation of One Step Anti-HIV 1+2 (Whole Blood) Test. It was prepared to demonstrate the evaluation results of analytical sensitivity, diagnostic sensitivity, analytical specificity, diagnostic specificity, accuracy, repeatability, reproducibility of the test.

4.0 List of Standards Applied

Directive 98/79/EC
EN 375:2001
EN 13612: 2002
EN 13640: 2002
Requirement for One Step Anti-HIV 1+2 Test (Colloidal Gold)
YZB/蓝 02-2004
Antibody quality control Standard (BC-HIV-TF-10)
Anti-HIV 1+2 Enterprise Quality Control Standard

5.0 Performance Study Records and Reports

5.1 Analytical Sensitivity and Specificity

The Criteria for Analytical Sensitivity is the lowest amount of Anti-HIV 1+2 antibodies which can be detected as positive by One Step Anti-HIV 1+2 (Whole Blood) Test. (the appearance of T1 and T2 line regardless of their color intensity).
The Criteria of analytical Specificity is to demonstrate that One Step Anti HIV 1+2 (Whole Blood) Test is specific only to anti-HIV 1+2 positive patient's sample. It should not show cross reactivity with normal sera or abnormal sera of other pathologies.

The study was conducted based on the **Requirement for One Step Anti-HIV 1+2 Test (Colloidal Gold),** which serves as a standard guideline for the production and quality control of this product before release to market.
The Sensitivity and specificity of One Step Anti-HIV 1+2 (Whole Blood) Test was first tested with In-House Negative sera panel N1-N20. Three lots were used for testing.
The sensitivity and specificity of this product were tested with a Sera panel provided by **the Chinese National Institute for the Control of Pharmaceutical and Biological Products**; a government body provides standard pharmaceutical and biological products. (http://www.nicpbp.org.cn/cmsweb/).

The sera panel from Chinese NICPBP contains 20 positive specimens labeled as P1 to P20; P1-P18 is standard solution for anti HIV 1 and P19-P20 is standard solutions for anti HIV 2. This panel also contains 20 negative specimens labeled as N1-N20; which were also used to assess the specificity of One Step Anti-HIV 1+2 (Whole Blood) Test. Three lots were used.

Specimen samples positive of anti-hepatitis B virus antibody, anti-HCV antibodies, anti-TP antibody, rheumatic factor and serum sample of high lipoidemia were also test for the specificity. Results are summarized in the following table:

Table 1 Analytical Specificity of One Step Anti-HIV 1+2 (Whole Blood) Test

Items			Lot 1：20001007	Lot 2：20001008	Lot 3：20001009
	Test sample				
Anti-HIV 1+2	Internal Negative serum	Negative (－/－) (20/20)	20/20	20/20	20/20
	NICPBP (sera panel)	Negative standard (－/－) (20/20)	20/20	20/20	20/20
		Positive standard for HIV 1 gp120, gp41 (＋/＋) (18/18)	18/18	18/18	18/18
		Positive standard for HIV 2 gp 36 (＋/＋) (2/2)	2/2	2/2	2/2
		Anti-TP (+)	－	－	－
		Anti-hepatitis B (+)	－	－	－
		Anti-HBV (+)	－	－	－
		Rheumatic Factor (+)	－	－	－
		High lipoidemia (+)	－	－	－

The Evaluation Criteria was set to be:
anti HIV 1 POSITIVE if T1 and control region showed color bands regardless of color intensity;
anti HIV 2 POSITIVE if T2 and control region showed color bands regardless of color intensity;
anti HIV 1+2 POSITIVE if T1, T2 and control region showed three color bands regardless of color intensity.
NEGATIVE if only control region showed color band.

Conclusion:
All samples showed positive red band at test region (T1) with P1-P18 positive standard specimens and positive red band at T2 region with P19-P20 from NICPBP panel. No false positive results were observed with In-house negative sera panel and with NICPBP negative standard specimens (N1-N20). It met the requirement of NICPBP standard. No cross reactivates were observed with anti HBV, anti-HCV, anti-TP, rheumatic factor and high lipoidemia. The analytic specificity of One Step Anti-HIV 1+2 (whole blood) Test meets the standard requirement of the national standard (NICPBP).

5.2 Diagnostic Sensitivity and Diagnostic Specificity:

The diagnostic sensitivity and specificity of One Step Anti-HIV 1+2 Test were determined by comparison studies with both anti HIV 1 and anti HIV 2 positive and negative clinical samples (Serum/Plasma/Whole Blood). These studies were conducted using One Step Anti-HIV 1+2 Test with leading commercially available reference ELISA methods including Abbott-1 from Abbott Laboratory and others at multiple clinical sites over a period of 2 years. A total number of 1815 clinical samples were tested. Test result was summarized in the following table:

Table 2 Clinical Accuracy of One Step Anti-HIV 1+2 Test

	Reference methods (Positive)	Reference methods (Negative)	Total
One Step Anti-HIV 1+2 Test (positive)	306 (a)	4 (b)	310
One Step Anti-HIV 1+2 Test (Negative)	0 (C)	1505 (d)	1505
Total	306	1509	1815

Diagnostic Sensitivity (Positive agreement) = a / (a+c) = 306/306 = **100%**

Diagnostic Specificity (Negative agreement) = d / (b+d) = 1505/1509 = **99.7%**

Total agreement = (a+d)/ (a+b+c+d) = (306+1505)/1815 = **99.8%**

The Evaluation Criteria was set to be:
anti HIV 1 POSITIVE if T1 and control region showed color bands regardless of color intensity;
anti HIV 2 POSITIVE if T2 and control region showed color bands regardless of color intensity;
anti HIV 1+2 POSITIVE if T1, T2 and control region showed three color bands regardless of color intensity.
NEGATIVE if only control region showed color band.

Conclusion: The positive agreement with a commercially available rapid test was 100% and negative agreement was at 99.7%. The total agreement was 99.8%.

As part of the clinical assessment, please review the attached translated version of clinical study report done at NATIONAL INSTITUTE FOR THE CONTROL OF PHARMACEUTICAL AND BIOLOGICAL PRODUCTS, during the period of September 2006.

Table 3 Clinical Accuracy of One Step Anti-HIV 1+2 Test (done at NICPBP-2006)

	Determine HIV-1/2 (Positive)	Determine HIV-1/2 (Negative)	Total
One Step Anti-HIV 1+2 Test (positive)	98 (a)	0(b)	98
One Step Anti-HIV 1+2 Test (Negative)	0 (C)	383 (d)	383
Uncertain (subsequent test proved to be negative)	0	12	12
Total	98	405	503

Please see report for details.

5.3 Repeatability and Reproducibility:

The performance of repeatability and reproducibility of One Step Anti-HIV 1+2 (Whole blood) Test were tested by conducting the Reproducibility Study and the Variability Study with the

standard solution (CV) from national sera panel from NICPBP mentioned above.
The study was conducted based on the **Requirement for One Step Anti-HIV 1+2 Test (Colloidal Gold).**
Three lots of One Anti-HIV 1+2 (Whole Blood) Test were used and 10 strips of each lot were tested with standard solution (CV) as well as one of the negative solutions (N1-N20).

Table 4 Precision study of One Step Anti-HIV 1+2 (Whole blood) Test

Sera Panel	Lot 1		Lot 2		Lot 3		Precision
	P	N	P	N	P	N	
N1 (negative)	0	10	0	10	0	10	100%
CV (weak positive)	10	0	10	0	10	0	100%

P: positive N: negative

The Evaluation Criteria was set to be:
anti HIV 1 POSITIVE if T1 and control region showed color bands regardless of color intensity;
anti HIV 2 POSITIVE if T2 and control region showed color bands regardless of color intensity;
anti HIV 1+2 POSITIVE if T1, T2 and control region showed three color bands regardless of color intensity.
NEGATIVE if only control region showed color band.

Conclusion: All samples were positive with standard solution CV and showed equal color intensity. All tests showed negative with the negative standard solution. No false and different results were observed. The precision of the color intensity in positive strips was excellent with all three lots. It indicates that One Step Anti-HIV 1+2 (Whole Blood) Test's reproducibility is good and its variability is very rare.

5.4 Real Time Stability Study

This study was done to demonstrate the real time stability of One Step Anti-HIV 1+2 (Whole Blood) Test and to ensure the shelf life of the product.
Enough test strips and cassettes in the sealed foil pouch were placed in 4-30 º C, which is the recommended storage temperature and in normal humidity status for 26 month (at least two months beyond the expiry period). These test strips were then tested for their performance characteristics (sensitivity, specificity and precision study) in a fixed date of every month and beyond the claimed expiry period until the test quality deteriorates.
The performance results were summarized in the following table.
The study was conducted based on the **Requirement for One Step Anti-HIV 1+2 Test (Colloidal Gold).** See attached Stability Study report for details.

Table 5 Stability Test for One Step Anti-HIV 1+2 (Whole Blood) Test

	Sensitivity (Positive Standard P1-P18 for HIV 1 and P19-20 for HIV 2)	Specificity (Negative standard N1-N20）	Precision study standard solution (CV)
Day 0	+	−	+
1st month	+	−	+
2nd month	+	−	+
3rd month	+	−	+
4th month	+	−	+
5th month	+	−	+
6th month	+	−	+
7th month	+	−	+
8th month	+	−	+
9th month	+	−	+
10th month	+	−	+
11th month	+	−	+
12th month	+	−	+
13th month	+	−	+
14th month	+	−	+
15th month	+	−	+
16th month	+	−	+
17th month	+	−	+
18th month	+	−	+
19th month	+	−	+
20th month	+	−	+
21st month	+	−	+
22nd month	+	−	+
23rd month	+	−	+
24th month	+	−	+
25th month	+	−	+
26th month	+	−	+

The Evaluation Criteria was set to be:
anti HIV 1 POSITIVE if T1 and control region showed color bands regardless of color intensity;
anti HIV 2 POSITIVE if T2 and control region showed color bands regardless of color intensity;
anti HIV 1+2 POSITIVE if T1, T2 and control region showed three color bands regardless of color intensity.
NEGATIVE if only control region showed color band.

Conclusion: No abnormal performance characteristics of the test strip were observed in the condition of room temperature (4-30° C) and test strips in the sealed foil pouch remained dry (the desiccant did not change color) throughout the entire 26 month test period. The test strips maintained in good quality and showed reliable stability for at least 24 month. It met the

quality control requirements.

5.5 Accelerated Stability Study (Aging Test)

Long time high temperature (beyond 4-30° C) may decrease the test's stability and deteriorate its quality. This study intended to demonstrate the test device's stability in high temperature within a certain period of time.
The study was conducted based on the **Requirement for One Step Anti-HIV 1+2 Test (Colloidal Gold),** which is done for batch release regularly.
Enough test cassettes were placed in 37° C for 20 days (within sealed foil pouch and with desiccant inside in an incubator). These cassettes were then tested for their performance characteristic everyday for 20 days. The results are summarized in the following table.

The Evaluation Criteria was set to be:
anti HIV 1 POSITIVE if T1 and control region showed color bands regardless of color intensity;
anti HIV 2 POSITIVE if T2 and control region showed color bands regardless of color intensity;
anti HIV 1+2 POSITIVE if T1, T2 and control region showed three color bands regardless of color intensity.
NEGATIVE if only control region showed color band.

Table 6 Accelerated Stability Study of One Step Anti-HIV 1+2 (Whole Blood) Test

	Sensitivity (Positive Standard P1-P18 for HIV 1 and P19-20 for HIV 2)	Specificity (Negative standard N1-N20）	Precision study standard solution (CV)
Day 0	+	−	+
Day 2nd	+	−	+
Day 3rd	+	−	+
Day 4th	+	−	+
Day 5th	+	−	+
Day 6th	+	−	+
Day 7th	+	−	+
Day 8th	+	−	+
Day 9th	+	−	+
Day 10th	+	−	+
Day 11th	+	−	+
Day 12th	+	−	+
Day 13th	+	−	+
Day 14th	+	−	+
Day 15th	+	−	+
Day 16th	+	−	+
Day 17th	+	−	+
Day 18th	+	−	+

Day 19th	+	−	+
Day 20th	+	−	+

Conclusion: No abnormal performance characteristics of the test device were observed in the condition of 37° C and in sealed foil pouch for the 20 days test period. The test device maintained in good quality and showed reliable stability in such condition for at least 20 days. It met the quality control requirements.

6.0 Summary

In summary, this report was prepared to focus on the description of the studies conducted, the content of the analysis incurred and conclusion drawn from each study to demonstrate mainly on the performance characteristics including analytical sensitivity, diagnostic sensitivity, analytical specificity, diagnostic specificity, repeatability, reproducibility, and stability study of One Step Anti-HIV 1+2 (Whole Blood) Test. It was made to validate the performance claims as One Step Anti-HIV 1+2 (Whole Blood) Test is an in vitro immunoassay for the rapid, qualitative detection of anti HIV 1 and anti HIV 2 virus antibody in human blood specimen. The test is for a visual, qualitative result and is intended for healthcare professional use as an aid in the clinical assessment of HIV infection.

7.0 References

1. Centers for Disease Control and Prevention (CDC) Universal Precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other blood borne pathogens in health-care settings. MMWR 1988; 37(24):377-388.

2. U.S. Department of Health and Human services. Biosafety in microbiological and Biomedical Laboratories. HHS Publication (NIH) 88-8395. Washington: U.S. Government Printing Office, May 1988.

3. World health Organization. Laboratory Biosafety manual. Geneva. World health organization, 1983.

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5. CDC: Updated U.S. Public Health Service Guidelines for the management of Occupational Exposures to HBV, HCV and HIV and recommendations for postexposure Prophylaxis. MMWR 2001; 50(RR-11): 1-42.

6. Essex, M. Human immunodeficiency viruses in the developing world. Adv Virus Res 53: 71-88.