Development of a Rapid HIV-1 Confirmatory Test

Keith Kardos*, Vijaya K Mokkapati, Geraldine Guillon and Bonnie Martinez

OraSure Technologies, Inc., 150 Webster Street, Bethlehem, PA 18015

*kkardos@orasure.com

OraSure Technologies, Inc.
diagnostic solutions for the new millennium

Abstract

In the United States, only the Western blot and Immuno fluorescent Antibody Assay (IFA) are approved by the FDA for confirmatory HIV testing. Both of these tests are labor intensive, expensive, slow to provide results and require skilled readers. The World Health Organization has recommended an algorithm for confirmation using combinations of simple/rapid tests with different HIV antigens.

We developed a rapid lateral flow, immunochromatographic assay with synthetic peptides and native protein antigens representing *env* (gp41, gp120, and gp160), *gag* (p24), and *pol* (p51/66) applied on the same nitrocellulose strip. The sample is applied to a disposable cassette which then flows onto the test strip, hydrates and mixes with a reporter conjugate, to which IgG antibodies in the specimen bind. Antibodies specific to a particular HIV antigen band bind and immobilize the colorimetric reporter at the site of that band on the strip. At completion (~30 min.), the device can be either read visually or by using a portable optical reader, which detects the intensities of the test bands.

Preliminary results using colloidal gold as the reporter with 4 positive and 2 negative serum specimens demonstrated detectable banding patterns comparable to results of the Western blot. Visible bands remained detectable with 1:1600 dilutions of HIV-positive serum, suggesting sensitivity is sufficient to detect the lower level of antibodies present in oral fluid.

A confirmatory lateral flow assay for HIV is feasible, and can be optimized for specimens with low antibody concentrations, such as oral fluid.

Background

- Only two confirmatory methods are FDA approved[1,2]: Western blot and IFA.
- Western blot[3] (WB) uses inactivated viral antigens, electrophoretically separated by molecular weight.
- These protein bands are transblotted on to nitrocellulose strip.
- In WB multi-step procedure, IgG antibodies to specific HIV-1 proteins in the sample bind to the protein bands on nitrocellulose.
- WB procedure is complex, requires specialized equipment, technical expertise, and 4 - 24 hours.
- A rapid, lateral flow HIV-1 confirmatory test based on similar principles could provide results in < 30 min.

Method

- We developed a lateral flow assay with multiple viral proteins or peptides immobilized as separate bands on a nitrocellulose membrane.
- Materials representing *env* (gp41, gp120, gp160), *gag* (p24) and *pol* (p51/66) were immobilized on nitrocellulose.
- The optimal order for the different bands on the nitrocellulose was determined empirically.

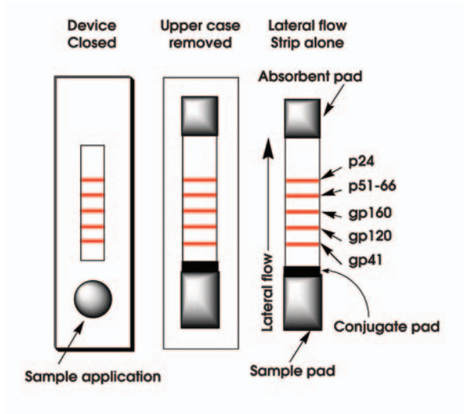

Figure 1: Test device with lateral flow strip.
The disposable test device will have two openings on top. One for sample application and one for reading the result. The strip will have a sample pad and a conjugate pad that contains the reporter on one end and an absorbent pad on the other end.

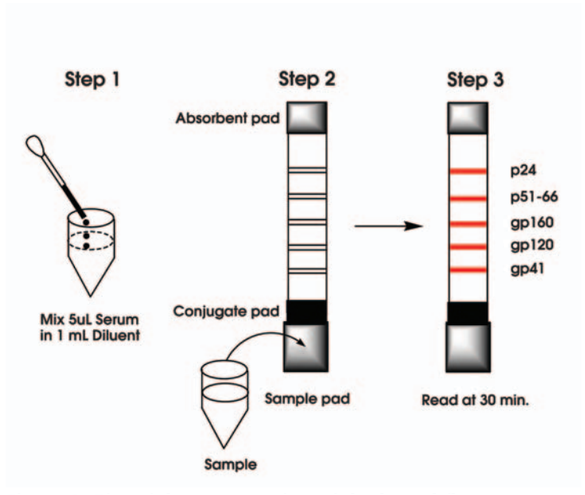

Figure 2: Pictorial representation of the lateral flow test.
Step 1: Five microliters (5 µL) of serum specimen is mixed with 1mL of a sample diluent.
Step 2: Add 200 µL of the mixture to the sample pad. Conjugate will bind to IgG antibodies in the specimen. It flows across the various HIV antigens, antibodies specific to a particular antigen band will bind.
Step 3: At the completion of the test (~30 min.), the device can be read.

Results

Sample ID	Western blot result					Rapid Lateral flow result				
	p24	p51/66	gp41	gp120	gp160	p24	p51/66	gp41	gp120	gp160
0729902	-	-	-	-	-	-	-	-	-	-
43973	-	-	-	-	-	-	-	-	-	-
D091036	+	+	+	+	+	+	+	+	+	+
43962	+	+	+	+	+	+	+	+	+	+
43965	+	+	+	+	+	+	+	+	+	+
43968	+	+	+	+	+	+	+	+	+	+

Table 1: Comparison of rapid lateral flow test with Western Blot.
The banding pattern in the lateral flow assay completely matched the Western blots.

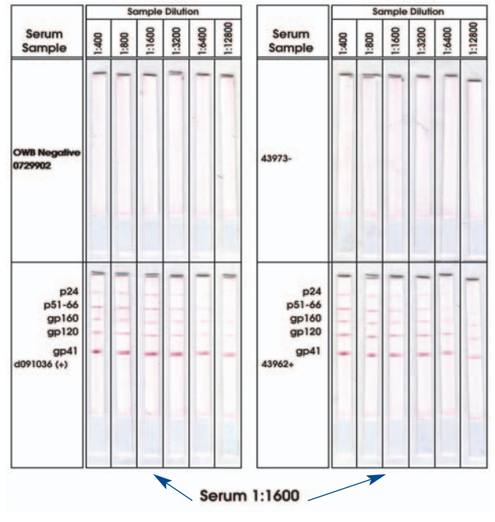

Figure 3: Dilutional sensitivity
Two HIV-1 positive and two negative serum specimens were run at dilutions of 1:400, 1:800, 1:1600, 1:3200, 1:6400 and 1:12800. All five bands were detectable for both the positive specimens down to 1: 1600 dilution.

Western blot Test	Rapid Lateral Flow test
1. Wash the strips in a diluted wash buffer for 5-10 min on a rocker. 2. Aspirate buffer. 3. Add Blot buffer and then add 30ul sample to the strips. 4. Incubate for 2 hrs on a rocker. 5. Aspirate the mixture out. 6. Add wash buffer, rock several times and aspirate. 7. Repeat the wash step 6 for 5 min. two more times. 8. Prepare and add conjugate-1 solution and rock for 60 min. 9. Aspirate and repeat wash steps 3 times as in steps 6-7. 10. Add conjugate-2 solution and rock for 60 min. 11. Aspirate and wash as in step 9. 12. Add substrate solution and rock for 15 min. 13. Aspirate substrate and wash the strips with water. 14. Air dry the strips and interpret as per manufacturer's instructions. 15. Total time is more than 5 hours.	1. Mix 5ul sample in 1ml of sample diluent. 2. Add 200ul of the mixture to the sample pad 3. Read the result after 30 min. 4. Total time is 30 min.

Table 2: Comparison of the number of steps.

Conclusions

- A confirmatory rapid lateral flow assay for HIV is feasible, and can be optimized for specimens with low antibody concentrations.
- The lateral flow assay is much faster and less labor intensive compared to the currently approved Western blot or IFA confirmatory tests.

REFERENCES

1. U.S. Food and Drug Administration, (CBER) Licensed / Approved HIV, HTLV, and Hepatitis Tests, January 10, 2005.
2. Interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. *MMWWR Morb Mortal Wkly Rep* 1989;38 (No.S-7):1-7.
3. Tsang VCW, Hancock K, Wilson M et al: Enzyme Linked immuno-electrotransfer blot technique (EITB) (Western blot) for HTLV-III / LAV antibodies. *Developmental Centers for Disease Control*, Atlanta; 1985.