A Multiplex Point-of-Care for Detection of HIV-1/HIV-2 and Hepatitis C Infection

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Introduction

There are over 3 million people chronically infected with Hepatitis C virus (HCV) and over 1 million infected with Human Immunodeficiency Virus (HIV) in the United States.[1,2] The expansion and improvement of HIV testing and linkage programs to high-risk groups, including those who abuse injectable drugs and men who have sex with men, are important for treatment and prevention of coinfections.[3] The ability to diagnose HIV and coinfections simultaneously can lead to more effective treatment decisions and improved linkage to care.[4] Prevention, education, and treatment are important because HIV and HCV coinfection is associated with higher rates of mortality due to accelerated liver disease compared to those with only HCV monoinfection.[5]

Currently, the diagnosis of HIV infection requires a testing algorithm which begins with an antibody and/or antigen test, followed by a HIV RNA detection test. For HCV testing, an antibody test is required and if positive; a nucleic acid test that detects HCV RNA. Generally, testing is laboratory-based or point-of-care rapid testing. Rapid tests can advantageously provide same day results and do not require highly-trained personnel.

Many of the HIV point-of-care rapid tests do not distinguish between HIV-1 and HIV-2 infection. Positive results at point-of-care is only preliminary and require supplemental testing to confirm HIV or HCV.[3]

Testing is a challenge as resources may be limited. Maxim's objective is to develop and evaluate a multiplex HIV and HCV which may offer convenient, quick, cost-effective, point-of-care screening for high-risk populations at health departments and substance abuse treatment facilities.

Methods

- Using lateral flow dispensing equipment, Control antibody, HIV-1 Antigen, HIV-2 Antigen, and HCV Core Antigens were dispensed onto nitrocellulose membrane. The positions are illustrated in Figure 1.
- Detection antibody was conjugated to colloidal gold and dispensed onto the conjugate pad.
- The processed nitrocellulose membrane, gold conjugate, wick and sample pad were then assembled onto a backing card, cut into strips and assembled into cassettes.
- Well-characterized, commercially available human plasma/serum specimens that are HIV/HCV negative, HIV monoinfected, HCV monoinfected, and the six genotypes (Genotype 1-6) of HCV were tested.
- Specimens were processed at ambient temperature (23-26ºC) on the benchtop. Serum/plasma specimens were diluted 1:200 into Running Buffer and 75µL of the diluted specimen was added to the sample well. The strip was assayed for 20 minutes then read visually.

Results

Figure 2 and 3 provides photographic results from the test. The strip was removed from cassette for photographic purposes. Table 1 and 2 lists the specimens tested and their visual result.
This multiplex assay was able to correctly identify the specimens as Negative, only HCV positive, HCV and HIV positive, or only HIV positive. Figure 1 shows initial testing results at 20 minutes with Running Buffer only, a negative specimen, HIV monoinfected specimens, HCV monoinfected specimens, and HIV/HCV coinfected specimens. In addition, this assay was able to detect the six genotypes of HCV, which are illustrated in Figure 3. Refer to Table 1 and 2 for the list of corresponding specimens tested and their serological status.

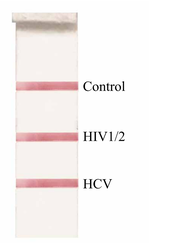

Figure 1: Schematic Representation of Multiplex HIV/HCV Test Strip. Three lines are striped on nitrocellulose.

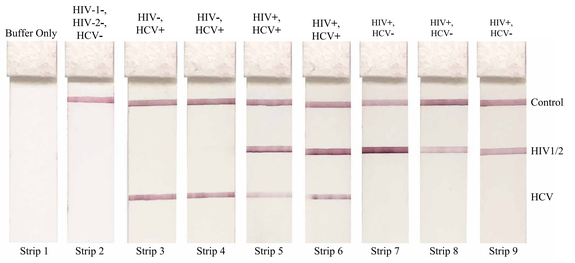

Figure 2: Multiplex Lateral flow results with specimens that are Negative, HIV monoinfected, HCV monoinfected, or HIV/HCV coinfected. Refer to Table 1 for each test's serological status, visual result, and interpretation.

Strip # / Sample #	Serological Status	Visual Result	Interpretation
1	Running Buffer Only	No Bands	No specimen added to buffer
2	HIV- ; HCV-	Only Control Band Visible	HIV- ; HCV-
3	HIV- ; HCV+	HCV Band Visible	HIV- ; HCV+
4	HIV- ; HCV+	HCV Band Visible	HIV- , HCV+
5	HIV+ ; HCV+	HIV-1 and HIV-2 band visible, HCV Band Visible	HIV+ ; HCV+
6	HIV+ ; HCV+	HIV-1 and HIV-2 band visible, HCV Band Visible	HIV+ ; HCV+
7	HIV+ ; HCV-	HIV-1 and HIV-2 band visible	HIV+ ; HCV-
8	HIV+ ; HCV-	HIV-1 and HIV-2 band visible	HIV+ ; HCV-
9	HIV+ ; HCV-	HIV-1 and HIV-2 band visible	HIV+ ; HCV-

Table 1: Specimens tested in Figure 2 with serological status, visual results and interpretations.

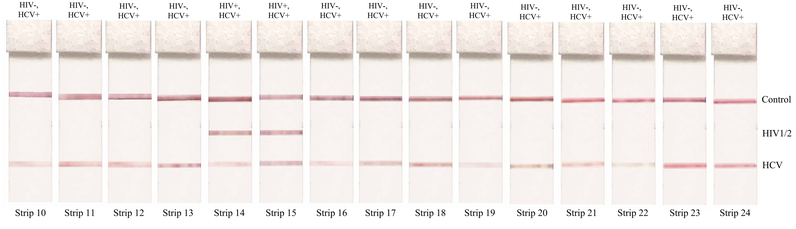

Figure 3: Further testing of Multiplex Lateral flow with HCV positive specimens including the six genotypes of HCV. Refer to Table 2 for each test's serological status, visual result, and interpretation.

Strip # / Sample #	Serological Status	Visual Result	Interpretation
10	HCV+	HCV Band Visible	HIV- ; HCV+
11	HCV+	HCV Band Visible	HIV- ; HCV+
12	HCV+, Genotype 1	HCV Band Visible	HIV- ; HCV+
13	HCV+, Genotype 1	HCV Band Visible	HIV- , HCV+
14	HIV+ and HCV+, Genotype 2	HIV-1 and HIV-2 band visible, HCV Band Visible	HIV+ ; HCV+
15	HIV+ and HCV+, Genotype 2	HIV-1 and HIV-2 band visible, HCV Band Visible	HIV+ ; HCV+
16	HCV+, Genotype 2	HCV Band Visible	HIV- ; HCV+
17	HCV+, Genotype 3	HCV Band Visible	HIV- ; HCV+
18	HCV+, Genotype 3	HCV Band Visible	HIV- ; HCV+
19	HCV+, Genotype 4	HCV Band Visible	HIV- ; HCV+
20	HCV+, Genotype 4	HCV Band Visible	HIV- ; HCV+
21	HCV+, Genotype 5	HCV Band Visible	HIV- ; HCV+
22	HCV+, Genotype 5	HCV Band Visible	HIV- ; HCV+
23	HCV+, Genotype 6	HCV Band Visible	HIV- ; HCV+
24	HCV+, Genotype 6	HCV Band Visible	HIV- ; HCV+

Table 2: Specimens tested in Figure 3 with serological status, visual results and interpretations.

Lessons Learned

The HIV-1 and HIV-2 antigens have been evaluated on lateral flow in over 1800 serum/plasma specimens with the CDC Approved Maxim Swift™ HIV Recent Infection Assay (RIA). The same antigens used for Maxim Swift™ RIA were used in the HIV-1/2 Test line. The sensitivity of the HIV-1 and HIV-2 Test line is 99.5% and specificity is 99.4%.
Based on initial evaluation, multiplex lateral flow for HIV-1/HIV-2 and HCV coinfection screening appears promising, but further testing is needed to evaluate sensitivity, specificity, cross-reactivity and interference. In addition, other sample types such as dried blood spots (DBS) and Oral Fluid will be evaluated.

References

[1] Schackman, Bruce R., et al. "Cost-Effectiveness of Rapid Hepatitis C Virus (HCV) Testing and Simultaneous Rapid HCV and HIV Testing in Substance Abuse Treatment Programs." Addiction, vol. 110, no. 1, 2014, pp. 129–143., doi:10.1111/add.12754.
[2] Centers for Disease Control. "U.S. Statistics." HIV.gov, 19 Feb. 2019, www.hiv.gov/hiv-basics/overview/data-and-trends/statistics.
[3] Integrating HIV and HCV Testing Toolkit." NASTAD. 27 Feb. 2019. 04 Mar. 2019 <https://www.nastad.org/resource/integrated-testing-toolkit>.
[4] Lochhead, M. J., K. Todorof, M. Delaney, J. T. Ives, C. Greef, K. Moll, K. Rowley, K. Vogel, C. Myatt, X.-Q. Zhang, C. Logan, C. Benson, S. Reed, and R. T. Schooley. "Rapid Multiplexed Immunoassay for Simultaneous Serodiagnosis of HIV-1 and Coinfections." Journal of Clinical Microbiology 49 (2011): 3584-590.
[5] Chen, Jennifer Y., et al. "HCV and HIV Co-Infection: Mechanisms and Management." Nature Reviews Gastroenterology & Hepatology, vol. 11, no. 6, 2014, pp. 362–371., doi:10.1038/nrgastro.2014.17.

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