ENHANCEMENT OF HIV-1 RNA DETECTION BUT NOT HSV-2 DNA FROM DACRON SWABS CONTAINING DNA/RNA PROTECT™

Tammy T. Evans-Strickfaden[1], Cheng Y. Chen[2], David L. Trees[2], Ronald C. Ballard[2], Clyde E. Hart[1]

[1]Laboratory Branch, Division of HIV/AIDS Prevention

[2]Laboratory Reference and Research Branch, Division of STD Prevention

National Center for HIV, STD, TB Prevention

Centers for Disease Control and Prevention, Atlanta, GA 30333

SAFER • HEALTHIER • PEOPLE™

Abstract

BACKGROUND/OBJECTIVES: An increasing number of clinical and epidemiological studies are beginning to investigate the impact of anti-viral therapies on the shedding of HIV-1 and HSV-2 in the genital secretions of co-infected women. The ability of women to collect self-administered genital tract swab specimens and adequately store them at home would greatly enhance the capacities of these studies to measure virus shedding as an outcome of therapy without increasing the number of clinic visits. Therefore, we investigated a non-toxic agent (DNA/RNA Protect™; Sierra Diagnostics) for its ability to protect the nucleic acids of HIV-1 and HSV-2 spiked on to swabs.

METHODS: Dry and DNA/RNA Protect™-infused sterile dacron swabs were spiked with a 25ul solution containing HIV-1 or HSV-2 at 1000, 500, 100, 50, 5, and 0 copies per swab. The spiked swabs were stored at ambient (22 – 25°C) or elevated (37°C) temperatures for less than 12 hours, 7 days, or 14 days. Following storage at test temperature, swabs were kept frozen (-70°C) until they were extracted for total nucleic acids using the NucliSens® lysis buffer (9ml) and isolation kit (bioMérieux, Inc.). HIV-1 RNA was quantified using Roche Amplicor Monitor (v1.5) and HSV-2 DNA was qualitated using a real-time PCR with TaqMan probe.

RESULTS: The presence of DNA/RNA Protect™ in the virus-spiked swabs enhanced the ability to detect HIV-1 RNA after short term storage (< 12 hrs.) at ambient and elevated temperatures. At ambient temperature, HIV-1 RNA detection was 10-fold more sensitive with DNA/RNA Protect™ swabs compared to dry swabs: 5 copies versus 50 copies, respectively. At the elevated temperature, detection was at least 2-fold more sensitive with DNA/RNA Protect™ swabs. When virus-spiked swabs were stored for 7 days at ambient temperature, DNA/RNA Protect™ swabs enhanced HIV-1 RNA detection over dry swabs by only 2-fold: 50 copies versus 100 copies, respectively. However, after 7 days at the elevated temperature, only DNA/RNA Protect™ swabs had detectable HIV-1 RNA (1000 copies). After 14 days at ambient temperature, DNA/RNA Protect™ swabs enhanced HIV-1 RNA detection over dry swabs by 20-fold: 5 copies versus 100 copies, respectively. In contrast, the presence of DNA/RNA Protect™ in the virus-spiked swabs did not produce a consistent enhancement of HSV-2 DNA detection at the ambient or elevated storage temperatures. Overall, DNA/RNA Protect™ swab stabilization of both HIV-1 RNA and HSV-2 DNA at 7days was more effective at ambient temperature.

CONCLUSIONS: These results indicate that DNA/RNA Protect™-infused swabs are potentially more effective than dry swabs at stabilizing HIV-1 RNA during short and long term storage at ambient and elevated temperatures. In addition, we recommend that for accurate HIV-1 and HSV-2 quantification from self-administered genital swabs, DNA/RNA Protect™-infused storage temperatures should not exceed 25°C.

Introduction

- Worldwide, most HIV infections in adults have been transmitted sexually through exposure to infected genital tract secretions.
- HIV co-infection with HSV-2 is common.
- HSV co-infection is associated with an increased risk of HIV transmission.
- The ability to monitor daily changes in HIV and HSV shedding in genital secretions is important for assessing the effectiveness of anti-viral therapies designed to lower virus loads in those secretions.
- Safe, convenient and reliable methods for home collection and storage of vaginal secretions are needed for a cost- effective methodology of monitoring daily shedding of HIV and HSV.
- This study evaluates the performance of dry and DNA/RNA Protect^{TM}-infused swabs to stabilize the nucleic acids of cell-free HIV-1 and HSV-2 at 25°C and 37°C.

Methods

- Serial dilute virus stocks in PBS
  - HIV-1 (subtype b) from human blood plasma
  - HSV-2 from cell culture suspensions

- Inoculate swabs (duplicates)
  - 25ul of HIV-1 or HSV-2
  - 1000, 500, 100, 50, 5, 0 copies per swab

- Incubate swabs
  - ambient or 37°C
  - < 12 hrs., 7 and 14 days
  - Store at -70°C

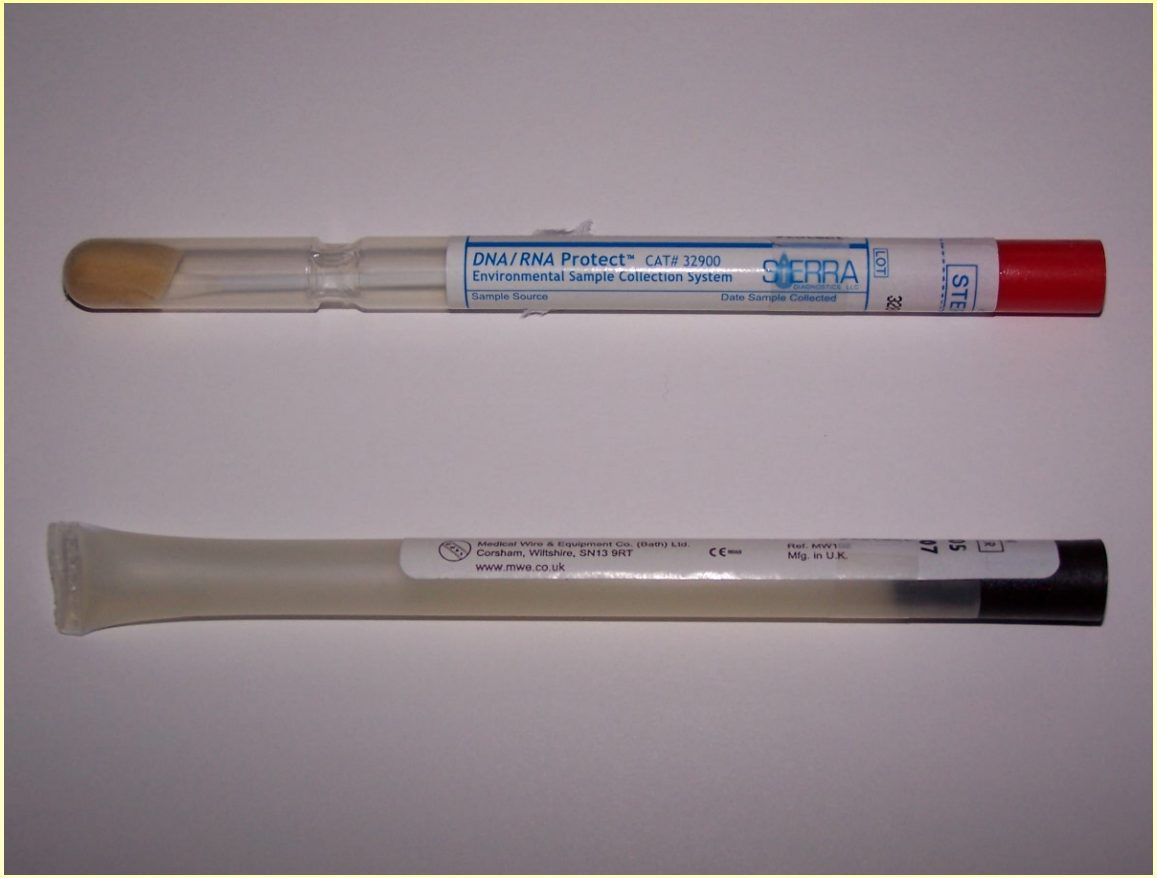
- Extract Nucleic Acids
  - 9-ml NucliSens® (bioMérieux, Inc.)

- Test Virus Load
  - HIV-1 Roche Monitor ® (v 1.5)
  - HSV-2 TaqMan (Rotor-Gene 3000)

Swabs

Top: DNA/RNA Protect™ (Sierra Diagnostics)

Bottom: Dry dacron swab



HIV-1 RNA Detection on Swabs

(+ = RT-PCR pos., - = RT-PCR neg.)

Incubation:	AMBIENT		37°C	
Swab: Protect™	DRY	DNA/RNA Protect™	DRY	DNA/RNA
DAY 0				
1000	++	++	++	++
500	++	++	ND[1]	ND
100	++	++	ND	ND
50	++	++	+ -	++
5	- -	+ + [2]	ND	ND
0	- -	- -	- -	- -
DAY 7				
1000	++	++	- -	++
500	++	+ -	ND	ND
100	++	+I [3]	ND	ND
50	+ -	++	- -	- -
5	- -	- -	ND	ND
0	+ - [2]	- -	+ - [2]	- -
Day 14				
1000	++	++	ND	ND
500	++	++	ND	ND
100	++	++	ND	ND
50	+ -	++	ND	ND
5	+ -	++	ND	ND
0	- -	- -	ND	ND

[1] Not done

[2] Results from virus load kits later recalled for possible false positives

[3] Invalid test

HSV-2 DNA Detection on Swabs

(+ = Real-Time PCR pos., - = Real-Time PCR neg.)

Incubation:	AMBIENT		37°C	
Swab: Protect™	DRY	DNA/RNA Protect™	DRY	DNA/RNA
DAY 0				
1000	++	++	++	++
500	++	++	++	++
100	++	++	++	++
50	++	++	++	++
5	++	+ -	- -	- -
0	- -	- -	- -	- -
DAY 7				
1000	++	++	++	++
500	++	++	++	++
100	++	++	- -	+ -
50	+ -	++	+ -	+ -
5	- -	- -	- -	- -
0	- -	- -	- -	- -

Summary

- DNA/RNA Protect™ swabs were more effective than dry swabs at preserving HIV-1 RNA for detection by RT-PCR.

- DNA/RNA Protect™ swabs were effective at preserving HIV-1 RNA detection at ambient temperatures for 14 days and at 37°C for 7 days (longest time tested).

- DNA/RNA Protect™ swabs provided no clear advantage over dry swabs for the of detection of HSV-2.

Conclusions

- DNA/RNA Protect™ swabs should be considered for use in protocols when daily, self-collected samples of genital tract secretions from HIV-1 and HSV-2 infected women are required.

- Storage of swabs (dry or DNA/RNA Protect™) should not exceed ambient temperature if possible.