Copan MSwab Rapid Extraction Paired with Altona RealStar® *alpha*Herpesvirus PCR For Detection of HSV1, HSV2 and VZV in Lesion Swabs

Kathy Luinstra[1], Candy Rutherford[1], and Marek Smieja[1,2]

[1]St. Joseph's Healthcare, Hamilton ON, Canada, [2]McMaster University, Hamilton ON, Canada.

ABSTRACT

Background: Direct fluorescent antibody (DFA) testing with or without shell vial culture, is a common laboratory method for detection of Herpes simplex virus (HSV) types 1 and 2 and Varicella zoster virus (VZV) from lesion swabs yielding results in 24 (HSV) to 72 hours (VZV). Additional typing with type specific monoclonal antibodies is required to distinguish HSV1 and HSV2. We examined the the value of pairing MSwab rapid nucleic acid extraction with the RealStar® *alpha*Herpesvirus PCR for molecular diagnosis of skin and mucous membrane lesions for HSV1, HSV2 and VZV.

Methods: Over the course of one year, lesion swab samples collected in Universal Transport Medium (UTM; Copan Italia) and submitted for *alpha*Herpesvirus detection by DFA/shell vial culture were saved. 74 positive and 61 randomly selected negative lesion swabs were stored for evaluation. The UTM was decanted from the collection tube and stored at -80ºC. Swabs were retained in their original collection tube at -80ºC. Residual material, retained in the swabhead was processed by a rapid extraction method. Swabs were thawed, placed in 1 mL of MSwab (Copan Italia) medium and vortexed vigorously for 30 seconds. A 200 µl aliquot was then heated at 97-98ºC for 3 minutes, briefly vortexed and centrifuged at 14 000 rpm for 2 minutes. As a comparator, 200 µl of the UTM-collection was extracted by the generic easyMag™ (bioMérieux) protocol and eluted in 55 µl. Ten µl of nucleic acid, obtained by MSwab rapid extraction or by UTM/easyMag™, was tested in the RealStar® *alpha*Herpesvirus PCR (Altona Diagnostics) assay according to the manufacturer's instructions. Discordants between PCR and DFA/shell vial culture were resolved by repeat culture and in-house PCR assays.

Results: UTM/easyMag™ and MSwab rapid extraction each confirmed 73/74 *alpha*Herpes DFA/shell vial culture positives (98%). UTM/easyMag™ detected 76 *alpha*Herpes viruses compared to 75 by MSwab rapid extraction. Of 61 DFA/shell vial culture negative samples, 47 were PCR negative by both extraction methods, 10 were PCR positive by both, 3 were positive by UTM/easyMag™ extraction only and 1 was positive by MSwab rapid extraction only. Both methods detected three dual VZV/HSV2 infections. UTM/easyMag™ and MSwab rapid extraction increased *alpha*Herpes virus detection over DFA/shell vial culture by 21% and 18%, respectively. PCR had a sensitivity of 99% (95% CI: 94-100), a specificity of 94% (95% CI: 84-98), a positive predictive value of 97% and a negative predictive value of 98%, compared to DFA/shell vial culture.

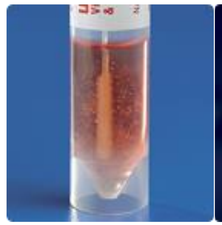
Conclusions: MSwab rapid extraction paired with the RealStar® *alpha*Herpesvirus PCR is more sensitive and specific than DFA/shell vial culture and provides same day results for HSV1, HSV2, and VZV. Mswab rapid extraction of lesion swab specimens is an inexpensive extraction approach which has high agreement with UTM/easyMag™ processing.

OBJECTIVE

- To evaluate molecular testing of lesion swabs for diagnosis of *alpha*Herpesvirus from skin and mucous membrane lesions using direct MSwab (Copan Italia S.P.A., Brescia, Italy) rapid nucleic acid extraction and RealStar® *alpha*Herpesvirus PCR (Altona Diagnostics GmbH Hamburg, Germany)

METHODS

- Lesion swab samples collected in UTM (Copan Italia) and submitted for *alpha*Herpesvirus detection by DFA/shell vial culture were saved
- 74 positive and 61 randomly selected negative lesion swabs were stored for this evaluation
- UTM was decanted from the collection tube and stored at -80ºC
- Swabs were retained in their original collection tube at -80ºC
- Residual material trapped in the swab was recovered by rapid extraction
- Swabs were thawed, placed in 1 mL of MSwab medium (Copan Italia), and vortexed vigorously for 30 seconds
- A 200 µl aliquot was heated at 97-98ºC x 3 minutes, briefly vortexed and centrifuged at 14 000 rpm x 2 minutes; the supernate was used for PCR
- As a comparator, 200 µl of the original UTM lesion swab collection was extracted by easyMag™ (bioMérieux Canada, St. Laurent, Q.C.) and eluted in 55 µl using the generic extraction protocol
- 10 µl of nucleic acid from each extraction method was tested in the RealStar® *alpha*Herpesvirus PCR assay (Altona Diagnostics) according to the manufacturer's instructions
- Discordant PCR/DFA/culture results were resolved by repeat culture and in-house PCR assays



RESULTS

Table. Identified *alpha*Herpes Viruses by DFA/Culture and PCR

DFA/Culture Results	Lesion Site	UTM/easyMag Extraction & PCR	MSwab/Rapid Extraction & PCR
44 HSV 1	29 Head and Neck, 10 Genital, 5 Unknown	43 HSV1 1 HSV1/VZV	44 HSV1
19 HSV 2	1 Lip, 1 Hand, 14 Genital, 3 Unknown	1 HSV1 16 HSV2 2 HSV2/VZV	1 HSV1 16 HSV2 2 HSV2/VZV
2 HSV untypeable	1 Mouth, 1 Back	1 HSV1 1 VZV	1 HSV1 1 VZV
9 VZV	Eye, Skin, Trunk, Buttocks	8 VZV 1 Negative	8 VZV 1 Negative
61 Negative	27 Head and Neck, 18 Genital, 15 Trunk, 1 Unknown	48 negative 7 HSV1 4 HSV2 1 HSV2/VZV; 1 VZV	50 negative 6 HSV1 4 HSV2 1 HSV2/VZV

- Both extraction methods confirmed 73/74 DFA/culture positives (98%)
- Of 61 DFA/culture negative samples, 47 were PCR negative by both extraction methods, 10 were PCR positive by both, 3 were positive by the UTM/easyMag™ only and 1 was positive by MSwab rapid extraction only
- Both methods detected 3 dual HSV2/VZV infections
- UTM/easyMag™ and MSwab rapid extraction increased *alpha*Herpes virus detection over DFA/culture by 21% (13/61) and 18% (11/61) respectively
- PCR had a sensitivity of 99% (95% CI: 94-100), a specificity of 94% (95% CI: 84-98), compared to DFA/culture

CONCLUSIONS

- Direct MSwab rapid extraction paired with the RealStar® *alpha*Herpesvirus PCR is sensitive and specific and provides same day results for HSV1, HSV2, and VZV
- Direct MSwab rapid extraction of lesion swab specimens is an inexpensive extraction approach which has high agreement with the UTM/easyMag™ processing