

A Comparison of Two Molecular Assays for the Detection of Herpes Simplex Virus (HSV)

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Abstract

Background: The herpes simplex virus (HSV) is a highly pervasive virus affecting millions of individuals worldwide and is categorized into two types. HSV-1 causes primarily oral herpes and HSV-2 causes lesions primarily in the genitalia. HSV can also affect other parts of the body and has the potential to cause serious complications in infants and immunocompromised individuals. Rapid and accurate detection is essential for prompt, optimal pharmaceutical intervention. The goal of this study was to evaluate the Luminex ARIES HSV 1 & 2 Assay (Luminex Corp, Austin, TX) and compare its performance with our standard of care procedure which uses analyte-specific reagents (ASR) for HSV 1 & 2 on the Focus DX Simplexa™ (Diasorin Molecular LLC, Cypress, CA). **Methods:** A total of 50 lesion swabs submitted to the laboratory for HSV testing were included in this comparison study. For our routine HSV testing, sample DNA was extracted using the EasyMag (bioMérieux, Marcy-l'Étoile, France) and eluted DNA was mixed 1:1 with the Simplexa reaction mix and loaded onto the Focus DX Simplexa™. Residual swab samples were stored at -80°C. For the ARIES HSV 1 & 2 assay, a 200 µl volume of previously frozen samples was added to individual test cassettes for automated extraction and PCR according to the manufacturer's instructions. Results from the two molecular tests were analyzed. **Results:** Out of 50 specimens evaluated, 48 samples were in agreement by both assays. A total of 16 samples were identified as positives for HSV-1, with a sensitivity and specificity of 100% between assays. A total of 8 out of 10 samples were identified as positives for HSV-2, with a sensitivity of 88.9% and specificity of 97.6% between assays. Only two discrepant results were observed between the two assays. **Conclusion:** The two HSV 1 & 2 assays evaluated presented an excellent overall agreement. Since our standard of care procedure involves an off-board extraction, the ARIES provides a faster analytical procedure for detection and differentiation of HSV 1 & 2, therefore reducing turnaround time and possible contamination due to sample handling.

Introduction

Herpes simplex virus (HSV) is categorized into two types: HSV-1 and HSV-2. Symptoms of the infection include painful blisters that can present on various parts of the human body. Infection can also be asymptomatic and pose a higher risk of transmission. Rapid accurate detection is optimal for treatment and molecular PCR testing is becoming the standard of care in diagnostic laboratories due to its sensitivity and specificity.

The Luminex ARIES HSV 1 & 2 Assay and Focus DX Simplexa™ are qualitative diagnostic tests for the detection and differentiation of HSV-1 and HSV-2 in cutaneous and mucocutaneous samples. While the Luminex ARIES is sample-to-answer, the Focus DX Simplexa™ procedure calls for an off-board extraction prior to testing samples. Both assays were compared for ease of use and efficiency.

Methods

Clinical Swab Sample Testing:

Evaluation of swab samples:

- A total of 50 unprocessed clinical universal transport medium swabs (UTM) from cutaneous or mucocutaneous sites were tested.
- After routine testing in lab on the Focus DX Simplexa™ 3M Integrated Cycler, residual UTM samples were stored at -80°C for testing on the Luminex ARIES.
- Analysis of PCR cycle threshold (Ct) values was performed to compare Ct value ranges between HSV-1 and HSV-2 for both the molecular tests.

Focus DX Simplexa™:

- 100 µl of fresh specimen was extracted using the EasyMag following manufacturer instructions.
- 5 µl of eluted DNA was mixed 1:1 with 5 µl of Simplexa reaction mix and loaded onto the Focus DX Simplexa™ 3M Integrated Cycler for PCR using a 96 well universal disc labeled with patient information
- Residual swab samples were stored at -80°C for testing on the Luminex ARIES.
- Run time: ~40 minutes for extraction, ~1 hour for PCR.

Luminex ARIES HSV 1 & 2 Assay:

- 200 µl volume of previously frozen samples was added to individual test cassettes for automated extraction and PCR.
- Patient information was labeled on test cassettes and typed into the instrument
- Up to 12 cassettes was loaded into 2 magazines and inserted into the Luminex ARIES.
- Run time: ~120 minutes for extraction and PCR.

Focus DX Simplexa™:



Luminex ARIES:



Table 1: Specimen Sources for testing on Luminex ARIES for HSV 1 & 2 Assay

	Genital mucosa	Mouth mucosa	Ocular	Skin	Unspecified
HSV-1	7	3	4	2	0
HSV-2	5	0	0	3	1

This study was supported by Luminex Corp (Austin, TX).

Results

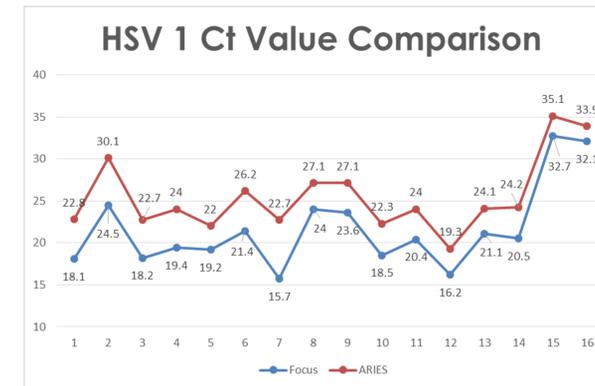


Table 2: Comparison of HSV-1 Ct value ranges on the Focus DX Simplexa™ and Luminex ARIES.

HSV-1 Ct	Simplexa	Luminex
15.0 – 19.9	7	1
20.0 – 24.9	7	9
25.0 – 29.9	0	3
30.0 – 34.9	2	1
35.0 – 39.9	0	2
Total	16	16

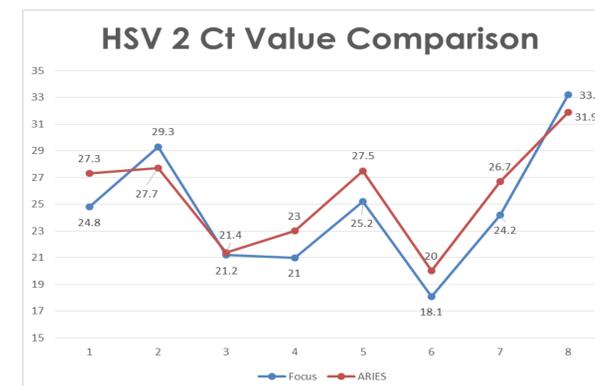


Table 3: Comparison of HSV-2 Ct value ranges on the Focus DX Simplexa™ and Luminex ARIES.

HSV-1 Ct	Simplexa	Luminex
15.0 – 19.9	1	0
20.0 – 24.9	4	3
25.0 – 29.9	2	4
30.0 – 34.9	2	1
35.0 – 39.9	0	1
Total	9	9

Table 4: Result agreement between Luminex ARIES HSV 1 & 2 Assay and Focus DX Simplexa™

Virus	True Positive (TP)	False Positive (FP)	True Negative (TN)	False Negative (FN)	Sensitivity (%)	Specificity (%)
HSV-1	16	0	34	0	100.00	100.00
HSV-2	8	1	40	1	88.9	97.6
Total	24	1	74	1	96.0	98.7

- The false negative sample was positive on the Focus with a Ct value of 33.2. The sample tested negative on the ARIES for both the original test and follow-up testing.
- The false positive sample was negative on the Focus. The sample tested positive on the ARIES with a high Ct value of 38.9. Follow-up analysis on the original extraction run on the Focus tested as a negative result.

Conclusions

- Both assays presented excellent overall agreement with only 2 discordant results out of 50 samples.
- Focus DX Simplexa™ provided efficient testing by the ability to load up to 96 samples per universal disc.
- Ease of use, turnaround time, and possible contamination due to sampling handling was improved using only one instrument, the Luminex ARIES, for extraction and PCR.
- HSV-1 and HSV-2 Ct values demonstrated 1 log greater sensitivity using the Simplexa assay.

