EVALUATION OF *ARIES® HSV 1 & 2* AND *SIMPLEXA™ HSV 1 & 2 DIRECT ASSAYS* FOR THE DETECTION AND DIFFERENTIATION OF HERPES SIMPLEX VIRUS FROM CEREBROSPINAL FLUID AND SWAB SPECIMENS

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Abstract

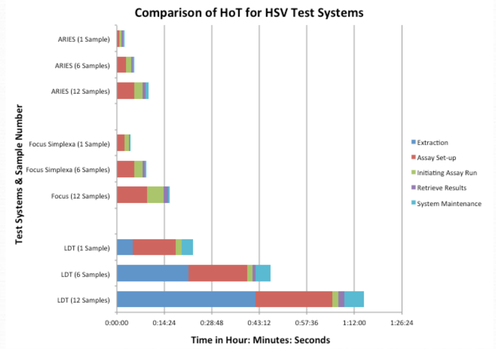
Background: Herpes simplex virus types 1 and 2 (HSV 1/2) are the leading cause of genital herpes infections throughout the world and are difficult to diagnosis due to the frequent asymptomatic presentation of disease within patients. Improved methods for the detection and differentiation of HSV 1/2 are important for the effective diagnosis and treatment of infections and several laboratory molecular tests have become available for clinical use to detect the presence of HSV in swab specimens, both cutaneous (CS) and mucocutaneous (MS), and cerebrospinal fluid (CSF) specimens. Molecular-based HSV assays have increased in popularity due to their increased sensitivity, specificity, and faster turn-around time over tests that rely on viral isolation or antigen detection.
Methods: Two FDA-approved molecular assays, the *ARIES® HSV 1 & 2 Assay (*ARIES*)* from Luminex Corp. and the Simplexa™ *HSV* 1 & 2 *Direct assay (*Simplexa*)* from Focus Diagnostics, used for the detection and differentiation of HSV 1/2, were compared side-by-side to current clinical methodologies, the ELVIS® HSV Culture test system (ELVIS) and a real-time HSV PCR laboratory-developed test (LDT) on the Roche LightCycler 2.0. 276 specimens (52 CS, 119 MS, 105 CSF) were run on the both the ARIES and Simplexa platforms. The HSV 1/2 results from both platforms were compared to ELVIS results for CS and MS specimens and LDT results for CSF specimens.
Conclusions: Both the ARIES and Simplexa assays showed good overall agreement with the two reference test methods. Based on the performance data, the ARIES assay was found to have an improved sensitivity and specificity for the detection of HSV 1/2 over the Simplexa assay when compared side-by-side to the ELVIS and LDT assays, excluding specificity of HSV 1 detection in CS specimens. The ARIES assay showed notably better performance over the ELVIS assay for detection of HSV 1 & 2 and a comparable level of performance to the LDT for the detection of HSV 1. The Simplexa assay also showed a higher level of performance over the ELVIS assay, but did not meet the performance of the LDT assay for detection of HSV 1/2 in CSF due to the incidence of 2 false negative results for both HSV 1 and 2. Further comparison of discrepant results from the ARIES and Simplexa assays needs to be performed using bidirectional sequencing for confirmation.

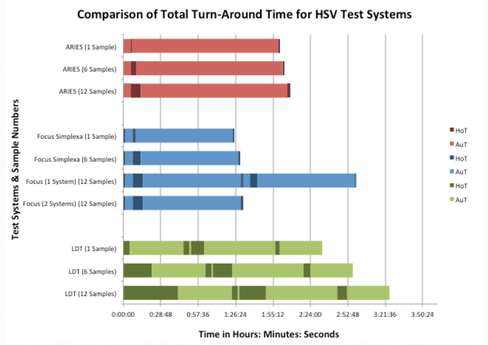
Background

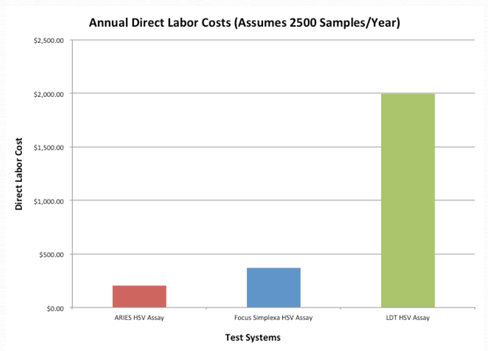
The herpes simplex virus (HSV1/2) is categorized into 2 types, HSV-1 and HSV-2 and cause lifelong infections in human individuals. HSV-1 is mainly transmitted by oral-to-oral contact which can cause symptoms like "cold sores" , but can also cause genital herpes infections. HSV-2 is almost exclusively a sexually transmitted infection that causes genital herpes in both genitalia and anal areas. Both HSV types can cause painful blisters or ulcers at the site of infection and are contagious regardless of symptoms being present. It is estimated by the World Health Organization that an estimated 3.7 billion (67%) people under the age of 50 have HSV-1 infections worldwide and an estimated 417 million (11%) people between the ages of 15-49 have HSV-2 infections worldwide. The infection with HSV-2 can increase the risk of acquiring and transmitting HIV infection among infected individuals. Other complications from HSV 1/2 infections can include neonatal herpes, in which a newborn is exposed to HSV in the genital tract during delivery, and psychosocial impacts for infected individuals. HSV 1/2 can also cause infections in the CSF of patients leading to viral meningitis and/or encephalitis which can often be a fatal condition if not promptly diagnosed and treated and can produce long-term brain damage for patients afterwards.

Methods and Workflow

Both the ARIES and Simplexa assays are real-time polymerase chain reaction (PCR) assays for the *in vitro* qualitative detection and differentiation of HSV 1/2 DNA from specimens of symptomatic patients suspected of having HSV infections. The ARIES assay is FDA-cleared for testing on cutaneous (skin lesions, genital-penis) and mucocutaneous (ocular, nasal, oral, anorectal/perianal, labia/vulva, vaginal/cervical, urethral) samples. The Simplexa assay has been FDA-cleared using genital swab and CSF samples. Of the 276 samples run on the ARIES and Simplexa platforms, 52 were CS, 119 were MS, and 105 were CSF samples. Both assays were compared side-by-side to the ELVIS HSV Culture test system for cutaneous and mucocutaneous samples and a LDT real-time PCR assay for CSF samples run on the Roche LightCycler 2.0.







A timed study was performed comparing the hands on time and automation time of the ARIES and Simplexa assays. All phases of testing were categorized in order to identify the handling and automation time of sample acquisition, extraction time, sample set-up and instrument loading time, on-board instrument time, data analysis, reporting results, and system maintenance. For a comparison to current molecular methods, the same criteria was used to show the amount of time necessary to complete each phase of the LDT assay used for CSF sample comparison. Each system's turn-around time was also compared on a basis of sample volume of 1, 6, and 12 samples respectively.

The ARIES proved to require less hands-on time than the Simplexa assay at every level of sample volume (< 14 minutes HoT for 12 sample maximum). Overall testing time for the ARIES system is longer than the Simplexa assay, but ARIES is capable of handling 12 samples per instrument versus the Simplexa assay 8 sample maximum. Due to the shorter hands-on time, the ARIES system helps lower labor costs to a greater extent than both the Simplexa and LDT assays.

The ARIES assays are designed with a Universal Assay Protocol which enables up to 12 different assays from multiple sample types to be run simultaneously on one instrument, offers increased productivity by running IVDs and LDTs in the same batch, simplifies workflow, and increased flexibility for testing throughout the day.

Results

HSV 1	ARIES			Simplexa™		
	Cutaneous	Mucocutaneous	CSF	Cutaneous	Mucocutaneous	CSF
True Positives	5	14	2	5	12	0
True Negatives	43	98	103	47	99	102
False Positives	4	7	0	0	6	1
False Negatives	0	0	0	0	2	2
Total	52	119	105	52	119	105
Sensitivity	100.00%	100.00%	100.00%	100.00%	85.71%	0.00%
Specificity	91.49%	93.33%	100.00%	100.00%	94.29%	99.03%
PPV	55.56%	66.67%	100.00%	100.00%	66.67%	0.00%
NPV	100.00%	100.00%	100.00%	100.00%	98.02%	98.08%
Agreement	92.31%	94.12%	100.00%	100.00%	93.28%	97.14%

HSV 2	ARIES			Simplexa™		
	Cutaneous	Mucocutaneous	CSF	Cutaneous	Mucocutaneous	CSF
True Positives	9	9	7	9	9	6
True Negatives	40	106	97	40	105	97
False Positives	3	4	0	3	5	0
False Negatives	0	0	1	0	0	2
Total	52	119	105	52	119	105
Sensitivity	100.00%	100.00%	87.50%	100.00%	100.00%	75.00%
Specificity	93.02%	96.36%	100.00%	93.02%	95.45%	100.00%
PPV	75.00%	69.23%	100.00%	75.00%	64.29%	100.00%
NPV	100.00%	100.00%	98.98%	100.00%	100.00%	97.98%
Agreement	94.23%	96.64%	99.05%	94.23%	95.80%	98.10%

As the tables above suggest, the ARIES assay has improved or equivalent sensitivity to the Simplexa assay for both HSV 1 and 2 across each specimen type when simultaneously compared to ELVIS and the LDT assay. Both assays had relatively high and comparatively similar specificities and agreement across all specimen types for both HSV 1/2 targets. Due to the low number of HSV 1/2 positive CSF samples used in the study, additional CSF samples need to be run for a more accurate comparison of the ARIES and Simplexa assays. The Simplexa assay had 6 false negatives (4 HSV 1, 2 HSV 2) and 15 false positives (7 HSV 1, 8 HSV 2) compared to the ARIES assay which had 1 false negative (HSV 2) and 18 false positives (11 HSV 1, 7 HSV 2) when compared to virology cultures for swabs and LightCycler assay results for CSF specimens. The total number of true positives for Simplexa was 17. Comparatively, the total number of true positives for ARIES was 18. Considering the overall higher sensitivity of molecular assays to viral culture, the increased rate of false positives for the ARIES assay could be attributed to higher greater sensitivity for detection of HSV 1/2 for the test system. When compared to an equivalent molecular LDT assay, the rate of false positive for the ARIES assay dropped to 0.00%.

Conclusions

- Both the *ARIES® HSV 1 & 2 Assay* and the Simplexa™ *HSV* 1 & 2 *Direct assay* offer improved testing performance and turn-around time compared to current HSV culture and real-time PCR detection methods. The total hands-on time for the ARIES assay was the shortest of all testing systems in the study.
- Both ARIES and Simplexa assays were shown to have a higher sensitivity and specificity over HSV viral cultures and comparable results to the LDT PCR assay. The ARIES either outperformed or was equivalent to the Simplexa assay in sensitivity and specificity for most specimen types with a high level of agreement for both HSV-1 and HSV-2.
- The ARIES assay can process 12 samples at a time individually compared to the Simplexa assay, which can process 8 samples on one disc. The ARIES test system is a highly flexible molecular assay for HSV 1/2 offering a simplified workflow and increased productivity throughout the day.
- Both the assays showed quicker turn-around times for testing compared to current methods. The hands-on time for the ARIES system (~11 min.) was slightly shorter than the Simplexa assay (~15 min.) and had an automation time approximately 38 minutes longer.

Acknowledgements

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