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 samples. Each system’s turn-around time was also compared on a basis of sample volume of 1, 6, and 12 samples respectively.

The ARIES assay 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. 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 culture results. 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.

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 per test system.
➢ 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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