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COMPARISON OF THREE ASSAYS FOR HSV 1 AND HSV 2 DETECTION: A LOOK AT FOCUS DIAGNOSTICS 3M INTEGRATED CYCLER, LUMINEX ARIES® SYSTEM, AND ROCHE LIGHTCYLER® 2.0

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INTRODUCTION

Herpes Simplex Virus type 1 (HSV 1) and Herpes Simplex Virus type 2 (HSV 2) are members of the alpha-herpesviridae subfamily. HSV 1 and HSV 2 cause infection in adults, infants, children, and immunocompromised individuals. HSV 1 is associated with the facial area, whereas HSV 2 is associated with genital infections. Herpes simplex virus can develop latency after primary infection. Since there is no cure, patients have to manage re-emerging infections with different viral drug therapies. Currently the HSV 1&2 testing method at Indiana University Health Pathology Lab (IUHPL) is the Roche LightCycler® 2.0 assay, which is a real-time polymerase chain reaction, non FDA-approved method. IUHPL has validated oral, genital, and cutaneous swabs, as well as plasma and cerebrospinal fluid (CSF). This method requires extraction of nucleic acid from patient specimens before performing the amplification and detection. The extraction procedure is a step that requires additional resources and specialized training that increases the turnaround time. Due to this workflow, specimens are batched to maximize efficiency of all instruments used. Therefore, the assay is performed once per day, which greatly prolongs results that can negatively impact patient health.

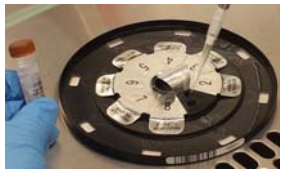
OBJECTIVE

The purpose of this study was to investigate two other molecular assays that are FDA-approved for all or some of the specimen types. Streamlining the method by direct amplification, improving lab workflow, and shortening turnaround time were other aspects taken into consideration. The Simplexa™ HSV 1&2 Direct Assay from Focus Diagnostics and the Aries® HSV 1&2 Assay from Luminex were the selected platforms that were evaluated.

MATERIALS AND METHODS

In this study, 109 patient samples were tested on both platforms; 53 swabs in universal transport media (UTM), 29 plasma, and 27 CSF. These samples were previously tested with the Roche LightCycler® 2.0 instrument using Roche HSV 1&2 ASR reagents.

Simplexa™ HSV 1&2 Direct Assay:
In the clean area, reaction mix was pulled from the freezer and thawed to room temperature. One vial of reaction mix was used per sample to be tested. Each wedge of the direct amplification disc (DAD) was uncovered and 50uL of reaction mix was pipetted into the corresponding well. In the processing area, 50uL of patient specimen was added to the DAD. After addition of the sample, each wedge was sealed and the tab removed.

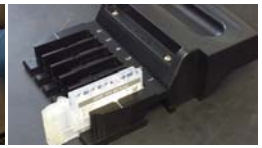


MATERIALS AND METHODS (CONT.)

The DAD was then loaded on the 3M Integrated Cycler in the post-PCR room. The total assay run time was approximately 65 minutes. Each disc can hold a maximum of eight samples.

Aries® HSV 1&2 Assay:

In the processing area, one Aries® cassette was used per sample to be tested. Each cassette was unwrapped and foil seal removed before placing in the sample prep tray. The cassette sample chamber was opened and 200uL of patient specimen was added. In the post-PCR room, the cassettes were loaded onto the magazine and inserted into the Aries® System. The total assay run time was approximately 1 hour and 55 minutes. Each module can hold a maximum of 6 samples with a total of two modules per tower.



Limit of detection:

Limit of detection was determined by using a known concentration of culture fluid from Zeptometrix. This study selected HSV type 1, strain MacIntyre and HSV type 2, strain MS for the sensitivity experiments. These dilutions were spiked into pooled negative CSF and plasma patient samples. Each limit of detection dilution series was performed in triplicate on both the 3M Integrated Cycler and the Aries® System. Only the CSF limit of detection (LOD) dilution series was run on the Roche instrument due to the inhibitory nature of the un-extracted plasma.

The dilution series was as follows:

HSV 1 McIntyre $TCID_{50}$/mL
3.4×10^{5}
3.4×10^{4}
3.4×10^{3}
3.4×10^{2}
3.4×10^{1}
3.4×10^{0}
3.4×10^{-1}

HSV 2 MS $TCID_{50}$/mL
5.0×10^{2}
5.0×10^{1}
5.0×10^{0}
5.0×10^{-1}
5.0×10^{-2}

CONCLUSION (CONT.)

Further investigation with a larger sample set is needed to help support the specificity and sensitivity of the two platforms. The Simplexa™ assay was more reliable for the UTM and CSF specimen types than for plasma. UTM and CSF are currently the only FDA approved specimen types for this assay. If plasma samples are to be used un-extracted, this matrix may need to be studied further to help decrease the error rate due to the inhibitory nature of the specimen type.

The Aries® System was also more reliable for the UTM and CSF specimen types than for plasma. While there were fewer errors with the Aries® System on plasma samples, plasma as a specimen type may need to be further evaluated. It may be worth investigating the ability to change the cycle threshold for the SPC. Cerebrospinal fluid will also need further validation since UTM is the only FDA approved specimen type at this time.

In comparing the two new molecular platforms, the difference in the limit of detection is probably not clinically significant. However, the LOD for each is more sensitive than the Roche assay. This is of particular importance in cerebrospinal infections where the viral load is low.

When evaluating workflow for the laboratory, it was found that both platforms decreased turnaround time. This is due to the fact that patient specimens do not have to be batched and can be tested as soon as they are received. The elimination of the extraction step also decreases total assay time. When comparing the two assays, one benefit of the Focus Diagnostics method is that only 50uL of patient specimen is required to perform the test. This is certainly beneficial for more difficult specimen types to collect such as CSF. Both assays have fewer and less technical steps which would minimize training for laboratory personnel.

RESULTS

Of the 109 patient samples that were tested, all of the UTM (100%) and 26 out of the 27 (96.3%) CSF specimens correlated between all three assays. These were the two most reliable specimen types across all three platforms. Plasma was found to be the least reliable specimen type for the two newer platforms; 27 out of the 29 (93.1%) specimens correlated. These percentages do not include samples that were previously resulted as indeterminate. Using our current method, if a result

Sample #	Roche	Focus	Aries
1	Indeterminate	HSV 1	Not Detected
2	Indeterminate	HSV 1	HSV 1
3	Indeterminate	HSV 1	HSV 1
4	Indeterminate	HSV 1	HSV 1
5	Indeterminate	HSV 1	HSV 1
6	Indeterminate	HSV 2	HSV 2
7	Indeterminate	HSV 2	HSV 2
8	Indeterminate	HSV 2	HSV 2
9	Indeterminate	HSV 1	HSV 1

RESULTS (CONT)

falls between the melting temperature of HSV 1 and HSV 2 it is reported as indeterminate. Below is the outcome of these specimens tested on the other platforms.

Of the 29 plasma specimens that were tested on the 3M Integrated Cycler, eight gave an invalid result (27.6%) due to the internal control's (IC) failure to amplify. These specimens were resolved by either repeating the sample again or extracting on the NucliSENS® easyMAG™ prior to testing. Considering the extracted DNA eliminated the IC failure, this led to the possibility that there was increased PCR inhibition in the plasma matrix. Focus Diagnostics recommends extracting plasma samples to increase success rate. The Aries® System had four invalid results for the plasma samples that were tested (13.8%). Three out of the four samples resolved after running the sample again. The fourth sample was not able to give a valid result even after repeating. These invalid results were due to the sample processing control (SPC) failure. The Aries® System SPC is equivalent to the internal control. Luminex technical support determined the failure was due to the SPC not meeting the preset cycle threshold (Ct).

Of the 109 patient samples, there were 3 cases of discrepant results. Discrepant samples were sent to Luminex technical support for further sequencing.

Summary of Discrepant Results

Sample #	Roche	Focus	Aries	Target for Sequencing	Sequencing Call
14	Not Detected	HSV 1	HSV 1	HSV 1	NEG
28	HSV 2	Not Detected	HSV 2	HSV 2	Poor POS
21	Not Detected	Not Detected	HSV 2	HSV 2	POS

Sensitivity experiments were performed to determine the limit of detection for HSV 1 and HSV 2 for both CSF and plasma specimen types. These results are shown in the following table.

Limit of Detection

	HSV 1			HSV 2		
Specimen Type	Simplexa	Aries	Roche	Simplexa	Aries	Roche
CSF	3.4×10^{2} $TCID_{50}$/mL	3.4×10^{3} $TCID_{50}$/mL	3.4×10^{4} $TCID_{50}$/mL	5.0×10^{0} $TCID_{50}$/mL		5.0×10^{2} $TCID_{50}$/mL
Plasma	3.4×10^{3} $TCID_{50}$/mL		N/A	5.0×10^{1} $TCID_{50}$/mL		N/A

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