Performance Comparison and Workflow Evaluation of Simplexa™ HSV 1 & 2 Direct and ARIES® HSV 1 & 2 Assay for the Detection of HSV 1 & 2 in Genital and Non-genital Specimens

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Abstract

Background: Rapid detection of Herpes simplex virus types 1 (HSV 1) and 2 (HSV 2) from genital and non-genital cutaneous or mucocutaneous lesions is of clinical importance for appropriate and prompt treatment of suspected Herpes infections. In this study we compare assay performance and workflow of two molecular assays for the rapid detection of HSV 1 & 2.

Methods: Sixty-six archived specimens (15 genital and 51 non-genital) were simultaneously tested by Simplexa™ HSV 1 & 2 Direct (Focus Diagnostics) and ARIES® HSV 1 & 2 Assay (Luminex Corp.) Specimens were previously identified as HSV 1, HSV 2, or negative by culture or DFA and HSV 1 & 2 monoclonal-antibody staining. Discordant results were repeated once and correlated to clinical presentation. Reproducibility studies were performed using Zeptometrix NaTrol HSV 1 & 2 controls at low, medium and high concentrations.

Results: Agreement between ARIES, Simplexa and culture or DFA was 100% (66/66) after repeat testing of discordant results and/or clinical correlation. Initial testing on ARIES revealed 1 invalid, 1 false negative (FN) (HSV 1), and 2 false positive (FP) results (one HSV 1 and 1 HSV 2). Simplexa reported 3 FP results (two HSV 1 and one HSV 2). Upon repeat testing, the ARIES results showed resolution of the invalid as negative (correct) and correct determinations for both the FP and FN HSV 1 results. Similarly, with Simplexa, repeat testing yielded a correct determination for the two FP HSV 1 results. The one culture negative specimen that was positive for HSV 2 by both instrument platforms initially, repeated positive for HSV 2 with both ARIES and Simplexa and was determined to be a true Herpes infection by clinical presentation and serology. Reproducibility studies for HSV 1 (low, medium, high) on ARIES and Simplexa was 100% and 96%, respectively. Reproducibility for HSV 2 (low, medium, high) on ARIES and Simplexa was 93% and 93%, respectively. Evaluation of workflow for pre-analytical hands on time was similar, whereas specimen volume, assay runtime and overall ease of use were dissimilar.

Conclusion: Performance was equal between the Simplexa HSV 1 & 2 Direct and ARIES HSV 1 & 2 Assay. Slight differences were observed for workflow evaluation and overall user preference. Both assays are rapid and performed direct from specimen with increased sensitivity over culture, allowing streamlined options for laboratories and critical benefits in decreased turn-around-time and increased clinical sensitivity.

Methods

Samples

- 66 archived clinical specimens (15 genital and 51 non-genital) collected between 2/2014 and 4/2016
- Previously identified as HSV 1, HSV 2, or negative by culture and/ or DFA, followed by HSV 1 & 2 monoclonal-antibody staining

Method Comparison

- Simultaneously tested by Simplexa™ HSV 1 & 2 Direct (Focus Diagnostics) and ARIES® HSV 1 & 2 Assay (Luminex Corp.)
- Compared to viral culture growth in MRC5 and A549 cells or DFA both stained with Light Diagnostics™ Simulfluor®HSV 1/2 Immunofluorescence Assay

Discordant Resolution

- Discordant results were repeated once and correlated to clinical presentation

Workflow Evaluation

- Pre-analytical procedure
- Test procedure (Specimen volume, Set up time, Run time)
- Post-analytical procedure
- Overall software ease of use

Results

Method Comparison for 15 Genital Specimens

Genital Specimens			ARIES		Simplexa	
Site	Source	Culture / DFA	HSV I	HSV II	HSV I	HSV II
Labia	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Vulva	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Vaginal	Vesicle	HSV type I	Not Detected	Not Detected	Detected	Not Detected
Vaginal-Repeat	Vesicle	HSV type I	**Detected**	Not Detected	Detected	Not Detected
Penis	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Penile Lesions	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Labia	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Cervical	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Penis	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Labia	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Penis	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Penis	Vesicle	Neg for HSV I & II	Not Detected	Detected	Not Detected	Detected
Penis-Repeat	Vesicle	Neg for HSV I & II	Not Detected	Detected	Not Detected	Detected
Penis	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Cervical Lesion	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Penile Ulceration	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Detected	Not Detected
Penile Ulceration-Repeat	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	**Not Detected**	Not Detected
Vaginal (no ves pres)	Skin	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected

Method Comparison for 51 Non-genital Specimens

Non-genital Specimens			ARIES		Simplexa	
Site	Source	Culture / DFA	HSV I	HSV II	HSV I	HSV II
Oral Lesions	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Mouth Ulcer	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Left Eye	Skin	HSV type I	Detected	Not Detected	Detected	Not Detected
Lower Lip	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Nose	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Gums	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Nostril	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Perirectal	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Eyelid	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Upper lip	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Throat	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Throat	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Cornea Scrapings	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Left Nostril	Vesicle	Neg for HSV I & II	Detected	Not Detected	Not Detected	Not Detected
Left Nostril –Repeat	Vesicle	Neg for HSV I & II	**Not Detected**	Not Detected	Not Detected	Not Detected
Mouth	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Mouth	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Pharynx	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Detected	Not Detected
Pharynx-Repeat	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	**Not Detected**	Not Detected
Mouth	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Oral	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Rectal	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Skin Lesion	Skin	HSV type I	Detected	Not Detected	Detected	Not Detected
Finger	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Neck	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Face	Vesicle	HSV type I	Detected	Not Detected	Detected	Not Detected
Right Buttock	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Back	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Left Inner Thigh	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Left Index Finger	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Buttock	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Back	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Thigh	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Chest	Vesicle	HSV type II	Not Detected	Detected	Not Detected	Detected
Ear	Skin	Neg for HSV I & II	Invalid	Invalid	Not Detected	Not Detected
Ear-Repeat	Skin	Neg for HSV I & II	**Not Detected**	**Not Detected**		
Right Arm	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Flank	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Face	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Axilla	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Finger	Vesicle	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Eyes, mouth, anus	Skin	HSV type I	Detected	Not Detected	Detected	Not Detected
Skin, Eyes Nose Rectum	Anus	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Skin, Eyes Nose Rectum	Skin	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Skin, Eyes Nose Rectum	Skin	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Skin, Eyes Nose Rectum	Skin	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Skin, Eyes Nose Rectum	Skin	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Skin, Eyes Nose Rectum	Skin	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Skin, Eyes Nose Rectum	Skin	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Eyes, mouth, rectum	Skin	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Eyes, nose, mouth, rectum	Skin	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Eye, nose, mouth, rectum	Skin	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Eyes, mouth, rectum	Skin	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected
Eye, mouth, rectum	Skin	Neg for HSV I & II	Not Detected	Not Detected	Not Detected	Not Detected

Method Comparison and Workflow Evaluation

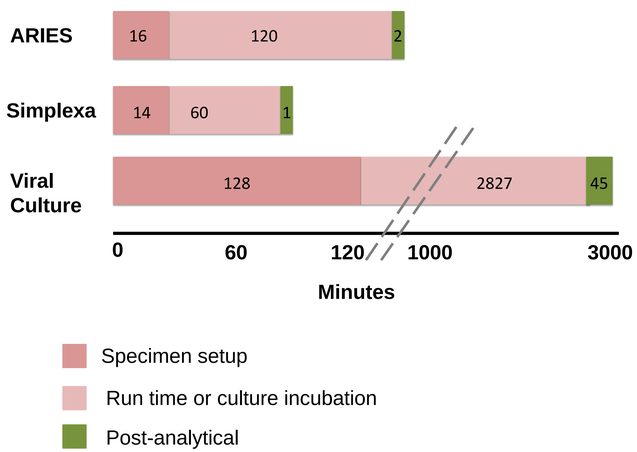
ARIES

- 95.4% agreement with culture/DFA
  - 1 Invalid
  - 1 False Negative (HSV 1)
  - 2 False Positive (HSV 1, HSV 2)
- 2 Corrected on repeat (HSV 1)
- 1 Corrected upon clinical review (HSV 2)

Simplexa

- 95.5% agreement with culture/DFA
  - 3 False Positives (2-HSV 1, 1-HSV 2)
- 2 Corrected on repeat (HSV 1)
- 1 Corrected upon clinical review (HSV 2)

Time Comparison of Molecular Assay to Viral Culture



	Luminex ARIES	Focus Simplexa
FDA Approved Specimens	Cutaneous and Mucocutaneous Lesions	CSF and Genital Lesions
# of Specimens on a Run	12	8
Storage for Consumables	Room Temperature	Room Temperature and -20°C
Specimen Volume	200uL	~50µL*
Specimen Setup	Very Easy	Very Easy
Run Time	~120 minutes	~60 minutes
Clean Up	Easy	Very Easy
Software Use	Easy	Very Easy
Interface	Bi-directional	Uni-directional

* Up to 50 µL, can be less

Conclusions

- 95% Agreement with viral culture/ DFA for both ARIES and Simplexa
  - All corrected upon repeat or clinical correlation
- 98% Agreement with viral culture/ DFA for non-genital specimens
- Comparable in pre-analytical, analytical and post-analytical test procedure complexity and hands-on time