



# Comparative Study of Simplexa<sup>®</sup> HSV 1&2 Direct with AmpliVue<sup>®</sup> HSV 1+2 Assay

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## Abstract (revised)

**Background:** Herpes simplex viruses 1 & 2 are common causes of a variety of clinical symptoms, influenced by the anatomic site of the infection, the age and immune status of the patient, and reactivation of latent infections. Sensitive and specific rapid tests can aid in appropriate patient management decisions including the choice of therapy. While cell culture was once the gold-standard diagnostic assay, molecular amplification assays are now commonly used due to their increased speed and sensitivity. Numerous PCR assays have been available for years, but more recently, new sample-to-answer PCR tests and isothermal assays have become available. We compared the Simplexa<sup>®</sup> HSV 1 & 2 Direct Assay (Focus Diagnostics) to the AmpliVue<sup>®</sup> HSV 1+2 Assay (Quidel Corp) using routine genital specimens sent to our laboratory.

**Methods:** This study was conducted on seventy-seven specimens collected in the Hershey, Pennsylvania area. The specimens were collected using three ml of UTM (Copan) and tested within twenty-four hours. Both assays were performed according to the kit instructions. Discordant results are to be adjudicated using the ELVIS<sup>®</sup> culture assay.

**Results:** Of the seventy-seven unique samples collected from genital lesions, twelve specimens were positive for HSV-1, and twenty-two were positive for HSV-2 by both methods. Thirty-eight specimens were negative for both viruses by both assays. Four specimens were positive for HSV-1 by the Simplexa<sup>®</sup> assay only, and there were two specimens that repeatedly showed inhibition by the AmpliVue<sup>®</sup> assay.

**Conclusion:** Both the AmpliVue<sup>®</sup> and Simplexa<sup>®</sup> HSV assays provided rapid and sensitive results. The Simplexa<sup>®</sup> assay requires the use of the Integrated Cyclor and is a sample-to-answer workflow. The AmpliVue<sup>®</sup> assay requires more hands-on time for specimen preparation, however it only requires a heated block to perform the test. Both assays can be completed in about the same period of time.

## Introduction

HSV 1 & 2 are common causes of genital infections. Cell culture has been used for many years to detect and identify these viruses, however PCR assays have mostly replaced cell culture due to their enhanced sensitivity and speed. Recently, sample-to-answer PCR assays that are simple to perform in any laboratory have been released. Another relatively recent development is the availability of isothermal molecular amplification assays as diagnostic alternatives to PCR. These assays do not require thermocyclers to perform and do not require technical expertise in molecular methods. This study compares three of these different types of assays, a sample-to-answer PCR method, an isothermal molecular method, and a centrifugation-enhanced cell culture method.

## Methods

Seventy-seven genital specimens were collected using flocked swabs that were placed in 3 mls of UTM. All specimens were then tested within 24 hours of collection by the following 3 methods.

The Simplexa<sup>®</sup> HSV 1&2 Direct Assay is a sample-to-answer PCR method that does not require any pretreatment or extraction of the specimen and is FDA approved for testing genital lesions. Up to eight specimens can be tested in a single run that takes approximately one hour to perform with minimal hands-on time.

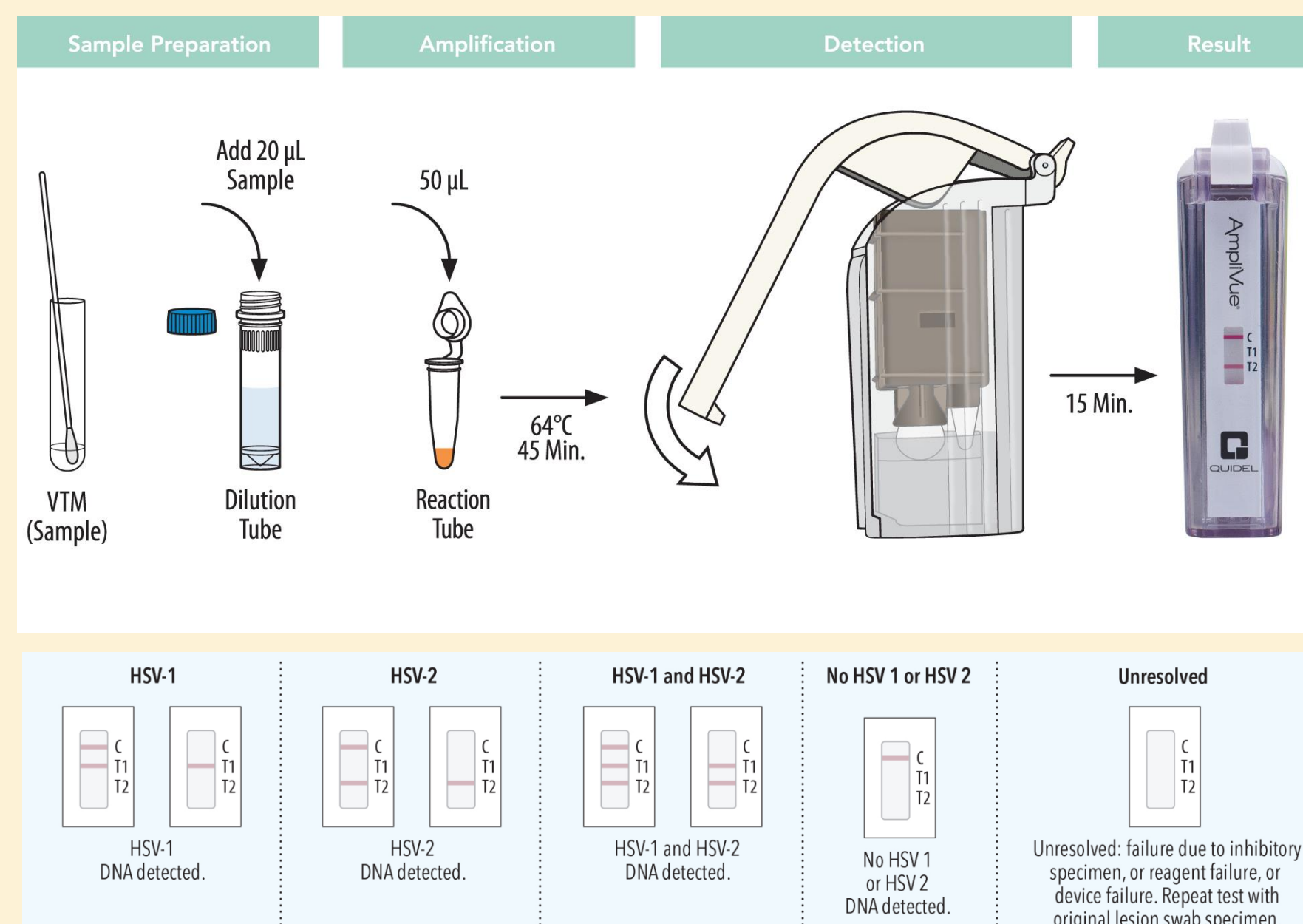
The AmpliVue HSV 1+2 Assay is an isothermal molecular assay that only requires a dilution step before being load into the heating block for extraction and amplification. Following a 45 minute incubation, the reaction tube is removed and placed in a disposable detection device for final identification.

Cell culture was performed using the ELVIS HSV ID Test System which is an enhanced centrifugation culture assay that is relatively simple to perform with the inoculated culture vials being incubated overnight before being stained for HSV 1 & 2 the following day.

## Simplexa<sup>™</sup> HSV 1&2 Direct



## Quidel AmpliVue<sup>®</sup> HSV 1+2 Assay Test Procedure



## Results

The results are presented in the following 2 figures. The Simplexa<sup>®</sup> PCR assay detected 38 HSV positive specimens and the AmpliVue<sup>®</sup> detected 34 positives. Two specimens were repeatedly inhibited with the AmpliVue<sup>®</sup> test which was not seen with the Simplexa<sup>®</sup> assay. Three of the positive specimens that were missed by the AmpliVue<sup>®</sup> had Ct values that were > 34. One of the indeterminate samples was also toxic for the cell culture. The ELVIS<sup>®</sup> assay did not detect any positive samples that were negative by the Simplexa<sup>®</sup> assay and only 1 that was negative by the AmpliVue<sup>®</sup> test. The cell culture was also negative for 5 samples that were positive by both the molecular methods.

### Performance of Simplexa and AmpliVue HSV 1 & 2 assays

	Negative	HSV-1	HSV-2	Total*
Simplexa	39	16	22	77
AmpliVue	41(+2)	12	22	77

### Comparison of Simplexa and AmpliVue HSV 1 & 2 assays

	Simplexa +	Simplexa -	Totals
AmpliVue +	34	0	34
AmpliVue -	4(+1)	38	43
Total	39	38	77

## Discussion

Both of these molecular assays were simple to perform and provided results in approximately one hour. The Simplexa<sup>®</sup> assay had greater sensitivity, however it requires the use of a thermocycler, can test up to 8 samples at a time, and is FDA cleared for genital lesions and CSF.

The AmpliVue<sup>®</sup> was not as sensitive for samples having high Ct values for HSV-1, however the only instrumentation required is a heating block, numerous samples can be tested at one time and it is FDA cleared for testing lesions from any site, including eyes.

Due to the simplicity, sensitivity and rapid time to result for the molecular assays, the cell culture method did not demonstrate any advantages over the other 2 assays.

## Conclusions

Overall, both molecular amplification assays performed well. Both are simple enough to be performed in any laboratory, both have minimal equipment requirements and provide sensitive results in about an hour.

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