# Comparison of Viral Culture to Altona Diagnostics RealStar® alpha Herpesvirus PCR Kit 1.0 for the Detection of HSV-1/2 & VZV in a Public Health Laboratory Setting

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## OBJECTIVE

Infection with herpes simplex virus (HSV) is commonly diagnosed by viral culture, where sensitivity is wholly dependent on viability of the virus. Molecular technology provides a valuable alternative to culture by increasing sensitivity and decreasing turn-around time. The purpose of this study is to compare Altona Diagnostics RealStar® alpha Herpesvirus PCR kit 1.0 (Hamburg, Germany) to traditional culture methods for the detection of HSV-1/2.

## METHODS

Swabs of suspect HSV-1 and HSV-2 lesions were sent to the lab for diagnostic purposes and aliquots of specimen were poured off and stored at -80°C until the time of nucleic acid extraction.

Specimens were inoculated into Vero and/or MRC-5 cells and observed for cytopathic effect (CPE) for seven days. Slides were prepared from cultures exhibiting CPE and cells were stained with LIGHT DIAGNOSTICS™ Simulfluor® HSV-1/2 Reagent (EMD Millipore, Billerica, MA). Slides were viewed with a fluorescent microscope to confirm the presence of HSV-1 or HSV-2.

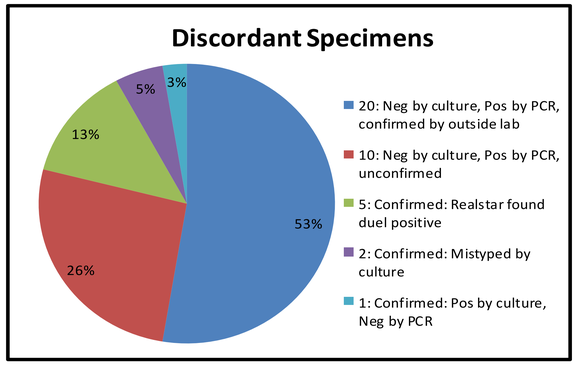
Nucleic acid extraction was performed using the Qiacube and QIAamp Viral RNA mini kit (Qiagen, Valencia, CA). PCR was performed using the Realstar® alpha Herpesvirus PCR Kit 1.0, which simultaneously detects and differentiates HSV-1, HSV-2, and varicella zoster virus (VZV) specific DNA (Altona Diagnostics). Results were compared to traditional culture and confirmatory testing was performed on discrepant samples by an external, independent laboratory using EraGen's MultiCode®-RTx HSV 1&2 kit (Austin, TX).

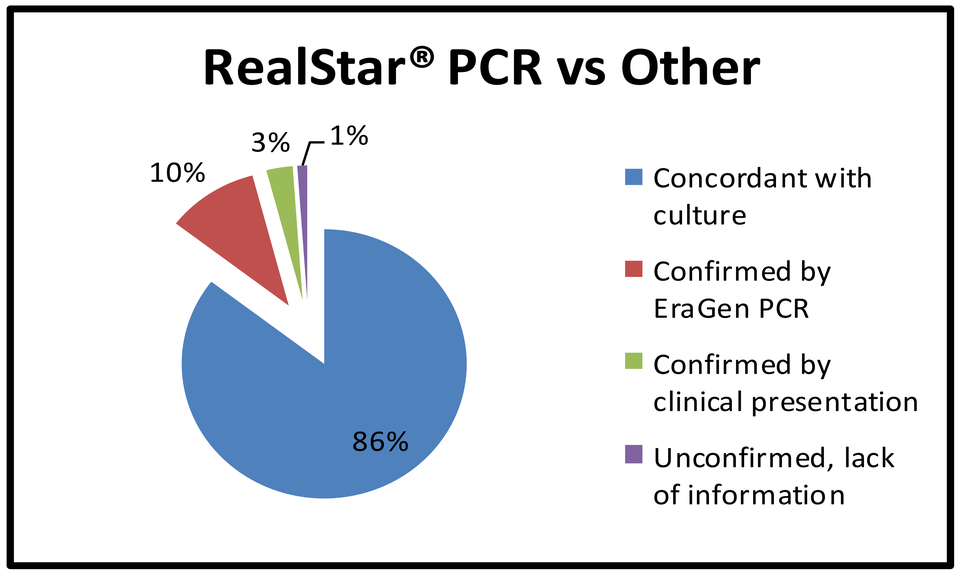
## RESULTS

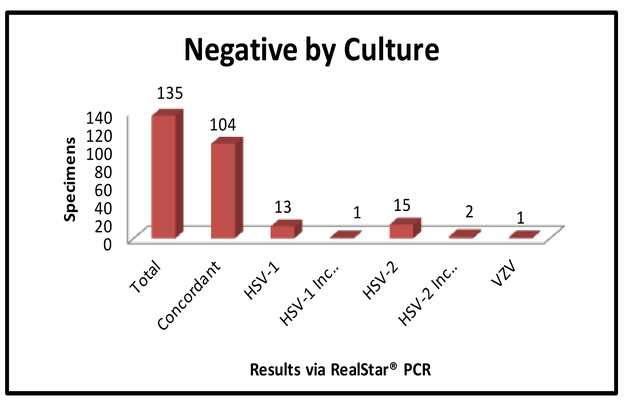
Results from 268 specimens were compared between traditional virus culture and the RealStar® alpha Herpesvirus PCR; 230 (86%) concurred (though one specimen was positive for VZV DNA) and 38 (14%) had discordant results.

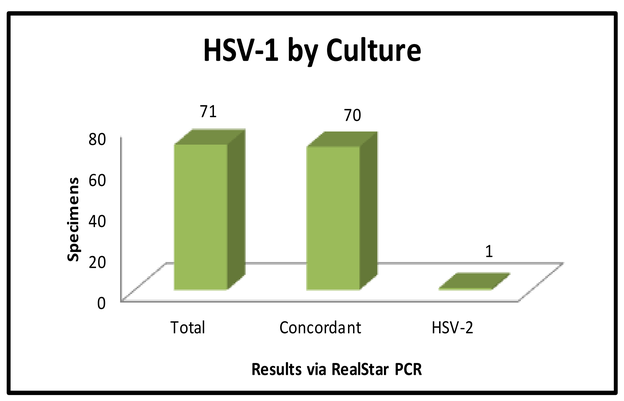
Of the 38 discrepant specimens, PCR results were confirmed by the independent laboratory in 28 (74%); 20 specimens were negative in culture but positive by PCR, five specimens were potential duel positives by PCR, two were discordant based on typing results (one HSV-1, one HSV-2), and one was positive for HSV-2 in culture but negative by PCR.

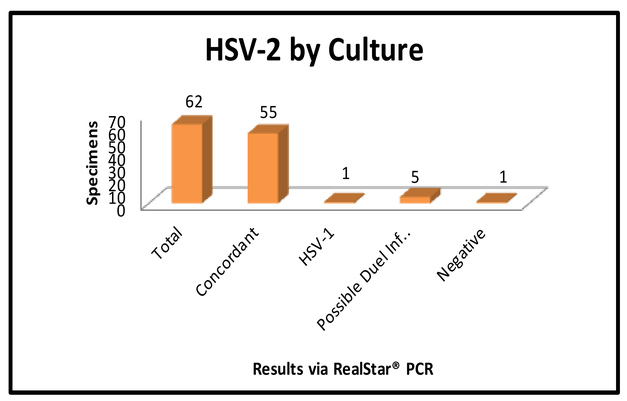
Of the 38 discrepant samples, the RealStar® assay detected DNA in 10 culture negative specimens which were also negative by the independent laboratory.




## DISCUSSION

The RealStar® alpha Herpesvirus PCR kit showed increased sensitivity when compared to culture. Of 268 specimens, 38 (14%) were discordant with culture results, of which 28 were confirmed by an outside laboratory and 8, though not confirmed, were consistent with clinical presentation and/or physician diagnosis. The remaining 2 had no clinical data available, however, based on the performance of the assay throughout the study, it would seem plausible that these two specimens are positive.

Based on the results of this study, and the lack of clinical data for the unconfirmed 2 samples, it was determined that crossing point thresholds >39 would be reported as inconclusive for clinical diagnosis. Further, the acceptable range of positive specimens was established at 11-39.

One major consideration when transitioning from traditional culture to PCR methodology is cost. This study reaffirms that the additional cost of performing PCR may be justified, when evaluated against the added sensitivity and reliability of results. In our laboratory, as a result of this evaluation, it was determined that the added cost was justified when evaluated against the improved result validity.

It should be noted that 15 of the 38 (39%) discrepant results were performed by a laboratorian with limited assay experience, during the initial evaluation trial run. Hence, these discrepancies should be evaluated with caution.

## CONCLUSION

The RealStar alpha Herpesvirus PCR kit 1.0 appears to be an impressive alternative to traditional virus culture because it has greatly increased sensitivity, with equal or better specificity.

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