·Clinical Research·

Cell culture isolation can miss the laboratory diagnosis of HSV ocular infection

Regis P Kowalski, Paul P Thompson, Tara H Cronin

The Charles T. Campbell Ophthalmic Microbiology Laboratory, UPMC Eye Center, Ophthalmology and Visual Sciences Research Center, the Eye and Ear Institute, Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Correspondence to: Regis P Kowalski. The Eye & Ear Institute, Ophthalmic Microbiology-Room 642203 Lothrop Street, Pittsburgh, PA 15213, USA. kowalskirp@upmc.edu

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Abstract

• AIM: We compared polymerase chain reaction (PCR) to cell culture isolation for the laboratory diagnosis of ocular herpes simplex virus (HSV) disease.

• METHODS: Laboratory and medical records of consecutive patients were reviewed for results of 1) HSV PCR testing, 2) HSV cell culture isolation, and 3) clinical diagnosis. PCR results were statistically compared to cell culture isolation and patients initially diagnosed for ocular HSV infection.

• RESULTS: Of 581 cases submitted for laboratory testing, 520 were PCR negative, cell culture negative (89.6%); 0 were PCR negative, cell culture positive (0%); 27 were PCR positive, cell culture negative (4.6%); and 34 were PCR positive, cell culture positive (5.8%). PCR tested more positive than cell culture isolation (McNemar's, P=0.0001). Of 47 HSV PCR positive cases with complete medical records, 19 were cell culture negative for HSV and 28 were cell culture positive for HSV. Fourteen of 19 cell culture negative cases (74%) (Without PCR, 5 cases of HSV would be missed) and 25 of the 28 cell culture positive cases (89%) (Laboratory testing was necessary for diagnosing 3 cases) were clinically diagnosed with HSV at the initial examination.

• CONCLUSION: PCR was a more definitive test for diagnosing HSV ocular infection than cell culture isolation. Cell culture isolation alone can miss an atypical presentation of HSV ocular infection.

• KEYWORDS: herpes simplex virus; polymerase chain reaction; cell culture isolation; HSV keratitis

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INTRODUCTION

Laboratory testing is a useful adjunct to clinical evaluation in guiding treatment of ocular herpes simplex virus (HSV) infection, especially when a patient presents with atypical clinical findings[1]. Traditionally, virus isolation has been considered the "gold standard" for detecting HSV, but HSV polymerase chain reaction (PCR) has been reported to be highly sensitive and specific [1-7]. A positive PCR result, however, may not necessarily parallel the clinical picture of ocular HSV infection.Kaufman *et al* [8] reported that 92.0% (46 of 50) of asymptomatic patients shed HSV-1 ("Non-Specific Shedders") in their tears at least once when tested via PCR twice daily over a 30 day period. Leigh *et al* [9] reported that asymptomatic shedding of HSV in the clinical setting did not contribute to an unacceptable rate of false-positive results by PCR. Both studies did not include cell culture isolation as a comparative test to confirm the presence of active HSV.

A positive HSV cell culture would indicate the presence of an active infection whereas a positive PCR would only indicate the presence of HSV DNA either from a live virus or from an inactive form of the virus. Consequently, the eye care professional encounters contrasting information when faced with an atypical HSV presentation in the setting of a positive PCR result and a negative cell culture. The immediate choices are: 1) The patient has HSV ocular infection and antiviral therapy should be commenced or continued (a negative cell-culture does not represent a non-active infection), or 2) There is residual HSV DNA from an ocular HSV infection that is no longer active (antiviral therapy is not necessary).

The goal of our study was to compare PCR results to cell culture isolation for the laboratory diagnosis of clinically-defined ocular HSV infection. We hypothesize that PCR will be more diagnostic than cell culture isolation for detecting HSV from ocular specimens. This hypothesis will be tested by: 1) Forming the subset of patients that tested positive with HSV PCR; 2) Determining the number of cell-culture positive and cell-culture negative patients from the PCR subsets; and 3) Comparing the PCR and cell-culture results to the clinical diagnosis of HSV infection by slit-lamp examination and treatment.

MATERIALS AND METHODS

HSV Laboratory Testing As a routine at The Charles T. Campbell Laboratory, University of Pittsburgh Medical Center, Pittsburgh, PA, all eye specimens used to diagnose HSV infection were submitted for cell-culture isolation and PCR testing (HSV types 1 and 2 DNA). Eye specimens from the cornea and conjunctiva were obtained with soft-tipped Dacron swabs or a kimura spatula and placed in 2mL of Chlamydia transport medium (CTM) (Bartels, Bellevue, WA). Intraocular specimens were obtained with a syringe and needle and also placed in CTM. The cell monolayer used for cell culture isolation was the A549 human lung carcinoma epithelial cell (Viromed, Minnetonka, MN). Routinely, 0.5mL of CTM was inoculated to the A549 cell monolayer and monitored every other day for viral cytopathic effect (CPE). ELVIS (enzyme linked virus induced system) (Diagnostic Hybrids, Athens, OH) was used to confirm any HSV CPE. Cell culture isolation was performed at the Charles T. Campbell Ophthalmic Microbiology laboratory at the University of Pittsburgh Medical Center, Pittsburgh, PA. For HSV PCR testing, 0.45mL of CTM was transported to the Division of Molecular Diagnostics at the University of Pittsburgh Medical Center, Pittsburgh, PA. Both laboratories are fully certified for clinical laboratory testing by independent (College of American Pathologists) and government (Pennsylvania Department of Health, Clinical Laboratory Improvement Amendment) agencies.

Patient Medical and Laboratory Record Data The medical and laboratory records of consecutive patients at the University of Pittsburgh Medical Eye Center from July 2004 to July 2007 were retrospectively reviewed for 1) PCR testing, 2) HSV cell-culture isolation, and 3) clinical diagnosis (University of Pittsburgh, IRB#: PRO07050204). A positive HSV clinical diagnosis required documentation of HSV by the examining clinician and supporting clinical signs. These signs specifically included: skin vesicles for dermatitis; conjunctival injection and follicles for conjunctivitis; dendrites, epithelial defects, or stromal haze for keratitis; cell or flare for uveitis; and retinal necrosis for acute retinal necrosis (ARN). Treatment initiation was recorded as a powerful supporting correlate to clinical diagnosis. All examinations were conducted by ophthalmologists at the UPMC Eye Center, including resident physicians. Patients were excluded from the study due to lack of documentation of clinical examination, diagnosis or treatment plan at time of culture. Patients were also excluded if the viral cultures were prematurely contaminated with bacteria and the results of PCR testing were reported as indeterminate.

Statistical Analysis The laboratory data were analyzed using McNemar's Test to compare paired proportions of PCR and cell-culture (CC) isolation {PCR+, CC+ versus PCR+, CC- versus PCR-, CC+ versus PCR-, CC-} (http://graphpad.com/quickcalcs/McNemars2.cfm). Randomization testing was analyzed using the Fisher's exact test (FE) http://www.langsrud.com/fisher.htm).

RESULTS

Laboratory Record Review Laboratory records determined that 581 patients were tested for the detection of HSV. Of the 581 cases, 520 were PCR negative, cell-culture negative (89.6%); 0 cases were PCR negative, cell-culture positive (0%); 27 were PCR positive, cell-culture negative (4.6%); and 34 were PCR positive, cell-culture positive (5.8%). Paired proportion testing determined that more cases tested positive for HSV by PCR than by cell-culture isolation (McNemar's, P=0.0001).

Medical Record Review Of the 61 cases that tested positive by PCR, complete medical and laboratory records were available on a subset of 47 (77%). Of the 47 PCR positive cases, 19 (40%) were cell-culture negative and 28 (60%) were cell-culture positive. Thirty-nine of 47 (83%) were initially diagnosed and treated for HSV infection. Of the initially diagnosed and treated for HSV infection, 14 of 19 (74%) were cell-culture negative. This would indicate that without PCR testing, negative cell-culture results would have missed the diagnosis of HSV infection in 5 cases. Of the initially diagnosed and treated for HSV infection, 25 of 28 (89%) were cell-culture positive. This would indicate that without laboratory testing (PCR or cell-culture isolation), the diagnosis of HSV infection would have been missed in 3 cases. Eighteen of 19 cell-culture negative cases (95%) and 28 of 28 (100%) cell-culture positive cases [total=98% (46 of 47)] were eventually diagnosed and treated as active HSV infection based on laboratory results and clinical data.

PCR testing determined the presence of HSV type 1 DNA, in all but one case where HSV type 2 DNA was detected in a case of ARN. All clinically diagnosed cases of HSV were treated with acyclovir, valacyclovir, or viroptic, except for two cases of HSV conjunctivitis that were treated with topical antibiotics alone and one case of HSV conjunctivitis in which antiviral treatment was recommended but deferred due to the pregnancy status of the patient.

Final diagnoses in the PCR positive, culture-negative group (n=19) included: 1) 14 cases of HSV keratitis (including 2 cases of keratouveitis and a single case of stromal keratitis without epithelial defect); 2) Three cases of HSV conjunctivitis, 3) One case of ARN; and 4) One case of kerato-conjunctivitis that tested PCR positive for both adenovirus and HSV.

Table 1 Sensitivity of HSV PCR testing and cell-culture isolation

Sensitivity Based on	PCR	Cell-Culture Isolation
Positive PCR	100% (47/47)	60% (28/47)
Positive Cell-Culture	100% (28/28)	100% (28/28)

Final diagnoses in the PCR positive, culture-positive group (n=28) included: 1) 23 cases of HSV keratitis (including 4 cases of keratouveitis); 2) One case of HSV conjunctivitis; 3) Three cases of HSV dermatitis, and 4) One case of ARN. The PCR positive, cell-culture positive keratitis group (70%, 16/23) presented significantly more with a classic dendritic appearance than the cell-culture negative group (29%, 4/14) (Fisher's Exact, P=0.015).

Table 1 summarizes the sensitivities of PCR and cell-culture isolation testing based on positive PCR and positive cell-culture. Based on positive PCR, PCR was more sensitive than cell-culture isolation for detecting HSV (Fisher's Exact, P=0.000002).

DISCUSSION

Laboratory testing needs to be definitive to support appropriate therapy. Contrasting laboratory results do not provide the confidence necessary to assure a successful prognosis. Our clinical ophthalmic microbiology laboratory has reported a significant number of patients with positive PCR testing, but cell-culture negative, for HSV. This presents several scenarios for discrepancy: 1) HSV DNA is present but no active virus, 2) HSV DNA is present but virus is inhibited or not propagating in cell-culture, 3) HSV DNA is present due to non-specific shedding, or 4) Testing was contaminated with HSV DNA. The fact that 98% of patients were diagnosed and treated for HSV infection indicates that the second scenario is most likely. The isolation of live virus from culture may have been attenuated by the host immune response triggered by the infection, and non-culturable HSV with intact DNA may still be present. The present study includes all positive PCR testing, and no false-positive results due to contamination have been noted. Our laboratory has also not documented false-positive PCR results in testing for adenovirus, Varicella zoster virus, acanthamoeba, and Chlamydia DNA. It is our experience, based on 5 years of PCR testing, that specimens for PCR testing were not externally contaminated by handling from medical or laboratory personnel.

In our study, the number of PCR positive patients was larger than the report from Leigh *et al*(61 versus 23). However, the percent of PCR positive was comparable, 10.4% (61/581) versus 11.2% (23/206) [9] It is unknown the number of patients that would have tested positive for cell-culture isolation in Leigh's study. The number of positive PCR testing in both studies was low. This is probably not an indication that ophthalmologist are not recognizing the symptoms of ocular HSV infection, but are more prudent, based on clinical experience, not to be fooled by atypical presentations. At our tertiary care facility, non-resolving keratitis is generally pan-cultured, and resident ophthalmologists are more likely to culture for HSV in most keratitis patients.

Positive PCR testing for the subset of patients with a HSV differential was well supported with the clinical diagnosis, thus allowing a more accurate comparison of laboratory testing with clinical judgment. Table 1 depicts PCR based on sensitivity to be more reliable than cell-culture isolation for detecting HSV infection. "Specificity Testing" could not be determined from the present study. Specificity is based on the testing of "true-negative" specimens; thus no sample from a patient with a herpetic differential diagnosis could truly be designated as a true-negative specimen. HSV PCR has already been demonstrated to be highly specific[1-7].

We did not review the records of patients with a possible herpetic differential diagnosis that were PCR negative, cell-culture negative, because our focus was on definitive laboratory diagnostic testing. This was a laboratory study supported with clinical data and not *vice versa* We reasonably assume and observed with our laboratory daily records that there were many patients tested in our laboratory for HSV but subsequently proven not to have herpetic infection. An in-depth chart review of PCR negative, cell-culture negative patients may demonstrate: 1) Patients diagnosed and treated with HSV anti-viral agents after initial clinical diagnosis, 2) Patients that resolved on anti-viral therapy, and 3) Patients that proved positive for other etiologic pathogenic agents. These parameters, though interesting, were not the focus of the current study.

In conclusion, we accept the hypothesis that PCR was a more definitive test for diagnosing HSV ocular infection than cell-culture isolation. PCR was less likely to miss the detection of HSV in the clinical laboratory and PCR was consistent with the clinical diagnosis. HSV infection cannot be ruled out by cell-culture isolation alone. In fact, without any laboratory testing, 17% (8 of 47) would not have been clinically diagnosed after the initial slit-lamp examination. Clinical judgment must direct therapy in atypical cases where there is a high suspicion of possible HSV infection. Although cell-culture positive testing is definitive of active infection, positive PCR will support the clinical diagnosis of HSV infection in cases of cell-culture negative testing. As PCR also offers timely results and is increasingly becoming more available in the medical community, we encourage greater utilization of this excellent diagnostic laboratory test.

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