Assessment of the Utility of Viral Culture of Cerebrospinal Fluid

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Nucleic acid amplification testing is the preferred method to detect enteroviruses and *Herpesviridae* in cerebrospinal fluid, but clinicians still request viral culture. Review of 22,394 viral cultures of cerebrospinal fluid samples found that <0.1% recovered nonenterovirus, non-*Herpesviridae* species, suggesting that, when nucleic acid amplification testing is performed, viral culture may have no additional benefit.

Nucleic acid amplification testing (NAAT) has become a standard part of the diagnostic evaluation of viral meningitis and meningoencephalitis [1, 2]. NAAT has superior sensitivity for the detection of herpes simplex virus (HSV) and enterovirus (EV) in CSF samples, and the rapidity of results can have an immediate impact on patient care [2, 3]. However, despite extensive clinical and laboratory data supporting the use of NAAT of CSF samples, clinicians often still request viral culture, with the expectation that viruses not specifically targeted by molecular tests might be recovered. Excluding EV, other viruses commonly implicated in CNS infections (e.g., HSV, varicella zoster virus, cytomegalovirus, and West Nile virus) are either poorly recovered from culture or better diagnosed by alternative methods (e.g., NAAT and serological testing) [1, 4–6]. We examined the utility of CSF culture to recover viruses not detected by routine NAAT, and we assessed the potential cost savings of eliminating viral CSF culture as a routine laboratory practice.

Methods. Results of viral culture from CSF samples submitted between 2 December 1994 and 1 December 2005 were retrospectively reviewed. Samples submitted between 2 January 2000 and 4 December 2005 were analyzed as a subset to determine the average time required to obtain EV and HSV results by culture and PCR and to compare the relative rates of detection for these viruses when performed using the same specimen.

Comprehensive viral cultures were performed by shell vial method, including 5 cell lines (rhesus monkey kidney, buffalo green monkey, A549, MRC-5, +/− rhabdomyosarcoma [April–September]) provided by ARUP Reagent Lab and Diagnostic Hybrids. Shell vials were monitored for viral cytopathic effect for at least 10 days. When requested, early antigen detection for cytomegalovirus was performed at 24 and 48 h. Cytopathic effect, virus type, and virus subtype were confirmed by virus-specific immunofluorescent antibodies (Chemicon International and Dako North America) when indicated. Cytopathic effect consistent with HSV was confirmed by polyclonal immunoperoxidase staining (ARUP Reagent Lab) and subtyped by HSV type–specific monoclonal DFA (Trinity Biotech). HSV PCR was performed by in-house assay using validated primer sets and real-time probe detection. HSV PCR assays with positive results with late crossing thresholds were performed again prior to reporting of results by standard laboratory protocol. EV PCR was performed by in-house assay, as described elsewhere [7].

Average annual and total study period costs for viral culture from CSF samples were calculated using an average laboratory charge for CSF viral culture of $52, which did not change significantly during the study period.

Results. During the 11-year period, viruses were recovered from 1270 (5.7%) of 22,394 viral cultures of CSF samples. The viruses isolated included 1249 (98.4%) EV, 16 (1.3%) HSV, 3 (0.2%) cytomegalovirus, 1 (0.08%) varicella zoster virus, and 1 (0.08%) adenovirus. Samples originated from 33 states, with all major geographic regions of the United States represented.

For the subset analysis comparing the performance of PCR and culture, both culture and EV PCR were performed for 929 CSF samples. Of these, 246 samples had positive results overall, with 124 (50.4%) of the samples having positive results by both methods, 4 (1.6%) having positive culture results only, and 118 (48.0%) having positive EV PCR results only. There were 1290 CSF samples for which both culture and HSV PCR were performed. Of these, 9 samples were HSV positive, and HSV was detected for all 9 (100%) only by PCR.

The mean time required to obtain a completed culture result positive for EV was 169 h ($n = 995$), a time period that was 7.2 times longer than the average time required to obtain a positive result from EV PCR (24 h; $n = 7085$). Similarly, the average time required to obtain a completed culture result positive for HSV was 149 h ($n = 14$), a period 3.9 times longer than the mean time required to obtain a PCR result positive for HSV (39

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h; $n = 2478$). The mean length of time required to obtain a negative result by culture was 277 h ($n = 16,887$), a period that was 12 times longer than that required to obtain a PCR result negative for EV (22 h; $n = 17,023$) or HSV (21 h; $n = 64,565$).

During the past 3 years, our laboratory received 3501 (in 2003), 3952 (in 2004), and 3443 (in 2005) requests for viral culture from CSF samples, resulting in a mean annual health care expenditure of $188,864 per year. For the 11-year period examined, the estimated health care expenditure resulting from the 22,394 viral CSF cultures performed was $1,164,488.

Discussion. Nucleic acid amplification–based methods have emerged as the recognized standard [1, 2] for the detection of EV and *Herpesviridae* from CSF samples and are increasingly requested as part of the diagnostic work-up of viral meningitis and meningoencephalitis for both immunocompetent and immunocompromised hosts. Yet, clinicians still commonly request viral culture of CSF samples in lieu of or as an adjunct to NAAT. One possible explanation for this practice is physicians' expectations that viral culture may recover viruses in addition to EV or *Herpesviridae*. Our 11-year review of viral cultures from CSF samples found that 99.6% of positive cultures recovered either EV or HSV. The remaining positive cultures (0.4%) isolated viruses (cytomegalovirus, varicella zoster virus, and adenovirus) that NAAT, if performed, would have detected more rapidly and with greater sensitivity [1, 2, 4, 5]. Although we were not able to obtain clinical history for the cultures positive for viruses other than EV and HSV, we suspect that the clinical presentations or predisposing conditions of these patients (e.g., congenitally infected newborns, solid-organ or hematopoetic stem cell transplant recipients, patients with lymphoma, and patients with advanced HIV disease) would lead experienced physicians to consider these viruses in their differential diagnosis and to specifically request molecular testing for them. Clearly, NAAT remains more expensive than culture (e.g., the approximate charges are $140 for EV PCR, $64 for HSV PCR, and $52 for viral culture), but with its superior sensitivity and faster results, NAAT generates more-meaningful results with a potentially greater impact on patient care [2, 3].

In this study, the yield of viral culture may have been reduced by the delay in specimen transport to a reference laboratory and might be better at laboratories with the opportunity to inoculate fresh specimens. Studies that have specifically examined this issue suggest that, for HSV and EV, recovery is generally adequate up to 72 h after collection when the sample is transported in appropriate media [8, 9], although poor recovery of HSV at low titers may be a relevant exception that further emphasizes the value of PCR [9]. We also acknowledge that clinical circumstances may arise for which performance of viral culture from CSF samples is indicated. For example, when rare but cultivable causes of viral meningoencephalitis (e.g., influenza, parainfluenza, measles, and mumps) are clinically suspected for which NAAT is unavailable, viral culture can serve as a useful adjunctive test [10, 11]. In fact, mumps, a common cause of meningitis and encephalitis in the prevaccine era, has recently resurfaced in several prominent outbreaks in the United Kingdom and the United States [11]. However, it should be emphasized that serological testing and NAAT have emerged as the diagnostic standard for the majority of recognized viral causes of meningitis and encephalitis for which diagnosis by culture-based methods is either not routinely possible (e.g., arboviruses) or not indicated because of poor sensitivity (e.g., HSV) [1, 2, 5, 6].

Although limited to the experiences of 1 laboratory, we demonstrate the potential costs incurred from routine performance of these cultures and, importantly, corroborate the previous findings of others that viral culture for EV and HSV is both insensitive and associated with marked diagnostic delay [2]. We conclude that routine submission of CSF samples for viral culture specifically to recover viruses not ordinarily detected by NAAT is costly and provides minimal, if any, additional benefit. We recommend that viral culture of CSF samples be eliminated as a routine laboratory practice.

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