To the Editor:

The polymerase chain reaction (PCR) technology offers many benefits to the study of infectious disease, but its use must be restrained by the limits of interpretation of the data generated. Wilborn et al recently described the results of a PCR-based prospective study of human herpesvirus-6 (HHV-6) infections in bone marrow transplant (BMT) recipients. Their basic conclusions were that HHV-6 infections correlate with graft-versus-host disease and that no other clinical correlations could be drawn, i.e., “No specific clinical condition could be associated with HHV-6.” Such a conclusion is unwarranted and does a disservice to the field of HHV-6 research. PCR is an inappropriate technology for such a study.

Various aspects of the use of the PCR technique to study HHV-6 infections are summarized in Fig 1. It is generally agreed that reactivations of HHV-6 infection occur frequently after BMT. Such a reactivation event and its aftermath is illustrated in the top of the figure. Reactivation of the latent virus occurs (panel 2), and the newly established productive infection expands (panel 3) until it is cleared by antiviral therapy and/or the patient’s immunologic responses. However, during the productive infection a whole new set of latently infected cells have been established (panel 4). Although such techniques as virus isolation, immunohistochemical staining, and serum PCR can distinguish between latent and productive infections, qualitative PCR such as that used by Wilborn et al cannot. Thus, the appearance of a new positive PCR reaction with

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**Fig 1. Schematic of HHV-6 reactivation from latency.** (○) Uninfected cell; (●) latently infected cell; (X) productively infected cell.
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a patient’s sample may reflect a virus reactivation event that occurred days or even weeks previously and that pushed the number of latently infected cells above the lower limit of sensitivity of the PCR assay. Productive infection of tissues with the virus may or may not be present. Because the existence or level of cells latently infected with HHV-6 or any other herpesvirus have never been correlated with clinical disease (Epstein-Barr virus-transformed cells are not truly latently infected), drawing conclusions concerning clinical correlations by comparing a positive PCR reaction with concurrent changes in the patient’s clinical status will inevitably lead to erroneous and misleading findings.

Some of the same limitations apply to quantitative PCR procedures such as that used by Cone et al1 in the analysis of HHV-6 infections in the lungs of BMT patients. Clearly, quantitative PCR can distinguish between a latent HHV-6 infection in an immunologically normal individual (panel 1) and a productive infection in an immunocompromised patient (panel 3). However, it may have difficulty distinguishing between a low-level productive infection (panel 2) and the increased load of latently infected cells in the tissue of an immunocompromised patient with a history of HHV-6 reactivations (panel 4). Because even low levels of productive infection by HHV-6 in a patient may cause clinical disease (immunopathological reactions, inflammation, cytokine production, BM suppression, etc), the distinction between low-level productive infection and high-level latent infection is important.

Another point of concern with the study by Wilborn et al1 must be raised. In that work they analyzed samples of blood, urine, and oral lavage fluids. It is known from work with cytomegalovirus (CMV) that detection of virus in samples of urine and saliva has little or no predictive value for the occurrence of CMV disease.4 Although detection of CMV in buffy coat cells is more predictive of disease, that positive predictive value is much less than 100%. There is no reason to believe that HHV-6 should be different. Thus, the choice of those patient specimens by Wilborn et al, while convenient, probably decreased dramatically the power of their study to detect HHV-6 disease. This point was not even addressed in their manuscript.

The inappropriate use of PCR and the analysis of patient specimens of dubious predictive value in attempts to draw conclusions with respect to the clinical manifestations of HHV-6 infections in BMT patients only serve to confuse the issue. The conclusion of Wilborn et al1 that no clinical correlations can be drawn between HHV-6 infections and clinical consequences reflects their poor choices of technology and patient specimens, not the reality of the patients. Methods must be used that can unequivocally differentiate between latent and productive infections and that do not depend on such surrogate samples as blood, urine, and saliva. To show HHV-6 suppression of BM function, productive infection of BM must be evaluated. Likewise, the demonstration of HHV-6 encephalitis required the demonstration of productive infection of the brain by the virus.5 Until investigators begin using methods that are able to show clinical correlates with HHV-6 infections, rather than those that are merely convenient, the scope of HHV-6 disease will remain a matter of speculation.

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REFERENCES

Human herpesvirus-6 and bone marrow transplantation [letter]

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