

New Viral Diagnostic Technique Can Prevent Misdiagnoses

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Over 90% of adults are carriers of latent human herpesvirus 6 (HHV-6). It is primarily a non-pathogenic infection, but can cause roseola infantum in young children and more severe disease in immunocompromised individuals such as transplant recipients. Active HHV-6 infection, resulting from reactivation of the latent virus, is generally detected by polymerase chain reaction (PCR) testing on plasma, serum, or whole blood. The latent HHV-6 genome exists as an extrachromosomal circular episome, however around 1% of the population possess an integrated copy of the HHV-6 genome in all of their cells. This can confound clinicians when attempting to diagnose active HHV-6 infection, and the result is that potentially toxic antiviral medication can be mistakenly administered to transplant patients without active HHV-6 infection who possess the integrated copy. A new method of detecting chromosomally integrated HHV-6 (ciHHV-6) in patient samples has recently been developed by Dr. Keith Jerome's Laboratory (Vaccine and Infectious Disease Division). Through the use of a precise quantitative PCR called droplet digital PCR (ddPCR), ciHHV-6 can be identified in patients with 100% sensitivity. The study was recently published in *Clinical Chemistry*.

Individuals with ciHHV-6 always exhibit high viral loads, which can be mistaken for actively replicating HHV-6. In fact, it has been shown that ciHHV-6 cannot be distinguished from active infection based solely on a plasma quantitative PCR (qPCR) for HHV-6. Currently, confirmation of ciHHV-6 requires fluorescence *in situ* hybridization or PCR testing of hair follicle cells, neither of which is a common assay in molecular diagnostics laboratories. The ability to accurately detect ciHHV-6 in patients using a standard laboratory assay would assist tremendously in preventing misdiagnoses and the unnecessary administration of drugs with severe side effects.

The newly developed droplet digital PCR assay provides a method for quantitating a DNA sequence of interest (e.g., HHV-6) with a very high degree of precision. This is accomplished by generating a large number of droplets from a test sample, which individually supply a digital readout (either yes or no) for the presence of the DNA sequence in each droplet. Software analysis on the number of positive droplets provides an exact quantitation of the specific DNA molecules without the need for a standard curve. Additionally, by duplexing the assay to also measure a chromosomal gene, the ratio of viral DNA copies per cell can be accurately determined.

Researchers in the Jerome Lab first utilized the new technique to study the virus per cell ratio detected from a cultured cell line possessing a single integrated copy of ciHHV-6. They found that the HHV-6 per cell ratio consistently had a value of about 1 (0.96 ± 0.03). Furthermore, whole blood samples from patients with known ciHHV-6 also showed virus per cell ratios to be near 1. They also confirmed that their test could be applicable to plasma samples, which contain cellular DNA, albeit in low amounts. They established a virus per cell cutoff range of 0.56–1.78 for identifying ciHHV-6. By these criteria, the assay showed 100% sensitivity, meaning that no sample from a ciHHV-6 patient was incorrectly identified as negative, as well as 82% specificity for ciHHV-6.

"This assay is going to be a big deal for our transplant patients who have ciHHV-6," said Jerome. "Previously, they've often been misdiagnosed with active HHV-6 disease, exposing them to unnecessary antivirals and ending their diagnostic workup too soon. Now we have a way to identify these patients and be sure they're getting appropriate care." The high accuracy of the test at identifying ciHHV-6 patients makes it a strong candidate for broad usage in molecular virology diagnostic labs around the world. Jerome envisions this assay to have great potential. "We hope this test will see quick adoption elsewhere so that advances from here in Seattle can soon be available worldwide."

[Sedlak RH, Cook L, Huang M-L, Magaret A, Zerr DM, Boeckh M, Jerome KR](#). 2014. Identification of Chromosomally Integrated Human Herpesvirus 6 by Droplet Digital PCR. *Clin Chem*. 60:765-72.

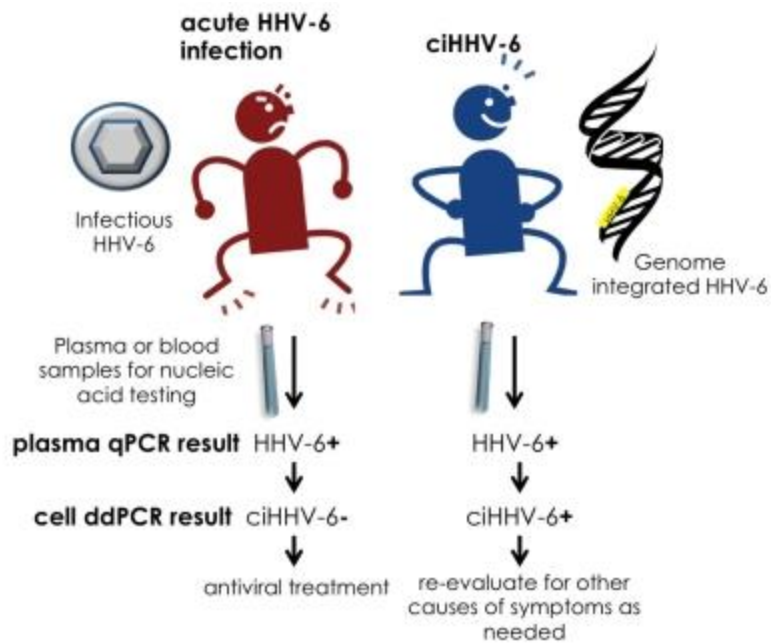


Image provided by Dr. Ruth Hall Sedlak.

Droplet digital PCR (ddPCR) can accurately identify ciHHV-6 patients while plasma qPCR oftentimes cannot distinguish between ciHHV-6 and acute HHV-6 infection.