Cyprinid herpesvirus 3 (CyHV-3) is the aetiological agent of a serious infective, notifiable disease affecting common carp and varieties. In survivors, infection is generally characterized by a subclinical latency phase with restricted viral replication. The CyHV-3 genome is difficult to detect in such carrier fish that represent a potential source of dissemination if viral reactivation occurs. In this study, the analytical and diagnostic performance of an alternative serum neutralization (SN) method based on the detection of CyHV-3-specific antibodies was assessed using 151 serum or plasma samples from healthy and naturally or experimentally CyHV-3-infected carp. French CyHV-3 isolate 07/108b was neutralized efficiently by sera from carp infected with European, American and Taiwanese CyHV-3 isolates, but no neutralization was observed using sera specific to other aquatic herpesviruses. Diagnostic sensitivity, diagnostic specificity and repeatability of 95.9%, 99.0% and 99.3%, respectively, were obtained, as well as a compliance rate of 89.9% in reproducibility testing. Neutralizing antibodies were steadily detected in infected carp subjected to restrictive or permissive temperature variations over more than 25 months post-infection. The results suggest that this non-lethal diagnostic test could be used in the future to improve the epidemiological surveillance and control of CyHV-3 disease.