HHV-6 virus stock preparation

High titer (1 X 10^6 - 10^7) purified virus stocks can be prepared from HSB-2 and Molt-3 suspension cells by the following method. 10^6 HHV-6 infected cells should be mixed with uninfected HSB-2 or Molt3 cells at a ratio of 10:1 in a large 150 sq cm flask in a total volume of 5ml and should be kept in a tilted position for 2hrs inside cell incubator at 37°C. For a high titre virus stock preparation, it is advisable to infect 5 such flask at a time. Fresh media should be added to the cells after 2hrs of infection and flasks should be transferred back to 37°C incubator. When more than 80% of cells show cytopathic effects visible under light microscope, infected cells together with the media should be collected in 50ml falcon tubes and should be kept on ice. Infected cells should then be lysed by mild sonication on ice for 1minute with 1 minute gap. Sonication should be repeated 3 times using a broad tip, 50% duty cycle and output power 8. Lysed cells with culture fluid should then be centrifuged at 3500rpm at 4°C for 1hr and the clear supernatant should then be filtered through a 0.45-µm filter, which contains viral particles. The virus particles should then be pelleted down by centrifugation at 25,000 x g for 3 h at 4°C. The virus pellet should then be re-suspended in cold IMDM media without any antibiotics and frozen at –80°C until further use. The HHV-6 titer expressed as the 50% tissue culture infective dose ($TCID_{50}$) should be determined by infecting fresh HSB-2 or Molt-3 cells at different dilutions and scoring the number of infected cells exhibiting cytopathic effects or by immunostaining the infected cells using a suitable antibody. Viral titer and $TCID_{50}$ value is calculated using Reed-Münch formula.