DNA Isolation for CMV viral load testing

Note: This protocol is for increased sensitivity for CMV monitoring by isolating DNA from 1 ml plasma or whole blood

Materials needed:
Qiagen Qiamp Blood DNA Midi Spin Kit (catalog # 51183)

Centrifuge that can hold 15 ml conical tubes and reach speeds of 4500 x g

Heat block/water bath set to 70°C

DEPC water

15 ml conical centrifuge tubes

100% Ethanol

Before beginning:
Prepare the following reagents according to protocol in Qiamp Blood Midi Spin Kit.
Protease: Add 4.4 ml DEPC water to the vial of lyophilized protease and swirl vial to reconstitute. Store at 2-8°C for short term storage, otherwise aliquot 1 ml per tube and store at -20°C.
Buffers AW1 and AW2: Add appropriate amount of 100% ethanol, as indicated on the bottle. Store at room temperature.

DNA isolation protocol using Qiamp Blood Midi Spin Kit:

1. Pipet 100 μl Qiagen protease into 15 ml conical tube.
2. Add up to 1 ml of sample to the 15 ml conical tube and mix by pipetting.
3. Add 1.2 ml buffer AL and mix by inverting 15 times, then vortex for 1 minute.
4. Incubate the samples at 70°C for 10 minutes.
5. Add 1 ml 100% ethanol to the tube and invert 10 times, then vortex for 1 minute.
6. Transfer the entire volume onto a spin column placed in a 15 ml (provided in kit) before centriguing the sample for 3 minutes at 1850 x g.
7. Discard the filtrate and place the column back into the original conical tube.
8. Add 2 ml Buffer AW1 to the spin column and centrifuge at 4500 x g for 1 minute (* a speed of 4000 x g is also sufficient). It is not necessary to discard flow-through at this step.
9. Add 2 ml Buffer AW2 to the spin colum and centrifuge at 4500 x g for 15 minutes.
10. Following the spin, remove the flow-through from the centrifuge tube and replace the column in the centrifuge tube. Incubate the column in the tube at 70°C for 10 minutes to evaporate any excess ethanol (this step is critical because residual ethanol can interfere with PCR).
11. Remove the spin column from the 15 ml centrifuge tube and inspect for condensation. If any condensation is visible, wipe the column with a clean absorbent tissue. Place the spin column in a new 15 ml conical tube.
12. Pipet 200 μl DEPC water directly onto the membrane of the spin column and incubate at room temperature for 5 minutes.
13. Centrifuge the column/conical tube for 5 minutes at 4500 x g and then transfer the eluate to an appropriate tube for storage.
14. DNA should be used for CMV VL QPCR (using the Taqman Fast Advanced Master Mix enzyme) or stored at -20°C.