Supplemental Materials

Optimization of HIV-1 RNA quantitation in CVL: Assay optimization and validation were performed using unfractionated CVL collected from HIV-negative donors using a protocol and informed consent approved by the UNC-CH Institutional Review Board. Frozen aliquots of the CVL were thawed, spiked with a known amount of HIV-1, and then treated with a specific pre-treatment (detailed below) before testing with the Abbott RealTime HIV-1 assay. A 0.6 ml aliquot of phosphate-buffered saline (PBS) was always spiked with the same amount of HIV-1 at the same time the CVL was spiked as a control and was tested with the CVL, albeit without any pre-treatment. Briefly, the different pre-treatment procedures were: (i) CVL was pelleted after thawing (1000×g for 5 min); (ii) CVL was treated as described for breastmilk [34] with the addition of RNA lysis buffer (Promega) and 60 ul proteinase K (Abbott), followed by incubation at 53°C for 20 min; (iii) CVL was diluted 1:2 with normal plasma; (iv) addition of ~600 cp of the RCAS virus [35] to increase the amount of (non-HIV) viral RNA as a carrier; (v) treatment of CVL with lysis buffer and proteinase K as above plus 5 min of heating at 95°C to inactivate the proteinase K; (vi) treatment of CVL with lysis buffer as above with double the proteinase K amount; (vii) treatment of CVL with PBS and proteinase K; (viii) or addition of only proteinase K to the CVL. This last pre-treatment procedure with only proteinase K was used for the rest of the testing as follows: 0.06 ml proteinase K (Abbott) was added to 0.74 ml CVL and manually mixed by pipetting, incubated at 53°C for 20 min, vortexed briefly, and spun at 3200×g for 5 min. The mixture was run on the Abbott m2000sp with the 0.6 ml plasma program. The small amount of dilution resulted in LLQ of 42 cp/ml.

Validation of HIV-1 quantification in unfractionated CVL. To ensure that the VL testing could be done successfully on unfractionated CVL from WIHS participants, unfractionated CVL from multiple uninfected donors was spiked with known levels of HIV-1 and tested on both a laboratory developed Droplet Digital PCR (ddPCR) HIV-1 assay and the Abbott RealTime HIV-1 assay. Both assays yielded less viral amplification in CVL than in plasma spiked with the same quantity of HIV-1 (data not shown). We tested the performance of the Abbott assay using pre-treatment procedures, which have been previously used successfully with other specimen types such as breastmilk and semen, to compare recovery of spiked HIV-1 in CVL to recovery of HIV-1 in PBS (all samples spiked with the same amount of HIV-1 at the same time and tested in parallel). As detailed in Supplemental Table S1, CVL was collected from 6 donors and spiked with HIV-1 at different levels from 100-500 cp/ml. CVL VL was always compared to the PBS VL obtained in the same run. HIV-1 recovery on the Abbott assay was poor under conditions of no pre-treatment and pelleting of the CVL cells and debris. Pre-treatment with Abbott Proteinase K and RNA lysis buffer with heating, a method previously successfully applied to breastmilk specimens [34] resulted in better, but inconsistent, recovery among donors. Other pre-treatments with poor and variable results included diluting with plasma, spiking with 6-fold more of a different retrovirus (RCAS) to increase the amount of viral RNA as carrier, heating to 95°C after proteinase K treatment, and doubling the amount of proteinase K. After replacing the lysis buffer with PBS during the proteinase K treatment we obtained consistent results with the 5 donors tested. We validated pre-treatment with and without PBS using a sample that previously gave inconsistent results (donor 1), and a set of samples spiked

with 12.5 to 100 cp/ml HIV-1 (Supplemental Table S1). This method of proteinase K treatment without any other additions was applied to all of the WIHS CVL samples tested in this study.