**Supplemental Digital Content 1 – Methods**

Quality assurance (QA) measures before deconvolution

The first QA measure implemented in the search and test algorithm is as follows: (1) Viral load values from the pools are transformed to a log10 scale; (2) Row and column pools are screened for values >5 log10 HIV RNA copies/mL; (3) If none of the pools have viral loads >5 log10 HIV RNA copies/mL, the search and test algorithm continues, as previously described16; (4) If a row OR column pool viral load (but not both) is larger than 5 log10 HIV RNA copies/mL, then the third contamination check described below is activated. (5) If any of the row pool values AND any of the column pool viral load values are larger than 5 log10 HIV RNA copies/mL, then the search and test algorithm identifies the individual sample(s) at the intersection(s) of the affected row and column pools to be tested first for an individual viral load; (6) The viral load value of each individual retested sample is divided by the number of samples in the original pool and then subtracted from its row and column pool values; (7) If the viral loads of the affected row and column pools are not reduced by more than 4.5 log10 HIV RNA copies/mL, then contamination is deemed likely and those rows and column pools are reconstituted, using the same samples that constituted them originally, and viral loads are then performed on the new row and column pools, and the new values incorporated in the search and test algorithm.

Second QA measure: (1) An assay standard deviation of sdassay = 0.12 (on a log10 scale) was chosen based on published data25. (2) The difference between the log averages of the column and row pool HIV RNA levels is calculated and divided by the assay standard deviation multiplied by an appropriate factor (sdassay\*sqrt(2)/sqrt(number of samples per row and column)) to determine if the column and row pool assay errors come from the same distribution; (3) If the absolute value of the difference divided by the above described factor is less than 2.58 log10 HIV RNA copies/mL (value corresponding to a two-sided test at alpha of 0.01), then the search and test algorithm continues, as previously described16; (4) If the absolute difference divided by the above described factor is 2.58 log10 HIV RNA copies/mL or more, the program recommends reconstitution and retesting of all of the pools; (5) If the absolute difference divided by the above described factor is less than 2.58 log10 HIV RNA copies/mL, the search and test algorithm continues using the new pool viral load values; (6) If the difference is again 2.58 or more, the program notes this and proceeds with the search and test algorithm. Of note, in this later case there is a potential that there is additional contamination during the re-constitution of one of the pools, but this is not investigated further in the QA approach at this time.

Third QA measure: (1) A check is made to determine if a row OR column (but not both) pool HIV RNA level is unusually high (i.e. >5 log10 copies/mL); (2) If no pool values are unusually high, then the search and test algorithm continues as previously described; (3) If a pool value is unusually high, the program recommends reconstitution of that row or column pool based on the same samples that were included in it before; (4) If the absolute value of the difference between the old and new pool values divided by the assay standard deviation multiplied by the sqrt(2) is less than 2.58 log10 HIV RNA copies/mL, the search and test algorithm continues as previously described; (5) If the absolute value of the difference divided by the assay standard deviation multiplied by the sqrt(2) is 2.58 log10 HIV RNA copies/mL or more, retesting of all of the individual samples constituting that pool is recommended, and these values are then incorporated into the algorithm to be used to complete the rest of the matrix, as previously described16.