CENTER FOR AIDS RESEARCH
University of California San Diego

Translational Virology Core Protocols

Quality Assurance

Our group employs a rigorous, multi-step data quality control procedure. All laboratory test results are independently checked for errors by a second lab technician. The raw data are entered and maintained in the study database, regulated by the Data Management and Information Systems Unit. All test result data are audited by Dr. Smith after data entry and prior to preparation for analysis.

Our QA program will incorporate several elements, including verification of specimen labeling, tracking of laboratory personnel performance, certification of laboratory instrumentation, reagents, and analytic test procedures, assessing precision and accuracy of final results, entry of final results into the study database, and complete documentation of all laboratory procedures. To assess precision of our methods, we will repeat assays in 5% of specimens. Specimens used in these QA assessments will be randomly selected on an annual basis. The results from these repeat measurements will be compared by three methods. First, we will compare laboratory measurements (blood and GS HIV viral loads, drug resistance and STI testing and percent clonal sequence analysis) with Pearson's correlation coefficient (r) and conclude that the 2 measurements are similar if r >0.75. Second, we will calculate the coefficient of variation (CV) for each duplicate measurement and will conclude that the 2 measurements are acceptably similar if the CV is less than 20%. Third, we will perform a paired t-test to assess for systematic differences (e.g., repeat values are consistently lower than initial values). For values outside the acceptable range, we will perform additional analyses to identify the source(s) of variability, which we will then correct. When aberrant lab results are identified, we will track their occurrence and also when such results require repeat processing. We will tabulate these results and review them quarterly to identify problems.

In addition, during each run of the viral load assay (Amplicor, Roche), we will run one high-positive control, one low-positive control and one negative control. Only when the controls are within the expected range (according to the manual), are results reported as valid.

For polymerase chain reaction (PCR) assays we will perform the following procedures:

Safeguards against contamination of amplification reactions: Because of the sensitivity of PCR amplification, a number of laboratory safeguards against contamination with exogenous DNA and RNA will be applied. These will include inclusion of negative control amplification reactions, use of aerosol resistant pipet tips, dedicated pipetmen for “clean” procedures, and the performance of all

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extraction and pre-PCR procedures in laminar flow hoods equipped with UV sources for decontamination. All work involving PCR amplified products will be performed in separate laminar flow hoods also equipped with UV lamps.

Sequencing quality control: The Core PI's laboratory will be a participant in the ENVA (Schurrman 1999) panel. Quarterly or semiannually, blinded samples of known sequence type will be processed and sequenced. Results will be compared with the actual sequences. The Core laboratory is certified by Applied Biosystems for use of the Viroseq HIV genotyping system. This includes satisfactory processing and sequencing of a panel of blinded samples, including low copy samples, provided by ABI. All sequences generated will be compared with existing sequences submitted to GENBANK using a BLAST algorithm. In addition, contaminating sequences will be excluded by Phylogenetic reconstruction of all sequences generated in the laboratory by Dr. Smith using a Neighbor Joining Algorithm (Phylip 5.4, Joseph Felsenstein, University of Washington).

Phylogenetic reconstruction and blast searches for contamination: The highly sensitive nature of PCR amplification makes these assays vulnerable to contamination with exogenous sequences. Contaminating sequences will be excluded first by comparison to all gene-specific sequences available through Genbank via Blast search and second by phylogenetic analysis along with all gene-specific sequences generated in the laboratory using PHYLIP 5.4. Phylogenetic trees for each investigated coding region will be obtained from a matrix of synonymous nucleotide distances, as is appropriate for analysis regions where there is strong selection against nonsynonymous changes.

Monitoring laboratory performance: Sequencing quality control and performance times will be reviewed quarterly. Quality Measure 1: successful amplification and sequencing of >90% of samples with viral loads >1000 copies/ml. Quality Measure 2: successful amplification and sequencing of >75% of samples with viral loads between 125 and 1000 copies/ml. Overall sequence contamination rate of <2%. 85% of samples will be processed within targeted performance times specified.