# VIROLOGY QUALITY ASSESSMENT PROGRAM

## STATISTICAL CENTER

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DATE: December 15, 1999

SUBJECT: 180-DAY STABILITY OF STORED RNA PROFICIENCY PANELS

The purpose of this study was to assess the stability of RNA proficiency panels through 180 days of storage in liquid nitrogen ($LN_2$) and at –80° C. The study was based on RNA Panel 004ru. The panel included six replicates of HIV spiked into serum at 50 copies/ml, five at 250 copies/ml, three at 1,250 copies/ml and three at 6,250 copies/ml. Three HIV-negative specimens were also included but they were not used in this analysis. To assess stability during storage, the panel was assayed at baseline and then after 30, 60, 90 and 180 days of storage in $LN_2$ and at –80° C. The Roche ultrasensitive HIV Monitor assay was employed for all assays.

Descriptive statistics for estimated RNA concentration and $\log_{10}$-transformed estimated RNA concentration are provided in Tables 1 and 2. Standard deviations are provided for the log-transformed data because the results were analyzed in the log-transformed scale. Log recovery was calculated as the difference between $\log_{10}$ estimated and $\log_{10}$ nominal concentration. Plots of log recovery against time in storage at –80° C and in $LN_2$ respectively are provided in Figures 1 and 2. Median log recovery at each time point is indicated with asterisks. There is no evidence of a downward trend in the medians in either plot.

Changes in estimated RNA concentration under each storage condition were assessed using regressions of $\log_{10}$ estimated RNA concentration on $\log_{10}$ nominal RNA concentration and storage time. The regressions explained most of the variance in $\log_{10}$ estimated RNA concentration (–80° C: $r^2$=0.93; $LN_2$: $r^2$=0.95). The estimated slope at –80° C corresponded to a 30% increase in RNA concentration over 180 days, while the estimated slope in $LN_2$ corresponded to a 13% loss over the same interval. However, neither slope was statistically significantly different from zero (–80° C: p=0.09; $LN_2$: p=0.29). The slight positive slope at –80° C may have been caused by slightly low recovery at 30 days and slightly high recovery at 180 days (Figure 1). However, this variation in recovery is within the limits that would be expected from inter-assay variation.

In summary, the results are consistent with the conclusion that RNA proficiency panels are stable for up to 180 days of storage at either temperature.

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TABLE 1. DESCRIPTIVE STATISTICS FOR ESTIMATED RNA CONCENTRATION IN THE RNA STABILITY STUDY.

| NOMINAL RNA COPIES/ML | N | STORAGE CONDITION | STORAGE (DAYS) | MEDIAN ESTIMATED RNA COPIES/ML |
|---|---|---|---|---|
| 50 | 6 | BASELINE | 0 | 54 |
| | | $LN_2$ | 30 | 58 |
| | | | 60 | 54 |
| | | | 90 | 36 |
| | | | 180 | 46 |
| | | -80° C | 30 | 37 |
| | | | 60 | 49 |
| | | | 90 | 55 |
| | | | 180 | 60 |
| 250 | 5 | BASELINE | 0 | 263 |
| | | $LN_2$ | 30 | 259 |
| | | | 60 | 276 |
| | | | 90 | 266 |
| | | | 180 | 187 |
| | | -80° C | 30 | 199 |
| | | | 60 | 254 |
| | | | 90 | 174 |
| | | | 180 | 238 |
| 1,250 | 3 | BASELINE | 0 | 1,298 |
| | | $LN_2$ | 30 | 1,132 |
| | | | 60 | 1,432 |
| | | | 90 | 1,719 |
| | | | 180 | 1,192 |
| | | -80° C | 30 | 1,057 |
| | | | 60 | 1,078 |
| | | | 90 | 1,194 |
| | | | 180 | 1,482 |
| 6,250 | 3 | BASELINE | 0 | 5,279 |
| | | $LN_2$ | 30 | 5,232 |
| | | | 60 | 6,152 |
| | | | 90 | 5,995 |
| | | | 180 | 5,529 |
| | | -80° C | 30 | 4,931 |
| | | | 60 | 5,205 |
| | | | 90 | 7,800 |
| | | | 180 | 8,530 |

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TABLE 2. DESCRIPTIVE STATISTICS FOR LOG10 ESTIMATED RNA CONCENTRATION IN THE RNA STABILITY STUDY.

| NOMINAL RNA COPIES/ML | N | STORAGE CONDITION | STORAGE (DAYS) | MEDIAN $LOG_{10}$ ESTIMATED RNA COPIES/ML | SD |
|---|---|---|---|---|---|
| 50 | 6 | BASELINE | 0 | 1.7214 | 0.4312 |
| | | $LN_2$ | 30 | 1.7631 | 0.2085 |
| | | | 60 | 1.7098 | 0.2324 |
| | | | 90 | 1.5589 | 0.1483 |
| | | | 180 | 1.6577 | 0.1300 |
| | | -80° C | 30 | 1.5682 | 0.1931 |
| | | | 60 | 1.6898 | 0.2005 |
| | | | 90 | 1.7127 | 0.3421 |
| | | | 180 | 1.7776 | 0.1869 |
| 250 | 5 | BASELINE | 0 | 2.4199 | 0.0155 |
| | | $LN_2$ | 30 | 2.4133 | 0.1432 |
| | | | 60 | 2.4409 | 0.0593 |
| | | | 90 | 2.4248 | 0.0859 |
| | | | 180 | 2.2718 | 0.2367 |
| | | -80° C | 30 | 2.2988 | 0.2282 |
| | | | 60 | 2.4048 | 0.2556 |
| | | | 90 | 2.2405 | 0.1853 |
| | | | 180 | 2.3765 | 0.1844 |
| 1,250 | 3 | BASELINE | 0 | 3.1132 | 0.2425 |
| | | $LN_2$ | 30 | 3.0538 | 0.0161 |
| | | | 60 | 3.1559 | 0.1423 |
| | | | 90 | 3.2352 | 0.0353 |
| | | | 180 | 3.0762 | 0.0353 |
| | | -80° C | 30 | 3.0240 | 0.1736 |
| | | | 60 | 3.0326 | 0.0399 |
| | | | 90 | 3.0770 | 0.0899 |
| | | | 180 | 3.1708 | 0.1630 |
| 6,250 | 3 | BASELINE | 0 | 3.7225 | 0.0609 |
| | | $LN_2$ | 30 | 3.7186 | 0.0810 |
| | | | 60 | 3.7890 | 0.0563 |
| | | | 90 | 3.7777 | 0.0449 |
| | | | 180 | 3.7426 | 0.1532 |
| | | -80° C | 30 | 3.6929 | 0.1022 |
| | | | 60 | 3.7164 | 0.1953 |
| | | | 90 | 3.8920 | 0.1128 |
| | | | 180 | 3.9309 | 0.1696 |

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FIGURE 1. LOG RECOVERY VS TIME IN DAYS AT –80° C. ASTERISKS INDICATE MEDIAN VALUES.

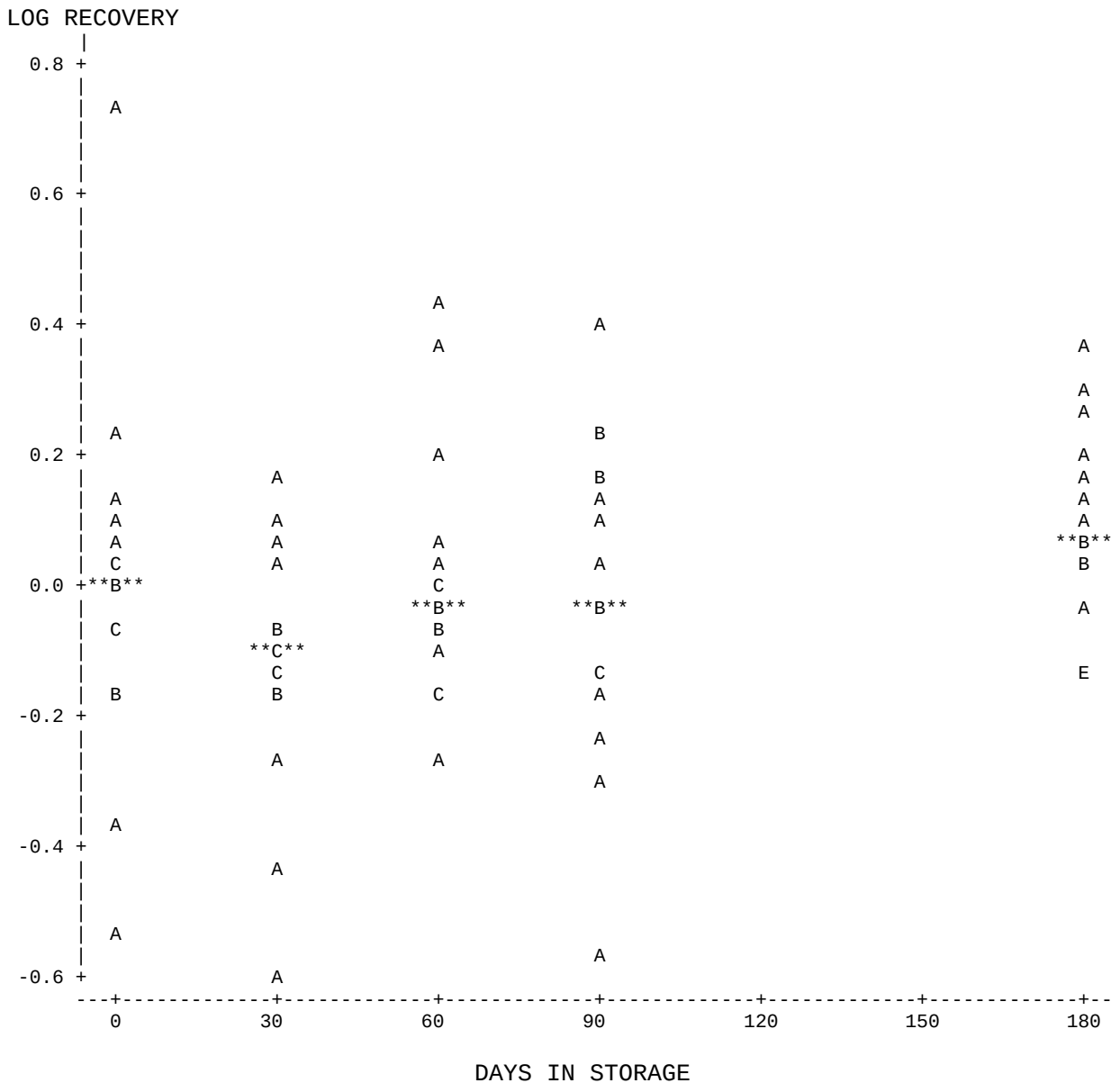


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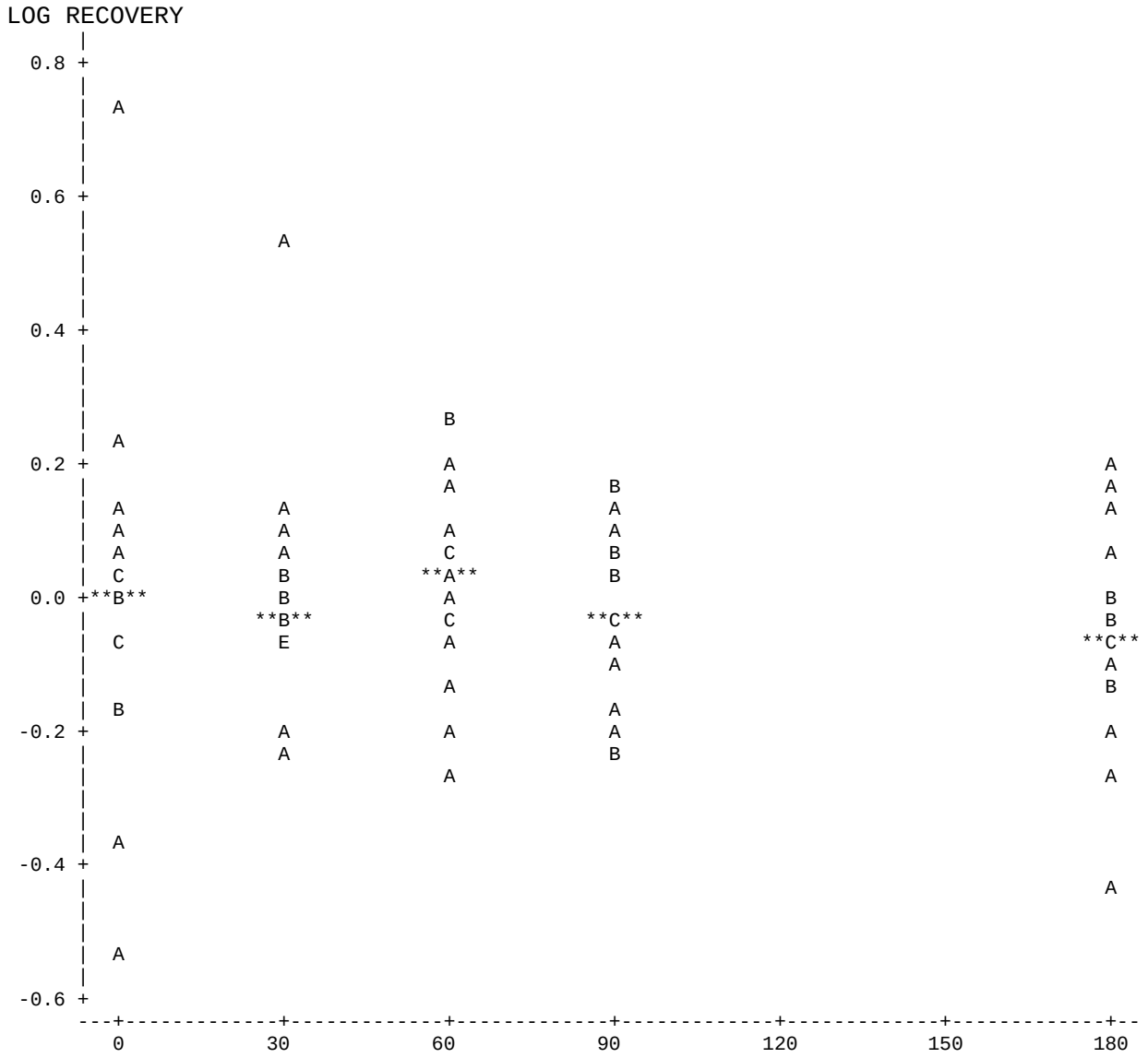
FIGURE 2. LOG RECOVERY VS TIME IN LIQUID NITROGEN IN DAYS. ASTERISKS INDICATE MEDIAN VALUES.

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