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Computer Science and Mathematics Division

Mathematical Sciences Section

RESULTS OF THE ANALYSIS OF THE BLOOD LYMPHOCYTE PROLIFERATION TEST DATA FROM THE NATIONAL JEWISH CENTER

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Date Published: March 1997

Research was supported by the Offices of Occupational Medicine, Environment, Safety and Health, U. S. Department of Energy

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RESULTS OF THE ANALYSIS OF THE BLOOD LYMPHOCYTE PROLIFERATION TEST DATA FROM THE NATIONAL JEWISH CENTER

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Abstract

A new approach to the analysis of the blood beryllium lymphocyte proliferation test (LPT) was presented to the Committee to Accredit Beryllium Sensitization Testing-Beryllium Industry Scientific Advisory Committee in April, 1994. Two new outlier resistant methods were proposed for the analysis of the blood LPT and compared with the approach then in use by most labs. The method based on a least absolute values (LAV) analysis of the log of the well counts was recommended for routine use. It was considered important to "field test" the method on a new data base from another laboratory, since results were obtained using data from a single laboratory—Oak Ridge Institute for Science and Education (ORISE).

The National Jewish Center (NJC) agreed to provide data (similar to that from ORISE in ORNL-6818) from a study that was underway at that time. Three groups of LPT data are considered; i) a sample of 168 beryllium exposed (BE) workers and 20 nonexposed (NE) persons; ii) 25 unacceptable LPTs, and iii) 32 abnormal LPTs for individuals known to have chronic beryllium disease (CBD). The LAV method described in ORNL-6818 was applied to each LPT. Graphical and numerical summaries similar to those presented for the ORISE data are given. Three methods were used to identify abnormal LPTs. All three methods correctly identified the 32 known CBD cases as abnormal. Results of applying the three methods to the BE-sample and Unacceptable data sets are presented, and results for each of the three methods for the 20 Unacceptable LPTs and retest results are given.

These results support the earlier recommendation that the LAV method is a simple and effective method for routine analysis of the blood beryllium LPT that is not effected by outliers.

1. Introduction

On April 22, 1994 a new approach to the analysis of the blood beryllium lymphocyte proliferation test (LPT) [1] was presented at the Committee to Accredit Beryllium Sensitization Testing-Beryllium Industry Scientific Advisory Committee meeting in Washington, D.C. The details of the method are described [2] by Frome *et al* in a research report (ORNL-6818) [4] and were presented on November 8, 1994 at the *Conference on Beryllium Related Diseases* [5]. At the meeting there was general satisfaction with the proposed methods, but it was considered important to "field test" the method on a new data base from another laboratory—results in ORNL-6818 were obtained using data from the Oak Ridge Institute for Science and Education (ORISE) laboratory. The National Jewish Center (NJC) agreed to provide data similar to that in ORNL-6818 from a study that was underway at that time.

In ORNL-6818 two outlier resistant methods were proposed for the estimation of the stimulation index (SI), which is the ratio of the response of beryllium stimulated cells to control cells. These outlier resistant methods were compared with the approach then in use by most labs. The method based on a least absolute values (LAV) analysis (Section 2.2) of the log of the well counts was recommended for routine use. **In this report all of the results are based on the LAV method. All LPTs showed an adequate response to concanavalin-A (ConA) and phytohemagglutinin (PHA) and those with obvious "laboratory error" (i.e. many wells with no response above background) have been eliminated.**

[1] Abbreviations used: AB,abnormal; Be,beryllium; BE,beryllium exposed; CBD,chronic beryllium disease; ConA,concanavalin-A; CV,coefficient of variation; df,degrees of freedom; LAV,least absolute values; LPT,lymphocyte proliferation test; NE,nonexposed; NJC,National Jewish Center; ORISE,Oak Ridge Institute for Science and Education; ORNL,Oak Ridge National Laboratory; PHA,phytohemagglutinin; SI,stimulation index; UN,unacceptable;

[2] On the Internet see URL: http://www.epm.ornl.gov/ frome/BeLPT/index.html

1.1. Description of Data From National Jewish Center

Three groups of LPT data are considered:

- LPTs for a sample of 168 beryllium exposed (BE) workers and 20 nonexposed (NE) persons.
- 25 unacceptable (UN) LPTs, and
- 32 abnormal (AB) LPT data sets.

The sample data consists of the first 168 persons whose blood arrived at NJC for beryllium testing as part of a recent study and are considered to be representative of the study cohort. One LPT from the beryllium exposed workers was removed because of laboratory error (eight of twelve control wells showed background counts). The nonexposed LPT data are from 20 people who have no known beryllium exposure or respiratory disorders. These 20 LPTs were performed by a single NJC technician.

The unacceptable data are from 25 patients who have "high variability" in their beryllium test results or control cells. Data are flagged as unacceptable for any of the following reasons:

- three or more of the six beryllium stimulated groups are excluded due to high variation
- more than five control well data values are excluded due to high variation,
- ConA and PHA SIs are low (indicating low cell viability), or
- cell control counts are judged to be too high or too low (indicating possible contamination, failure to pulse, or other laboratory error).

High variation is defined in several ways. For beryllium stimulated quadruplicates (groups of four at a particular beryllium concentration), values are rank ordered and the coefficient of variation (CV) is calculated. If the CV is greater than 30%, the value farthest from the mean is dropped and the CV is recalculated. If the CV for the remaining three values is still above 30%, the group is excluded. Three or more excluded groups is considered high variation. High variation for cell control groups is

the same as for beryllium stimulated groups with one exception. It is defined as more than five control well counts excluded from the group without achieving an acceptable CV.

The abnormal data are from 32 patients who have clinically confirmed beryllium disease or beryllium sensitivity. The data are considered abnormal if two or more SIs exceed the technician's cut-off value. The cut-off value is two standard deviations above the mean peak SI for nonexposed people.

2. Estimation of SIs Using Least Absolute Values Method

Results in this report are based on the LAV method described in detail in ORNL-6818. The main results are summarized here.

2.1. Regression Model for the LPT Data

Let y_{jk} denote the well count for the k^{th} replicate of the j^{th} set of culture conditions. The expected count in each well can be represented by a log-linear regression function:

$$E(y_{jk}) = \lambda_j = exp(\boldsymbol{X_j}\boldsymbol{\beta}), \tag{1}$$

where $j = 1, \ldots, 10$ and $k = 1, \ldots, 12$ for the controls and $k = 1, 2, 3, 4$ for the beryllium stimulated cells and the positive controls. In (1), $\boldsymbol{X_j}$ is a row vector of indicator variables and $\boldsymbol{\beta}$ is the vector of regression parameters (see below). It is further assumed that the variance of the well counts is proportional to the square of the expected count:

$$Var(y_{jk}) = (\phi\lambda_j)^2. \tag{2}$$

Equations 1 and 2 together are referred to as a generalized linear model with constant coefficient of variation ϕ (see ORNL-6818 for more details)

2.2. Least Absolute Value Regression on Log(y)

The first step in this approach is to take the log of the counts since this is the variance-stabilizing transformation and leads to a linear model in say $z_{jk} = \log(y_{jk})$, i.e.

$$E(z_{jk}) = \boldsymbol{X_j}\boldsymbol{\beta} - \phi^2/2 \text{ and } Var(z_{jk}) \simeq \phi^2.$$

In this report all logs are natural (base e) logarithms. If outliers are not present, applying ordinary least squares to the transformed data will yield consistent estimates for the log(SI) parameters [7]. The effect of outliers is minimized by using least absolute values (or some other robust method) on the z_{jk}. Least absolute value regression—also known as L_1 norm, least absolute deviations and minimum sum of absolute errors—is well known to be resistant to outliers and is an important particular case of a general class of robust methods known as M-estimators [10, 9]. In general, LAV regression requires special computational resources to calculate parameter estimates [1]. In this situation, however, it is only necessary to find the median of the log of the well counts for each set of design conditions and then subtract the control median for each harvest day from the beryllium stimulated medians (see Appendices ORNL-6818). Let $\tilde{z}_j$ denote the median for the j^{th} beryllium concentration and $\tilde{z}_o$ denote the median of the log well counts for beryllium stimulated cells and the corresponding control wells. The LAV estimate of the j^{th} log(SI), $\tilde{\beta}_j$, is

$$\tilde{\beta}_j = \tilde{z}_j - \tilde{z}_o. \tag{3}$$

A resistant estimate of the coefficient of variation (ϕ) can then be obtained as

$$\tilde{\phi} = C \times median\{|z_{jk} - \tilde{z}_j|\}, \tag{4}$$

where $C = 1.48 \times \sqrt{n/(n-p)}$, n = number of wells, and p = the number of medians. On the log scale ϕ corresponds to the standard deviation of the log counts. For the overall pooled estimate $n = 48$ and $p = 8$ in this report. The value of C is chosen to make the estimate consistent for the standard deviation for a Gaussian error model and for consistency with the usual least squares results in which the estimated variance is multiplied by the correction factor $n/(n-p)$ – see [6] and S-PLUS function `mad` in [12]. Alternative approaches to estimating ϕ have been discussed in the context of LAV regression (see e.g., [11, 9]) and there is no consensus as to the best approach. In addition to the fact that this parameter is of direct interest in this situation, it is also needed to obtain an estimate of the parameter covariance matrix

$$w^2(\boldsymbol{X}'\boldsymbol{X})^{-1},$$

where $w^2 = [2f(0)]^{-2}$ is the asymptotic variance of the sample median [2]. Following the approach of [8] we assume that the underlying error distribution is Gaussian in the center and use $\tilde{w} = \sqrt{\pi/2}\tilde{\phi}_L$ to obtain an estimate of the standard deviation of the log of the stimulation indices. The appropriate diagonal term from $(\boldsymbol{X'X})^{-1}$ is 4/12, and consequently the estimated standard deviation of log(SI) is $1.25\tilde{\phi}_L(0.58) = 0.72\tilde{\phi}_L$.

3. Identification of LPTs With Large SIs

This section describes three approaches to the the problem of identification of an "abnormal" LPT. Each of these methods uses the LAV estimates $\tilde{\beta}_j$, j= 1,...,6, of the log(SI)s and resistant estimates of ϕ. **Note that $\tilde{\beta}_j$ is a statistical shorthand for the LAV estimate of the j^{th} log(SI).**

3.1. Method 1- Using Distribution of Maximum SI From Nonexposed Controls and/or Historical Population of Beryllium Exposed Workers

This approach parallels that currently in use for identification of LPTs with large SIs. The procedure is to use the distribution of the maximum log(SI) in a *reference data base* of LPT data sets to determine a "cut point". The reference data base could be composed of LPTs for a group of nonexposed individuals, or nonexposed plus historical data from beryllium exposed workers with no indication of beryllium sensitivity. In this report there are LPTs for 20 nonexposed individuals, and these, alone or in combination with the sample of 167 beryllium exposed workers, will serve as the reference data base. The individuals with abnormal LPTs **have not** been removed. The methods we use are outlier resistant and should be effective as long as the proportion of abnormal LPTs in the study population is not too large. [3] An LPT is considered abnormal if **at least two** log(SI)s exceed this cut point. The steps for this procedure are as follows:

1. Find $\beta_i^* = max[\tilde{\beta}_{ij}, j = 1, \ldots, 6]$ for $i = 1, \ldots, N$, where N= number of LPTs in *reference data base.*
2. Find $M = median[\beta_i^*, i = 1, \ldots, N]$ and S the median absolute deviation (MAD) estimate of the standard deviation of the β_i^*.

[3] If the reference data base is restricted to NE individuals, then the moment estimates of location and scale could be used instead of the resistant estimates. This approach is based on the assumption that there are no beryllium sensitive individuals in the NE group.

3. Then calculate $cut = \mathrm{M} + z_p\mathrm{S}$, where z_p is the p^{th} quantile of the standard normal distribution. If $p = 0.975$ then $z_p \approx 2$.

4. An LPT is defined to be *abnormal if at least two log(SI)s exceed cut.*

The probability of a statistical false positive for this procedure is less than 1-p.

3.2. Method 1A- Using Distribution of Second Largest SI From Nonexposed Controls and/or Historical Population of Beryllium Exposed Workers

This approach is the same as Method 1 except the second largest log(SI) in a *reference data base* of LPT data sets is used to determine the "cut point". An LPT is considered abnormal if **the second largest** log(SI) exceeds this cut point. The steps for this procedure are as follows:

1. Find $\beta_i^\dagger = secondlargest[\tilde{\beta}_{ij}, j = 1, \ldots, 6]$ for $i = 1, \ldots, N$, where N= number of LPTs in *reference data set.*

2. Find $M = median[\beta_i^\dagger, i = 1, \ldots, N]$ and S the median absolute deviation (MAD) estimate of the standard deviation of the $\beta_i^\dagger$.

3. Then calculate $cut = \mathrm{M} + z_p\mathrm{S}$, where z_p is the p^{th} quantile of the standard normal distribution. If $p = 0.975$ then $z_p = 1.96$.

4. An LPT is defined to be *abnormal if at least two log(SI)s exceed cut.*

The probability of a false positive for this procedure should be about 1-p.

3.3. Method 2- Using The Empirical Distribution of log(SI)s For Each Day and Each Beryllium Concentration

The third approach is the one proposed in Section 3.6 of ORNL-6818. It is based on the assumption that the log(SIs) are approximately normally distributed (see Figures 5 and 6). **In this report the reference data base consists of all available LPTs in the BE-sample data set and the NE data set.** *In practice this data set would change during the course of a study as new data becomes available.* The first step is to convert each log(SI) into a standardized deviate

$$u_{ij} = \frac{\tilde{\beta}_{ij} - \tilde{\mu}_j}{\tilde{s}_j}$$

using the values of $\tilde{\mu}_j$ and $\tilde{s}_j$ given in Table 3. These standardized deviates can be compared with the quantiles of the standard normal distribution, i.e. $\Pr[u < z_p] = p$. If we assume that the log(SIs) are independent then the binomial distribution can be used to calculate an approximate probability of at least k out of six "large" SIs for a given value of z_p. The probability of at least one large SI is $1 - p^6$, and the probability of at least two is $1 - [p^6 + 6(1-p)p^5]$.

In fact, the log(SIs) are positively correlated, so this probability should be a lower bound on the chance of finding a false positive LPT.

4. Results

4.1. Descriptive Statistics for Control Wells

An important assumption is that the standard deviation of the well counts is proportional to the mean as implied by Equation (2). Since each of the LPTs contains 12 replicate control wells on both day 5 and day 7 we can evaluate this assumption by computing location and scale estimates for the control wells for each assay on day 5 and day 7. Figure 1 (top) shows the relationship between the moment estimator of location ($\bar{y}$, the sample mean) and the moment estimator of scale (s, the sample standard deviation) for the day 5 control wells. This plot also shows the resulting line when the standard deviation is regressed on the mean. The least squares equation for this line is $\hat{\sigma} = 0.29\bar{y}$, and the slope (0.29) is an estimate of the coefficient of variation for day 5. Figure 1 (bottom) is a similar plot but resistant estimates are used in place of moment estimates. Specifically, the sample median ($\tilde{y}$) replaces the sample mean, the MAD estimate ($\tilde{\sigma}$) replaces s, and LAV is used to regress $\tilde{\sigma}$ on $\tilde{y}$. The solution to this resistant fit is $\tilde{\sigma} = 0.22\tilde{y}$ and the slope (0.22) is a resistant estimate of the coefficient of variation.

Figure 2 shows the relationship between the resistant estimates of location and scale for the day 5 control wells (top) and the day 7 control wells (bottom) on a log-log scale. If the standard deviation is proportional to the mean (i.e. constant coefficient of variation), the log-log plot should be linear with a slope of 1. The LAV fit is $\tilde{\sigma} = 0.262\tilde{y}$ for the day 7 control wells and the solid line in Figure 2 (bottom) is $\log(\tilde{\sigma}) = \log(0.262) + \log(\tilde{y})$. Comparing this fit to the resistant fit for the day 5 control wells ($\tilde{\sigma} = 0.216\tilde{y}$) reveals that the results on both days are quite similar. The main

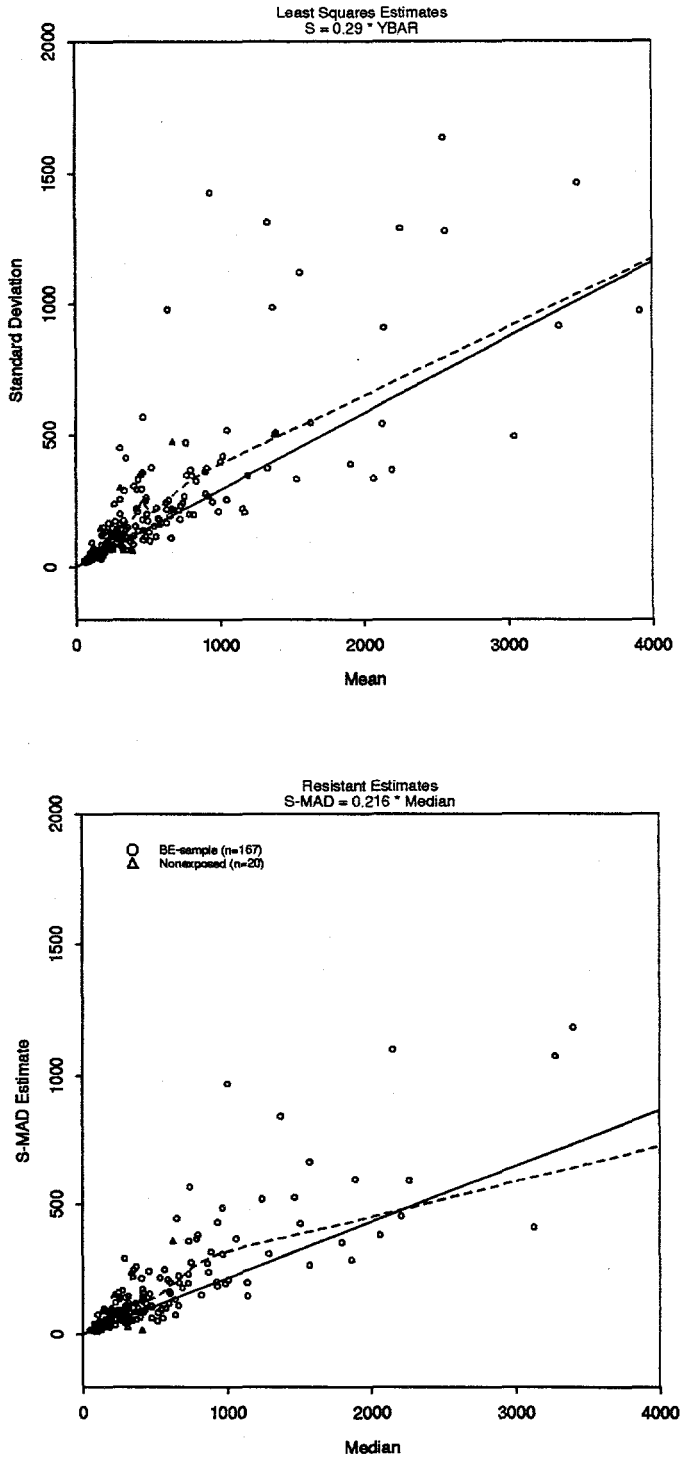

Figure 1: (Top) Relationship between the mean, $\bar{y}$, and the standard deviation, $\hat{\sigma}$, for day 5 control wells. The solid line is the ordinary least squares fit, $\hat{\sigma} = 0.29\bar{y}$. (Bottom) Relationship between the Median, $\tilde{y}$, and the MAD estimate, $\tilde{\sigma}$. The L_1 solution, the solid line, is $\tilde{\sigma} = 0.216\tilde{y}$. The dashed lines are the result of applying scatterplot smoothers to the data.

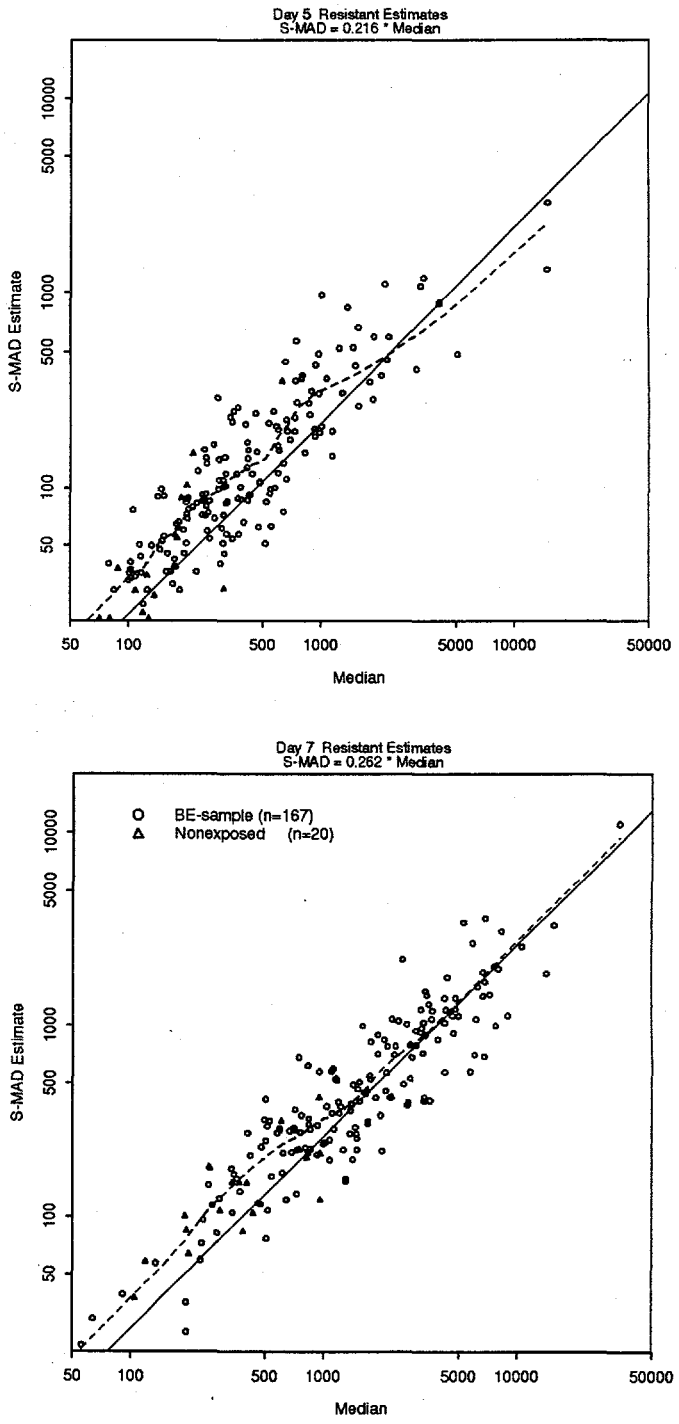

Figure 2: Results for Nonexposed and Beryllium Exposed Workers-Sample. Relationship between the Median, $\tilde{y}$, and the MAD estimate, $\tilde{\sigma}$ of the standard deviation for the control wells on harvest day 5 (top) and harvest day 7 (bottom). The dashed lines are the result of applying scatterplot smoothers to the data. Since results are shown on log-log scale the slope of the line is 1.

difference in the day 5 and day 7 results is that the day 7 results are shifted to the right since the control well counts are generally higher on day 7 than those on day 5. The median of the $\tilde{y}$s on day 5 is 378 compared with 1552 for day 7. **These results are consistent with the laboratory observation that day 7 results are generally higher and show greater variability than well counts on day 5.** They also support the regression model assumption discussed in Section 2.1 that the variance of the well counts is proportional to the square of the expected counts.

Figure 3 is identical to Figure 2 except the results for Abnormal and Unacceptable LPTs have been included.

4.2. Graphical Summaries for SIs For Beryllium Exposed Workers and Non-exposed People

Figure 4 shows the SIs for the sample of beryllium exposed (BE) workers and nonexposed (NE) controls (compare with Fig. 4 for the ORISE-AC Data in Frome *et al.* [5]). The vertical scale on the right hand side of the plots is in SI units. The SIs for both the NE controls and the beryllium exposed workers decrease as the beryllium concentration in the test wells increases. This may be due to a toxic effect of high beryllium concentration that results in "cell killing". There is considerably more variability in the log(SI)s for the BE LPTs than for the NE LPTs at each concentration on both day 5 and day 7.

Figure 5 shows normal (Gaussian) probability plots for the combined BE and NE SIs for each of the three beryllium concentrations on day 5 and day 7. In each of the six plots, the data (ordered values of the log(SIs)) are shown on the vertical scale on the left, and the quantiles of the standard normal distribution are shown on the horizontal scale. A detailed account of the construction and interpretation of normal probability plots is provided by Chambers *et .al* [3]. In this situation statistical theory indicates that the log(SIs) should be approximately normally distributed, and the large sample standard deviation should be about 0.28 if the coefficient of variation is 0.4. If the relation between the empirical quantiles (on the vertical axis) and theoretical quantiles (on the horizontal axis) is linear, this indicates that the distribution is Gaussian. Each plot includes the median (labeled M) and a resistant estimate of the standard deviation (labeled S) for the log SIs. The solid line in each plot shows the relation that is

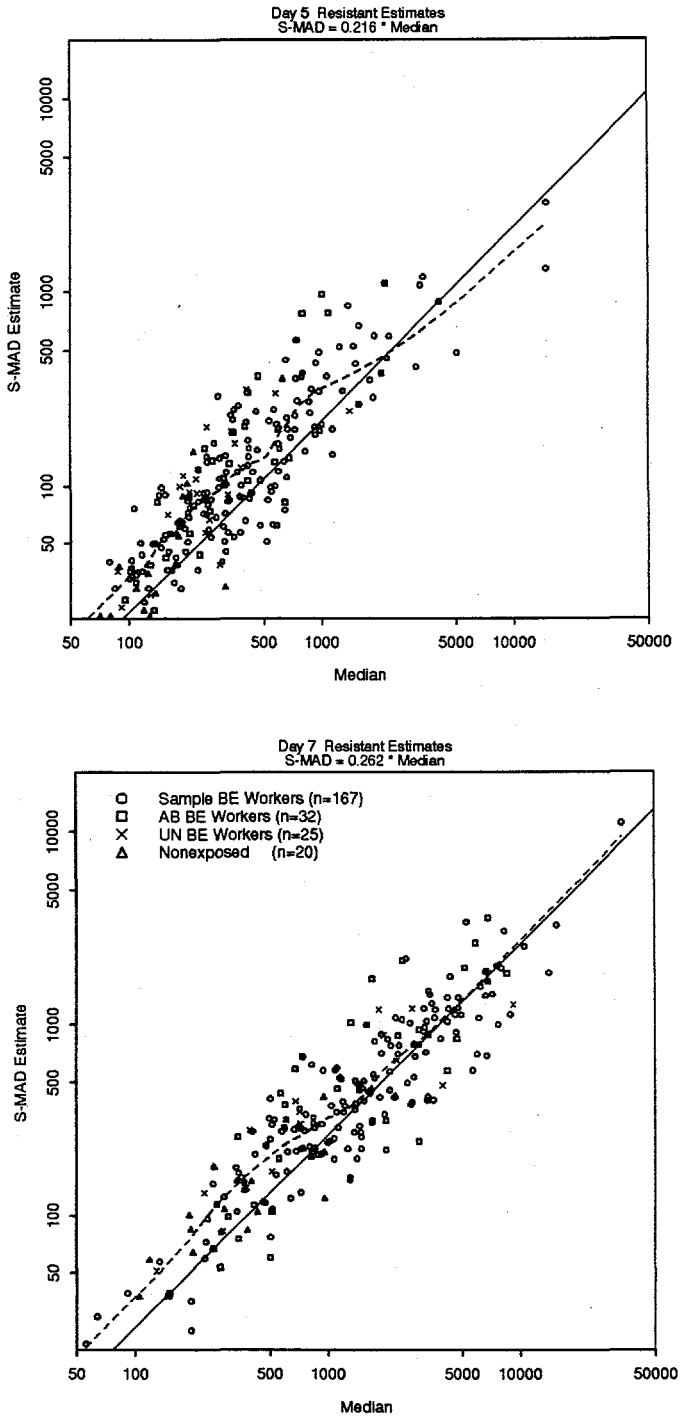

Figure 3: Results for Nonexposed, Beryllium Exposed Workers- Sample, Abnormal, and Unacceptable LPTs. Relationship between the Median, $\tilde{y}$, and the MAD estimate, $\tilde{\sigma}$ of the standard deviation for the control wells on harvest day 5 (top) and harvest day 7 (bottom). The dashed lines are the result of applying scatterplot smoothers to the data. Since results are shown on log-log scale the slope of the line is 1.

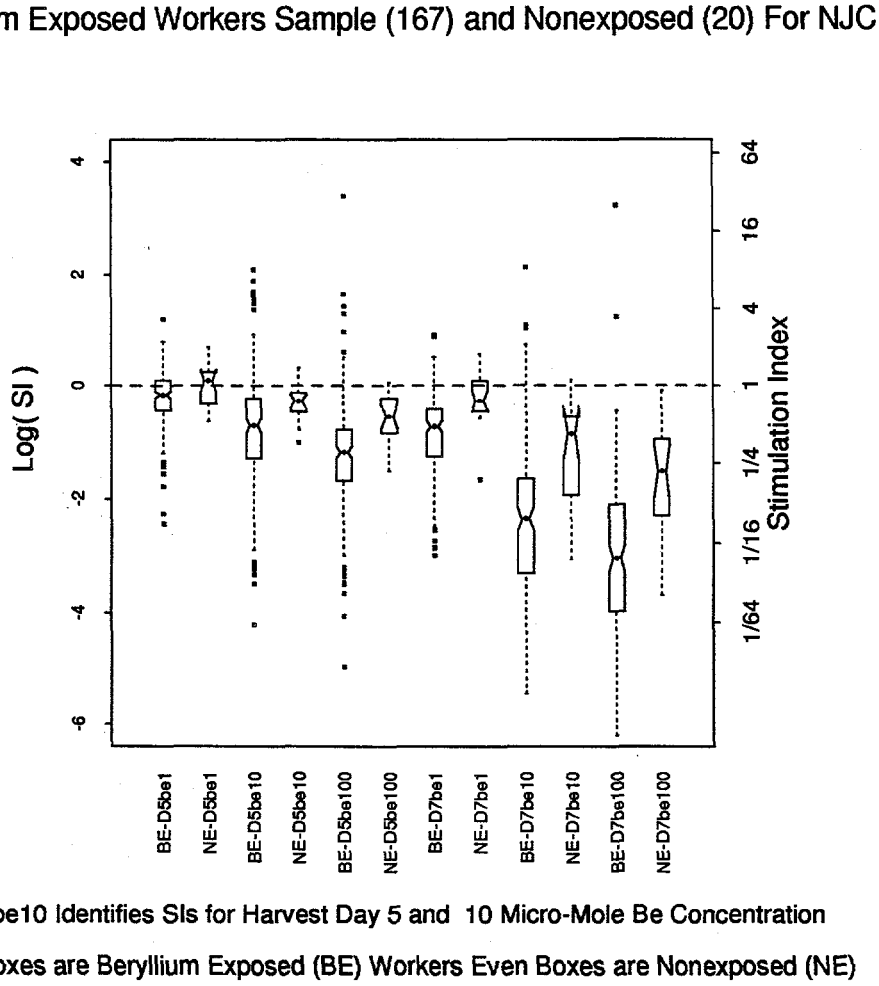

Figure 4: Boxplots of SIs for Sample of Beryllium Exposed Workers and Nonexposed Controls. There are Three Beryllium Concentrations on Harvest Days 5 and 7.

expected if the log SI values are from a normal distribution with location parameter M (which determines the intercept) and standard deviation S (which determines the slope). Resistant methods were used to estimate the location and scale parameters for the combined data from the BE and NE groups. This reflects the assumption that most of beryllium exposed workers do not show an abnormal response, i.e. they look like the nonexposed group. For example, consider the plot for day 5 Be-10 in Figure 5. The log(SIs) appear to be approximately normal in the center, but there are several values that are larger than expected (these are the points above the line). These "outliers" are SIs that indicate hypersensitivity to beryllium. Compare these results to similar plots for the ORISE data—see [5] Fig. 5.

Normal probability plots for the **nonexposed** workers alone are shown in Figure 6. Boxplots for each of the data sets considered in this report are shown in Figure 7. The top panel contains results for day 5 and the bottom panel for day 7. The NE controls are shown on the left side of each panel. The LAV estimates of the log(SI)s for the 32 Abnormal LPTs are clearly increased and show a different profile than the NEs and BE-sample.

4.3. Resistant Estimates of the Coefficient of Variation ($\tilde{\phi}$)

Resistant estimates of $\tilde{\phi}$, the coefficient of variation, for the NE LPTs on day 5 and day 7 and for the BE-sample are shown in Figure 8. For example, the boxplot labeled BE5-T is based on a pooled estimates of $\tilde{\phi}$ from the beryllium treated wells on day 5 from the sample of 167 BE workers. The dotted line corresponds to a value of 0.3 for ϕ. Figure 8 suggests that the internal variability may be slightly greater on day 7. There is, however, no indication of increased variability in the beryllium stimulated wells.

Log normal probability plots for the $\tilde{\phi}$ values are shown in Figure 9. These plots indicate that $\log(\tilde{\phi})$ is approximately normally distributed with very similar parameters.

Figure 10 shows the distributions of the maximum SIs for the NE, BE (sample), AB, and UN data sets. The values for the NE and/or BE-sample are used as the reference group in identification of abnormal LPTs as described in Section 3.1.

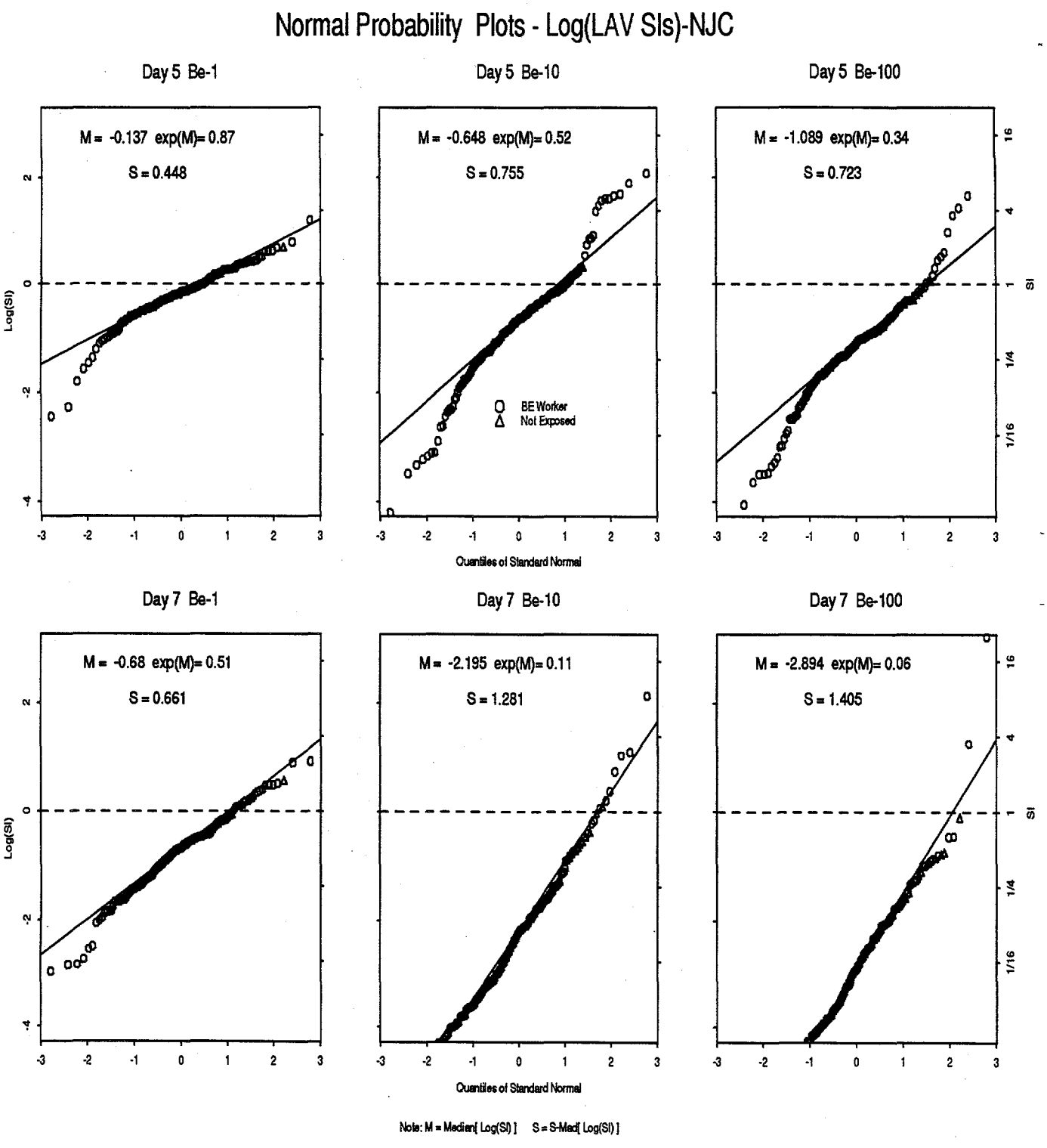

Figure 5: Normal Probability Plots of LAV Estimates of Log(SI)s ($\tilde{\beta}_j$) for Each Beryllium Concentration on Day 5 and Day 7. The Data Values Are Shown on the Vertical Axis. The Median (M) of $\tilde{\beta}_j$ and of the SIs = exp(M) are Listed on Each Plot.

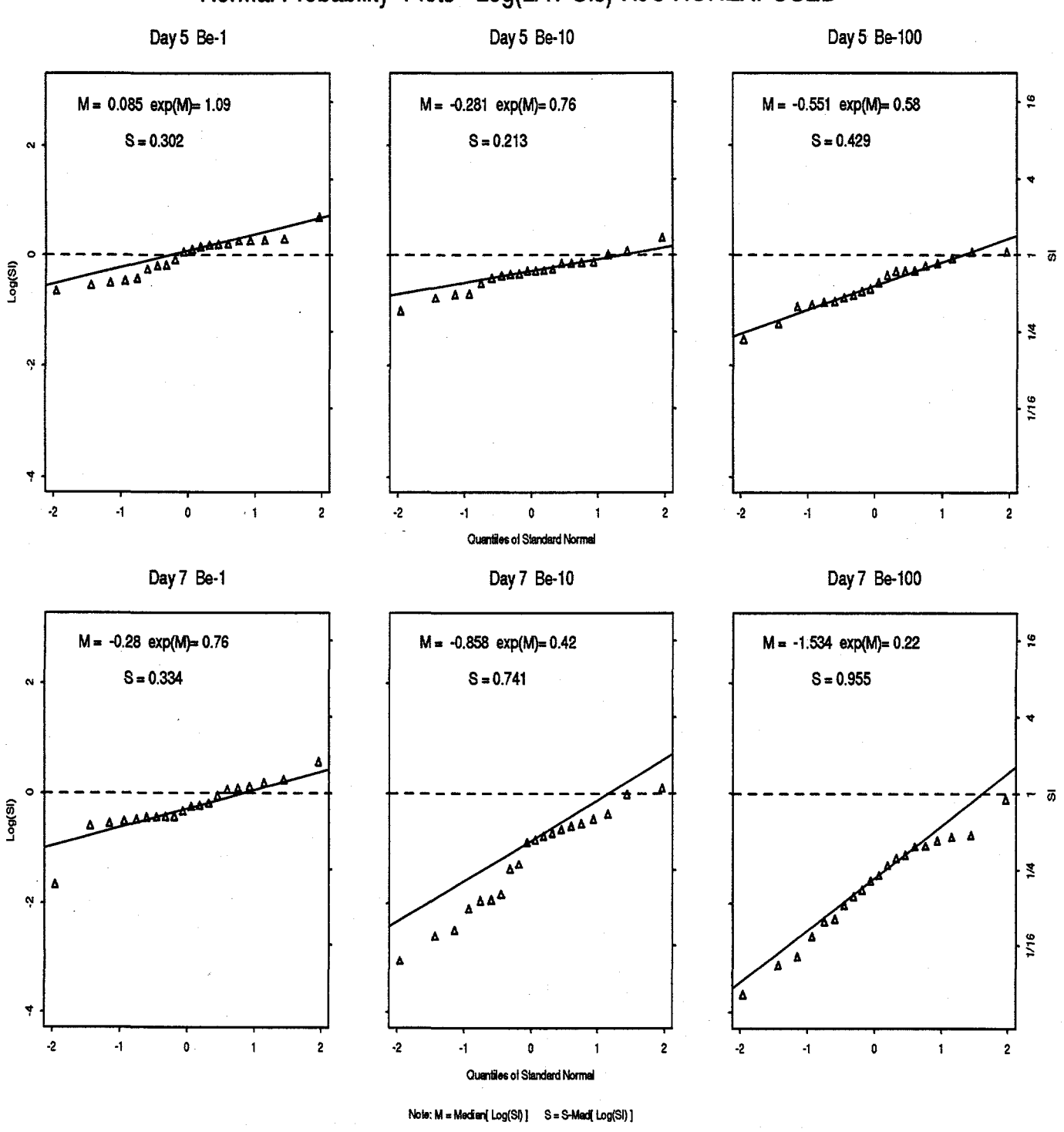

Figure 6: Normal Probability Plots of LAV Estimates of Log(SI)s ($\tilde{\beta}_j$) for Each Beryllium Concentration on Day 5 and Day 7 for **nonexposed** individuals. The Data Values Are Shown on the Vertical Axis. The Median (M) of $\tilde{\beta}_j$ and of the SIs = exp(M) are Listed on Each Plot.

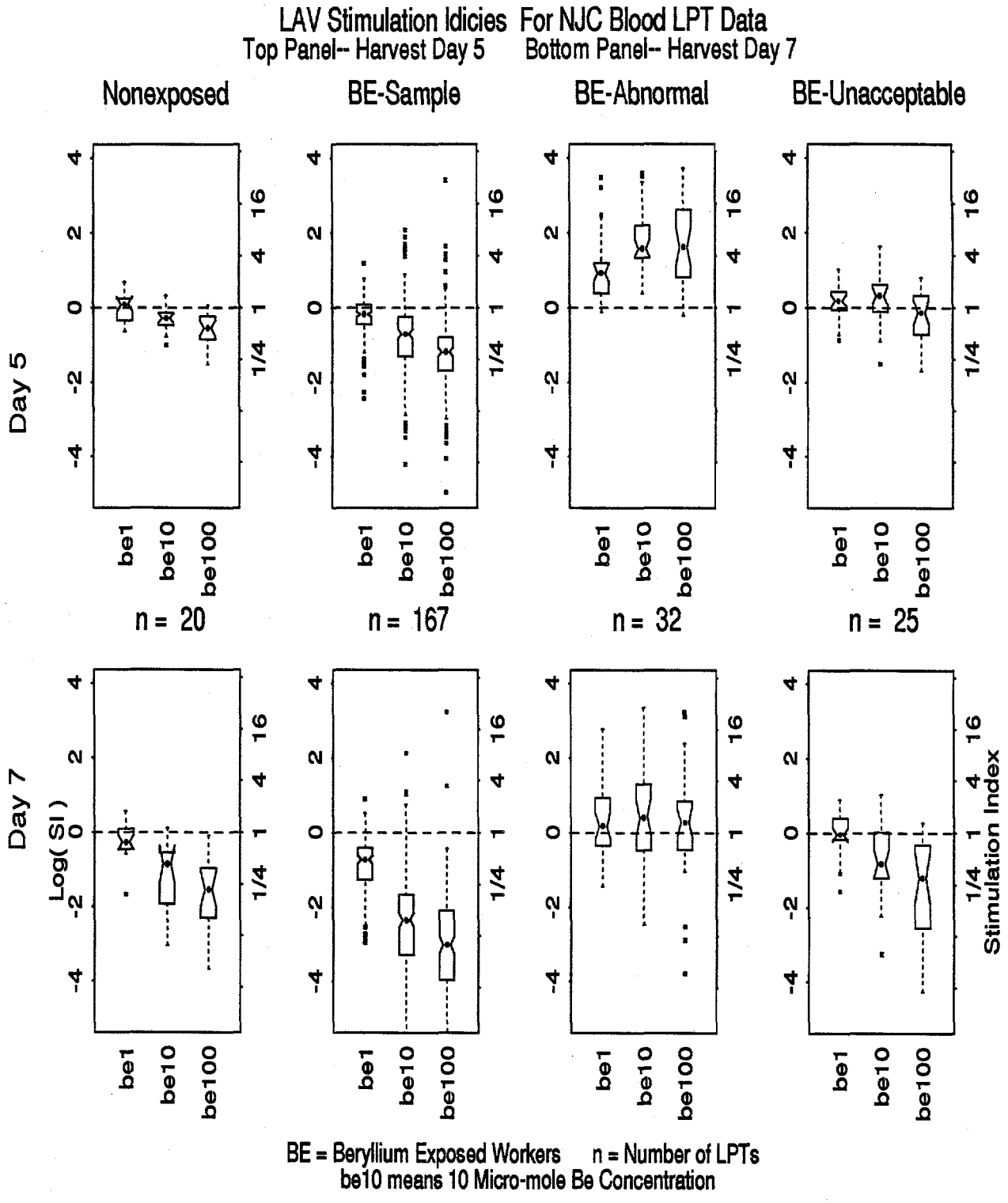

Figure 7: SI Boxplots For NE (Controls) BE-Sample BE-Abnormal and BE-Unacceptable Data Sets

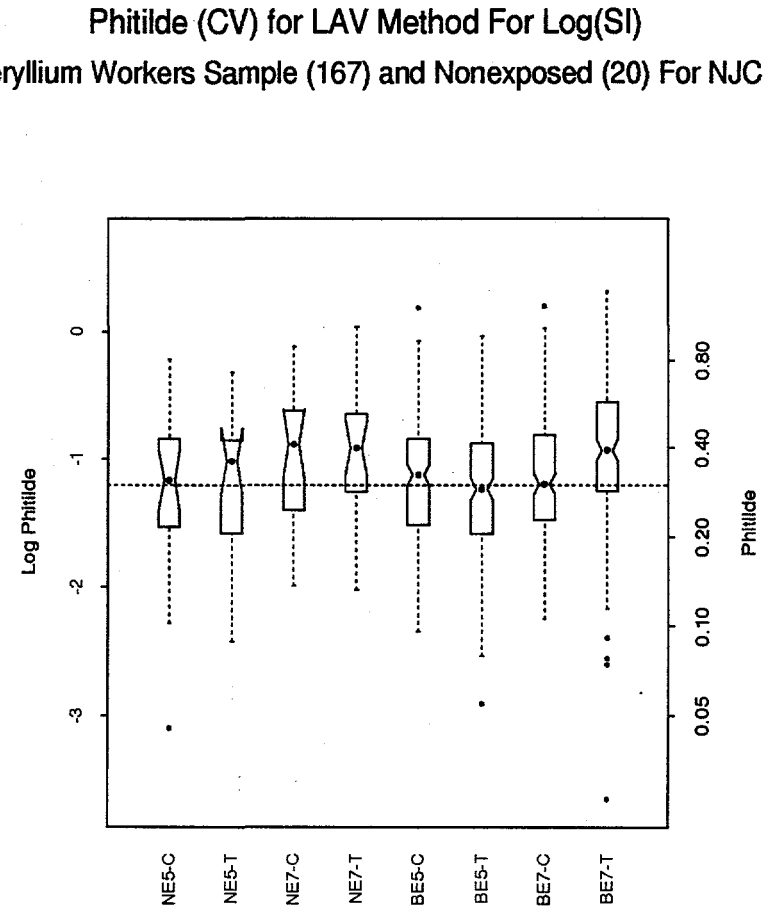

Figure 8: Resistant Estimates of $\tilde{\phi}$ (CV) For Nonexposed and Sample of 167 Beryllium Exposed Workers

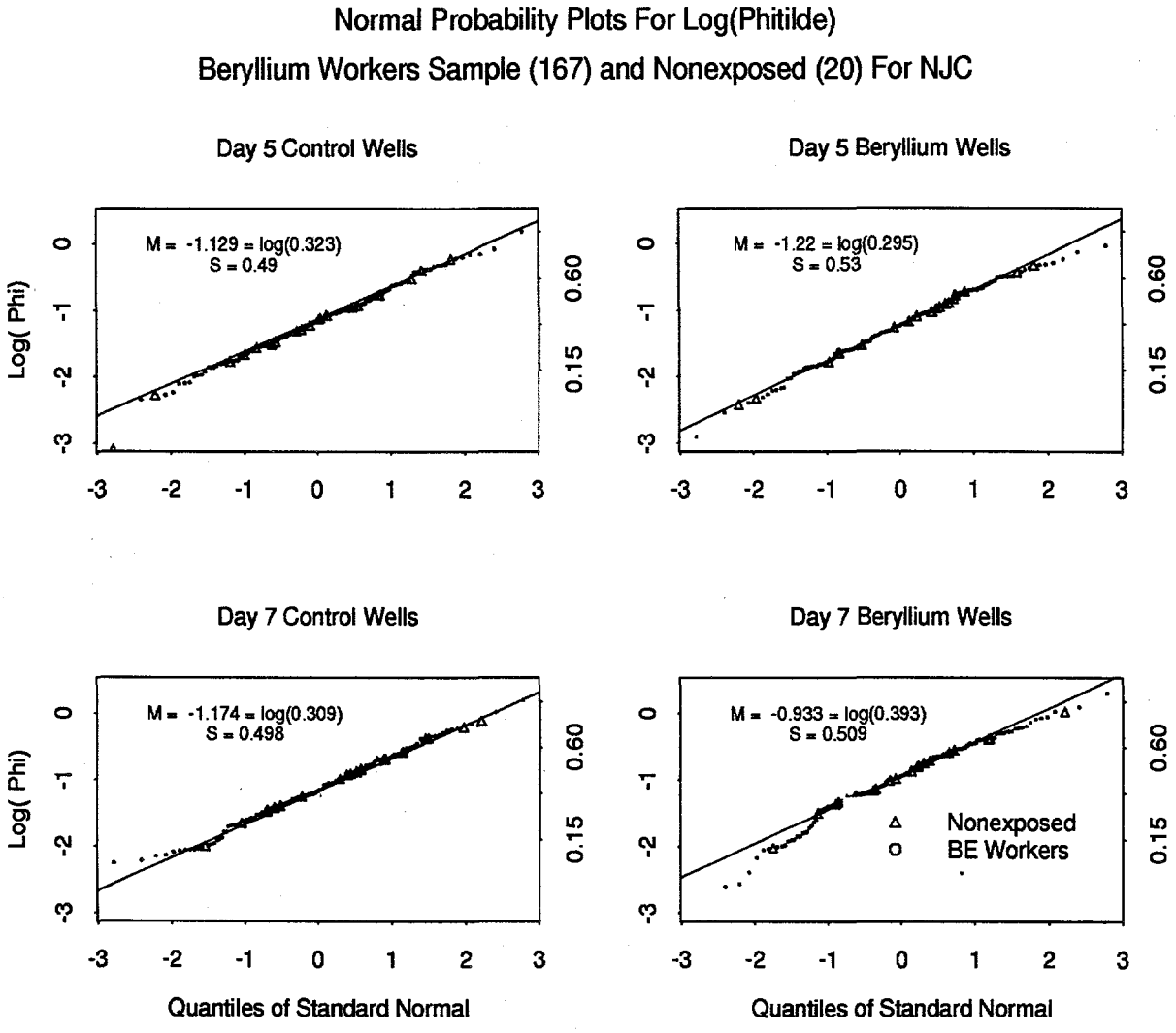

Figure 9: Resistant Estimates of $\tilde{\phi}$ (CV) For 20 Nonexposed and Sample of 167 Beryllium Exposed Workers

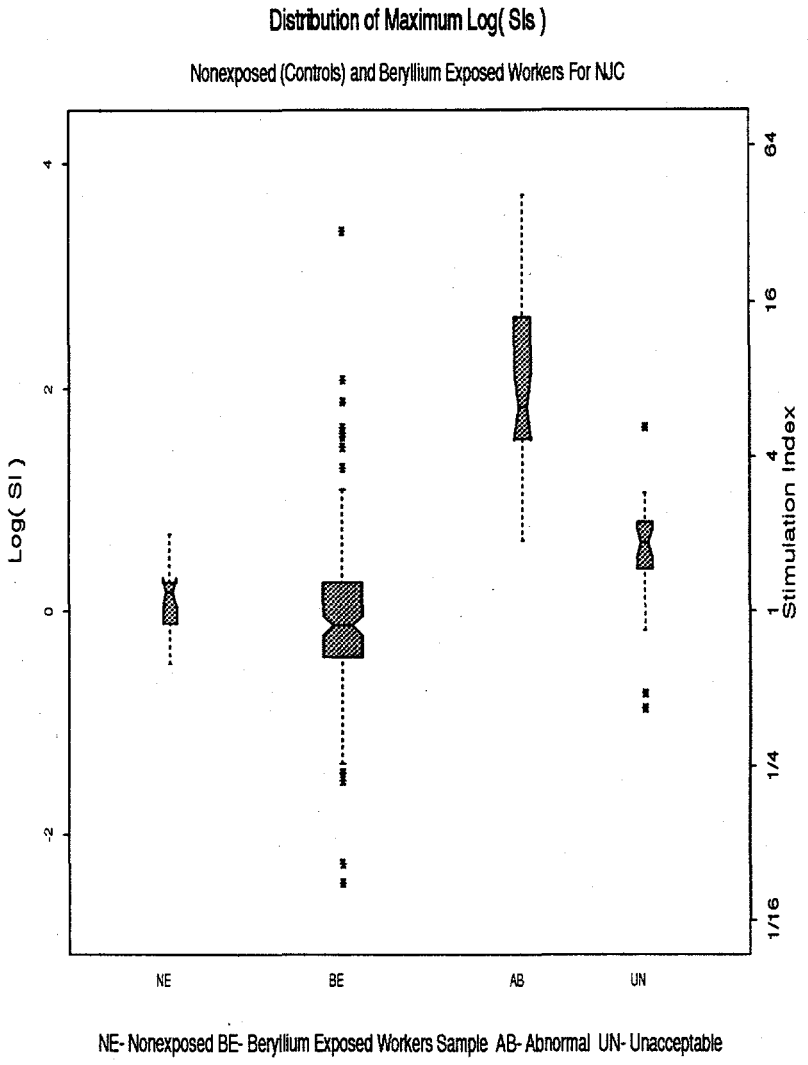

Figure 10: Distributions of β_i^* The Maximum of Log(SI)s For NJC Data Sets. There are 32 LPTs in The "Abnormal" Group That Are Confirmed Cases of CBD and 25 LPTs That Were Considered to Be Unacceptable.

4.4. Identification Of LPTs With Large SIs

Table 1 lists the LPTs with large SIs based on Method 1 (Section 3.1). The reference data base used was the nonexposed controls. There were 11 LPTs in the BE-sample and 4 LPTs in the unacceptables that were identified as having at least two large SIs. **All 32 of the abnormal LPTs were identified as having at least two large SIs** so they were not included in Table 1.

Table 2 lists the SIs for LPTs with large SIs based on Method 1A (Section 3.2). The reference data base used was the nonexposed controls. There were 19 LPTs in the BE-sample and 14 LPTs in the unacceptables that were identified as having at least two large SIs. **All 32 of the abnormal LPTs were identified as having at least two large SIs** so they were not included in Table 2.

Table 3 gives the **standardized deviates** defined in Section 3.3 for LPTs with large SIs based on Method 2. The reference data base used was the nonexposed controls combined with the BE-sample, and z_p was $z_{.9568} = 1.715$. The values of $\tilde{\mu}_j$ and $\tilde{s}_j$ are given at the bottom of Table 3. The SI value that had to be exceeded for each Be concentration is in the last row of Table 3. An LPT is abnormal if at least two of the standardized deviates exceed 1.715. There were 13 LPTs in the BE-sample and 9 LPTs in the unacceptables that were identified as having at least two large SIs. **All 32 of the abnormal LPTs were identified as having at least two large SIs** so they were not included in Table 3. An approximate lower bound (see Section 3.3) on the probability of a false positive is $1 - [p^6 + 6(1-p)p^5] = .025$.

Table 1: Method 1 Results: Log(SI)s (top panel) and SIs (bottom panel) for Individuals from the BE Sample (167) and Unacceptables (25) with "abnormal" LPTs. The values for M and S (Section 3.1) are 0.170 and 0.179, respectively, *cut*= 0.521 is the cut point for a large Log(SI)– exp(.521)=1.68. The *reference data base* used was the 20 LPTs for Nonexposed individuals. **ALL abnormal LPTs had at least two large SIs and are not listed.**

ID	Day 5			Day 7		
	be 1	be 10	be 100	be 1	be 10	be 100
Log(SI)s						
BS1033	0.19	1.65	0.15	-0.07	1.03	-1.35
BS1034	0.31	0.92	0.60	-0.50	-1.29	-1.68
BS1035	0.63	1.57	0.97	-1.43	-2.47	-3.78
BS1091	-0.10	1.37	1.65	-0.42	0.20	1.25
BS1259	0.62	-0.71	-1.42	0.90	-2.09	-4.08
BS1269	1.20	1.60	1.43	-0.55	-0.72	-0.44
BS1271	0.68	0.86	0.03	0.20	1.09	-2.30
BS1315	0.00	0.74	1.29	-0.76	-0.80	-1.32
BS1316	-0.12	1.70	3.40	0.49	2.13	3.24
BS1321	0.80	1.47	-0.20	-0.72	-2.30	-2.89
BS472	0.38	1.89	0.45	0.26	0.74	-0.99
BU1033	0.19	1.65	0.15	-0.07	1.03	-1.35
BU2685	1.05	1.06	0.02	0.57	-0.42	-1.20
BU3064	0.36	0.77	0.59	0.29	-0.15	0.03
BU3287	0.96	0.07	0.61	0.47	-0.32	0.25
SIs						
BS1033	1.20	5.22	1.16	0.93	2.80	0.26
BS1034	1.36	2.50	1.82	0.61	0.28	0.19
BS1035	1.88	4.80	2.63	0.24	0.08	0.02
BS1091	0.90	3.95	5.22	0.66	1.22	3.47
BS1259	1.87	0.49	0.24	2.47	0.12	0.02
BS1269	3.31	4.94	4.19	0.58	0.49	0.64
BS1271	1.98	2.37	1.03	1.22	2.98	0.10
BS1315	1.00	2.10	3.64	0.47	0.45	0.27
BS1316	0.89	5.45	29.85	1.63	8.45	25.41
BS1321	2.21	4.37	0.82	0.49	0.10	0.06
BS472	1.46	6.62	1.56	1.30	2.10	0.37
BU1033	1.20	5.22	1.16	0.93	2.80	0.26
BU2685	2.85	2.88	1.02	1.77	0.66	0.30
BU3064	1.43	2.16	1.80	1.34	0.86	1.03
BU3287	2.61	1.07	1.84	1.61	0.73	1.28

Table 2: Method 1A Results: SIs for Beryllium Workers Sample (167), Unacceptables(25) LPTs For NJC Data. The values for M and S (Section 3.2) are -0.197 and 0.240, respectively, and *cut*= 0.274 is the cut point for a large Log(SI) and exp(0.274)= 1.32. The *Reference Data Base* used was made up of the 20 LPTs for Nonexposed and $p = 0.975$, with a false positive rate of about 2.5 percent. **ALL abnormal LPTs had at least two large SIs and are not listed.**

ID	Day 5			Day 7		
	be 1	be 10	be 100	be 1	be 10	be 100
BS1027	0.57	8.02	0.56	0.62	1.45	0.10
BS1033	1.20	5.22	1.16	0.93	2.80	0.26
BS1034	1.36	2.50	1.82	0.61	0.28	0.19
BS1035	1.88	4.80	2.63	0.24	0.08	0.02
BS1087	1.48	1.27	1.67	0.20	0.14	0.20
BS1091	0.90	3.95	5.22	0.66	1.22	3.47
BS1259	1.87	0.49	0.24	2.47	0.12	0.02
BS1269	3.31	4.94	4.19	0.58	0.49	0.64
BS1271	1.98	2.37	1.03	1.22	2.98	0.10
BS1315	1.00	2.10	3.64	0.47	0.45	0.27
BS1316	0.89	5.45	29.85	1.63	8.45	25.41
BS1321	2.21	4.37	0.82	0.49	0.10	0.06
BS425	1.44	0.90	0.54	1.62	0.86	0.28
BS472	1.46	6.62	1.56	1.30	2.10	0.37
BS731	1.32	0.78	0.31	1.46	0.14	0.07
BS818	1.54	1.03	0.96	1.49	0.14	0.11
BS819	1.65	1.20	0.65	2.42	0.51	0.42
BS824	1.53	0.29	0.32	1.38	0.06	0.05
BS826	1.48	1.73	1.35	0.66	0.68	0.63
BU1033	1.20	5.22	1.16	0.93	2.80	0.26
BU1960	1.34	2.24	0.50	0.97	0.42	0.06
BU2167	1.54	1.84	1.01	1.35	1.43	0.59
BU2172	0.63	1.37	0.89	1.50	1.71	0.72
BU2282	1.03	1.46	1.38	1.88	1.00	1.30
BU2685	2.85	2.88	1.02	1.77	0.66	0.30
BU2947	1.45	1.65	1.45	0.89	1.15	0.80
BU3064	1.43	2.16	1.80	1.34	0.86	1.03
BU3068	2.59	1.46	0.93	0.92	0.35	0.18
BU3175	1.15	2.06	1.50	1.47	1.35	0.96
BU3251	1.37	1.09	1.82	0.51	0.11	0.11
BU3287	2.61	1.07	1.84	1.61	0.73	1.28
BU3470	0.61	2.02	0.71	1.60	0.44	0.81
BU819	1.65	1.20	0.65	2.42	0.51	0.42

Table 3: Method 2 Results: Standardized Deviates (see Section 3.3) For Beryllium Exposed Workers and Unacceptable LPTs. The value of $p = 0.9568$ so the false positive rate is about 2.5 percent. **ALL abnormal LPTs had at least two large SIs and are not listed.**

ID	Day 5			Day 7		
	be 1	be 10	be 100	be 1	be 10	be 100
BS1027	-0.94	3.62	0.70	0.31	2.00	0.41
BS1033	0.72	3.05	1.71	0.92	2.52	1.10
BS1034	0.99	2.07	2.33	0.28	0.71	0.86
BS1035	1.72	2.94	2.85	-1.14	-0.22	-0.63
BS1091	0.08	2.68	3.79	0.39	1.87	2.95
BS1261	-0.20	2.97	0.75	0.21	1.75	0.57
BS1269	2.98	2.97	3.49	0.20	1.15	1.74
BS1271	1.83	2.00	1.54	1.33	2.57	0.42
BS1315	0.31	1.84	3.29	-0.13	1.09	1.12
BS1316	0.04	3.10	6.20	1.77	3.38	4.36
BS1321	2.08	2.81	1.22	-0.06	-0.08	0.00
BS472	1.15	3.36	2.12	1.43	2.29	1.35
BS826	1.18	1.58	1.92	0.40	1.41	1.73
BU1033	0.72	3.05	1.71	0.92	2.52	1.10
BU2172	-0.72	1.27	1.34	1.64	2.13	1.83
BU2282	0.36	1.36	1.95	1.98	1.72	2.25
BU2685	2.64	2.26	1.53	1.89	1.39	1.21
BU2947	1.14	1.52	2.02	0.85	1.82	1.90
BU3064	1.10	1.88	2.31	1.47	1.60	2.08
BU3175	0.61	1.82	2.07	1.61	1.95	2.03
BU3287	2.45	0.95	2.35	1.75	1.47	2.24
BU3470	-0.79	1.79	1.03	1.74	1.07	1.91
Med	-0.14	-0.65	-1.09	-0.68	-2.19	-2.89
Smad	0.45	0.75	0.72	0.66	1.28	1.41
Cut SI	1.88	1.91	1.16	1.57	1.00	0.62

Table 4: Beryllium Exposed Workers Classified as Abnormal by at Least One Method[a]

	Method			NJC	
ID	1	1A	2	Result	Retest[b]
BS 425	N	A	N	N	
BS 731	N	A	N	N	
BS 818	N	A	N	N	
BS 819	N	A	N	U	N
BS 824	N	A	N	N	
BS 826	N	A	N	N	
BS1027	N	A	A	B	B A (BE Sensitized)
BS1033	A	A	A	U	N A U (Equivocal)
BS1034	A	A	A	B	B N
BS1087	N	A	N	B	B U N
BS1259	A	A	N	N	
BS1261	N	N	A	B	Unknown
BS1271	A	A	A	U	N

[a]Seven additional workers that are known CBD cases in the BE sample were called abnormal by all three methods and are not listed.

[b]NJC retest results: A-abornmal, B-borderline, N-normal, U-unacceptable.

5. Comparison of The Three Methods

All three methods (1 1A and 2) for identifying abnormal LPTs, based on the LAV method for estimating the SIs, correctly identified the 32 known CBD cases as abnormal.

All three methods (see Tables 1-3) also correctly identified the seven BE workers with CBD. They are BS1035, BS1091, BS1269, BS1315, BS1316, BS1321, BS472. Method 1 (see Table 1) called an additional 4 BE workers abnormal. Method 2 (see Table 3) called an additional 6 BE workers abnormal. Method 1A (see Table 2) called an additional 12 BE workers abnormal, some of whom were included in Table 1 and Table 3. Thirteen beryllium exposed workers were called abnormal by at least one of the three methods—see Table 4 for a summary of their results.

5.1. Classification of Unacceptable LPTs

Table 5: Summary For NJC Unacceptable LPTs

A-abnormal U-unacceptable B-borderline N-normal

ID	CV	Meth-1	Meth-1A	Meth-2	RETEST	RESULTS
BU0819	0.86	n	A	n	N	
BU0606	0.34	n	n	n	N	
BU3470	0.69	n	A	A	N	
BU3454	0.45	n	n	n	N	
BU3287	0.47	A	A	A	A	Be Sen
BU3251	0.35	n	A	n	N	
BU3175	0.46	n	A	A	A B B	Equivocal
BU3163[a]	0.70	n	n	n	4Us	CBD see BU1960
BU3068	0.47	n	A	n	N	
BU3064[b]	0.61	A	A	A	N ?	CBD
BU3056	0.56	n	n	n	N	
BU2947	0.58	n	A	A	A B	Equivocal
BU2685	0.53	A	A	A	A	Be Sen
BU2680[c]	0.51	n	n	n	A A	Not Evaluated
BU2282	0.81	n	A	A	A B	Equivocal
BU2172	0.44	n	A	A	A B B	CBD
BU2167	0.47	n	A	n	N B	
BU1960[a]	0.68	n	A	n	4Us	CBD see BU3163
BU1805	0.91	n	n	n	N U	
BU1033	0.58	A	A	A	N A U	Equivocal

[a]BU3163 and BU1960 are for the same person.

[b]BU3064 repeat was N, but this person was abnormal (x2) at the other laboratory involved in this study.

[c]BU2680 re-tested abnormal. This person's LPT had very high phitilde values: day 5 controls = 0.565 day 7 control= 0.569, and would clearly be a candidate for the designation unacceptable due to too much variability (if I had one).

Table 5 summarizes the results for each of the unacceptable LPTs that were due to "high variability". Five of the original LPTs are not shown because they were not called unacceptable because of high variability. BU877, BU537, and BU475 were unacceptable due to high control counts; BU3532 had very low counts; and BU2629 was not unacceptable by any criteria. The results in Table 5 are of interest since each of the workers with unacceptable LPTs had at least one additional LPT at NJC and

the retest results are given in Table 5. Two of these (BU3163 and BU1960) are for the same person, a confirmed CBD case. Both of the $\tilde{\phi}$ values are very high (0.70 and 0.68) and BU1960 was called abnormal by Method 1A.

One LPT that was called abnormal (BU3064) using LAV SIs by all three methods was normal on retest by NJC, but was called abnormal twice at a second lab. BU2680 was called normal using LAV estimates by all three methods, and was called abnormal in two retests at NJC. The CBD status of this patient has not been evaluated.

5.1.1. Method 1 Results Based On Maximum SI

Table 6 summarizes the results of the retest LPTs that were done for each of the original unacceptable LPTs using Method 1. For example, row two indicates that 3 of the Method 1 abnormal LPTs were abnormal on retest, and row five shows that 5 of the normal LPTs were abnormal. This suggests that Method 1 may be missing some of the beryllium sensitized individuals.

5.1.2. Method 1A Results Based On Second Largest SI

Table 7 summarizes the results of the retest LPTs that were done for each of the original unacceptable LPTs using Method 1A. Row two indicates that seven of the Method 1A abnormal LPTs were abnormal on retest, and only one of the normal LPTs was abnormal on retest (see row 5). The first row of Table 7 shows that seven of the abnormals were normal on retest (based on NJC method), suggesting that this method may have more false positive results.

5.1.3. Method 2 Results Based On Each Day/Concentration

Table 8 summarizes the results of the retest LPTs that were done for each of the original unacceptable LPTs using Method 2. Seven of the eight abnormals were called abnormal on retest, and only three of the abnormals were called normal.

Note that some NJC Unacceptables had more than one RETEST (see Table 5) , and all of the retest results were used to obtain Tables 6-8.

Table 6: RETEST Results For Method 1 Based on Maximum SI Distribution (See Section 3.1 And Column 3 of Table 5)

LPT Evaluated	NJC Repeat	Number
A	N	2
A	A	3
A	U	1
N	N	9
N	A	5
N	U	3

Table 7: RETEST Results For Method 1A Based on Second Largest SI Distribution (See Section 3.2 And Column 4 of Table 5)

LPT Evaluated	NJC Repeat	Number
A	N	7
A	A	7
A	U	2
N	N	4
N	A	1
N	U	1

Table 8: RETEST Results For Method 2(see Section 3.3 And Column 5 of Table 5)

LPT Evaluated	NJC Repeat	Number
A	N	3
A	A	7
A	U	1
N	N	8
N	A	1
N	U	3

6. Criteria For Unacceptable LPTs

One feature of the LAV approach is that it is not necessary to declare LPTs as unacceptable because of "high variability" in the well counts based on the CV. There are however situations that may result in an unacceptable LPT. Data may be considered unacceptable if any of the following situations occur:

1. Control well counts are too low or too high relative to plate background due to laboratory error. Sources of technical error might include mistakes in pipetting, such as failures to add appropriate numbers of cells to individual wells, lack of addition or double addition of tritiated thymidine to specific wells, or improper washing of filters resulting in residual counts of unincorporated thymidine, or smearing of radiolabel across the filter paper.
2. Positive control SIs for ConA or PHA SIs are low (indicating low cell viability).
3. The internal variability for a quadruplicate of Be stimulated cells is "too high" for at least two Be concentrations, provided this is due to at least two counts that are "two low", i.e. close to background for the plate indicating laboratory error— See Section 6.1.
4. The internal variability for the control wells is too high on day 5 or day 7. An approximate critical value for $\tilde{\phi}_L$ can be obtained using an empirical or theoretical approach—see Section 6.1.
5. At least four SIs are "too low" indicating cell killing. An SI is too low if it is significantly below the null value of one (zero on the log scale). In Section 2.2 the theoretical standard deviation of log(SI) is $1.25\phi_L(0.58) = 0.72\phi_L$. If $\phi_L = 0.30$, then the standard deviation of a log(SI) is 0.216. Since the $\Pr[log(SI) < 0.216 z_p] = p$, then for p= 0.001,

   $\Pr[log(SI) < -3.1 * 0.216] = Pr[log(SI) < -0.67] = 0.001,$

   i.e $\Pr[SI < 0.51] = 0.001.$

The last item above may not be needed if it can be demonstrated that cell killing only occurs among individuals who are not sensitized to beryllium.

6.1. Criteria To Determine If Internal Variability Is Too High

The resistant estimate of the CV ($\tilde{\phi}_L$) is too high if it exceeds a critical value CV*. The value of CV* can be obtained using an empirical or theoretical approach.

6.1.1. Empirical Approach

The empirical approach uses the distribution of ϕ_L estimates available from previous data (see e.g. Frome *et al.*[4] page 13 and page 26). This method could be applied to the control well counts on day 5 and day 7 (12 replicates per set). It could also be applied to the CV-mad estimates obtained from the Be stimulated wells (4 replicates per set).

6.1.2. Theoretical Approach

Assume that the log (base e) counts follow the Gaussian distribution with **known** ϕ. Then use either mathematical analysis or simulation to determine a percentage point for the sampling distribution of $\tilde{\phi}_L$. Recall that ϕ is the standard deviation of the log of the counts, and corresponds to the CV on the original scale under the assumption that the standard deviation is proportional to the mean on the original scale (see ORNL-6818 Section 2.1).

6.1.3. Mathematical Analysis

This approach can be applied to the usual moment estimate of the standard of the log counts, i.e., NOT to the MAD estimate, $\tilde{\phi}_L$. If SD is the moment estimate of the standard deviation for a sample of size n of z(i)'s that are normally distributed with **known variance** (i.e. ϕ^2), then the chi-square distribution can be used to determine a critical value of the moment estimate of the standard deviation.

This should be a lower bound for the distribution of the resistant estimate $\tilde{\phi}_L$. For example, if ϕ =0.3, then for control wells (df= 11),

pr[SD > 0.401] is about 0.05,

pr[SD > 0.450] is about 0.01,

and, pr[SD > 0.506] is about 0.001.

For the Be stimulated wells (df= 3), and

and pr[SD > 0.48] is about 0.05,

and pr[SD > 0.70] is about 0.001

The problem with this approach is that the distribution of $\tilde{\phi}_L$ will be more spread out than the distribution of the moment estimate of the standard deviation when the log counts follow the Gaussian distribution.

6.1.4. Simulation Based Approach

An alternative is to use simulation to generate the sampling distribution of SD and $\tilde{\phi}_L$. For a given value of ϕ generate say 10,000 samples of size n (n = 4, 8, or 12). Calculate the value of SD and $\tilde{\phi}_L$ for each sample and calculate the desired quantiles of the sampling distribution. In the absence of outliers this should match the results based on the chi-square distribution for SD, but the null distribution of $\tilde{\phi}_L$ will be more spread out.

This same procedure can then be repeated **with outliers being added**, say ten percent of the time, to each of the samples of size n. This leads to a specified critical value for $\tilde{\phi}_L$ that will depend on the value of ϕ that is used, and on the proportion of outliers that is assumed.

7. Conclusions

Three methods were described for identification of an abnormal blood LPT using LAV estimates of the SIs. These methods were applied to the BE-sample, Unacceptable, and Abnormal data sets. All three methods correctly identified the 32 known CBD cases as abnormal, and identified the seven known CBD cases in the BE-sample.

Results of applying the three methods to the BE-sample and Unacceptable data sets were presented. Table 5 summarizes the results for each method for the 20 NJC Unacceptable LPTs, gives the retest results and the evaluation of the patients' CBD status. Both Method 2 and Method 1A were effective at classifying beryllium sensitized individuals. Method 1A had more results that were normal on retest by NJC using their usual criteria, i.e. the retest results were not based on Method 1A using LAV approach. Method 2 used the combined data from the NE (control) group and the BE worker group. Consequently, we cannot determine how this method would have classified the unacceptable LPTs in a "real time" situation, since the *reference data base* changes as new data becomes available.

Figure 4 suggests that there was "cell killing" present at the two highest Be con-

centrations on Day 5 and 7. Figure 5 and Figure 6 support the assumption that the log(SI)s are Gaussian in the center.

Distributions of resistant estimates ($\tilde{\phi}$) of the CV were presented—Figure 8. These results show that the internal variability is similar for the BE-sample and NE-sample for control wells and treated wells on days 5 and 7. These distributions are similar to those seen at ORISE and are centered at about $\tilde{\phi}$ =0.30.

8. Acknowledgments

This research was supported by the Offices of Occupational Medicine, Environment, Safety and Health, U. S. Department of Energy under contract DE-AC05-96OR22464 with Lockheed Martin Energy Research Corp. The authors thank R. L Schmoyer, L. G. Littlefield, and S. P. Colyer for reviewing this report, and R. L. Neubert for technical assistance with data preparation.

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A. Detailed Report For LPT Data

The following page describes the data from day 5 and day 7 for the LPT. All statistics in the top of the report are based on the outlier resistant approach described in Section 2.2. The results based on the method currently in use at NJC are listed at the bottom of the report.

The left hand side of the top panel describes the treatment groups and lists the observed well counts. Column 6 gives the fitted value obtained using the LAV method. Smad is computed using Equations 4 (see below). The residuals rounded to the the nearest logarithmic percent units (L%) are obtained as

$$Residual(L\%) = 100 \times \frac{count}{fit}$$

For example, day 5 control well 4: 100*log(231/252.4) = -9 L%.

The LAV estimates of the log(SI)s from Equations 3 and the corresponding SIs are listed in the second panel. Since this report was originally set up for ORISE data where positive controls are counted on day 5, and NJC runs their positive controls on day 3, we have not reported theses results. **All LPTs in this report showed an adequate response to ConA and PHA**

The estimates of the coefficient of variation ($\tilde{\phi}$) are listed near the middle of the page and were calculated as describe in Section A.2 of ORNL-6818. A separate estimate is calculated for the control wells and the treated wells on days 5 and 7. These same numbers are listed in column 7 of the top panel in L% units (i.e. they have been multiplied by 100). Pooled estimates are calculated for day 5, day 7, and overall.

```
      DATE  NJCID
BS472 31093 BE-472
BLPT ANALYSIS USING LAV METHOD-- see ORNL-6818 Report  for   BS472
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Treatment Grp	Well Counts				FIT	Smad(%)	Residuals (L% Units)			
day5 controls	66	125	157	231	252.4	57.2	-134	-70	-47	-9
day5 controls	397	334	276	313	252.4	57.2	45	28	9	22
day5 controls	354	220	426	169	252.4	57.2	34	-14	52	-40
day5 be1	342	475	332	396	368.0	15.1	-7	25	-10	7
day5 be10	1284	4171	531	2175	1670.7	100.9	-26	92	-115	26
day5 be100	136	181	861	11696	394.7	158.0	-106	-78	78	339
day7 controls	445	356	691	520	457.6	29.5	-3	-25	41	13
day7 controls	378	604	471	645	457.6	29.5	-19	28	3	34
day7 controls	401	379	367	471	457.6	29.5	-13	-19	-22	3
day7 be1	365	607	1656	585	595.9	43.5	-49	2	102	-2
day7 be10	1156	2644	501	800	962.0	71.6	18	101	-65	-18
day7 be100	173	1104	99	167	170.0	47.8	2	187	-54	-2
pha	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
candida	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

FIT = Fitted Value = exp(median) for each treatment group

Stimulation Indices

	Day 5			Day 7			Positve Controls	
	be1	be10	be100	be1	be10	be100	pha	candida
log(SI)	0.377	1.890	0.447	0.264	0.742	-0.991	NA	NA
SI	1.457	6.618	1.563	1.302	2.101	0.371	NA	NA

Summary Statistics for BS472

Phitilde (Coef. of Variation)*

Overall:	0.428					
Day 5 Control:	0.572	Day 5 Treated:	0.894	Day 5 Pooled:	0.6	
Day 7 Control:	0.295	Day 7 Treated:	0.577	Day 7 Pooled:	0.309	

N:	48
Nprime:	42.888
N > z(.995):	4
N > z(.9995):	2

* Phitilde is MAD est. of the std. dev. on log scale (corresponds to CV on orig. scale)

METHOD 1 - CURRENT NJC METHOD BS472

	Reps	Time	Avg	CV	log(SI)	SI
day5 controls	8	4	256.750	0.289	NA	NA
day5 be1	4	4	386.250	0.170	0.408	1.504
day5 be10	3	4	1330.000	0.619	1.645	5.180
day5 be100	3	4	392.667	1.034	0.425	1.529
day7 controls	12	4	477.333	0.241	NA	NA
day7 be1	3	4	519.000	0.258	0.084	1.087
day7 be10	3	4	819.000	0.400	0.540	1.716
day7 be100	3	4	146.333	0.281	-1.182	0.307

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