Development and Evaluation of a Screening Test for Beryllium Sensitization

E. L. Frome
Oak Ridge National Laboratory
Computer Science and Mathematics Division
D. L. Cragle, S. P. Colyer
Oak Ridge Institute for Science and Education
P. F. Wambach,
U. S. Department of Energy

Key Words:beryllium; least absolute values; lymphocyte proliferation test; outliers; resistant methods; electronic notebook

Abstract:

Beryllium[1] sensitivity testing is used as a screening tool for possible chronic beryllium disease, as a surveillance tool in indicating hazardous working conditions, and as part of the diagnostic criteria for the disease. The blood lymphocyte proliferation test (BeLPT) is used to determine if an individual is sensitized to beryllium. The Department of Energy (DOE) has developed a directive that describes the laboratory procedure and statistical analysis for use in this work. This document and the review process that was used to provide comments and improvements are available in an "electronic notebook". The new procedure standardizes the interpretation of BeLPT results by using a metric that controls for the inter-laboratory and host differences inherent to this test. The statistical analysis takes into account the biological variability in the patient test result and the distribution of the maximum test result in a reference data set of nonexposed (control) individuals. Results obtained using this procedure for a group of beryllium workers are presented. For additional information see the BeLPT Notebook (BeLPT-NB) [2].

1. Introduction

The potential hazards of exposure to beryllium compounds were first reported in the 1930s. The clinical syndrome of chronic beryllium disease (CBD) was first described in 1946 [6]. Initial speculation on the immunologic basis of CBD occurred in the 1950s. The first *in vitro* observation of beryllium-specific cell proliferation was demonstrated in the 1970s. A review of the development of the blood BeLPT is provided by Newman [7]. In response to concerns over "outliers " the least absolute values (LAV) method on the Ln of the well counts was developed by Frome *et al* [4]. In the standard protocol for the BeLPT a stimulation index (SI) is calculated for each of three beryllium concentrations on two harvest days. Once SIs have been calculated it is necessary to determine if the results indicate an "abnormal" response to beryllium. Three methods were considered in previous reports that utilize a "cut point" that is established using a reference data base of BeLPTs (see Frome *et al* [1]).

The U.S. Department of Energy is operating a screening program for CBD that will eventually include approximately 30,000 current and former beryllium-exposed workers at 20 DOE sites. In June of 2000 the DOE decided that a Specification for the BeLPT was needed to support the beryllium workers and former beryllium worker surveillance programs. A working group was established to write an initial draft version of the BeLPT Specification and an "electronic notebook"[2] was used to document the development, review, and revision of the BeLPT Specification (see BeLPT-NB, Frome and Cragle [2]). Figure 1 shows the first page of this electronic notebook. The final version of the BeLPT Specification [3] was completed in April, 2001 [9] and is available on page 21 of the BeLPT-NB. Previous methods for identification of an abnormal BeLPT were considered unacceptable by the working group for either statistical or practical reasons. A new method is proposed that combines clinical judgment and statistical analysis to identify an abnormal test. This new method described in Section 2.3 is referred to as the statistical-biological positive (SBP) method. Data from a group of beryllium workers at the Y-12 plant in Oak Ridge is used to evaluate new SBP method.

[1] **From the 2001 Proceedings of the Statistics in Epidemiology Section of the American Statistical Association.**

[2] See Geist and Nachtigal [5],or DOE 2000 Electronic Notebook Project at www.csm.ornl.gov/enote/.

[3] At tis.eh.doe.gov/techstds/standard/spec1142/SPEC11422001.pdf

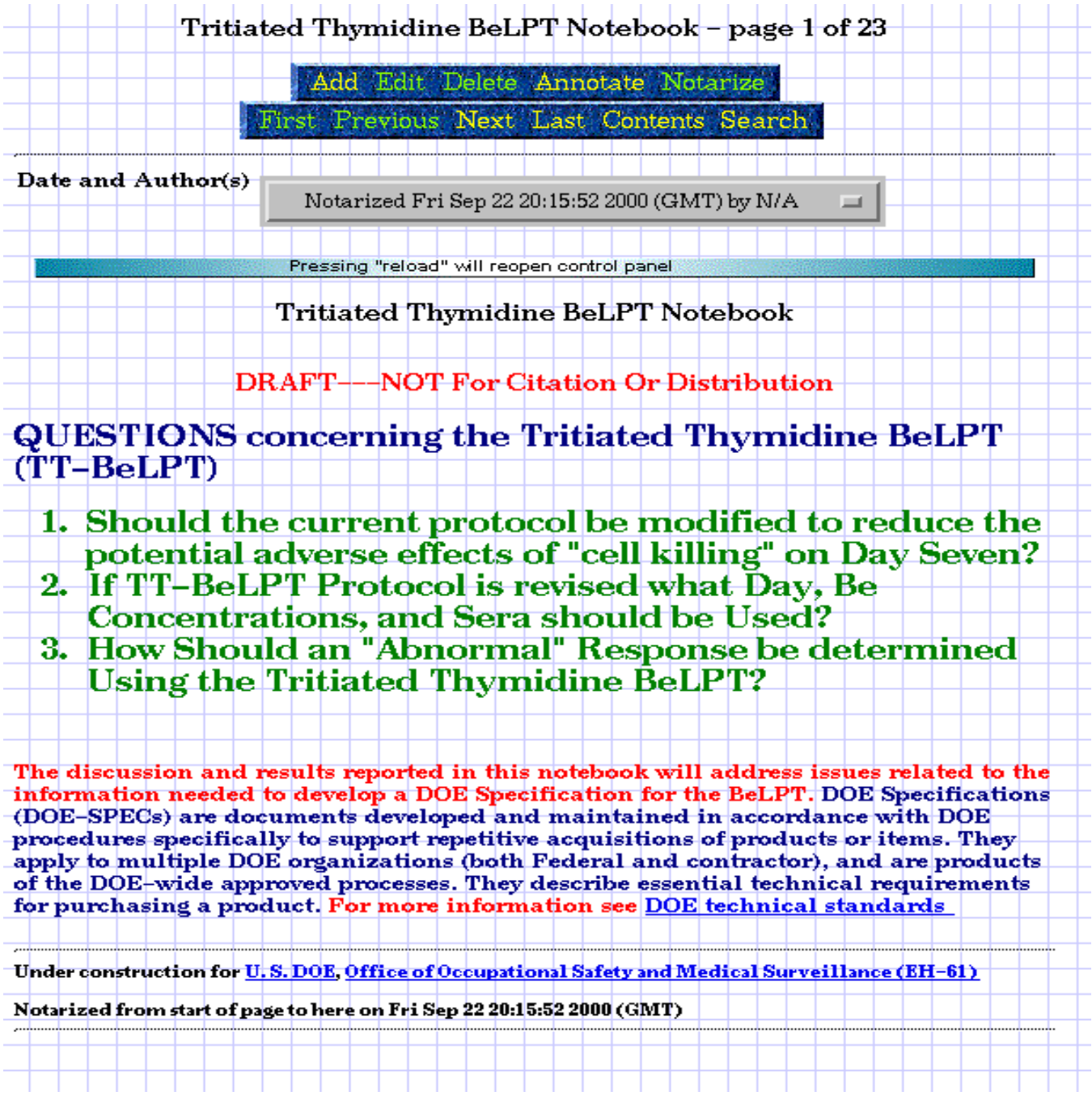

Figure 1: First page in the Tritiated Thymidine Beryllium Lymphocyte Proliferation Test Electronic Notebook (see BeLPT-NB, Frome and Cragle [2])

2. Materials and Methods

2.1 Beryllium Lymphocyte Proliferation Test

The tritiated thymidine BeLPT has followed a standard protocol for laboratory procedure and data collection since the late 1980s. A detailed description of lymphocyte culture methods, quality control measures, and examples of plate maps and printouts of raw data in use at the ORISE BeLPT laboratory was provided by Frome *et al* [4]. This and several alternative assay designs are also described in the BeLPT-NB (see [2] pages 2-5). The details of this procedure and the equipment used vary at different laboratories that are performing the BeLPT.

2.2 Statistical Analysis of The BeLPT

As the result of biological variability in the well counts there are different levels of uncertainty present in each BeLPT. This internal variability is described by the standard deviation of the Ln well counts, and is equivalent to the coefficient of variation on the original scale. This "internal analysis" of the BeLPT is based on estimates of the Ln(SI)s and their standard errors. These estimates are calculated using the least absolute values (LAV) method as described by Frome *et al* [4]. This approach only requires the ability to calculate medians and can be done in a spread sheet (e.g., Excel, see page 14 of the BeLPT-NB [2]) or a statistical program (e.g., Splus or R). The LAV analysis is based on the assumptions that i) the Ln of the well counts are normally distributed; ii) the standard deviations of Ln counts are constant within harvest days; iii) multiple outliers may be present in the Ln well counts; and iv) if "responder cells" are present, an increase in cell proliferation relative to the control wells will occur in cultures with beryllium. The steps in the LAV analysis are described in detail and a numerical example is provided in BeLPT-NB [2] and in the Appendix of [1] and [9] .

[9]

2.3 Identification of an Abnormal BeLPTs Using the SBP Method

This new SBP method reflects the clinical judgment that i) at least two sets of beryllium stimulated wells should show a response (statistical positive), and ii) the requirement that the maximum SI must exceed a cut point (biologically positive) that is determined from a reference data set of normal individuals. A BeLPT is considered abnormal if both the statistical and biological criteria are satisfied (see Frome *et al* [1] [3] for details).

If only one of these criteria is met, and the data is otherwise acceptable, then the test is considered to be a "borderline" test. If neither criteria is meet the test is normal. If a patient's first test is not normal then a second evaluation is requested, and two repeat BeLPTs are evaluated in different laboratories or in the same laboratory using different sera. If at least two of the three BeLPTs are abnormal the patient is deemed beryllium sensitized. A person may be a "sensitized responder" and not have CBD. If a person is identified as sensitized, then further medical evaluation (see Stokes and Rossman [8]) is available to determine if the worker has CBD.

3. Results

Data from a group of beryllium workers at the Y-12 plant in Oak Ridge is used here to evaluate new SBP method. The raw data for 1113 BeLPTs (1080 workers and 33 controls) was obtained in electronic form from the Oak Ridge Institute for Science and Education (ORISE) LPT laboratory (see [1] for details).

3.1 Results For Beryllium Workers and Nonexposed BeLPTs

Histograms and normal q-q plots for the SIs for each day and Be concentration (beryllium workers and nonexposed combined) for the BeLPT data are provided in Frome *et al*,[1]. For the serum supplement used in this study SIs above three were abnormally high, indicating a response to beryllium. The q-q plots indicates that the SIs are best described by the normal distribution on the log scale. This is further supported by Figure 2 which shows lognormal q-q plots for the beryllium workers and nonexposed control 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 Ln(SI)s—are shown on the vertical scale on the left, and the quantiles of the standard normal distribution are shown on the horizontal scale. The relation between the empirical quantiles and theoretical quantiles is approximately linear in the center of the distribution for each panel in Figure 2, indicating that the distribution is Gaussian. This reflects the assumption that most 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 2. The Ln(SI)s appear to be approximately normal in the center, for both the nonexposed controls and the beryllium workers. There are several values that are larger than expected (these are the points above the lines) for the beryllium workers. These "outliers" are SIs that indicate hypersensitivity to beryllium. There are also several points below the line which indicate cell killing.

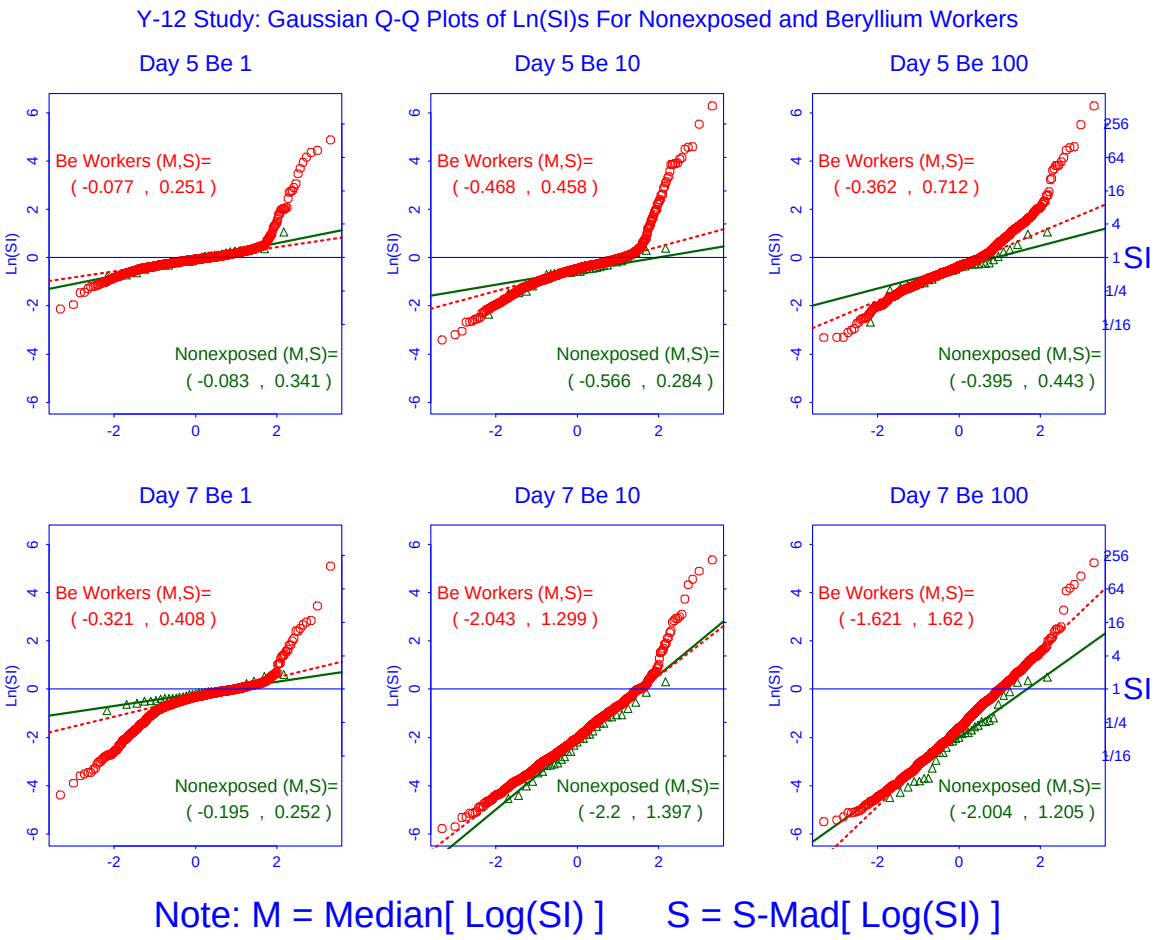

Figure 2: Normal q-q plots of Ln(SI)s for each beryllium concentration on Day 5 and Day 7 for beryllium workers and nonexposed controls. The data values are shown on the vertical axis. The Median (M) estimate of location, and MAD (S) estimate of scale for the Ln(SI)s are listed on each plot. Values of M and S for beryllium workers (circles) are in upper left and nonexposed controls (triangles) are in lower right of each panel. The lines in each plot (solid for nonexposed and dotted for beryllium workers) show the relation that is expected if the Ln(SI) values are from a normal distribution with location parameter M (which determines the intercept) and standard deviation S (which determines the slope).

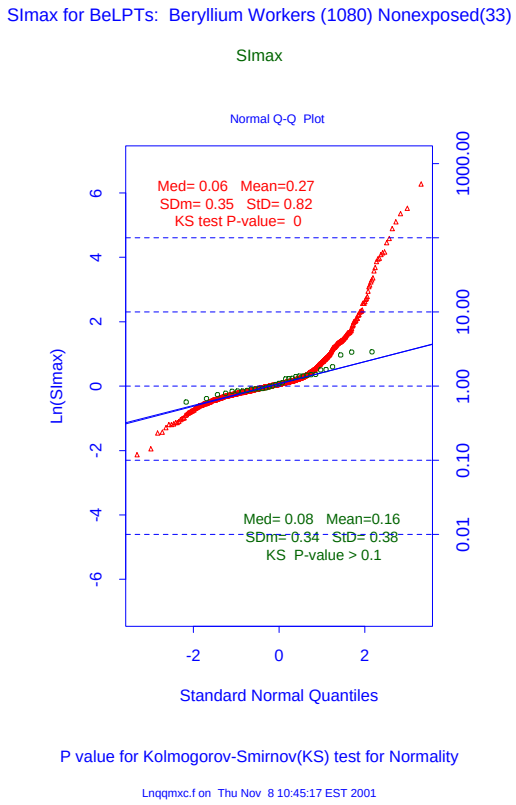

Figure 3: Normal q-q plots for Ln(SImax). Summary statistics for nonexposed controls (circles) are shown in lower right, and for beryllium workers (triangles) in upper left of q-q plot. Small P value for Kolmogorov-Smirnov (KS) test indicate departure from normal distribution for LN(SImax).

3.2 Identification of Abnormal BeLPTs Using the SBP Method

The SBP method described in Section 2.3 was used to evaluate each BeLPT. The first step was to determine if the BeLPT was a statistical positive test. This occurs if at least two standardized Ln(SI)s are large (see [1] [9] [2] for details and an example). The second step was to determine if the BeLPT was a biological positive test. This requires estimates of the location and scale parameters for the reference data set (RDS). **The BeLPTs from the nonexposed controls were used as the RDS**. The Ln(SImax) values for the nonexposed controls and beryllium workers are shown in normal q-q plots in Figure 3. The outlier resistant estimates of M and S from the RDS are 0.0812 and 0.34, respectively (see [9] Appendix B) . A test is biological positive (see criteria 2 in Section 2.3 and [1]) if Zmax = [Ln(SImax) - M]/S is greater than 3.09. An example is provide in the Appendix of [1] and [9].

3.3 Identification of Cases

All of the BeLPTs in the Y-12 study were done before July, 1996, and all of the workers with a positive test and most of the 944 workers with an initial normal test were followed and re-tested over the next five years. The results of this follow-up are shown in Columns 2-7 of Table 1. A total of 132 BeLPTs had an initial positive test by at least one of the criteria in Section 2.3. There were 80 BeLPTs that were abnormal, 36 tests with Zmax greater than 3.09 (biological positive only), 16 tests with at least two SLsis greater than 2.53 (statistical positive only), and 948 normal tests. These groups are identified in the first column of Table 1. **The classification of individuals in the columns 2-6 of Table 1 was based on the criteria being used by the ORISE LPT laboratory at the time the tests were done** (see Section 3 of Frome *et al* [1] for details).

The results of the SBP method summarized in Table 1 can be used to estimate the true positive rate (TPR) and the specificity (1 - false positive rate) for a **first abnormal BeLPT in a specific serum**. The results in Table 1 were further summarized by assuming that i) individuals follow-up status reflects their condition at the time the first test was done; ii) individuals with unknown status were normal (these are mostly retired workers with a normal first test that are asymptomatic); iii) individuals that have CBD are sensitized ; and iv) individuals that were not sensitized to beryllium are normal. The TPR of the first BeLPT in Serum 3040083 is 48/56 or 85.7 percent, and the specificity is 992/1024 or 96.9 percent. The ORISE LPT laboratory identified abnormal BeLPTs using the methods and criteria in place at the time that each test was done. Using the information from

Table 1 Summary Follow-Up Data For Y-12 Group

	Follow-UP Results					
Group	B[a]	N	UN	SENS	CBD[b]	Total
Abnormal Test	7	21	4	27	21	80
Biological Positive	6	22	6	1	1	36
Statistical Positive	0	10	4	2	0	16
Normal	6	629	309	3	1	948
Total	10	682	323	33	23	1080

[a] B-Borderline N-Normal Un-Unknown SENS-sensitized

[b] CBD see text for detailed explanation.

the ORISE historical data base the TPR was 78.6 percent and the specificity was 98.3 percent. If individuals with unknown status are not included (see ii above) in the calculations the specificity for the SBP method is 96.0 percent, and the specificity for ORISE historical method is 92.7 percent.

4. Discussion

Figure 2 and results in Section 3 of Frome *et al* [1] provide empirical evidence that the assumptions described in Section 2.2 are reasonable. The results in Table 1 indicate that the SBP method, using the LAV approach to estimate the SIs, is at least as good as current methods for evaluating the BeLPT. The "outlier rejection method" that is used by some laboratories has no logical statistical basis (see page 16 of the BeLPT notebook [2] for further discussion). Further evaluation of the SBP method is currently underway using results from ORISE obtained in several different sera after 1996, and using data from at least two additional laboratories.

5. Acknowledgments

This research was supported by the Offices of Occupational Medicine, Environment, Safety and Health, U. S. Department of Energy under contract DE-AC05-00OR22725 with UT-Battele, LLC. The authors thank ORISE, Center for Epidemiologic Research and ORNL, Computer Science and Mathematics Division staff for assistance in preparing this report.

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof. The U.S. Government retains a nonexclusive, royalty-free license to publish or reproduce the published form of this work, or to allow others to do so for U. S. Government purposes.

References

[1] E.L. Frome, D. L. Cragle, L. G Littlefield, and S. P. Colyer. Results of the analysis of the blood lymphocyte proliferation test data from the Oak Ridge Y-12 study. Technical Report ORNL/TM-2001/79, Oak Ridge National Laboratory, 2001.

[2] E.L. Frome and D.L. Cragle, editors. *BeLPT-NB:Notes Concerning The Tritiated Thymidine Beryllium Lymphocyte Proliferation Test at http://www.csm.ornl.gov/~frome/BE/tnote.html*. Oak Ridge National Laboratory, Oak Ridge, Tn, 2001.

[3] E.L. Frome, L.S. Newman, D.L Cragle, and S.P. Colyer. Identification of an abnormal beryllium lymphocyte proliferation test. *Manuscript*, 2001.

[4] E.L. Frome, M.H. Smith, L.G. Littlefield, R.L. Neubert, and S.P. Colyer. Statistical methods for the blood beryllium lymphocyte proliferation test. *Environmental Health Perspectives Supplement*, 104:957–968, 1996.

[5] A. Geist and N. Nachtigal. *Oak Ridge National Laboratory Electronic Notebook Project*. http://www.csm.ornl.gov/~geist/java/applets/enote/, 2000.

[6] H.L. Hardy and J.R. Tabershaw. Delayed chemical pneumonitis occuring in workers exposed to beryllium compounds. *Indust. Hygine Toxicol.*, 28:197–211, 1946.

[7] L.S. Newman. Significance of the blood beryllium lymphocyte proliferation test (BeLPT). *Environmental Health Perspectives Supplement*, 1996.

[8] R.F. Stokes and M.D. Rossman. Blood cell proliferation response to beryllium: Analysis by receiver-operating characteristics. *Journal of Occupational Medicine*, 33:23–28, 1991.

[9] P. W. Wambach, editor. *DOE-SPEC-1142-2001:BERYLLIUM LYMPHOCYTE PROLIFERATION TESTING*. U.S. Department of Energy,Washington, D.C., 2001.