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ORIGINAL ARTICLE

Performance of the beryllium blood lymphocyte proliferation test based on a long-term occupational surveillance program

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Abstract
Objective Data from surveys of the general workforce and new employees at a beryllium manufacturer were used to evaluate the performance of the beryllium blood lymphocyte proliferation test (BeBLPT).
Methods Over 10,000 results from nearly 2,400 participants collected over 12 years were analyzed using consistent criteria to describe the performance characteristics of the BeBLPT.
Results Approximately 2% of new employees had at least one positive BeBLPT result at the time of hire, and approximately 1% of new employees with no known potential occupational or possible take-home exposures to beryllium were confirmed positive (two positive results) from the time of hire. Positive results were observed in some workers within weeks or months of initial exposure, and the median time to the first positive result in confirmed positive individuals was 5 months. The prevalence of positive BeBLPT results was greatest during the first year of employment with an apparent peak in months 4–8. At least one negative or borderline/negative result was observed in 100% of new workers who underwent follow-up testing after they had been confirmed positive. There was no correlation between time of employment and an increasing prevalence of confirmed positive BeBLPT results in individual surveys; however, the cumulative incidence of confirmed positive results in subsets of workers that participated in multiple surveys increased over time.
Conclusion The detection of confirmed positive results in non-occupationally exposed persons, the apparent reversions of previously confirmed positive results, the identification of a positive BeBLPT peak prevalence period, and the variation in intra- and inter-laboratory test methods and interpretation should be considered when interpreting results from studies utilizing the BeBLPT, especially when considering worker-specific interventions. Additional research to refine the BeBLPT or develop a new test is needed to properly characterize the relationship between sensitization and subclinical or clinical indicators of chronic beryllium disease.

Keywords Beryllium · Lymphocyte · Surveillance · Hypersensitivity · Industrial hygiene

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Introduction

Chronic beryllium disease is a hypersensitivity disorder that has been studied among workers in a variety of beryllium manufacturing settings for more than 60 years (Kreiss et al. 1993; Deubner et al. 2001a; Johnson et al. 2001; Kelleher et al. 2001; Stange et al. 2001; Schuler et al. 2005). Clinical chronic beryllium disease can be characterized by symptoms of dry cough, wheezing, shortness of breath, fatigue, weight loss, or decline of pulmonary function. Subclinical chronic beryllium disease is microscopically characterized by a profusion of non-necrotizing granulomas and is differentiated from clinical chronic beryllium disease by the absence of physical symptoms or detection of X-ray or pulmonary function changes typically

associated with clinical chronic beryllium disease pathology (Saltini and Amicosante 2001). It was first suggested in 1951 that chronic beryllium disease was an immune-mediated disease, and a beryllium-sensitized state was initially defined by the beryllium skin patch test (Curtis 1951, 1959; Sterner and Eisenbud 1951). Beryllium skin patch testing using soluble beryllium salts has also been utilized more recently on small numbers of individuals for diagnostic and research purposes (Bobka et al. 1997; Fontenot et al. 2002), but the test is not currently used for the widespread surveillance of beryllium workers due to concerns that the test might induce sensitization or exacerbate existing chronic beryllium disease (Curtis 1951; Waksman 1959; Hanifin et al. 1970).

The beryllium blood lymphocyte proliferation test (BeBLPT) is an in vitro assay that has been used as an indicator of beryllium sensitization in occupational surveillance programs since it was described in its modern form in the late 1980s (Kreiss et al. 1989). The BeBLPT is typically performed using mononuclear cells collected from venous blood by gradient centrifugation. A fixed number of cells are suspended in tissue culture medium and incubated in microtiter plates with 1, 10, or 100 μM beryllium sulfate. The cells are harvested at two time points (typically on Day 4 or 5 and again on Day 6 or 7), and [^{3}H]-thymidine is added during the day prior to collection. A stimulation index, believed to correlate with beryllium sensitization, is calculated by comparing the extent of thymidine incorporation in DNA from beryllium-treated lymphocytes to that of lymphocytes cultured in the absence of beryllium. Positive control samples are incubated with non-specific mitogens, such as phytohemagglutinin A or concanavalin A, or antigen-specific mitogens, such as tetanus toxoid or *Candida albicans*. A test is determined to be positive (abnormal), negative (normal), or borderline based on the total number of wells with stimulation indices above a threshold value.

Unfortunately, the BeBLPT has a number of shortcomings that limit its usefulness and complicate the interpretation of worker surveillance programs and studies. It is widely recognized that the BeBLPT can yield false negative results, and the test is known to be subject to substantial intra- and inter-laboratory variability (Kreiss et al. 1997; Deubner et al. 2001b; Maier 2001; Stange et al. 2004; Pott et al. 2005; Schuler et al. 2005). A co-operative effort led by the Beryllium Industries Scientific Advisory Committee recommended to the United States Department of Energy a consensus standard on BeBLPT assay materials and procedures (DOE 2001). Despite this recommended standard, there continues to be no uniform protocol that commercial laboratories use to perform the test nor is there a quality control program that ensures that various testing laboratories generate consistent findings for the same sample. The differences in the way the BLPT has been performed and interpreted have recently been reviewed (Borak 2006). Intra- or inter-laboratory differences that have the potential to affect the outcome of the BeBLPT include the concentrations of cells, media, or serum; the types of sample wells (i.e., flat-bottom vs. round-bottom); the number of replicate wells; length of incubation; blood sample collection and shipping procedures; the stimulation index threshold that is used to define a positive result; and the statistical methods used to analyze data. Consequently, samples are often split and sent to different laboratories for analysis in order to more reliably confirm test results or to assess inter-laboratory variability (Deubner et al. 2001b).

Sensitization in some individuals that are not occupationally exposed to beryllium might be expected because it is ubiquitous in nature, is present in common materials such as tobacco or coal, and is used in some dental applications (Kolanz 2001; ACGIH 2002; ATSDR 2002), and data suggestive of a background prevalence of sensitization in people with no known occupational or incidental exposure to beryllium have been reported in multiple studies. In a study of United States Department of Energy beryllium facilities, 1.5% (7/458) of workers with no known exposure yielded single positive BeBLPT results (Stange et al. 2004); however, none of those initial positive results were confirmed based on the repeat testing protocol utilized in that study of a single blood draw tested at two labs. In another study, positive BeBLPT results were observed between 1992 and 1995 in 2/159 (1.2%) newly hired workers (Yoshida et al. 1997). An update of the Japanese facility studied by Yoshida covering the period 1992 to 2005 found positive BeBLPT results in 4/350 (1.1%) newly hired workers (NGK Metals, personal communication 2006). In addition, analytical laboratories performing BeBLPT assays have reported a baseline level of positive results of approximately 1% among the control population they determined to be unexposed for purposes of calculating their stimulation index value for defining normal versus abnormal results (Kolanz 2001). In an occupational study that utilized the beryllium skin patch test, a positive rate of 3.8% was observed in 420 workers with no known exposure to beryllium (Shima 1966). In a more recent patch test research study, the prevalence of positive responses among controls with no known exposure to beryllium was 1/20 (5%) (Bobka et al. 1997).

Materials and methods

Since 1992, Brush Wellman Inc. has collected BeBLPT data at various beryllium mining, manufacturing, or processing facilities as part of its medical surveillance program (Table 1). The program has included surveys of the general workforce at four facilities between 1992 and 2004, repeat

Table 1 Summary of the Brush Wellman BeBLPT general workforce surveys

Facility	Workforce surveys/test periods		New employee program		Processes
	Year(s)	Participants	Initiation	Participants	
Elmore	1993–1994	627	August 1999	343	Processing of beryllium hydroxide for beryllium metal, alloys, and ceramics; manipulation of ceramics; dry pressing; research and development; ceramic production (historical)
	1999	730			
	2000–2001[a]	622			
	2002–2004[a,b]	415			
Tucson	1992	135	January 1999	132	Ceramic production
	1998	159			
	1999–2000[a]	127			
	2001–2004[a,b]	77			
Reading	2000	152	June 2000	64	Finishing of beryllium alloys
	2001–2004[b]	123			
Delta	1996	75	–	–	Mining and milling of beryllium ore

[a] Period includes data from limited surveys or follow-up testing

[b] Data collected through May 30, 2005, were included in the analysis of data from the 2001–2004 test period

testing data from a new employee program at three of these facilities, and any associated follow-up testing. For the purposes of the current study, these data were analyzed according to consistent criteria to describe the performance characteristics of the BeBLPT. The analysis included more than 10,000 BeBLPT results that were obtained from nearly 2,400 participants.

Each testing laboratory typically reported six SI values, corresponding to three concentrations of beryllium and two incubation periods, for each BeBLPT. Because the criteria used to define a positive BeBLPT varied over time or between testing laboratories, BeBLPT results ('positive', 'negative', 'borderline', 'technically inadequate', or 'uninterpretable') were considered as they were reported by the individual testing laboratories for the purpose of this analysis. Individuals with more than one positive BeBLPT over any time interval (including two positive results from a split sample) were designated to be 'confirmed positive' and were considered to be beryllium sensitized. For the purpose of characterizing the time course of BeBLPT-positive results, the date of the first positive result was considered to be the point at which a person became BeBLPT positive.

Facilities

BeBLPT results from four Brush Wellman beryllium facilities in the United States were evaluated.

Elmore

The beryllium manufacturing facility near Elmore, Ohio, has been in operation since 1953 and has in recent years employed approximately 600–800 workers. Until 1969, beryl ore was converted to beryllium hydroxide which in turn was processed into one of three major product lines: metallic (pure) beryllium, alloys containing beryllium (typically copper-beryllium or nickel-beryllium containing 2% or less beryllium by weight), and beryllium oxide. Over the years, the facility has produced a variety of beryllium metals, beryllium alloys, and beryllium ceramic materials and products. Ceramic product fabrication was relocated from the Elmore facility to the Tucson facility between 1980 and 1982. The results of a BeBLPT survey conducted at the Elmore facility in 1993–1994 were previously described (Kreiss et al. 1997).

Tucson

The Tucson, Arizona, plant began production in 1980. The beryllium oxide powder produced at Elmore is shipped to Tucson, where it is used for the production of beryllia ceramics. Processing operations include powder preparation, pressing, firing furnaces, and machining. The results of worker surveys conducted at the Tucson facility in 1992 and 1998 have been previously reported (Kreiss et al. 1996; Henneberger et al. 2001).

Delta

The Delta, Utah, mining and milling facility began operation in 1969. At this location, beryllium ore is extracted from bertrandite and beryl ores. The primary potential for beryllium exposure at the Delta mine is from inhalation of ore dust; mists containing soluble beryllium salts; or the plant's end product, hydrated beryllium hydroxide. The results of a worker survey conducted at the Delta facility in 1996/1997 have been previously described (Deubner et al. 2001a).

Reading

The Reading, Pennsylvania, facility processes copper or nickel alloys containing less than 2% beryllium by weight. Semi-finished alloy strip and wire are converted into finished strip, wire, and rod products using rolling and drawing in conjunction with heat treatment, annealing, and pickling. The results of the Reading facility survey conducted in 2000 have been previously reported (Schuler et al. 2005).

General workforce surveys

Blood samples collected from workers at the Tucson facility in 1992 and 1998, the Elmore facility in 1993–1994 and 1999, the Delta facility in 1996, and the Reading facility in 2000 were analyzed with the BeBLPT. Blood samples were divided into aliquots ("split samples") that were analyzed at two of four commercial laboratories (Labs 1, 2, 3, and 4). Additional testing of select workers outside of the formal survey periods was performed to follow up on earlier positive, borderline, or difficult-to-interpret BeBLPT results or in response to other issues pertaining to specific employees.

New employee program

A complementary program designed to characterize the rate of positive BeBLPT results in newly hired employees at the Tucson, Elmore, and Reading facilities was initiated in 1999–2000. Questionnaires or interviews designed to ascertain information on prior occupational or secondary beryllium exposures were administered to all participants. At least 75% of the new employee program participants at each facility were directly involved in beryllium manufacturing or production processes, and approximately 7% of participants in the new employee program worked previously for Brush Wellman or in other jobs where they may have been exposed to beryllium. The BeBLPT was administered to program participants prior to any work in areas with potential beryllium exposure and after approximately 3, 6, 12, 24, and 48 months of employment. In most cases, a blood sample was sent to one laboratory at each of these time points. When a test was positive or borderline, an additional blood sample was collected and sent to two laboratories for confirmation. A split-sampling design was also used for the baseline test when a new worker was known to have previously worked at Brush Wellman as a temporary or contract employee. The average length of participation in the new employee program at the time of this analysis was 18 months.

Data analysis

An electronic database of the BeBLPT results from the general workforce surveys, the new employee program, and all additional follow-up testing has been maintained by Brush Wellman since 1992. SAS version 9.2 software (SAS Institute, Inc.; Cary, NC, USA) was used to identify confirmed BeBLPT-positive workers (defined for the purpose of this study as those with two or more positive BeBLPT results within any time period). Borderline, uninterpretable, or technically inadequate results (as defined and reported by the testing laboratory) were not included in this determination except as noted in the analysis of results obtained during the new employee program. It is important to note that the use of consistent criteria to define confirmed positive BeBLPT individuals for the purpose of this program-wide evaluation (i.e., two positive BLPT results over any interval of time) was expected to result in some apparent inconsistencies with previously published analyses of subsets of these data that considered trends in laboratory performance or other factors in the analysis (Schuler 2005).

Results

General workforce surveys

The results from this analysis of all available BeBLPT data from the Brush Wellman facility surveys are summarized in Table 2. In the 1998 Tucson, 1999 Elmore, and 2000 Reading surveys (those that routinely included workers employed less than 1 year), the rate of testing confirmed positive on the BeBLPT was greatest during the first year of exposure. The rate of confirmed BeBLPT positives in workers employed less than 1 year was 13% in the 1998 Tucson survey, 13% in the 1999 Elmore survey, and 15% in the 2000 Reading survey. In comparison, the rate of confirmed positive BeBLPT results in workers employed more than 1 year ranged from 7.4% in the 1998 Tucson survey to 11% in the 2000 Reading survey. Overall, the rate of confirmed BeBLPT-positive results in these surveys was 54% greater for those workers employed less than 1 year than for workers employed more than 1 year.

Additional trends were apparent when the survey data from workers employed less than 1 year were stratified into 4-month intervals (Table 2). A peak in the prevalence of confirmed positive BeBLPT results was consistently observed between 4 and 8 months of employment. The prevalence of confirmed BeBLPT-positive workers during the 4–8-month interval in the 1999 Elmore, 1998 Tucson, and 2000 Reading surveys was 19% (5/27), 19% (3/16), and 38% (3/8), respectively. When the results of these surveys were combined, there was a nearly threefold difference between the prevalence of confirmed BeBLPT positives among workers with 4–8 months of employment (22%) and those who had worked at Brush Wellman more than 12 months at the time of their tests (8.8%). It should be

Table 2 Prevalence of confirmed BeBLPT positives in Brush Wellman general workforce surveys

Time employed[c]	Elmore 1993–94[a]			Elmore 1999			Tucson 1992[a]			Tucson 1998			Reading 2000[b]			Delta 1996		
	n	Positive BeBLPT	Prevalence (%)	*n*	Positive BeBLPT	Prevalence (%)	*n*	Positive BeBLPT	Prevalence (%)	*n*	Positive BeBLPT	Prevalence (%)	*n*	Positive BeBLPT	Prevalence (%)	*n*	Positive BeBLPT	Prevalence (%)
0–4 months	–	–	–	44	4	9.1	–	–	–	5	0	0	10	0	0	–	–	–
4–8 months	–	–	–	27	5	19	3	0	0	16	3	19	8	3	38	1	0	0
8–12 months	1	0	0	15	2	13	6	0	0	17	2	12	2	0	0	–	–	–
1–5 years	29	3	10	191	18	9.4	40	3	7.5	41	2	4.9	27	6	22	2	0	0
5–10 years	183	15	8.2%	15	2	13	72	5	6.9	13	1	7.7	9	0	0	12	0	0
10–20 years	205	19	9.3	235	22	9.4	10	0	0	63	6	9.5	37	2	5.4	44	1	2.3
20–30 years	103	10	9.7	78	8	10	1	0	0	3	0	0	45	5	11	15	1	6.7
>30 years	106	4	3.8	125	6	4.8	3	0	0	1	0	0	14	1	7.1	1	0	0
<1 year	1	0	0	86	11	13	9	0		38	5	13	20	3	15	1	0	0
>1 year	626	51	8.1	644	56	8.7	126	0	0	121	9	7.4	132	14	11	74	2	2.7
Overall	627	51	8.1	730	67	9.2	135	8	5.9	159	14	8.8	152	17	11	75	2	2.7

Confirmed BeBLPT positives defined as occurrence of two positive BeBLPT results within any time period

Some workers participated in multiple surveys

[a] Workers employed for less than 1 year were not routinely included in the 1993–1994 Elmore and 1992 Tucson surveys

[b] In a previously published analysis of these data, nine confirmed positive BeBLPT results in the 2000 Reading survey were attributed to technical problems at one of the testing laboratories and were excluded from the reported rate of confirmed positive BeBLPT results (Schuler et al. 2005). These nine results were included in the present analysis for consistency purposes and therefore result in a substantial difference in the reported rate of confirmed positive BeBLPT results at the Reading facility

[c] Time of continuous full-time employment at time of sampling of the first positive BeBLPT result (prior to confirmation upon subsequent sampling)

noted that nine confirmed positive BeBLPT results in the 2000 Reading survey were attributed to technical problems at one of the testing laboratories and were so noted and excluded from the reported rate of confirmed positive BeBLPT results in a previously published analysis of these data (Schuler et al. 2005). These nine results were included in the present analysis for consistency purposes and therefore result in a substantial difference in the reported rate of confirmed positive BeBLPT results at the Reading facility when compared to the previously reported results (Schuler et al. 2005). However, our decision to include these results did not change the outcome of this analysis.

Results from multiple surveys of the Elmore facility showed no association between time of employment and the prevalence of confirmed positive BeBLPT results after the first year (Fig. 1). The rate of confirmed BeBLPT-positive results in the first Elmore survey ranged from 8.2 to 10% in workers employed 1–30 years, and the lowest prevalence of positive BeBLPT results (3.8%) was observed in workers employed more than 30 years (Table 2). Notably, in workers employed more than 1 year, there was no increase in the prevalence of positive BeBLPT results with years worked in this survey, in the subsequent Elmore survey, or in surveys conducted at the smaller facilities (Table 2).

The prevalence of positive BeBLPT results among workers at the Elmore facility was nearly constant during each period of the program and was independent of time worked (Table 2). However, because additional long-tenured workers were identified as BeBLPT positive each time the test was administered, an increase in the cumulative incidence of confirmed BeBLPT positive workers was observed over time This was most clearly demonstrated in a subset of workers at the Elmore facility that were tested on at least four occasions between 1993 and 2004. The mean prevalence of confirmed BeBLPT workers in the 1993–1994 Elmore survey was 8.1%, and the cumulative incidence of confirmed BeBLPT-positive workers for this subset through May 30, 2005, was 21% (Fig. 2).

New employee program

BeBLPT results obtained from participants in the Brush Wellman new employee program are presented in Tables 3, 4, 5. Thirteen of the 538 participants (2.4%) had at least one positive BeBLPT result (or a borderline result which was followed by at least two positive results) at or near the time they started work at Brush Wellman. Nine of these individuals (1.7%) were confirmed to be positive during subsequent testing (Table 3). A known potential for previous occupational or possible take-home exposures to beryllium was identified in the histories of three of these nine workers, and the background prevalence of confirmed BeBLPT-positive responses was calculated to be 1.1% (6/535) when they were excluded from the analysis. In addition to the use

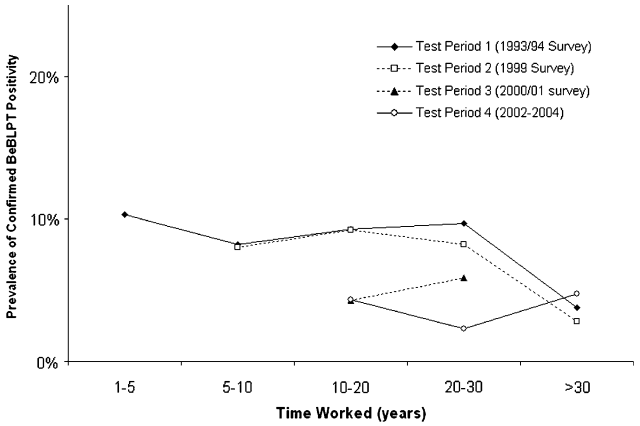

Fig. 1 Rate of confirmed BeBLPT positive results in successive Elmore plant surveys by time of employment. The prevalence of confirmed BeBLPT positive individuals among a subset of workers at the Elmore facility during each of the indicated time periods shows no relation to either initial prevalence or time worked. Only data from workers that participated in the 1993–1994 survey and were also tested during each of the subsequent periods were included in this analysis. Workers were considered to be confirmed BeBLPT positive when they accumulated two positive tests during any period of time. For the purpose of this analysis, workers were excluded from the calculation in subsequent time periods, and the time worked calculation was based on the time of the first positive test

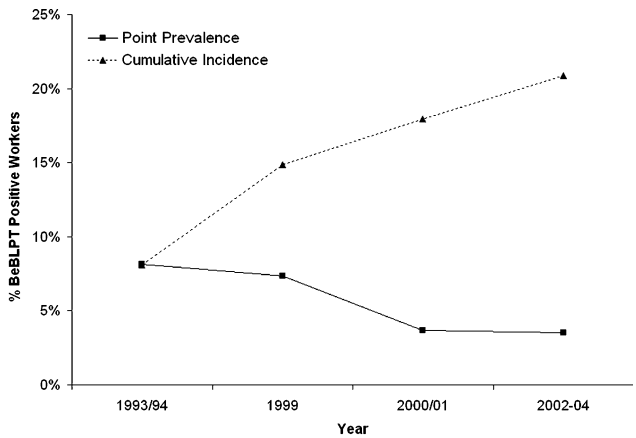

Fig. 2 Comparison of the cumulative incidence of newly BeBLPT positive workers and point prevalence during successive surveys of workers at the Elmore facility. Only data from workers that participated in the Elmore 1993–1994 survey and in all subsequent survey periods were included in this analysis. The cumulative incidence of confirmed positive BeBLPT workers (derived from a life table analysis) increases over time while the point prevalence of BeBLPT positive results in each survey remains relatively constant. Estimates of point prevalence for the 1999, 2000–2001, and 2002–2004 survey periods were based on the number of newly positive workers in each period who had participated in previous surveys

Table 3 Brush Wellman new employee program participants with confirmed BeBLPT positive results at time of hire

ID no.	Lab #	Time of employment		Test result
		Days	Months	
NEP-1[a]	1	1	–	Negative
	3	1	–	Positive
	1	50	1.7	Negative
	3	50	1.7	Positive
	2	84	2.8	Negative
	1	183	6.1	Negative
	1	399	13.3	Tech inadequate
	1	456	15.2	Negative
	1	744	24.8	Negative
NEP-2	1	1	–	Positive
	1	22	0.7	Positive
	3	22	0.7	Positive
	2	119	4.0	Positive
NEP-3	1	1	–	Neg/borderline
	1	28	0.9	Neg/borderline
	2	50	1.6	Negative
	1	50	1.6	Positive
	2	65	2.1	Negative
	1	65	2.1	Neg/borderline
	2	78	2.6	Negative
	1	78	2.6	Neg/borderline
	1	175	5.8	Positive
	1	351	11.5	Neg/borderline
NEP-4[a]	1	2	–	Negative
	3	8	0.3	Positive
	1	44	1.5	Negative
	3	44	1.5	Neg/borderline
	1	56	1.9	Neg/borderline
	3	56	1.9	Positive
	2	147	4.9	Negative
	1	434	14.5	Negative
NEP-5	1	2	–	Positive
	2	23	0.8	Positive
	1	23	0.8	Negative
	1	30	1.0	Negative
NEP-6[a]	1	1	–	Positive
	1	23	0.8	Positive
	2	23	0.8	Negative
	2	63	2.1	Negative
	1	105	3.5	Tech inadequate
	1	184	6.1	Negative
	1	372	12.4	Negative
NEP-7	1	0	–	Positive
	1	20	0.7	Positive
	2	21	0.7	Positive
	1	84	2.8	Positive
	1	167	5.6	Neg/borderline
NEP-8	1	0	–	Positive
	2	18	0.6	Negative
	1	18	0.6	Neg/borderline
	1	41	1.4	Negative
	1	89	3.0	Positive
	1	186	6.2	Negative
	1	363	12.1	Neg/borderline
NEP-9	1	1	–	Neg/borderline
	1	37	1.2	Positive
	2	58	1.9	Positive
	1	58	1.9	Negative
	2	92	3.0	Negative

Includes workers that had at least one positive or borderline BeBLPT result at or near the time of hire and that were subsequently confirmed to be BeBLPT positive

See text for explanation of test results

[a] The potential for prior occupational or possible take-home exposures to beryllium was identified in the case of participants NEP-1, NEP-4, and NEP-6

of a questionnaire, the work history of each new employee confirmed as beryllium-sensitized was individually reviewed by an occupational physician for possible previous beryllium exposure via family, contractor, household or prior work experience. In cases where additional potential exposure links were identified, further inquiry and personal interviews were completed with the new employee by the occupational physician with the support of certified industrial hygienists. However, as in all evaluations of the background rate of beryllium sensitization, the possibility of unidentified occupational or household exposures to beryllium from industrial sources cannot be completely excluded.

A total of 12 new employee program participants from three facilities that did not test positive at the time of hire were confirmed to be BeBLPT positive during subsequent testing (Table 4). One of these workers (NEP-20) had an initial positive BeBLPT result after 24 months of employment, but the remaining 11 individuals had at least one positive BeBLPT within their first year of employment. When workers with at least one positive test at the time of hire were excluded, the median time to the initial positive BeBLPT result in the new employee program among workers who were subsequently confirmed to be positive was 5 months (Table 5).

Numerous instances of positive-to-negative reversions in confirmed BeBLPT positive individuals were evident in the Brush Wellman surveillance program. Normal BeBLPT results in individuals that were previously confirmed to be positive were first noted when the 1993–1994 and 1999

Table 4 Individual results from workers determined to be BeBLPT positive during the Brush Wellman new employee program

ID no.	Lab #	Time of employment		Test result[a]
		Days	Months	
NEP-10	1	2	–	Negative
	1	86	2.8	Neg/borderline
	1	113	3.7	Positive
	1	184	6.1	Negative
	2	184	6.1	Negative
	1	198	6.5	Positive
	2	198	6.5	Negative
NEP-11	1	2	–	Negative
	1	86	2.8	Negative
	1	175	5.8	Positive
	2	198	6.5	Positive
	1	198	6.5	Positive
	1	205	6.7	Positive
	2	205	6.7	Positive
	2	211	6.9	Positive
	1	211	6.9	Positive
	2	245	8.1	Positive
	2	352	11.6	Negative
	2	644	21.2	Negative
NEP-12	1	1	–	Negative
	1	79	2.6	Negative
	1	182	6.0	Positive
	2	204	6.7	Positive
	1	204	6.7	Positive
	2	226	7.4	Positive
	2	358	11.8	Negative
NEP-13	1	1	–	Tech inadequate
	1	44	1.4	Negative
	1	98	3.2	Positive
	2	121	4.0	Positive
	1	121	4.0	Positive
	2	128	4.2	Negative
	1	128	4.2	Positive
	1	184	6.1	Neg/borderline
	2	239	7.9	Negative
NEP-14	1	2	–	Negative
	1	147	4.8	Positive
	2	163	5.4	Positive
NEP-15	1	3	–	Negative
	1	72	2.4	Neg/borderline
	1	133	4.4	Negative
	1	177	5.8	Positive
	1	205	6.7	Positive
NEP-16	1	1	–	Negative
	1	91	3.0	Positive
	2	120	3.9	Positive
	1	120	3.9	Positive
	2	128	4.2	Positive
	1	128	4.2	Positive
	2	183	6.0	Positive
	1	364	12.0	Neg/borderline
NEP-17	1	2	–	Negative
	1	85	2.8	Negative
	1	189	6.2	Negative
	1	303	10.0	Positive
	2	351	11.5	Negative
	2	373	12.3	Positive
	1	373	12.3	Negative
NEP-18	1	1	–	Negative
	1	93	3.1	Negative
	1	197	6.5	Neg/borderline
	1	217	7.1	Neg/borderline
	2	260	8.6	Positive
	1	260	8.6	Neg/borderline
	2	281	9.2	Positive
	2	331	10.9	Positive
	1	344	11.3	Positive
NEP-19	1	2	–	Negative
	1	77	2.5	Negative
	1	155	5.1	Positive
	1	184	6.1	Positive
	2	197	6.5	Negative
	2	231	7.6	Positive
NEP-20	1	1	–	Negative
	1	93	3.1	Negative
	1	176	5.8	Negative
	1	365	12.0	Negative
	1	726	23.9	Positive
	2	743	24.4	Positive
	1	743	24.4	Positive
	2	1430	47.0	Neg/borderline
	1	1465	48.2	Positive
NEP-21	1	3	–	Negative
	1	87	2.9	Positive
	2	122	4.0	Positive
	3	191	6.3	Negative

Confirmed BeBLPT positives defined as occurrence of two positive BeBLPT results within any time period

[a] See text for explanation of test results

Elmore survey results were compared. The 1999 survey included 18 workers who continued to work in beryllium operations after they were confirmed to be BeBLPT posi-

Table 5 Time to first positive BeBLPT result in the Brush Wellman new employee program

Plant	Incidence of first positive BeBLPT by months employed[a,b]						
	Pre-employment[c]	0–4[d,e]	4–8[e]	8–12[e]	12–24[e]	24–48[e]	>48[e]
Elmore	5 (327)	4[f] (278)	4 (245)	2 (149)	0 (92)	0 (32)	0 (17)
Reading	0 (48)	1 (58)	1 (55)	0 (26)	0 (17)	0 (6)	–
Tucson	2 (101)	1[f] (99)	0 (85)	0 (57)	1 (46)	0 (19)	0 (5)

[a] Includes only workers that were confirmed to be BeBLPT positive by a second positive result during subsequent testing

[b] Number in parentheses indicates number of participants surveyed during interval

[c] Includes samples collected on Days 1–4 of employment

[d] Excludes samples obtained on Days 1–4 of employment

[e] Excludes workers that had at least one positive BeBLPT upon hire

[f] Total includes one worker that had a borderline result at the time of hire and an initial positive result during the 0–4 month interval

tive based on 1993–1994 survey results (Table 6). Normal BeBLPT results were reported in ten of these individuals (56%) when blood samples were sent to at least two different laboratories in 1999. Data from the new employee program provided additional evidence of reversion from confirmed positive to negative BeBLPT results and suggested that such discrepancies can often be observed during the first year of exposure (Table 4). For example, worker NEP-11 had normal BeBLPT results upon hire and at 3 months of employment. Positive results were subsequently obtained in 8/8 tests performed at two different laboratories after 6–8 months of employment, and the employee was therefore considered to be confirmed BeBLPT positive. However, normal BeBLPT results were observed when the same worker was tested again after 12 and 21 months of employment. Overall, at least one reversion in BeBLPT results (defined by positive-to-negative or positive-to-borderline results) was observed in 100% (9/9) of new employee program participants that underwent follow-up testing after they were previously classified as confirmed BeBLPT-positive following workplace exposure to beryllium at Brush Wellman (Table 4).

Discussion

This analysis of data obtained during the Brush Wellman BeBLPT surveillance program provides valuable insights into the performance characteristics of the BeBLPT that may have important implications with regard to research, industrial practice, and regulation. Some of these findings were made possible only because of the unique features of the program, which included multiple surveys of long-tenured workers as well as testing of new employees prior to occupational exposure to beryllium and repeat testing during the first years of employment.

It was found that the prevalence of confirmed BeBLPT-positive results in beryllium workers was highest during the first year of employment, and a peak in the prevalence of positive BeBLPT results was observed during 4–8 months of employment. Normal BeBLPT results were observed in many workers that were previously determined to be confirmed BeBLPT-positive. Although reversions from positive to normal BeBLPT results have been observed in other studies and surveillance programs, such inconsistencies have typically been attributed to false positive or false negative results (Newman et al. 2001; Stange et al. 2001, 2004; Newman et al. 2005; Middleton 2006). Consequently, the reversibility of the BeBLPT has not been widely acknowledged in the literature. Based on this analysis of data from the Brush Wellman workforce surveys and the new employee program, we hypothesize that intra-individual differences in the number or responsiveness of circulating lymphocytes during various stages of the immune response or other physiological changes may underlie much of the variability associated with use of the BeBLPT over time (Fontenot et al. 2005). If this is the case, the predictive value of the BeBLPT can be expected to be variable and may be influenced by individual or population characteristics (e.g., time worked or exposure conditions), and therefore data from surveys using the BeBLPT may be expected to be inaccurate or incomplete.

Data obtained from the new employee program indicated that the prevalence of confirmed positive BeBLPT results in new employees with no known occupational or possible take-home exposure to beryllium was approximately 1%. This value is comparable to the rate of single positive BLPT or beryllium skin patch test results observed in populations identified as unexposed in previous studies (Shima 1966; Bobka 1997; Stange 2004; Yoshida 1997); however, differences between the testing protocols make direct comparisons between these studies difficult. Additional studies would be helpful to better characterize the background rate of beryllium sensitization in the general population and to determine if rates vary in different geographic areas due to genetic or environmental differences.

Table 6 Individual results from confirmed positive BeBLPT Elmore plant workers that participated in both the 1993–1994 and 1999 general workforce surveys

ID no.	Time of employment		Test result
	Lab #	(Years)	
SUR-1	2	25.3	Negative
	4	25.3	Positive
	2	25.6	Negative
	4	25.6	Positive
	2	26.1	Negative
	2	26.7	Neg/borderline
	4	26.7	Negative
	1	30.8	Negative
	2	30.8	Negative
	1	33.4	Negative
SUR-2	4	13.3	Negative
	2	13.3	Positive
	2	13.4	Positive
	4	14.6	Neg/borderline
	2	14.6	Negative
	4	14.6	Negative
	4	15.2	Negative
	2	15.2	Negative
	1	19.3	Neg/borderline
	2	19.3	Neg/borderline
	1	19.4	Negative
	2	19.4	Negative
	1	20.9	Neg/borderline
	1	21.0	Negative
	1	24.0	Positive
SUR-3	2	26.7	Negative
	4	26.7	Positive
	2	26.9	Neg/borderline
	4	26.9	Tech inadequate
	2	26.9	Positive
	4	26.9	Tech inadequate
	4	28.5	Positive
	2	28.5	Positive
	1	32.2	Negative
	2	32.2	Positive
SUR-4	4	22.9	Negative
	2	22.9	Positive
	4	23.0	Negative
	2	23.0	Negative
	2	23.9	Positive
	1	28.8	Positive
	2	28.8	Positive
	1	34.1	Positive
SUR-5	2	9.6	Negative
	4	9.6	Positive
	2	9.6	Negative
	4	9.6	Tech inadequate
	4	9.7	Positive
	2	10.1	Negative
	2	10.8	Negative
	4	10.8	Negative
	1	14.7	Negative
	2	14.7	Negative
	1	18.5	Negative
SUR-6	4	9.1	Neg/borderline
	2	9.1	Neg/borderline
	2	9.2	Negative
	4	9.2	Positive
	4	10.3	Neg/borderline
	2	10.3	Negative
	2	10.4	Positive
	4	10.4	Positive
	2	14.2	Negative
	1	14.2	Negative
	3	14.7	Tech inadequate
	1	15.2	Negative
SUR-7	4	8.5	Neg/borderline
	2	8.5	Positive
	4	8.5	Neg/borderline
	2	8.5	Neg/borderline
	4	8.6	Neg/borderline
	2	8.6	Negative
	2	8.7	Negative
	4	8.7	Positive
	2	9.0	Negative
	2	10.5	Negative
	4	10.5	Positive
	2	10.7	Negative
	4	10.7	Positive
	2	13.1	Neg/borderline
	1	14.0	Negative
	3	14.0	Negative
	1	14.5	Negative
	2	14.5	Negative
	3	15.8	Positive
SUR-8	4	9.4	Negative
	2	9.4	Positive
	2	9.5	Negative
	4	9.5	Negative
	2	9.6	Neg/borderline
	2	9.7	Negative

Table 6 continued

ID no.	Time of employment		Test result
	Lab #	(Years)	
	2	11.2	Negative
	4	11.2	Positive
	2	11.3	Negative
	4	11.3	Positive
	2	15.3	Negative
	1	15.3	Negative
	1	16.0	Negative
SUR-9	4	34.4	Negative
	2	34.4	Positive
	4	34.5	Positive
	2	34.5	Tech inadequate
	2	35.6	Neg/borderline
	4	35.6	Negative
	2	39.6	Negative
	1	39.6	Positive
	2	39.8	Neg/borderline
SUR-10	2	14.0	Negative
	4	14.0	Positive
	4	14.1	Positive
	2	14.1	Positive
	4	15.7	Positive
	2	15.7	Positive
	2	15.9	Positive
	4	15.9	Positive
	1	19.8	Positive
	2	19.8	Positive
	2	20.0	Positive
	1	21.1	Positive
SUR-11	4	11.0	Negative
	1	11.0	Negative
	2	13.1	Negative
	4	13.1	Positive
	2	13.2	Negative
	4	13.2	Positive
	2	14.1	Negative
	4	14.1	Tech inadequate
	1	14.2	Negative
	4	14.2	Tech inadequate
	1	17.8	Negative
	2	17.8	Negative
SUR-12	2	14.3	Negative
	4	14.3	Positive
	2	14.5	Negative
	4	14.5	Negative
	4	14.6	Negative
	2	16.2	Positive

Table 6 continued

ID no.	Time of employment		Test result
	Lab #	(Years)	
	4	16.2	Positive
	2	16.3	Negative
	4	16.3	Tech inadequate
	2	16.5	Neg/borderline
	4	16.5	Negative
	2	20.0	Neg/borderline
	1	20.0	Positive
SUR-13	2	30.5	Neg/borderline
	4	30.6	Positive
	2	30.7	Negative
	4	30.7	Positive
	2	31.9	Positive
	4	31.9	Positive
	2	32.0	Negative
	4	32.0	Positive
	2	32.1	Negative
	4	32.1	Positive
	2	35.7	Negative
	1	36.1	Neg/borderline
	2	36.1	Negative
	3	36.2	Neg/borderline
	1	36.2	Negative
	3	36.3	Negative
SUR-14	2	28.0	Negative
	4	28.0	Positive
	2	28.1	Negative
	4	28.1	Positive
	2	28.5	Neg/borderline
	2	29.2	Neg/borderline
	4	29.2	Negative
	1	33.2	Negative
	2	33.2	Negative
	1	33.4	Negative
	2	33.4	Tech inadequate
	2	34.1	Negative
SUR-15	4	13.0	Negative
	2	13.0	Positive
	2	13.1	Negative
	4	14.1	Neg/borderline
	2	14.1	Neg/borderline
	4	14.2	Positive
	2	14.2	Tech inadequate
	4	14.3	Negative
	2	14.3	Negative
	2	15.2	Negative
	4	15.2	Negative

Table 6 continued

ID no.	Time of employment		Test result
	Lab #	(Years)	
	1	19.1	Tech inadequate
	2	19.1	Tech inadequate
	2	19.2	Positive
	1	19.5	Negative
	3	19.5	Negative
	1	20.2	Negative
SUR-16	2	6.5	Negative
	4	6.5	Tech inadequate
	4	6.9	Positive
	4	7.0	Negative
	2	7.0	Negative
	4	7.1	Tech inadequate
	4	7.2	Positive
	2	7.7	Negative
	4	8.1	Negative
	2	8.1	Negative
	1	12.3	Negative
	2	12.3	Negative
	1	13.9	Negative
SUR-17	2	5.6	Neg/borderline
	4	5.6	Negative
	4	6.0	Neg/borderline
	2	6.0	Negative
	2	6.2	Neg/borderline
	4	6.2	Negative
	4	6.6	Neg/borderline
	2	6.6	Positive
	2	7.1	Positive
	4	7.7	Negative
	2	7.7	Positive
	2	7.8	Negative
	4	7.8	Negative
	4	7.9	Neg/borderline
	2	7.9	Negative
	4	8.1	Negative
	2	8.1	Negative
	1	10.1	Positive
	2	10.1	Positive
	1	10.3	Positive
	2	10.3	Positive
	1	11.6	Positive
	2	11.6	Positive
SUR-18	2	14.3	Negative
	4	14.3	Positive
	2	14.4	Negative
	4	14.4	Tech inadequate
	4	14.6	Positive
	4	15.8	Neg/borderline
	2	15.8	Negative
	1	19.8	Negative
	2	19.8	Negative
	2	21.0	Neg/borderline
	1	21.0	Positive

Confirmed BeBLPT positive defined as occurrence of two positive BeBLPT results within any time period

Includes data from 1993–1994 and 1999 surveys and associated follow-up testing

The possibility that a substantial fraction of participants may have been erroneously classified as sensitized or non-sensitized in previous studies based on BeBPLT results alone has important implications with regard to the true prevalence of beryllium sensitization in worker or community populations. Such errors would also have the potential to affect the interpretation of studies that serve as the basis for our current understanding of the mechanisms that underlie the development of beryllium sensitization and chronic beryllium disease.

Despite the large number of participants and the long duration of the Brush Wellman BeBLPT surveillance program, and despite the fact that repeat testing, split-sampling, and serial testing protocols were utilized in an attempt to better understand the results, significant gaps remain in our understanding of the true prevalence and natural history of beryllium sensitization in the company's workers, especially as it relates to predicting the likelihood that a given worker will progress to subclinical or clinical chronic beryllium disease. It is important for researchers, industrial hygienists, human resource managers, risk assessors, and regulators to appreciate the limitations of the BeBLPT when interpreting results from occupational surveillance programs or experimental studies that utilized the test. For example, the apparent likelihood that some beryllium-sensitized individuals will be misclassified based on the results from BeBLPT screening studies may reduce the effectiveness of protection programs that rely on worker-specific intervention mechanisms (i.e., removing or reassigning sensitized workers to areas with no potential exposure to beryllium). Although, the BeBLPT can be used to help characterize beryllium sensitization in worker populations, it does not appear to be a reliable indicator of sensitization in individual workers, especially when it is administered in the context of a screening study. The

American Conference of Governmental Industrial Hygienists Biological Exposures Indices Committee has assessed the BeBLPT and did not recommend its use as a biological indicator to assess exposure and health risk to workers (ACGIH 2002, 2005).

The findings from our analysis of the Brush Wellman BeBLPT database suggest that improvements in the methods used to identify sensitized individuals will be required in order to properly evaluate the potential risk that sensitization poses to beryllium workers or to accurately determine the rate of beryllium sensitization in non-occupationally exposed populations. Future research evaluating new methods based on enzyme-linked immunoassays or other techniques may be more reproducible and well suited for use in worker surveillance programs (Pott et al. 2005). Such alternative assays or genetic markers may also have the potential to differentiate sensitized workers from those with chronic beryllium disease or to identify sensitized workers who are more likely to progress to subclinical or clinical chronic beryllium disease (Fontenot and Maier 2005).

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References

Agency for Toxic Substances and Disease Registry (ATSDR) (2002) Toxicological profile for beryllium (PB2003–100135). US Department of Health and Human Services, Public Health Service. Atlanta, GA

American Conference of Governmental Industrial Hygienists (ACGIH) (2002) Biological exposure index feasibility assessment for beryllium and inorganic compounds. Cincinnati, OH

American Conference of Governmental Industrial Hygienists (ACGIH) (2005) ACGIH TLV-BEI booklet. Cincinnati, OH

Bobka CA, Stewart LA, Engelken GJ, Golitz LE, Newman LS (1997) Comparison of in vivo and in vitro measures of beryllium sensitization. J Occup Environ Med 39:540–547

Borak J (2006) The beryllium occupational exposure limit: historical origin and current inadequacy. J Occup Environ Med 48(2):109–116

Curtis GH (1951) Cutaneous hypersensitivity due to beryllium; a study of thirteen cases. AMA Arch Derm Syphilol 64:470–482

Curtis GH (1959) The diagnosis of beryllium disease, with special reference to the patch test. AMA Arch Ind Health 19:150–153

Deubner D, Kelsh M, Shum M, Maier L, Kent M, Lau E (2001a) Beryllium sensitization, chronic beryllium disease, and exposures at a beryllium mining and extraction facility. Appl Occup Environ Hyg 16:579–592

Deubner DC, Goodman M, Iannuzzi J (2001b) Variability, predictive value, and uses of the beryllium blood lymphocyte proliferation test (BLPT): preliminary analysis of the ongoing workforce survey. Appl Occup Environ Hyg 16:521–526

Fontenot AP, Maier LA (2005) Genetic susceptibility and immune-mediated destruction in beryllium-induced disease. Trends Immunol 26:543–549

Fontenot AP, Maier LA, Canavera SJ, Hendry-Hofer TB, Boguniewicz M, Barker EA, Newman LS, Kotzin BL (2002) Beryllium skin patch testing to analyze T cell stimulation and granulomatous inflammation in the lung. J Immunol 168:3627–3634

Fontenot AP, Palmer BE, Sullivan AK, Joslin FG, Wilson CC, Maier LA, Newman LS, Kotzin BL (2005) Frequency of beryllium-specific, central memory CD4+ T cells in blood determines proliferative response. J Clin Invest 115:2886–2893

Hanifin JM, Epstein WL, Cline MJ (1970) In vitro studies on granulomatous hypersensitivity to beryllium. J Invest Dermatol 55:284–288

Henneberger PK, Cumro D, Deubner DD, Kent MS, McCawley M, Kreiss K (2001) Beryllium sensitization and disease among long-term and short-term workers in a beryllium ceramics plant. Int Arch Occup Environ Health 74:167–176

Johnson JS, Foote K, McClean M, Cogbill G (2001) Beryllium Exposure Control Program at the Cardiff Atomic Weapons Establishment in the United Kingdom. Appl Occup Environ Hyg 16:619–630

Kelleher PC, Martyny JW, Mroz MM, Maier LA, Ruttenber AJ, Young DA, Newman LS (2001) Beryllium particulate exposure and disease relations in a beryllium machining plant. J Occup Environ Med 43:238–249

Kolanz ME (2001) Introduction to beryllium: uses, regulatory history, and disease. Appl Occup Environ Hyg 16:559–567

Kreiss K, Newman LS, Mroz MM, Campbell PA (1989) Screening blood test identifies subclinical beryllium disease. J Occup Med 31:603–608

Kreiss K, Mroz MM, Zhen B, Martyny JW, Newman LS (1993) Epidemiology of beryllium sensitization and disease in nuclear workers. Am Rev Respir Dis 148:985–991

Kreiss K, Mroz MM, Newman LS, Martyny J, Zhen B (1996) Machining risk of beryllium disease and sensitization with median exposures below 2 micrograms/m3. Am J Ind Med 30:16–25

Kreiss K, Mroz MM, Zhen B, Wiedemann H, Barna B (1997) Risks of beryllium disease related to work processes at a metal, alloy, and oxide production plant. Occup Environ Med 54:605–612

Maier LA (2001) Beryllium health effects in the era of the beryllium lymphocyte proliferation test. Appl Occup Environ Hyg 16:514–520

Middleton DC, Lewin MD, Kowalski PJ, Cox SS, Kleinbaum D (2006) The BeLPT: algorithms and implications. Am J Ind Med 49(1):36–44

Newman LS, Mroz MM, Maier LA, Daniloff EM, Balkissoon R (2001) Efficacy of serial medical surveillance for chronic beryllium disease in a beryllium machining plant. J Occup Environ Med 43:231–237

Newman LS, Mroz MM, Balkissoon R, Maier LA (2005) Beryllium sensitization progresses to chronic beryllium disease: a longitudinal study of disease risk. Am J Respir Crit Care Med 171:54–60

Pott GB, Palmer BE, Sullivan AK, Silviera L, Maier LA, Newman LS, Kotzin BL, Fontenot AP (2005) Frequency of beryllium-specific, TH1-type cytokine-expressing CD4+ T cells in patients with beryllium-induced disease. J Allergy Clin Immunol 115:1036–1042

Saltini C, Amicosante M (2001) Beryllium disease. Am J Med Sci 321:89–98

Schuler CR, Kent MS, Deubner DC, Berakis MT, McCawley M, Henneberger PK, Rossman MD, Kreiss K (2005) Process-related risk of beryllium sensitization and disease in a copper-beryllium alloy facility. Am J Ind Med 47:195–205

Shima S (1966) Beryllium hazards and the sensitization phenomenon due to beryllium exposure, with special reference to the patch test.

In: Proceedings of the 15th international congress on occupational health, Vienna

Stange AW, Hilmas DE, Furman FJ, Gatliffe TR (2001) Beryllium sensitization and chronic beryllium disease at a former nuclear weapons facility. Appl Occup Environ Hyg 16:405–417

Stange AW, Furman FJ, Hilmas DE (2004) The beryllium lymphocyte proliferation test: Relevant issues in beryllium health surveillance. Am J Ind Med 46:453–462

Sterner JH, Eisenbud M (1951) Epidemiology of beryllium intoxication. AMA Arch Ind Hyg Occup Med 4:123–151

US Department of Energy (DOE) (2001) Beryllium lymphocyte proliferation testing. Washington, DC

Waksman BH (1959) The diagnosis of beryllium disease, with special reference to the patch test; discussion of paper by Dr. Curtis. AMA Arch Ind Health 19:154–156

Yoshida T, Shima S, Nagaoka K, Taniwaki H, Wada A, Kurita H, Morita K (1997) A study on the beryllium lymphocyte transformation test and the beryllium levels in working environment. Ind Health 35:374–379