

# Significance of the Blood Beryllium Lymphocyte Proliferation Test

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The blood beryllium lymphocyte proliferation test (BeLPT) is an *in vitro* measure of the beryllium antigen-specific cell-mediated immune response. This response to beryllium is now understood to play a central role in the immunopathogenesis of chronic beryllium disease (CBD). Although there remain some unresolved methodologic issues with testing, the blood BeLPT has already undergone sufficient development and field assessment to lead to a number of important conclusions: a) The BeLPT identifies beryllium sensitization and CBD earlier and better than any other clinical test presently available. b) The CBD cases identified with the blood test are clinically significant. c) A subset of the people identified by the BeLPT who do not yet have clinical disease will progress and require treatment with corticosteroids for impairing illness. d) The BeLPT can be used to improve clinical diagnostic accuracy and to correct mistaken diagnoses. e) The blood test can be used in screening large numbers of exposed workers because it is sensitive and specific and has high positive and negative predictive value for CBD. f) In every workforce studied to date, the BeLPT has identified beryllium sensitization and CBD that had been missed by conventional screening efforts. g) Worker populations that have been characterized using the BeLPT can help to elucidate the role of exposure genetics and dysregulated inflammation in the genesis of occupational lung disease. — *Environ Health Perspect* 104(Suppl 5):953–956 (1996)

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## Introduction

The first clinical demonstration of the immunologic basis of chronic beryllium disease (CBD) occurred in the 1950s when researchers showed that beryllium salts could elicit a typical delayed-type hypersensitivity reaction (1,2). While application of the beryllium fluoride patch test showed that it was possible to identify individuals who were sensitized to beryllium and who had CBD, skin testing has not been widely used in the United States since the 1960s because of concern that such testing could either exacerbate disease or induce sensitization (3). Patch testing is

both time consuming and impractical as a screening test, and has since been supplanted by other methods of detecting the cell-mediated immune response to beryllium.

In 1970, Hanifin and colleagues demonstrated that blood cells obtained from patients who had positive beryllium patch tests would proliferate *in vitro* when stimulated with beryllium sulfate or oxide (4). Their work set the stage for the next 25 years of research into the utility of *in vitro* assays for detecting CBD. Other investigators verified their observation of a cell-mediated immune response to beryllium

both in the blood and in cells retrieved from the lung via bronchoalveolar lavage (5–15). The lymphocyte response to beryllium in these cell cultures is specific for beryllium salts and specific for CBD, forming the basis for use of lymphocyte proliferation as a clinical tool, as discussed below (12,14,15). The immunopathogenesis and the role of cell-mediated immunity in CBD have been the subjects of two recent reviews (16,17).

CBD illustrates how our increased understanding of immunology can lead to clinical application. As shown in Table 1, immunologic assessment of beryllium sensitization provides diagnostic information, serves as a biomarker for screening large numbers of exposed workers for disease, and has made it possible to examine the role of occupational exposure and of genetic predisposition in the development of occupational disease. Blood lymphocyte proliferation testing for beryllium sensitization has revolutionized both the clinical and research approaches to CBD in the last decade.

## Development of Methods

Although the first *in vitro* observations of beryllium-specific cell proliferation were made in the 1970s, it was not until the early 1980s that the first studies were done to test whether the blood BeLPT could be used as a clinical diagnostic tool (7,8,10). Initial studies from the United Kingdom demonstrated that while the blood test could detect individuals with beryllium sensitization and disease, it was too insensitive and too difficult to reproduce for clinical use. The test fell out of favor until our group reevaluated the test parameters, made additional efforts to optimize culture conditions, and then field tested the BeLPT at a nuclear weapons plant (13,15,18). We demonstrated that by further optimizing assay conditions using a radiolabeled DNA precursor (tritiated thymidine) as the marker of proliferation, the blood test can be made more easily reproducible and validated as a superior means of detecting disease. The assay, as used today, relies on mononuclear cells isolated from heparinized venous blood. The cells are placed in primary culture, in the presence and absence of beryllium sulfate, across a three-log range of salt concentrations. Cell proliferation is measured by the incorporation of the tritiated thymidine into the dividing cells after 3, 5, and 7 days in culture. Cells are harvested from microtiter plates and

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Abbreviations used: CBD, chronic beryllium disease; BeLPT, beryllium lymphocyte proliferation test; BAL, bronchoalveolar lavage.

**Table 1.** Significance of the blood beryllium lymphocyte proliferation test.

Type of assessment	Function	Potential advantages
Diagnosis	Screen clinical cases for CBD Correct mistaken diagnoses of sarcoidosis and interstitial lung disease Improve diagnostic accuracy over the available tests Identify individual cases of CBD as "sentinel health events"	Recognition of disease in new segments of the beryllium industry
Workplace screening	Improve sensitivity over other available screening tools (e.g., chest X-ray, spirometry, symptom reporting, physical examination)	High positive and negative predictive value
	Improve specificity over other available tests	
	Early detection of beryllium sensitization (sensitized individuals develop CBD)	Possibility for early intervention
	Early detection of CBD (subclinical cases progress to clinical CBD)	Possibility for early intervention
Research	Identify individuals with clinically active CBD who have been misdiagnosed and incorrectly treated	
	Targeting engineering control efforts	Reduce exposure of beryllium workers and others
	Epidemiologic investigations	Identify high-risk work tasks, high-risk exposures, and personal risk factors in the workplace
	Immunogenetic investigations	Population-based examination of genetic risk factors and their association with exposure
	Development of new biomarkers of disease risk, activity, severity, or response to therapy	
	Facilitate <i>in vitro</i> studies of immunologic and inflammatory mechanisms (e.g., T-cell and macrophage cytokine release and dysregulation)	

the amount of radiolabel that has entered the cells measured in a liquid scintillation counter. Results are expressed as a "stimulation index": the ratio of the counts per minute of radioactivity in cells stimulated by beryllium salts divided by the counts per minute for unstimulated cells. Each laboratory sets its own normal range for the test, based on data from normal nonexposed control subjects whose blood has been tested by the same method.

In reworking the BeLPT, we showed that the blood test is reproducible between laboratories, that it can identify 94% of patients who had abnormal bronchoalveolar (BAL) BeLPT plus granulomatous lung pathology (CBD), and that it reliably identifies patients with CBD at very early stages of disease or when they are sensitized and on their way to developing clinical disease (15,19). Subsequent work with the test suggests that it detects somewhat less than 94% of cases. Further efforts to standardize the assay have been undertaken by several laboratories, with varying success.

The BeLPT, like other cell culture assays, is associated with significant intra-test, intertest, and interlaboratory variability. The BeLPT's "growing pains" are in

evidence at this conference, as industry has encouraged multiple laboratories to quickly increase the volume of testing at multiple centers. Laboratories continue to differ in their ability to identify cases of CBD because of variability in serum, culture conditions, methods of cell harvesting, radiolabel counting, and data analysis; and lack of consistent positive control in each assay. Thus, it is advisable that prior to launching a clinically invasive evaluation for CBD based on an abnormal blood BeLPT, the positive test should be reconfirmed with a second venipuncture sample (16). The technical challenges of the blood BeLPT do not negate the observations that the test detects beryllium sensitization and CBD at earlier stages than any other clinical test available (20) and has proven validity, having been used to detect clinically significant lung disease, as discussed below.

### Blood BeLPT in Diagnosis

Several case series have demonstrated that the BeLPT, as an indicator of beryllium-specific cellular immune response, is abnormal in patients with CBD but not elevated in normal subjects, beryllium-exposed normal subjects, beryllium-exposed subjects

with other (nongranulomatous) lung diseases, and patients with no beryllium exposure but who have granulomatous diseases such as sarcoidosis and hypersensitivity pneumonitis (9,11,12,15). Therefore, the BeLPT can be used to help diagnose CBD in patients who have compatible lung pathology, helping to differentiate it from similar-appearing lung diseases. This assay has become part of the standard clinical repertoire in CBD diagnosis (16,18). Although the test is performed reliably in only a few centers in the United States, it can be obtained by shipping blood via overnight carrier. If handled properly, blood can be tested up to 24 hours after venipuncture.

It has been observed that the level of beryllium stimulation of blood cells is lower than that seen in BAL cells in many patients with CBD (9,12,21). This is of little significance except that it reflects the difference in the number of beryllium-reactive lymphocytes present in a blood specimen compared with the pulmonary compartment (21). The blood test is less invasive, more practical, nearly as predictive of beryllium-related lung granulomas as BAL BeLPT, and better standardized (15,16). Therefore, individuals with suspected CBD should first be screened with the blood test. Under certain circumstances, BAL BeLPT can give false negative results, such as when there is a marked excess of alveolar macrophages in the lavage, e.g., in cigarette smokers. Blood can be readily shipped in heparinized tubes to one of the laboratories that performs the BeLPT, whereas BAL must be centrifuged and the cells resuspended in complete culture medium prior to shipping. Under some circumstances, such as when there is an abnormal blood BeLPT and typical clinical, radiographic, and physiologic findings, it may be reasonable to diagnose CBD even without biopsy. Some individuals with CBD may have negative blood responses, so in instances in which the index of suspicion is high but the blood BeLPT is normal, BAL and transbronchial lung biopsies should be performed to determine if there is reactivity to beryllium by lung lymphocytes and compatible histopathology.

When should clinicians order the BeLPT? Based on the epidemiologic studies of exposed work forces and on clinical case series, the diagnosis should be considered in any individual with either direct or bystander exposure to beryllium. This includes secretaries, maintenance workers, security guards, spouses of beryllium

workers, and others with seemingly trivial exposure (13,15,18–20,22). CBD can masquerade as other lung diseases, including sarcoidosis (23), pulmonary fibrosis, hypersensitivity pneumonitis, and asthma. Thus, the diagnosis should be considered and the BeLPT drawn in any patient with lung disease who has had any direct or indirect contact with beryllium, as elicited in the occupational or environmental history. Physicians should not prejudge the significance of past beryllium exposure (20,22,23). An abnormal blood BeLPT proves that the exposure was sufficient to cause the body's immune system to recognize beryllium as antigenic. The diagnosis may be missed if the physician assumes that the exposure was "too low" to cause disease, assumes that exposure was too remote, or fails to take a sufficiently detailed occupational or environmental history of exposure.

### Blood BeLPT in Screening for Sensitization and Disease

The BeLPT was assessed in the 1970s as a potential screening test, but at that time the read-out was highly subjective, based on the morphologic appearance of "lymphoblasts" in culture (6,24). Reproducibility of that method was poor and workers with abnormal blood tests did not show spirometric and radiologic abnormalities suggestive of CBD, leading the investigators to discard it as a screening test (24). We now know that spirometry and chest X-ray were insensitive tools by which to gauge the ability of the blood test to identify CBD (13).

Following methodologic improvements in the assay, the BeLPT was given a fresh chance in four population-based studies conducted over the past 9 years. Additional studies are on-going. Since 1986, the use of the blood BeLPT has been reported in screening populations of workers from the nuclear weapons, ceramics, and foundry industries (11,13,20,22). In a cohort of beryllium ceramics workers, confirmed abnormal blood BeLPTs predicted granulomatous lung disease on biopsy in every case. In the nuclear industry, not all workers with confirmed abnormal tests had beryllium disease on biopsy at the time of screening. One-third of those with abnormal blood tests had no granulomas on biopsy initially, but half of this "sensitized-only" group developed granulomas on biopsy during a short, 2-year follow-up period. We conclude that the blood BeLPT can identify individuals with CBD

and also can identify beryllium-sensitized individuals who do not have disease but who are at high risk of developing CBD. Longitudinal follow-up of these groups is in progress (25).

While our initial BeLPT studies of the active workforce at the nuclear weapons plant identified many individuals with so-called "subclinical" or early-stage CBD, later, more detailed examination of the pulmonary physiology in these patients showed that most of them had one or more abnormalities of lung function and gas exchange even if they were only minimally symptomatic (26). Moreover, since the nuclear weapons plant started to use the BeLPT to screen retired workers, many cases of symptomatic, physiologically impaired but often misdiagnosed cases of CBD have been identified. The proportion of clinical to subclinical cases may reflect, in part, the degree to which other screening tests have been applied. We conclude that any individual with confirmed abnormal blood BeLPT warrants close examination for evidence of early disease, that all pulmonary diagnoses in these patients should be carefully reexamined because of the risk of misdiagnosis, and that patients with abnormal blood tests should remain under medical surveillance due to the risk of progression to clinical illness.

The cross-sectional workforce studies demonstrate that the BeLPT has higher positive and negative predictive value for CBD than other tests conventionally used in screening beryllium workers, including clinical examination, symptom reporting on questionnaires, spirometry, and chest radiography (20). These studies also show that the BeLPT is not perfectly sensitive, although it is highly specific. In both ceramics and nuclear worker cohorts we identified cases of CBD among some individuals with abnormal chest radiographs who had normal blood BeLPTs. A negative blood test does not fully exclude CBD. Recent evidence suggests that exposed workforces should undergo periodic rescreening with the BeLPT because additional cases will be detected. This may reflect either the imperfections in the BeLPT or newly emerging sensitization due to on-going exposure, changes in host immunity, or other factors affecting immune responsiveness.

### The BeLPT as a Catalyst for Research on Exposure, Mechanism, and Prevention

The merit of a biomarker of disease hinges on whether the consequence being

identified results in the prevention of disease. The screening of large numbers of beryllium-exposed individuals may lead to secondary and tertiary prevention. Patients who are sensitized or who have CBD can receive earlier treatment and may be less likely to progress to end-stage fibrosis, cor pulmonale, and death. Preliminary evidence from an on-going longitudinal study of these patients indicates that earlier intervention with corticosteroids helps reduce morbidity and may prevent progression to end-stage disease (25). Confirmation of this hypothesis merits formal study.

The use of the blood BeLPT in population-based studies has opened the door to two fruitful areas of investigation that may lead to primary prevention: studies of the relation between exposure and disease and studies of the relation of genetics to exposure. Such research avenues could not have been pursued effectively until large numbers of workers were screened with the BeLPT. Knowing who is sensitized and diseased has enabled us to begin to examine three important questions: *a*) What is different about beryllium exposure for those who develop sensitization and disease than for those who do not? *b*) Are there genetic differences in those who develop sensitization and CBD compared with those who do not? *c*) What is the interaction between exposure and genetics that results in sensitization and CBD? These questions go beyond CBD, and are important for our broader understanding of how environmental and host factors interact in producing disease (27). Several conclusions can be drawn thus far from these studies. First, individuals who are detected by the BeLPT differ from other workers in their job titles, tasks, and dates of beryllium exposure in beryllium industries. There is an exposure–disease relationship (20,22). Second, individuals who develop sensitization and disease share one or more genetic risk factors for this condition (16,28). Third, as collaborative work between groups in Denver and Modena, Italy shows, beryllium sensitization and disease are the result of an interaction between the nature of the beryllium exposure and an individual's inheritance.

How should such information be used in prevention? Even though CBD is a result of a hypersensitivity to beryllium, controlling exposure can make a difference. Armed with information about the types of jobs and exposures that result in disease, a company can institute engineering controls that reduce exposures for all workers,

especially targeting work conditions that have been identified as hotspots for CBD in the plant. Will the genetic data allow us to screen for susceptible workers? It is unlikely that such genetic tests will prove useful at this stage in our understanding.

Such tests are not warranted if exposures can be adequately controlled. Future research centered on how to reduce the rate of sensitization and disease in industry through environmental control is still the best path to prevention of new cases of

CBD. Medical surveillance with the blood BeLPT can guide us in assessing the efficacy of these interventions and of other preventive strategies.

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