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Optimizing BeLPT Criteria for Beryllium Sensitization

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Background *The beryllium lymphocyte proliferation test (BeLPT) is used to identify persons sensitized to beryllium. ATSDR convened an expert panel of physicians and scientists in April 2006 to discuss this test and to consider what BeLPT test results actually establish beryllium sensitization. The three criteria proposed by panel members were*
(1) one abnormal result,
(2) one abnormal and one borderline result, and
(3) two abnormal results.
Methods *Complete algorithms were developed for each of the three proposed criteria. Using single-test outcome probabilities developed by Stange et al. [2004. Am J Ind Med 46:453–462], we calculated and compared the sensitivity, specificity, and positive predictive values (PPVs) for each set of criteria.*
Results *The overall sensitivity and specificity of the three criteria were similar. When the criteria required confirmation of an abnormal result the PPV was higher—whether the requirement was satisfied by a borderline result, or only by another abnormal result. Confirmation also reduced the likelihood of false positives. The differences between the three criteria decreased as the prevalence of sensitization increased.*
Conclusions *A single unconfirmed abnormal is usually insufficient to establish sensitization for an apparently healthy person. When the prevalence of beryllium sensitization in a group is high, however, even a single abnormal BeLPT can be a strong predictor.* Am. J. Ind. Med. 51:166–172, 2008. *© 2008 Wiley-Liss, Inc.*

KEY WORDS: beryllium; proliferation; sarcoidosis; sensitization; chronic beryllium disease; sensitivity; specificity; positive predictive value; algorithm; berylliosis; beryllium lymphocyte proliferation test (BeLPT)

INTRODUCTION

The beryllium lymphocyte proliferation test (BeLPT) is used for screening and surveillance of persons exposed to beryllium. Although the BeLPT also distinguishes between chronic beryllium disease (CBD) and sarcoidosis [Middleton, 1998; Müller-Quernheim et al., 2006], this article does not address the BeLPT's clinical uses.

Despite the lack of a ''gold standard'' for the BeLPT, an article by Stange et al. [2004] has provided credible estimates for various single test characteristics. Middleton et al. [2006] applied this information to calculate the epidemiologic parameters for common BeLPT screening algorithms.

While abnormal test results suggest beryllium sensitization (BeS) and an increased risk for CBD, the specific criteria for establishing beryllium sensitization vary [Deubner et al., 2001; Welch et al., 2004]. On April 25, 2006, in Oak Harbor, Ohio, the Agency for Toxic Substances and Disease Registry (ATSDR) convened an expert panel to discuss the BeLPT. The panel included physicians associated with local and

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federal government, with environmental activism, and with the beryllium industry. A summary report of the expert panel's comments is available at http://www.atsdr.cdc.gov/sites/brushwellman/docs/experts_panel.pdf.

Panel members were asked: *What BeLPT results establish beryllium sensitization?*

Panel members suggested three different criteria for establishing Be sensitization:

Criteria A—one abnormal BeLPT result,
Criteria B—one abnormal BeLPT result *and* one borderline (or abnormal) BeLPT result, or,
Criteria C—two abnormal BeLPT results.

GOALS AND OBJECTIVES

The goal is to compare and contrast the performance of these three criteria for beryllium sensitization to address the question, ''What BeLPT results establish beryllium sensitization?'' For each criteria proposed, we develop a complete algorithm and calculate the associated sensitivity, specificity, and positive predictive values (PPVs) for testing exposed and apparently healthy persons.

METHODS

The probabilities of a single BeLPT being *abnormal* (P_{AB}), *normal* (P_{NL}), or *borderline* (P_{BL}) are based on serial test results (1992–2001) for 7,820 current and former employees at the Rocky Flats Environmental Technology Site (RFES) [Stange et al., 2004]. Participants with an abnormal BeLPT result were categorized as ''sensitized'' if a concurrent or subsequent BeLPT confirmed the result. These confirmed results were considered indicative of the participant's true sensitization status; the probabilities of specific individual test results were then calculated for persons truly sensitized and for persons *not* truly sensitized [Stange et al., 2004; Middleton et al., 2006].

We have not referred to single test probabilities as sensitivity, specificity, or PPV, preferring to reserve these terms for the three sensitization *criteria*. *Sensitivity* refers to the likelihood that a set of criteria will correctly identify persons who are truly sensitized to beryllium. *Specificity* refers to the likelihood that criteria for beryllium sensitization will correctly identify persons who are not truly sensitized to beryllium. *PPV* refers to the likelihood that a person who meets the criteria is truly sensitized to beryllium. Unlike sensitivity and specificity, the PPV varies with the prevalence of beryllium sensitization in the population tested.

The BeLPT

To perform the BeLPT, T-lymphocytes are incubated at three concentrations of beryllium sulfate over two different time periods [Stange et al., 2004]. When beryllium is present, sensitized lymphocytes are stimulated to take up tritiated thymidine, thereby increasing the radioactivity of the incubations. Six ratios are generated by comparing the radioactivity of incubations with beryllium sulfate to that of incubations without beryllium sulfate. If none of the six ratios are elevated, the test is considered *normal*. One elevated ratio is considered a *borderline* abnormal result, and two or more elevated ratios are considered an *abnormal* test result. If the blood sample the laboratory receives is not adequate for testing, the test is considered *indeterminate*. *Indeterminate* test results are not meaningful results; the test is simply repeated with a new blood sample, regardless of the criteria for sensitization.

Traditionally, initial testing with the BeLPT is done by sending a single blood sample to a single laboratory. This is not the most sensitive approach; Middleton et al. [2006] showed that splitting the initial sample between two laboratories could enhance the sensitivity of testing. Still, the single initial sample remains more common than a split initial sample. This is probably because the single-sample test has been standard practice in occupational settings, the test is expensive, and similar outcomes can be achieved by testing periodically over time. Because the single initial blood sample is still common practice, each of the three algorithms presented here begins with a single BeLPT.

To facilitate discussion, we have chosen to refer to normal, borderline, and abnormal BeLPT test results (respectively) simply as *normals*, *borderlines*, and *abnormals*. A *normal* is interpreted as an indication that the person is not currently sensitized to beryllium. Hence, no further tests are advised for that person during that screening effort. Exposed persons may nonetheless decide to participate in periodic screenings.

An *abnormal* can be used either to establish sensitization for the person tested or to prompt additional testing for confirmation. If additional testing occurs, the blood specimen is split and sent to two laboratories for testing.

A *borderline* can be interpreted as either an indication to repeat the test, or as a final result. When seen as a final result, *borderlines* are treated much like *abnormals*; that is, an initial *borderline* prompts the collection of a second blood sample to split for confirmatory testing.

The probabilities of the single test outcomes for persons truly sensitized to beryllium are

(1) $P_{AB} = 0.5970$;
(2) $P_{NL} = 0.2770$; and
(3) $P_{BL} = 0.1260$.

Among 19,396 total tests, there were approximately 970 tests performed on persons found to be sensitized [Stange et al., 2004]. Therefore, there were 18,426 tests performed on

persons not truly sensitized. Among these 18,426 tests on persons not truly sensitized, there were 291 BL; that is, $P_{BL} = 291/18{,}426 = 0.0158$. This figure (0.0158) is slightly more accurate than the figure reported in the Middleton et al. [2006] article (0.0154), though the difference (0.0004) is trivial. The probabilities, then, of the single test outcomes for persons *not* truly sensitized to beryllium are

(1) $P_{AB} = 0.0109$;
(2) $P_{NL} = 0.9733$; and
(3) $P_{BL} = 0.0158$.

Criteria A (see Fig. 1) requires one *abnormal* BeLPT result to establish sensitization. If the initial test is *abnormal*, sensitization is established. If the initial test yields a *borderline*, *it* is not considered a final result; the test is repeated until a *normal* or an *abnormal* is obtained.

Criteria B (see Fig. 2) requires one *abnormal* plus one *borderline (or abnormal)* to establish sensitization. If the initial test result is *abnormal* or *borderline*, an additional blood sample is collected, split, and sent to two laboratories for testing.

Criteria C (see Fig. 3) requires two *abnormals* to establish sensitization. If the initial test is *abnormal*, a second blood sample is split between two laboratories for confirmatory testing. If the initial test result is *borderline*, it is repeated until a *normal* or an *abnormal* is obtained. If an *abnormal* is obtained, a second specimen is collected and sent to two separate laboratories for testing.

Approach to Analyses

The combinations of results that lead to establishing sensitization are developed for each criteria. The single test probabilities for persons truly sensitized are used to calculate *sensitivity* for the respective criteria. The single test probabilities for persons *not* truly sensitized are used to calculate the proportion of false positives (FP). Specificity is the complement of this proportion (i.e., Specificity = 1 − FP).

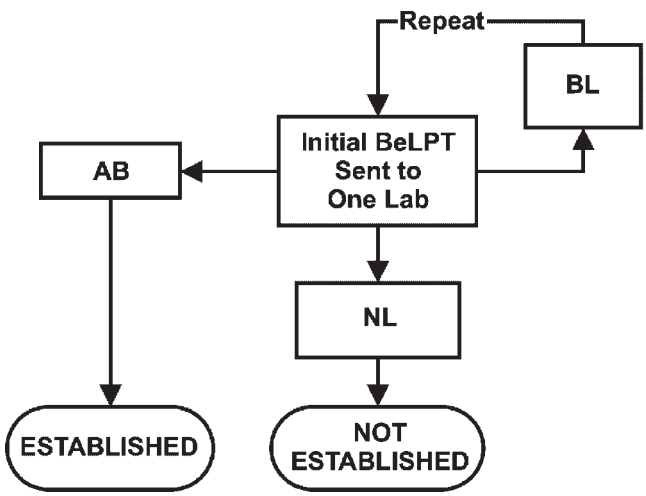

FIGURE 1. One abnormal BeLPT establishes sensitization.

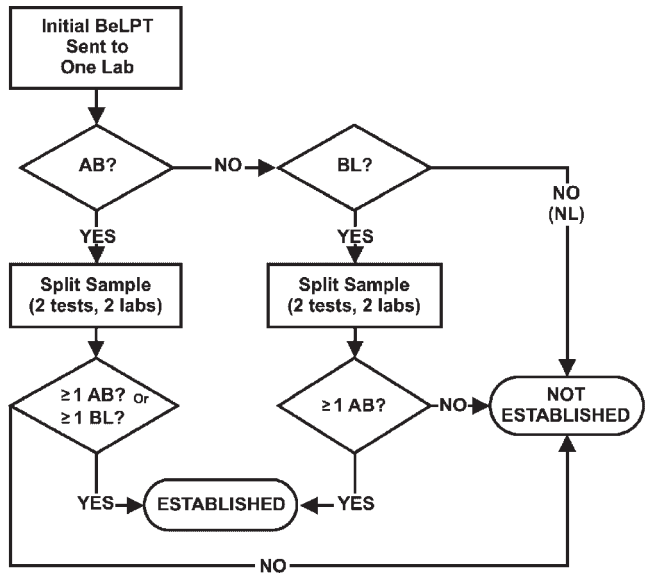

FIGURE 2. One abnormal and one borderline establish sensitization.

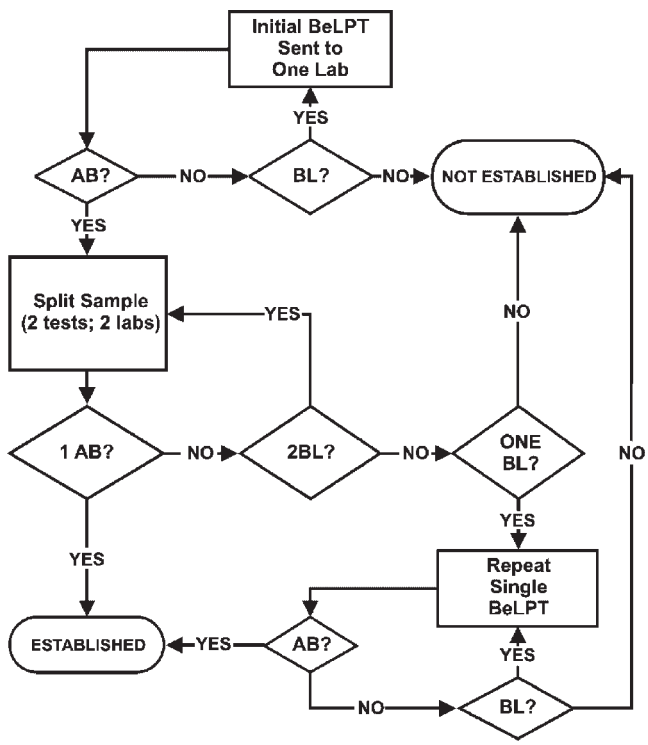

FIGURE 3. Two abnormal BeLPTs establish sensitization.

The PPV and negative predictive values (NPVs) are calculated as follows [Fleiss et al., 2003]:

$$\text{PPV} = \frac{\text{Sensitivity} \times \text{Prevalence}}{\text{Sensitivity} \times \text{Prevalence} + (1 - \text{Specificity})(1 - \text{Prevalence})}$$

$$\text{NPV} = \frac{\text{Specificity} \times (1 - \text{Prevalence})}{(1 - \text{Sensitivity}) \times \text{Prevalence} + \text{Specificity} \times (1 - \text{Prevalence})}$$

RESULTS

Referring to Table I, the likelihood that the first test will be *borderline* and then the second will be *abnormal* is $0.126 \times 0.597 = 0.075$. The likelihood of a *borderline*, a *borderline*, and then an *abnormal* is $0.126 \times 0.126 \times 0.597 = 0.009$. The likelihood of three *borderlines* followed by an *abnormal* (not shown) is only 0.001. So, while the "*borderline–retest–borderline–retest*" loop could continue indefinitely, it becomes trivial as a probability—more than two or three borderline-retest cycles in a row would be rare. The sensitivity of Criteria A was calculated to be 68.2%.

The totals for each combination of BeLPT results in Table I (i.e., the rows) for persons who are not truly sensitized are then summed to determine the false positive rate of 0.0111, or 1.11%. The specificity of Criteria A for establishing sensitization is calculated as follows:

$$\text{Specificity} = 1 - \text{FP} = 1 - 0.0111 = 0.9889, \text{ or } \sim 98.9\%$$

The PPV for Criteria A at 2% prevalence is calculated as follows:

$$\text{PPV}_\text{A} = \frac{0.682 \times 0.02}{0.682 \times 0.02 + 0.0111 \times 0.98} \sim 55.6\%$$

Summary Results

Tables II and III parallel Table I and provide similar information for Criteria B and C. Table IV provides a summary of results for all three criteria. The sensitivities for Criteria A, B, and C (respectively) are 68.2%, 65.7%, and 61.2%. If 10,000 persons who are *not* truly sensitized are tested, Criteria A, B, and C are expected to produce (respectively) 111 FP, 8 FP, and 2 FP; the corresponding specificities (respectively) are 98.89%, 99.92%, and 99.98%.

PPVs vary with the prevalence of sensitization (Table IV); the PPVs for Criteria A, B, and C (respectively) are each calculated for prevalences from 1% (0.383, 0.893, 0.968) to 10% (0.872, 0.989, 0.997). Negative predictive values also vary with the prevalence of sensitization, but remain high (>95%) for all three criteria as prevalences vary from 1% to 10%.

DISCUSSION

Sensitivity and specificity were similar for the three criteria. Not surprisingly, the PPV improved with confirmation, whether by an *abnormal* and a *borderline* or by *two abnormals*. Confirmation helps to ensure that an individual determination of beryllium sensitization is correct, especially in populations with lower prevalences of true beryllium sensitization (e.g., 5% or less). Differences in criteria performance are also evident in the rates of false positives, which are lower with confirmation. Our findings overall support the common medical practice of requiring confirmation before identifying a person as sensitized to beryllium. We do recognize that in some settings, higher exposures have produced prevalences of beryllium sensitization of 10% or higher [Kreiss et al., 2007]. As the prevalence rises, the PPV of a single abnormal BeLPT rises dramatically and the differences among the PPVs for different criteria become smaller (Fig. 4). In other words, confirmation is less important when the prevalence of beryllium sensitization is high.

The single test results used for these analyses are based on data from the four laboratories in the United States that currently perform the BeLPT. These parameters are not

TABLE I. Likelihood of Meeting Sensitization Criteria of One Abnormal BeLPT, by True Sensitization Status

BeLPT results that meet the criteria			Probability calculations[a]	Likelihood of meeting the criteria	
Blood samples					
1	2	3	$P_1 \times P_2 \times P_3$	Truly sensitized	Not truly sensitized
AB	—	—	P_{AB}	0.5970	0.0109
BL	AB	—	$P_{BL} \times P_{AB}$	0.0752	0.0002
	BL	AB	$P_{BL} \times P_{BL} \times P_{AB}$	0.0095	0.0000
Overall likelihood of meeting the criteria. . .				0.6817	0.0111

Based on the true status of sensitization, the single test probabilities are: truly sensitized: $P_{AB} = 0.5970$, $P_{BL} = 0.1260$, $P_{NL} = 0.2770$. Not truly sensitized: $P_{AB} = 0.0109$, $P_{BL} = 0.0158$, $P_{NL} = 0.9733$.

TABLE II. Likelihood of Meeting Sensitization Criteria of One Abnormal and One Borderline (or Abnormal) BeLPT, by True Sensitization Status

BeLPT results that meet the criteria		Probability calculations[a,b]	Likelihood of meeting the criteria	
Blood samples				
1	2	$P_1 \times P_2$	Truly sensitized	Not truly sensitized
AB	AB, AB	$P_{AB}(P_{AB} \times P_{AB})$	0.2128	0.0000
	AB, NL	$P_{AB}(P_{AB} \times P_{NL} \times 2)$	0.1975	0.0002
	AB, BL	$P_{AB}(P_{AB} \times P_{BL} \times 2)$	0.0898	0.0000
	BL, NL	$P_{AB}(P_{BL} \times P_{NL} \times 2)$	0.0417	0.0003
	BL, BL	$P_{AB}(P_{BL} \times P_{BL})$	0.0095	0.0000
BL	AB, AB	$P_{BL}(P_{AB} \times P_{AB})$	0.0449	0.0000
	AB, NL	$P_{BL}(P_{AB} \times P_{NL} \times 2)$	0.0417	0.0003
	AB, BL	$P_{BL}(P_{AB} \times P_{BL} \times 2)$	0.0190	0.0000
Overall likelihood of meeting the criteria. . .			0.6569	0.0008

[a]Based on the true status of sensitization, the individual test probabilities are: truly sensitized: $P_{AB} = 0.5970$, $P_{BL} = 0.1260$, $P_{NL} = 0.2770$. Not truly sensitized: $P_{AB} = 0.0109$, $P_{BL} = 0.0158$, $P_{NL} = 0.9733$.
[b]The factor "2" was added as needed to consider order (e.g., a,b or b,a).

TABLE III. Likelihood of Meeting Sensitization Criteria of Two Abnormal BeLPT's, by True Sensitization Status

BeLPT results that meet the criteria				Probability calculations[a,b]	Likelihood of meeting the criteria	
Blood samples						
1	2	3	4	$P_1 \times P_2 \times P_3 \times P_4$	Truly sensitized	Not truly sensitized
AB	AB, AB	—	—	$P_{AB}(P_{AB})^2$	0.2128	0.0000
	AB, BL	—	—	$P_{AB}(P_{AB} \times P_{BL} \times 2)$	0.0898	0.0000
	AB, NL	—	—	$P_{AB}(P_{AB} \times P_{NL} \times 2)$	0.1975	0.0002
	BL, NL	AB	—	$P_{AB}(P_{BL} \times P_{NL} \times 2)P_{AB}$	0.0249	0.0000
		BL	AB	$P_{AB}(P_{BL} \times P_{NL} \times 2)P_{BL} \times P_{AB}$	0.0031	0.0000
	BL, BL	AB, AB	—	$P_{AB}(P_{BL})^2 \times (P_{AB})^2$	0.0034	0.0000
		AB, BL	—	$P_{AB}(P_{BL})^2 \times (P_{AB} \times P_{BL} \times 2)$	0.0014	0.0000
		AB, NL	—	$P_{AB}(P_{BL})^2 \times (P_{AB} \times P_{NL} \times 2)$	0.0031	0.0000
		BL, NL	AB	$P_{AB}(P_{BL})^2 \times (P_{BL} \times P_{NL} \times 2) \times P_{AB}$	0.0004	0.0000
		BL, BL	AB, AB	$P_{AB}(P_{BL})^2 \times (P_{BL})^2 \times (P_{AB})^2$	0.0001	0.0000
			AB, BL	$P_{AB}(P_{BL})^2 \times (P_{BL})^2 \times (P_{AB} \times P_{BL} \times 2)$	0.0000	0.0000
			AB, NL	$P_{AB}(P_{BL})^2 \times (P_{BL})^2 \times (P_{AB} \times P_{NL} \times 2)$	0.0000	0.0000
BL	AB	AB, AB	—	$P_{BL} \times P_{AB}(P_{AB})^2$	0.0268	0.0000
		AB, BL	—	$P_{BL} \times P_{AB}(P_{AB} \times P_{BL} \times 2)$	0.0113	0.0000
		AB, NL	—	$P_{BL} \times P_{AB}(P_{AB} \times P_{NL} \times 2)$	0.0249	0.0000
		BL, NL	AB	$P_{BL} \times P_{AB}(P_{BL} \times P_{NL} \times 2)P_{AB}$	0.0031	0.0000
		BL, BL	AB, AB	$P_{BL} \times P_{AB}(P_{BL})^2 \times (P_{AB})^2$	0.0004	0.0000
			AB, BL	$P_{BL} \times P_{AB}(P_{BL})^2 \times (P_{AB} \times P_{BL} \times 2)$	0.0002	0.0000
			AB, NL	$P_{BL} \times P_{AB}(P_{BL})^2 \times (P_{AB} \times P_{NL} \times 2)$	0.0004	0.0000
	BL	AB	AB, AB	$P_{BL} \times P_{BL} \times P_{AB}(P_{AB})^2$	0.0034	0.0000
			AB, BL	$P_{BL} \times P_{BL} \times P_{AB}(P_{AB} \times P_{BL} \times 2)$	0.0014	0.0000
			AB, NL	$P_{BL} \times P_{BL} \times P_{AB}(P_{AB} \times P_{NL} \times 2)$	0.0031	0.0000
Overall likelihood of meeting the criteria. . .					0.6115	0.0002

[a]Based on the true status of sensitization, the individual test probabilities are: truly sensitized: $P_{AB} = 0.5970$, $P_{BL} = 0.1260$, $P_{NL} = 0.2770$. Not truly sensitized: $P_{AB} = 0.0109$, $P_{BL} = 0.0158$, $P_{NL} = 0.9733$.
[b]The factor "2" was added as needed to consider order (e.g., a,b or b,a).

TABLE IV. Epidemiologic Parameters, by Sensitization Criteria

Criteria[a]	Sensitivity/ specificity[b]	False positives per 10,000[c]	Positive predictive values (PPVs), population prevalence of Be sensitization						
			1%	2%	3%	4%	5%	7%	10%
1 AB	0.682/0.9889	111	0.383	0.556	0.655	0.719	0.764	0.822	0.872
1 AB + 1 BL	0.657/0.9992	8	0.893	0.944	0.962	0.972	0.977	0.984	0.989
2 AB	0.612/0.9998	2	0.968	0.984	0.990	0.992	0.994	0.996	0.997

[a]More than the minimum criteria is also acceptable.
[b]Specificity = 1 − FP proportion (e.g., 1.000 − 111/10,000 = 0.9889).
[c]Number *falsely* established as sensitized for each 10,000 tested who are truly not sensitized.

precise for any individual laboratory. Neither do these results apply directly to any individual person.

The results presented were based on data from a large number of apparently healthy, exposed workers. We believe that they provide reasonable estimates of what to expect when exposed groups of asymptomatic adults are tested with the BeLPT.

As is common with medical data, our analyses depend on certain assumptions not likely to be universally true. For example, there is an expectation that the state of sensitization is constant over time, that the state of sensitization is truly bipolar (i.e., fully present versus not present), and that special circumstances have not affected the person's immune system, as might occur with chronic disease or immunosuppressive drugs. Also, these results are not applicable to the clinical evaluation of persons with granulomatous lung disease (e.g., sarcoidosis) or persons being evaluated for respiratory symptoms.

Despite its limitations, the beryllium industry has used the BeLPT routinely for years [Kreiss et al., 2007]. Although the test has not been used extensively outside the workplace, examples of its successful use in other settings are now available [ATSDR, 2006]. Cases have been identified among household contacts and among persons who live near beryllium facilities [Newman and Kreiss 1992; Kreiss et al., 2007].

Consideration should be given to the purpose of the testing, the potential benefits and risks, the likelihood of exposure, the expected prevalence of sensitization, and the informed wishes of those who may be tested. Careful forethought is important before testing with the BeLPT—such testing can lead to bronchoscopy, lavage, and lung biopsy [Borak et al., 2006].

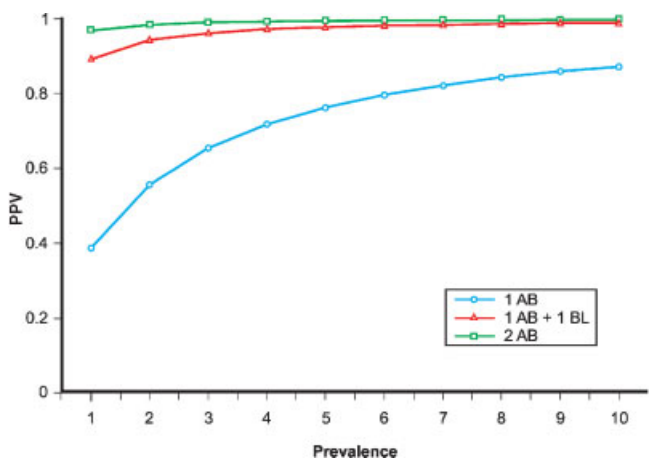

FIGURE 4. Positive predictive values (PPVs) for each criteria, by the prevalence of Be sensitization in the population.

CONCLUSIONS

Confirmation of BeLPT results—whether as 1 *abnormal* and 1 *borderline* or as 2 *abnormals*—enhances the test's PPV and protects the persons tested from unnecessary and invasive medical procedures. In most settings, a single unconfirmed *abnormal* is insufficient for establishing sensitization among apparently healthy persons. However, in situations where exposure has led to an elevated prevalence of beryllium sensitization, even a single abnormal BeLPT is likely to be a strong predictor of sensitization.

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