The Beryllium Lymphocyte Proliferation Test (BeLPT)

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National Jewish Health
Recognition of Beryllium Health Effects

1940s: Reports of adverse health effects associated with beryllium exposure.
  – Acute disease with high exposures.
  – Chronic disease present.

1950s: Be exposure standard (2 μg/m³) implemented but cases of CBD continue to be reported.
1980s: New test (BeLPT) identifies beryllium sensitization, a precursor to CBD.

2000: Department of Energy mandates CBD protection programs

2001: EEOICPA: Compensates employees injured during employment in the nuclear weapons development effort
Extent Of Beryllium Use

• NIOSH estimates
  – At risk: 134,000 in the US
    (J Occup Environ Hyg 2004 1:648)
  – Probably conservative- Doesn’t include “downstream users”

• Study of Construction Trades Workers at 3 DOE sites
  – Estimate 230,000 exposed (Welch 2004)
Importance of an Accurate Diagnosis

• Intervene early
  – If CW, remove/reduce exposure
  – Reduce exposures to workers in same job

• If CBD
  – Follow closely for progression of symptoms
  – Advise to maintain health (exercise, preventive vaccines)
  – Treat to preserve lung function
Beryllium Lymphocyte Proliferation Test (BeLPT)

- Measures blood cells response to beryllium
- Identifies beryllium sensitization
- Further clinical testing needed to diagnose CBD
BeLPT Characteristics

• Detects a beryllium-specific cell mediated immune response Identify CBD at early stages
• Differentiates CBD from lung disease of other etiology (sarcoidosis, COPD, HP)
• Increased sensitivity and specificity to diagnose BeS/CBD compared with other tests
  – Occupational History
  – CXR or CT scan
  – Spirometry
The BeLPT is Specific to Be-Related Health Effects
How is the Test Performed

- Culture peripheral blood or BAL cells with and without beryllium
- Measure incorporation of $^3$H-Td for proliferation
- Stimulation index or SI: ratio of Be-stimulated to unstimulated cpm
- Use positive controls to ensure cells are able to proliferate
Lymphocyte Proliferation Test: Peripheral Blood

Patient Name:
ID Number:  
OS#:
Date of Test:  
Accession#:
Referring Physician:
Technician:

Results

Mean Stimulation Index
Mitogens:  
Day 3  Day 4  Day 6
Phytohemagglutinin  431.3
Antigens:
Candida  107.8
Beryllium Sulfate:
1X10-4 M  1.1  0.6
1X10-5 M  1.5  0.8
1X10-6 M  1.1  0.6

Normal response to mitogen.
Normal response to antigen. (Normals: >3.0)
Normal lymphocyte proliferation to beryllium sulfate.

Note: A normal blood result does not exclude the diagnosis of Chronic Beryllium Disease. If the clinical setting is compatible, a beryllium lymphocyte proliferation test using bronchoalveolar lavage lymphocytes is recommended.

A normal result is all values equal or below the cutoff value of 2.5.
**Raw Data for Normal Blood LPT**

Calculate Stimulation Index (D4B4) \( \frac{267.8}{242.3} = 1.1 \)
### Lymphocyte Proliferation Test: Peripheral Blood

**ABNORMAL LPT**

<table>
<thead>
<tr>
<th>Patient Name:</th>
<th>ID Number:</th>
<th>OS#:</th>
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<tr>
<th>Date of Test:</th>
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<tr>
<th>Referring Physician:</th>
<th>Technician:</th>
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#### Results

<table>
<thead>
<tr>
<th>Mean Stimulation Index</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitogens:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phytohemagglutinin</td>
<td>125.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antigens:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida</td>
<td></td>
<td>172.6</td>
<td></td>
</tr>
<tr>
<td>Beryllium Sulfate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1X10-4 M</td>
<td></td>
<td>8.0</td>
<td>39.0</td>
</tr>
<tr>
<td>1X10-5 M</td>
<td></td>
<td>5.0</td>
<td>30.0</td>
</tr>
<tr>
<td>1X10-6 M</td>
<td></td>
<td>1.9</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Normal response to mitogen.
Normal response to antigen. (Normals: >3.0)
Abnormal lymphocyte proliferation to beryllium sulfate.

Note: An abnormal result is two or more values above the cutoff value of 2.5.
Raw Data for Abnormal LPT

Calculate Stimulation Index (D6B4) \( \frac{24312.7}{623.6} = 39.0 \)
Lymphocyte Proliferation Test: Peripheral Blood

UNINTERPRETABLE

Patient Name: 
ID Number: OS#: 
Date of Test: Accession#: 
Referring Physician: 
Technician: 

Results

<table>
<thead>
<tr>
<th>Mean Stimulation Index</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitogens:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phytohemagglutinin</td>
<td>36.0</td>
<td></td>
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</table>

Antigens:

<table>
<thead>
<tr>
<th>Candidate</th>
<th>4.0</th>
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</table>

Beryllium Sulfate:

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1X10-4 M</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1X10-5 M</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1X10-6 M</td>
<td>---</td>
<td>---</td>
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</tbody>
</table>

Normal response to mitogen.
Normal response to antigen. (Normals: >3.0)
Uninterpretable lymphocyte transformation to beryllium sulfate.
Why is a Test Uninterpretable

- Cells “over” respond in all conditions, possibly masking a true lymphocyte proliferation in Be stimulated condition(s)
- Positive controls not positive
- Lab error
# Specificity and Sensitivity of the BeLPT

Maier AOEH, 2001

<table>
<thead>
<tr>
<th>Study</th>
<th>Individuals Enrolled, n</th>
<th>BeS/CBD, n (%)</th>
<th>Estimated Sensitivity</th>
<th>Estimated Specificity</th>
<th>Positive Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mroz et al, JACI 1991</td>
<td>35</td>
<td>17 (49%)</td>
<td>94%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Stokes et al, JOEM 1991</td>
<td>57</td>
<td>27 (47%)</td>
<td>38%</td>
<td>97%</td>
<td></td>
</tr>
<tr>
<td>Kreiss et al, JOEM 1993*</td>
<td>505</td>
<td>9 (2%)</td>
<td>89%</td>
<td>NA</td>
<td>100%</td>
</tr>
<tr>
<td>Kreiss et al, ARRD 1993*</td>
<td>895</td>
<td>18 (2%)</td>
<td>100%</td>
<td>NA</td>
<td>100%</td>
</tr>
<tr>
<td>Stange et al, Environ Health Perspect 1996*</td>
<td>4268</td>
<td>101 (2%)</td>
<td>97%</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>
# Predictive Value of Clinical Tests in the Diagnosis of CBD

Kreiss et al. JOEM 1989

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td>1.6-10.5%</td>
<td>98.3-99%</td>
</tr>
<tr>
<td>FEV₁</td>
<td>2.9%</td>
<td>98.3%</td>
</tr>
<tr>
<td>FVC</td>
<td>3.3%</td>
<td>98.2%</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>3.4%</td>
<td>98.5%</td>
</tr>
<tr>
<td>Chest Xray</td>
<td>12.9%</td>
<td>98.9%</td>
</tr>
<tr>
<td>LPT</td>
<td>100%</td>
<td>99.8%</td>
</tr>
</tbody>
</table>
Alternative Screening Tests for CBD

• Chest X-ray
  – PV+ = 12.9% (Kreiss et al, 1993)
  – PV+ = 0.8% (Stange et al, 1996)

• Spirometry
  – PV+ of FEV$_1$ = 2.9% (Kreiss et al, 1993)
  – PV+ of FVC = 3.3% (Kreiss et al, 1993)

• Symptoms
  – PV+ of COUGH = 3.2% (Kreiss et al, 1993)
  – PV+ of DYSPNEA = 5.0% (Kreiss et al, 1993)
Interlaboratory Agreement

• Previous report 85-96% between labs, 30-60% for abnormal tests (Stange, Env Health Perspec 1996)

• Prelim evaluation split test from 2 labs (n=825)
  – 716/825 agreed (87%)
  – 703/716 negative (98% of agreed, 85% of total)
  – Total positive: 44 (5%)
<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Follow-up test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast CA Mammography</td>
<td>75 – 88%</td>
<td>83 – 99%</td>
<td>Biopsy</td>
</tr>
<tr>
<td>Colon CA Fecal Occult Blood</td>
<td>26 – 92%</td>
<td>90 – 99%</td>
<td>Colonoscopy</td>
</tr>
<tr>
<td>Neural Tube Defects AFP</td>
<td>56 – 91%</td>
<td>5 – 10%</td>
<td>Amniocentesis</td>
</tr>
<tr>
<td>Prostate CA PSA</td>
<td>29 – 80%</td>
<td>75%</td>
<td>Biopsy</td>
</tr>
<tr>
<td>BeS/CBD BeLPT*</td>
<td>74 – 91%</td>
<td>96 – 99%</td>
<td>Bronchoalveolar lavage/biopsy</td>
</tr>
</tbody>
</table>
Considerations in using the BeLPT

• Borderline/uninterpretable
• False negative
• False positives
• Lack of concordance between some labs
• At least 7 days for results
• Does not discriminate between BeS and CBD
Efforts to Standardize Testing

- CABST
- ICPT
- DOE Specification, April 2001 (update expected)
  - Minor differences remain:
    - Days of culture
    - Cut-off value determination
    - Serum lots
What if There are Problems with the BeLPT?

- Sometimes the cells fail to respond to beryllium. A negative BeLPT does not exclude the diagnosis of CBD
  - More common in smokers
- Beryllium skin patch testing can be used to demonstrate an immune response to beryllium
Benefits of the BeLPT

- Redefined and broadened our understanding of beryllium related health effects:
  - Can detect health effects at an early stage
  - Higher sensitivity and specificity than other screening tests
- Can be used in screening and surveillance mode to help evaluate processes/tasks
Applications of the BeLPT

• Diagnosis of beryllium related health effects
  – Beryllium Sensitization
  – CBD

• Workplace screening and surveillance
  – Prevalence of health effects
  – Work practices and job titles with increased risk
Summary

- BeLPT provides non-invasive test to screen for Be-health effects
  - Specific and sensitive
  - Used in evaluation of process/exposure risks
- Studies needed of other tests
  - Discriminate BeS and CBD
  - Enhance performance of BeLPT
  - Increased specificity/sensitivity
Questions ?