OAK RIDGE INSTITUTE
FOR SCIENCE AND EDUCATION

Beryllium Lymphocyte Proliferation Testing

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The Beryllium Lymphocyte Proliferation Test (Be-LPT)

- Measures beryllium-specific cellular immune response
- Useful in medical surveillance of beryllium-exposed populations
- Identifies people at elevated risk of developing chronic beryllium disease (CBD)

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Procedure

- Requires 30 ml blood sample in sodium heparin
- Density gradient centrifugation to isolate lymphocytes
- Standard cell culture methods
- Control wells and beryllium-stimulated wells

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Procedure
(continued)

- DNA precursor, thymidine, labeled with tritium (radioactive isotope of hydrogen) added to media
- Cell growth measured by how much radioactive thymidine is incorporated into the cultured cells
- Cells harvested on two days (5 & 7) or (4 & 6)
- Positive controls are utilized to ensure that the cells are healthy

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Plate Map

(5-day test)

	Counts	Counts	Counts	Counts	Median (C.V.)
Control	641	532	834	513	
Control	624	568	379	598	
Control	563	452	533	448	548 (0.17)
1 μ M	473	363	888	422	447 (0.23)
10 μ M	4232	12565	250	4506	4367 (0.50)
100 μ M	2128	5515	2655	999	2377 (0.82)

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Test Measures

- Counts measures the cell growth rate
- Coefficient of Variation measures the consistency of the duplicate counts in the replicate sets
- Stimulation Index (SI) measures the rate of growth in the beryllium wells compared to the rate of growth in the control wells
  - External reference comparison to determine abnormal results
- Standardized Log SI measures the statistical probability that the rate of growth in the beryllium wells is the same as the growth in the control wells
  - Internal comparison to determine abnormal results

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Calculating the Results
Least Absolute Values (LAV) Method

- Developed in 1994
- Uses outlier resistant methods for calculating stimulation indexes
- Considers internal variability
- Uses external reference data
- Considers statistical and biological variation

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Results

(from the 5-day example)

	Median Counts	Coefficient of Variation	Stimulation Index (SI) [>2.9=high]	Standardized Log SI [>2.5=high]
Control	548	0.17		
1 μ M	447	0.23	0.82	-1.44
10 μ M	4367	0.50	7.97	14.71
100 μ M	2377	0.82	4.34	10.40

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Results Categories

- Normal = All 6 SI values are below the reference level
- Borderline = One SI above the reference level
- Abnormal = Two or more SI values above the reference level
- Unsatisfactory = Uninterpretable for a variety of reasons

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Unsatisfactory Categories

- Cell Killing = Growth in the beryllium wells is significantly below growth in the control wells (44%)
- High Coefficient of Variation = CV of one set of control data or two SI values exceeds a reference level (31%)
- Low Growth = Control wells do not exhibit counts at least 3 time background measurements (15%)
- Insufficient Cells = Inadequate lymphocytes in the sample to set up a complete test (9%)
- Low Response in Positive Control = Positive control wells did not show at least a 3 fold increase in growth over control values (1%)

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Distribution of 1st Test Results

(ORISE Lab 1997 – 2004)

	All Tests (N=13,057)	Local Tests (N=3,839)	Shipped Tests (N=3,693)
Abnormal	236 (1.81%)	73 (1.90%)	74 (2.00%)
Borderline	187 (1.43%)	64 (1.67%)	46 (1.24%)
Normal	11,941 (91.45%)	3,539 (92.18%)	3,373 (91.33%)
Unsatisfactory	693 (5.31%)	163 (4.24%)	200 (5.42%)

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Repeat Tests

(A, B, and U tests repeated immediately; N tests repeated after at least 1 year)

	First Test A (N=171)	First Test B (N=106)	First Test U (N=410)	First Test N (2,236)
Second Test A	70 (40.94%)	8 (7.55%)	6 (1.46%)	24 (1.07%)
Second Test B	13 (7.60%)	8 (7.55%)	11 (2.68%)	36 (1.61%)
Second Test N	80 (46.78%)	88 (83.01%)	339 (82.68%)	2,075 (92.80%)
Second Test U	8 (4.68%)	2 (1.89%)	54 (13.17%)	U (4.52%)

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Laboratory Agreement
for Simultaneous Tests

- Overall agreement on all tests is usually between 90 and 98 percent
- Agreement on abnormal results ranges from 36 to 65 percent
- Serum differences may account for many discrepancies

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Known Abnormal 2 Sera

	Counts Serum1	SI Serum1	StdLNSI Serum1	Counts Serum2	SI Serum2	StdLNSI Serum2
5-D Cont	192			604		
1 μ M	574	2.98	3.97	13717	22.70	16.06
10 μ M	4107	21.34	11.10	31739	52.53	20.38
100 μ M	1158	6.02	6.51	18775	31.07	17.68
7-D Cont	252			3854		
1 μ M	931	3.69	2.47	54203	14.06	17.56
10 μ M	10055	39.82	6.96	107695	27.94	19.96
100 μ M	556	2.20	1.49	73385	19.04	32.06

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Known Abnormal 2 Sera

	Counts Serum1	SI Serum1	StdLNSI Serum1	Counts Serum2	SI Serum2	StdLNSI Serum2
5-D Cont	372			214		
1 μ M	741	1.99	1.92	271	1.26	1.34
10 μ M	1528	4.10	3.95	190	0.89	-0.68
100 μ M	1430	3.84	3.77	158	0.74	-1.74
7-D Cont	2401			598		
1 μ M	4372	1.82	2.31	348	0.58	-2.56
10 μ M	5112	2.13	2.92	136	0.23	-7.02
100 μ M	2273	0.95	-0.21	163	0.27	-6.14

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Can We Estimate Missed Abnormals for 'One Test' Screening?

- Used quality control split samples to estimate
- All people who had two tests on initial visit
- Outcomes:
  - +/+ = Sensitized
  - +/- = One abnormal—retest to determine status
  - -/- = Normal—rescreen in 3-5 years

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Assumptions for Calculations

- Under a ‘one test’ screening scenario
  - +/+: would have an abnormal test and would be brought back for a retest that would be abnormal
  - +/-: half of this group would have an abnormal test and return for retest, yielding half the number of second abnormal tests
  - -/-: would return in 3-5 years for second test

Missed Abnormal Rate Calculation

[based on split tests]

Site	# with split test	Result	# with result	# Sens	% Sens	Final Sens rate	Sens rate w/ one test	Missed
RFETS	2811	+/+	49	49	100.0%	2.85%	2.29%	19.65%
		+/-	111	31	27..93%			
		-/-	2652	13	0.49%			
KCP	577	+/+	3	3	100.0%	2.43%	1.47%	39.51%
		+/-	27	11	40.74%			
		-/-	547	??	0.00%			

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Testing Recommendations

- Each person tested should receive at least two tests
  - Sequential testing—recommended in the workplace or if beryllium work was in the recent past
  - Simultaneous testing—recommended if beryllium work was in the distant past or if it may be difficult to retest in the future

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