

The Reliability of the Beryllium Lymphocyte Proliferation Test (BeLPT) The «Québécoise» Experience

P. Brousseau ¹, M. Rossignol ², B. Mazer ³, C. Dion ⁴

- 1. Biophage Inc.**
- 2. Direction de santé publique Montréal**
- 3. Meakins Christie Laboratories, Mc Gill University**
- 4. Institut de recherche Robert-Sauvé en santé et en sécurité du travail**

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Introduction

- ✓ Before 2002, BeLPT was not available in Quebec or in Canada
- ✓ Controversies about BeLPT



Validation

Objectives

- ❖ **Establishment of a BeLPT based on reference protocols**
- ❖ **Harmonization of the BeLPT between the two laboratories**
- ❖ **Using true split samples with $n = 500$**
 - ❖ **Evaluate the level of variations of the raw data**
 - ❖ **Evaluate the level of agreement of interpretation within each category (normal, borderline, abnormal)**
 - ❖ **Evaluate the intra-lab reproducibility for the same worker tested at various times (longitudinal follow-up)**

Establishment of a BeLPT based on Reference Protocols

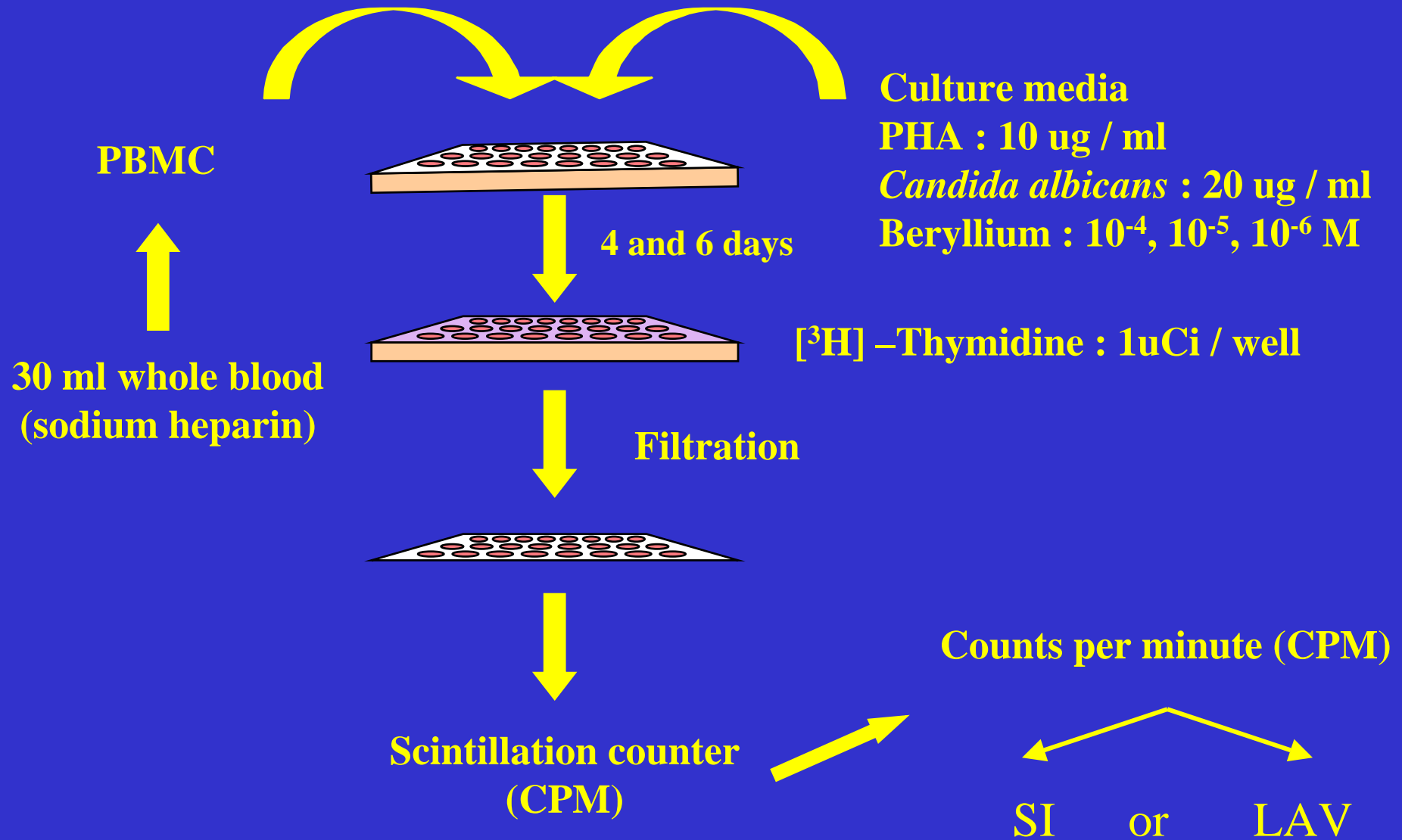
Standard Protocols

- ✓ **SOP from National Jewish**
- ✓ **DOE (Specifications, Avril 2001)**

Non Harmonized Parameters

- ✓ Time of incubation (days): 4 and 6 / 5 and 7
- ✓ Time of thymidine pulse (hours) : 6 / 18
- ✓ Report : Least Absolute Value (LAV)
Stimulation index (SI)

Methodology for BeLPT



Harmonized Methods

Parameters	Lab A	Lab B
Volume of blood	30 ml	30 ml
Anticoagulant	sodium heparin	sodium heparin
Transit time	24 hours	24 hours
Gradient	Ficol paque	Lympholyte-H
Medium preparation	PBS	PBS
Culture medium	RPMI-1640	RPMI-1640
Mitogen	PHA (10 µg/ml)	PHA (10 µg/ml)
Incubation	4 days	4 days
Antigen	<i>Candida albicans</i> (20 µg/ml)	<i>Candida albicans</i> (20 µg/ml)
Incubation	6 days	6 days
Beryllium sulphate	10 ⁻⁴ M, 10 ⁻⁵ M, 10 ⁻⁶ M	10 ⁻⁴ M, 10 ⁻⁵ M, 10 ⁻⁶ M
Serum	10% AB	10% AB
Beryllium replicate	4	4
Mitogen replicate	4	4
Antigen replicate	4	4
Medium replicate	12	12
Number of cells	2,5 10 ⁵ / well	2,5 10 ⁵ / wells
³ H-thymidine	1µCi / 20µl	1µCi / 20µl
³ H-thymidine pulse	18 hours	18 hours
Scintillation Cocktail	Betaplate Scint	Microscint 20
Report	Stimulation index	Stimulation index
Cut off value	<i>f</i> (serum)	<i>f</i> (serum)
% CV	35%	35%
Software	Microsoft Excel	Microsoft Excel

Calculations

Treatment Group	Well 1	Well 2	Well 3	Well 4
Day 4 control	560	527	826	752
Day 4 control	1023	1001	905	1045
Day 4 control	969	1099	843	809
Day 4 Be 10^{-6} M	809	861	743	887
Day 4 Be 10^{-5} M	532	462	391	400
Day 4 Be 10^{-4} M	468	420	382	343
PHA	219305	178394	187729	183828
Day 6 control	4811	4517	5568	4182
Day 6 control	5188	6321	5918	5101
Day 6 control	5961	5387	6422	5179
Day 6 Be 10^{-6} M	6307	4989	6774	6867
Day 6 Be 10^{-5} M	535	446	632	360
Day 6 Be 10^{-4} M	307	199	349	207
<i>Candida albicans</i>	78644	86530	91295	82494

LAV

Ln transformation of CPM

Median, SD, %CV, SE

Ln(SI) : Median – median of –ve control

Standardized Ln(SI) : Ln(SI) / SE

SI

No transformation

Mean, SD, %CV

SI : Mean / mean of –ve control

LAV

6 standardized Ln(SI) for Be

1 standardized Ln(SI) for PHA

1 standardized Ln(SI) for *Candida albicans*

Standardized maximum Ln(SI)

SI

6 SI for Be

1 SI for PHA

1 SI for *Candida albicans*

Results

LAV

SI

Candida albicans

Standardized $\text{Ln}(\text{SI}) \geq 3$

$\text{SI} \geq 3$

PHA

Standardized $\text{Ln}(\text{SI}) \geq 3$

$\text{SI} \geq 50$

Results

LAV

SI (*f* serum)

Beryllium

A) Stan. Maximum Ln(SI) > 3.1

Lab A ≥ 2.6

B) Stan. Ln(SI) > 2.5

Lab B ≥ 2.8

Abnormal : A and 2xB satisfied

Borderline: either A or B satisfied

Normal : neither A or B satisfied

Abnormal : 2 IS ≥ cut off

Borderline: 1 IS ≥ cut off

Normal : 6 IS < cut off

Statistical Analysis

Raw Data : ANOVA for repeated measures (F -test statistic)

Interpretation : Z -test on Kappa values :

Chance-corrected measure of agreement
between pairs of observers

Data Base

Id	Lab	Day	Location	Month	Smoker	C-	PHA	Candida	Be 1	Be 10	Be 100
1	A	4	Mtl	7	y	C ₁ ...C ₁₂	P ₁ ...P ₄	C ₁ ...C ₄	BeA ₁ ...BeA ₄	BeB ₁ ...BeB ₄	BeC ₁ ...BeC ₄
1	A	6	Mtl	7	y						
1	B	4	Mtl	7	y						
1	B	6	Mtl	7	y						
2	A	4	Out Mtl	12	n						
2	A	6	Out Mtl	12	n						
2	B	4	Out Mtl	12	n						
2	B	6	Out Mtl	12	n						

- Each sample
- ❖ 2 sets of 12 readings (negative control at days 4 and 6)
 - ❖ 2 sets of 4 readings (Positive controls at days 4 and 6)
 - ❖ 6 sets of 4 readings (Be 10⁻⁴, 10⁻⁵, 10⁻⁶ M at days 4 and 6)

Quality Control

- ✓ Double blind clinical study
- ✓ Quality control samples

Raw Data

Parameters	Lab A	Lab B	F-test	P
Negatif controls / Day 4	415 (cpm)	687 (cpm)	41,75	< 0,001
Negatif controls / Day 6	1185 (cpm)	2455 (cpm)		
PHA / Day 4	SI : 261,05	SI : 339,47	26,33	< 0,001
<i>Candida albicans</i> / Day 6	SI : 2,07	SI : 42,5		
Beryllium 10 ⁻⁶ M / Day 4	SI : 1,11	SI : 1,14	1,60	0,21
Beryllium 10 ⁻⁶ M / Day 6	SI : 0,99	SI : 1,07		
Beryllium 10 ⁻⁵ M / Day 4	SI : 1,41	SI : 1,10	7,38	< 0,01
Beryllium 10 ⁻⁵ M / Day 6	SI : 1,00	SI : 0,76		
Beryllium 10 ⁻⁴ M / Day 4	SI : 1,76	SI : 1,07	25,70	< 0,001
Beryllium 10 ⁻⁴ M / Day 6	SI : 1,22	SI : 0,56		

Summary of Analysis

Inter laboratory Reproducibility	Lab A	Lab B
Agreement	Normal	Normal
	Borderline	Borderline
	Abnormal	Abnormal
Relative discordant	Normal	Borderline
	Abnormal	Borderline
	Borderline	Normal
	Borderline	Abnormal
Absolute discordant	Normal	Abnormal
	Abnormal	Normal

Inter Laboratory Reproducibility using LAV

Lab B	Lab A		
	Abnormal	Borderline	Normal
Abnormal	5	1	3
Borderline	5	1	13
Normal	8	17	63

n = 116

Agreement : 69 / 116 (59,5%)

Absolute discordant : 11 / 116 (9,5%)

Relative discordant : 36 / 116 (31,0%)

❖ **Borderline – Normal:** 30 / 116 (25,9%)

❖ **Borderline – Abnormal :** 6 / 116 (5,2%)

Inter Laboratory Reproducibility using SI

Lab B	Lab A		
	Abnormal	Borderline	Normal
Abnormal	3	2	2
Borderline	1	1	4
Normal	5	9	100

n = 127

Agreement : **104 / 127 (81,9%)**

Absolute discordant : **7 / 127 (5,5%)**

Relative discordant : **16 / 127 (12,6%)**

❖ **Borderline – Normal:** **13 / 127 (10,2%)**

❖ **Borderline – Abnormal :** **3 / 127 (2,4%)**

Lab A

Intra Laboratory Reproducibility using LAV and SI

LAV	SI		
	Abnormal	Borderline	Normal
Abnormal	12	7	1
Borderline	0	4	16
Normal	0	0	89

n = 129

Agreement : 105 / 129 (81,4%)

Absolute discordant : 1 / 129 (0,8%)

Relative discordant : 23 / 129 (17,8%)

❖ **Borderline – Normal:** 16 / 129 (12,4%)

❖ **Borderline – Abnormal :** 7 / 129 (5,4%)

Lab B

Intra Laboratory Reproducibility using LAV and SI

LAV	SI		
	Abnormal	Borderline	Normal
Abnormal	5	2	2
Borderline	0	4	12
Normal	0	0	87

n = 112

Agreement : 96 / 112 (85,7%)

Absolute discordant : 2 / 112 (1,8%)

Relative discordant : 14 / 112 (12,5%)

❖ **Bordeline– Normal:** 12 / 112 (10,7%)

❖ **Borderline– Abnormal :** 2 / 112 (1,8%)

Analysis for the Follow Up

	1 st analysis	2nd analysis
Agreement	Normal	Normal
	Borderline	Borderline
	Abnormal	Abnormal
Relative discordant	Normal	Borderline
	Abnormal	Borderline
	Borderline	Normal
	Borderline	Abnormal
Absolute discordant	Normal	Abnormal
	Abnormal	Normal

Follow up

n = 246

Agreement : **191 / 246 (76,7%)**

Absolute discordant : **11 / 246 (4,5 %)**

Relative discordant : **44 / 246 (17,9%)**

Bordeline – Normal: **35 / 44 (79,5 %)**

Normal – Bordeline: **5 / 44 (11,4 %)**

Borderline- Abnormal: **3 / 44 (6,8 %)**

Abnormal – Bordeline : **1/ 44 (2,3 %)**

Conclusions

- ✓ The methodologies are fully harmonized.
- ✓ Some significant variations between the labs are observed at the level of the raw data. However these variations do not prevent a high level of concordance in the interpretation of the results with a percentage of 81.9 % using the stimulation index.
- ✓ Using both LAV and SI the intra-laboratory agreement is higher than 80 % for both laboratories.
- ✓ Using a cohort of 246 workers, 76,7 % of the results are in agreement for the longitudinal follow up.

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