



***CRITÉRIOS ANALITICOS NA QUANTIFICAÇÃO DE  
CELULAS TCD4+/CD8+ POR CITOMETRIA DE FLUXO***

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*Field Application Specialist*  
*BD Biosciences*

12 de Dezembro, 2018

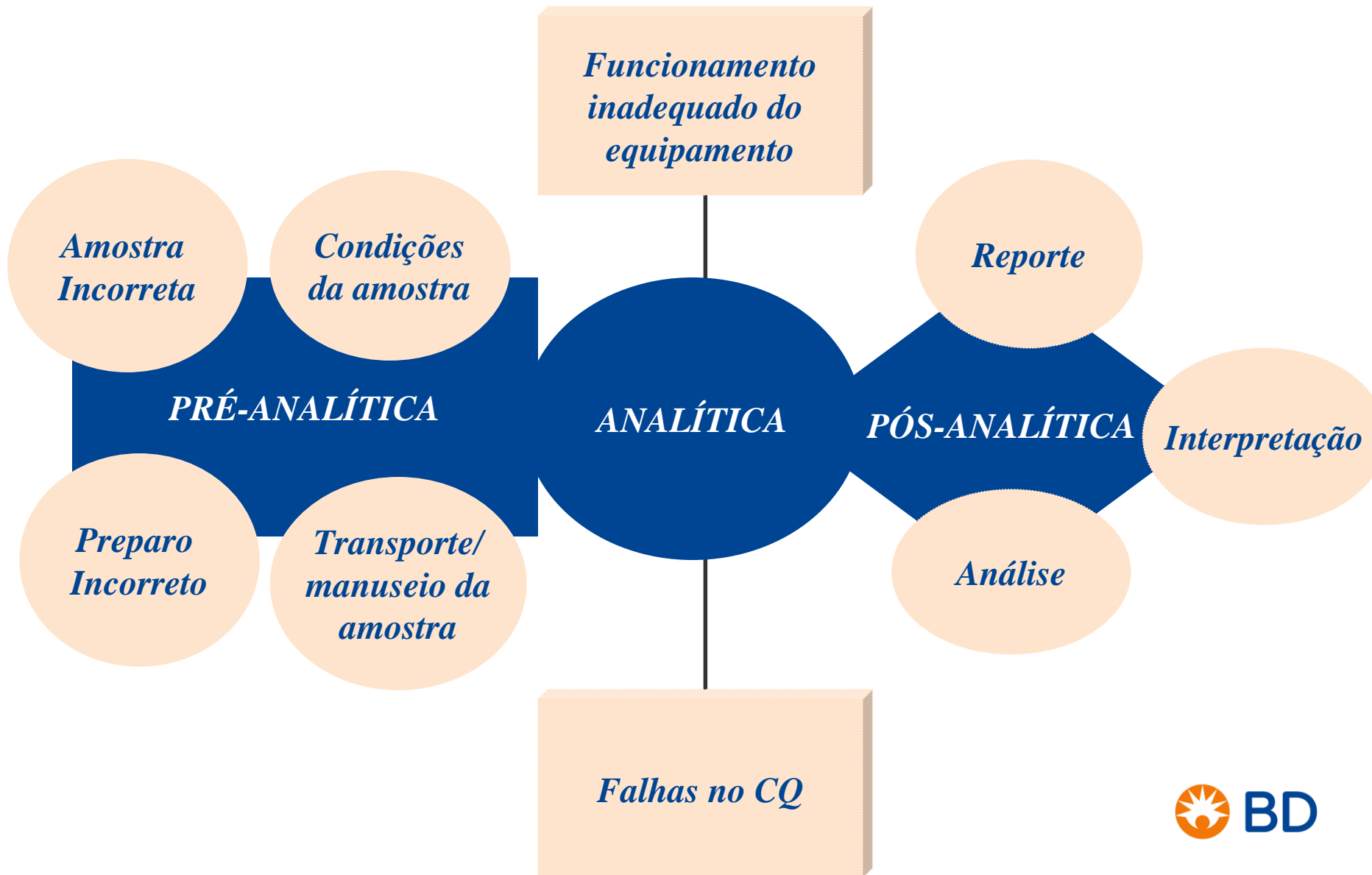
# A rotina de Contagem de Linfócitos TCD4<sup>+</sup>/CD8<sup>+</sup>

## AGENDA

- ❖ *Controle de Qualidade na Rotina Laboratorial*
- ❖ *Fase Pré-Analítica*
- ❖ *Fase Analítica*
- ❖ *Fase Pós-Analítica*
- ❖ *AEQ*
- ❖ *BD FACSVia*



# Controle de Qualidade na Rotina Laboratorial





# *FASE PRÉ-ANALÍTICA*

# Fase Pré-Analítica

## Coleta/Conservação da Amostra

- ✓ Tubos de coleta **EDTA K<sub>2</sub>** ou **K<sub>3</sub>** (*o reagente MultiTEST não foi validado para utilização em amostras coletadas com heparina ou ACD*).
- ✓ Se armazenada entre **20°C a 25°C** poderá ser processada em até **48 horas** após a coleta.
- ✓ Após o processamento, se armazenada entre **20°C a 25°C** sob o abrigo da luz, poderá ser analisada em até **24 horas**.

## Critérios de Rejeição

- ✓ Amostras que não atendem as condições acima.
- ✓ Amostras coaguladas e/ou com fibrina.
- ✓ Amostras com volume inadequado.
- ✓ Amostras hemolisadas

*\*CONTEUDO RETIRADO DA APOSTILA DO TREINAMENTO*

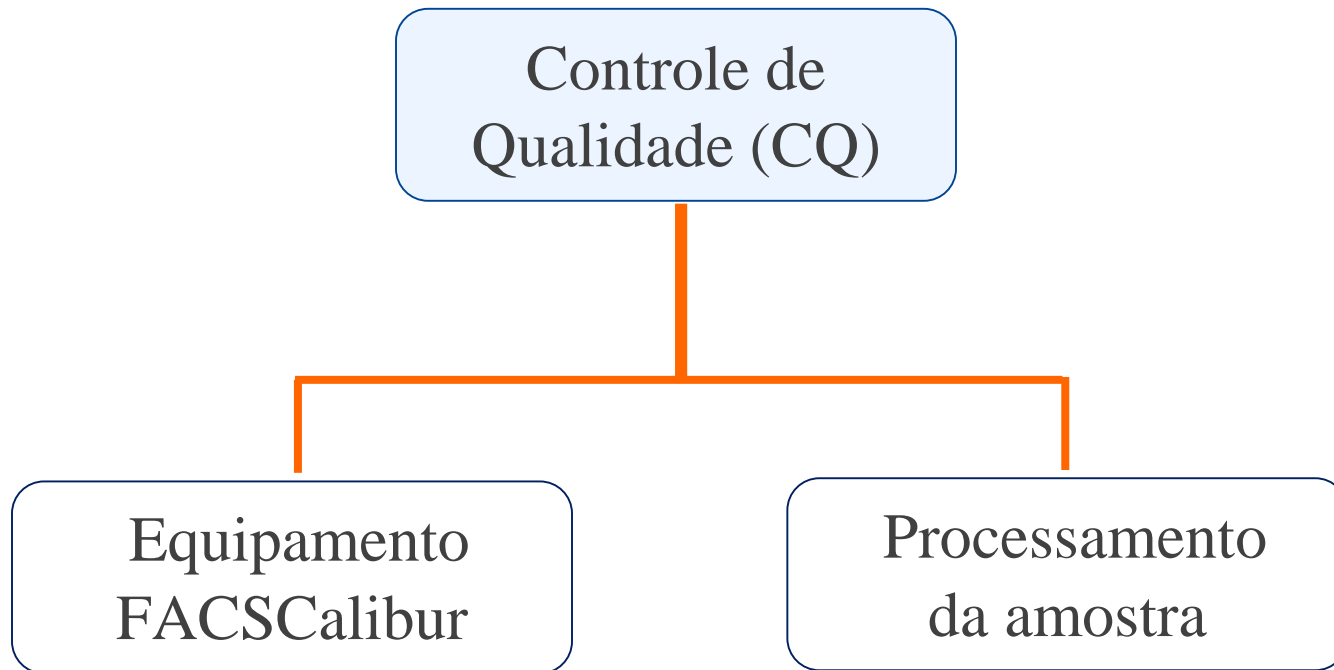


**Fonte:** Guidelines for Performing Single-Platform Absolute CD4+ T-Cell Determinations with CD45 Gating for Persons Infected with Human Immunodeficiency Virus (2003) – CDC (Centers for Disease Control and Prevention).



*FASE ANALÍTICA*

# Fase Analítica



**RESULTADOS CONFIÁVEIS,  
COMPARÁVEIS E REPRODUTÍVEIS.**



# *Fase Analítica*

Avaliar a *Performance Diária do Instrumento*

- ✓ Parâmetros de dispersão de luz (FSC e SSC).
- ✓ Parâmetros de Fluorescência (FL1, FL2, FL3 e FL4).
- ✓ Corrigir sobreposição de espectros (compensação)
- ✓ Gerar *settings* aplicáveis a análise de linfócitos humanos (Calibração).

## **BD CaliBRITE™**

**Não processar amostras antes da certificação de que o equipamento foi aprovado no CQ.**





# Fase Analítica

Passos da calibração	Tubo
<ol style="list-style-type: none"><li>1. Ajuste das voltagens dos PMTs</li><li>2. Calibração do <i>Time Delay</i></li></ol>	A ( <i>beads</i> não marcadas e <i>beads</i> APC)
<ol style="list-style-type: none"><li>3. Ajuste das compensações</li><li>4. Estabelecimento do <i>threshold</i> em FL3</li><li>5. Teste de sensibilidade</li></ol>	B ( <i>beads</i> não marcadas, <i>beads</i> FITC, <i>beads</i> PE, <i>beads</i> PerCP, <i>beads</i> APC)



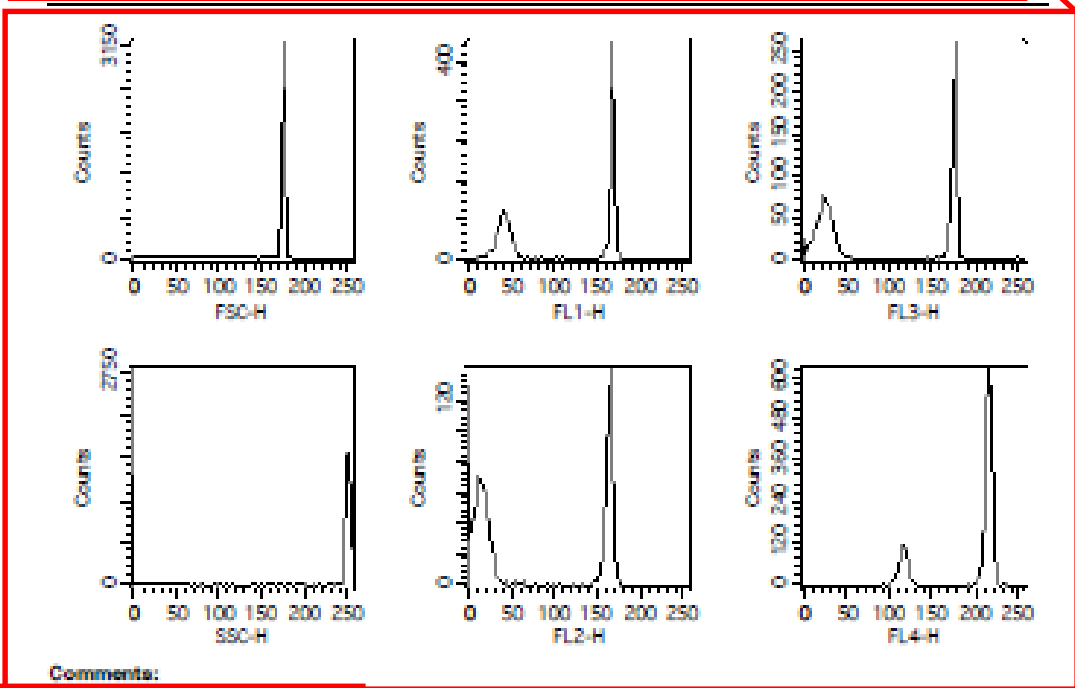
# 4-Color Lyse/No-Wash FACSCComp Report

Institution:   
 Director:   
 Operator:   
 Date: 11 de dezembro de 2012 09:15   
 Software: FACSCComp 6.0   
 Cytometer: FACSCalibur 0123456789

Parameter	High	Low	Separation	Minimum	Result	Lot ID
FSC	175	4	171	100	Pass	52584I
SSC	246	1	245	210	Pass	52584I
FL1	165	42	123	100	Pass	57587H
FL2	161	14	147	105	Pass	52583L
FL3	172	24	148	135	Pass	38745L
FL4	211	114	97	56	Pass	18850H

Parameter	Detector	Amplifier	Threshold	Blue Laser Current	Blue Laser Power
FSC	E00	2.00		5.88 Amps	15.00 mWatts
SSC	348	1.00			
FL1	523	Log			
FL2	465	Log			
FL3	539	Log			
FL4	656	Log			

Compensation	FL1-%FL2	FL2-%FL1	FL3-%FL3	FL3-%FL2	FL3-%FL4	FL4-%FL3
	2.6	13.1	0.0	7.7	1.2	7.6



Comments:   
 Time Delay Calibration Passed.

Teste de sensibilidade

Corrente e potência do laser azul

Voltagens dos detectores

Threshold   
 Valores de compensação

Teste de sensibilidade

Time Delay

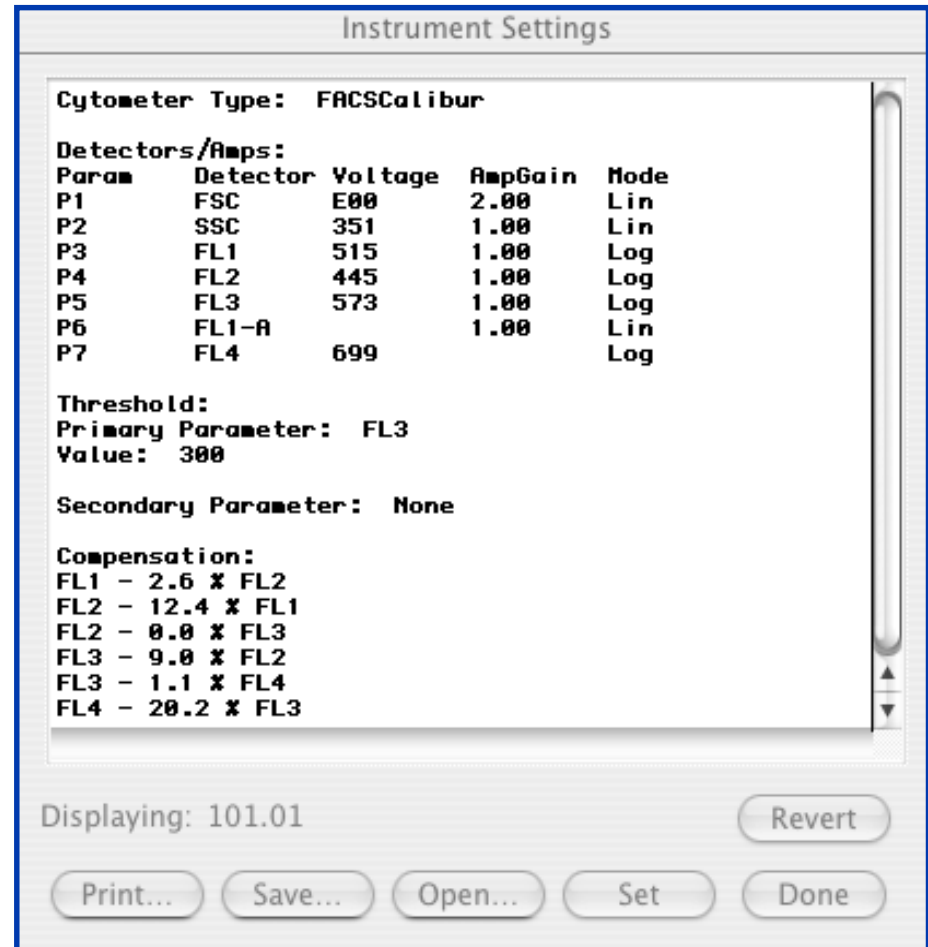


# Fase Analítica

Arquivo (CalibFile.LNW) que contém:

- ✓ Valores de voltagem dos PMTs;
- ✓ Valores de compensação;
- ✓ Threshold

**Não contém o ajuste do *Time Delay* nem do *Teste de Sensibilidade*;**



# Fase Analítica

*Tubo TruCOUNT*



← 50µl Sangue  
← 20µl Multitest

Incubação de 15 min/ escuro  
Vórtex

Após a diluição 1:10, utilizar em 7 dias\*

+ 450µl de solução de lise 1:10

Incubação de 15 min/ escuro  
Vórtex

CITÔMETRO

*Utilizar Pipetagem Reversa para o sangue*



\*CONTEUDO RETIRADO DA APOSTILA DO TREINAMENTO

# Fase Analítica

## Tubo TruCOUNT



- ← 450 µl de Solução de Lise
- ← 50µl Sangue (doador sadio ou CD4 ≥ 500)
- ← 20µl Multitest CD3/8/45/4

+ beads  
TruCOUNT Control



No máximo  
24 horas



Citômetro

Pipetagem  
Reversa



50µl  
Low

50µl  
Medium

50µl  
High



\*CONTEUDO RETIRADO DA APOSTILA DO TREINAMENTO

# Fase Analítica

## Plataforma única (sem necessidade de leucograma):

- ✓ CD45 – Linfócitos Totais
- ✓ Tubos Trucount – Contagens absolutas

## Protocolo

- ✓ Seguir protocolo de processamento indicado pela BD
- ✓ **MultiTEST – CD3/CD4/CD8/CD45**
- ✓ Prosseguir com a marcação das amostras em no máximo **48h após a coleta.**
- ✓ Após a marcação, as amostras devem ser lidas em no máximo **24h.**
- ✓ Quando necessário, procedimentos de lavagens da amostra podem ser realizados

**Fonte:** Guidelines for Performing Single-Platform Absolute CD4+ T-Cell Determinations with CD45 Gating for Persons Infected with Human Immunodeficiency Virus (2003) - CDC (Centers for Disease Control and Prevention).



# Fase Analítica

<b>Reagente</b>	<b>Temperatura ambiente (20°C a 25°C)</b>	<b>Geladeira (2°C a 8°C)</b>	<b>Freezer (-20°C)</b>
BD Calibrite		X	
Tubos TruCOUNT	X	X	
Multitest		X	
BD TruCOUNT Controls		X	
BD FACS Lysing Solution	X	X	



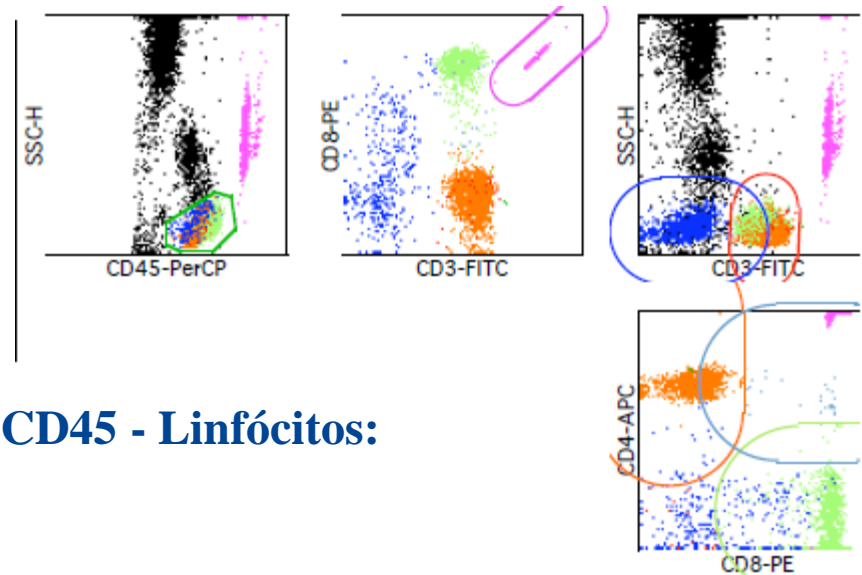
# *FASE PÓS-ANALÍTICA*



# Fase Pós-Analítica

## 1. Avaliar posicionamento das populações celulares.

**PRINCIPALMENTE GATE DE LINFÓCITOS E BEADS**



## 2. Avaliar posicionamento do gate do CD45 - Linfócitos:

- ✓ Alta intensidade de CD45;
- ✓ Baixa granulosidade (SSC);
- ✓ Não deve incluir a população de basófilos (baixo SSC e baixo CD45);
- ✓ Não deve incluir população de monócitos (intermediário SSC e CD45);

# Fase Pós-Analítica

## 3. Avaliar – Linfócitos e Beads:

***O NUMERO DE EVENTOS É DIRETAMENTE INFLUENCIADO PELO POSICIONAMENTO DOS GATES***

- ✓ *Lymph Events*  $\geq 2000$
- ✓ *Beads Events*  $\geq 500$

## 4. Análise de consistência:

- ✓ A soma dos valores percentuais (% Lymph) e absolutos (Abs Cnt) das células CD3+CD4+ e CD3+CD8+ deve ser igual as contagens das células CD3+ ( $\pm 10\%$ ).

**\*Se MAIOR que CD3 – presença de LTDP**

**\*Se MENOR que CD3 – presença de LTDN**

# *Fase Pós-Analítica*

- ✓ Valores dos TruCount Controls
- *Planilha Levey Jennings*
- ✓ Aplicar Regras de Westgard
- *Fluxograma*

# Fase Pós-Analítica

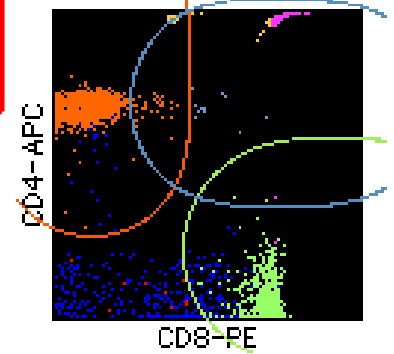
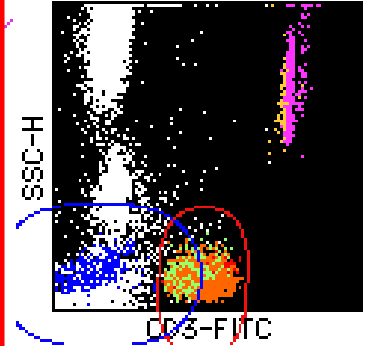
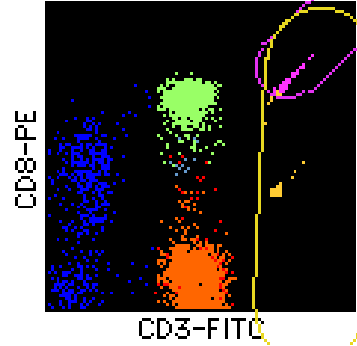
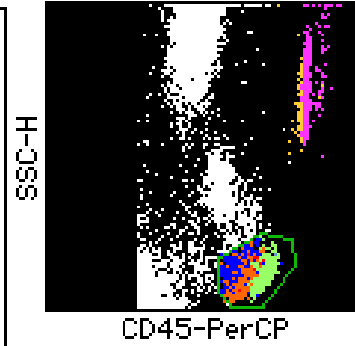
CD3/CD8/CD45/CD4 TruC Ctrl L

Data Set [ 1 ] Data File: 202.01

Reagent Lot ID: 00481 Events Acquired: 15000 Abs Cnt Bd Lot ID: 90919 Attr Def File: 3/8/45/4 MLT/TruC C v2.0

File ID: CE60965A- E604-11DC-94B5-000D9362 Beads Per Pellet: 52179 Ctrl Bead Lot ID: 97194 Mean Count: 51 Std Dev: 8.10

Lymph Events	5318
Bead Events	1852
Control Bead Events	101
CD3+ %Lymph	74
CD3+ Abs Cnt	2204
CD3+CD8+ %Lymph	25
CD3+CD8+ Abs Cnt	738
CD3+CD4+ %Lymph	47
CD3+CD4+ Abs Cnt	1406
CD3+CD4+CD8+ %Lymph	0
CD3+CD4+CD8+ Abs Cnt	8
CD45+ Abs Cnt	2995
T H/S Ratio	1.91
Control Bead Abs Cnt	57

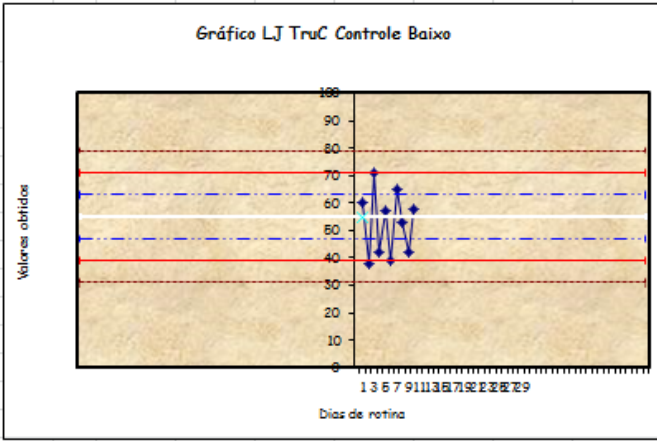


*Confira os valores*



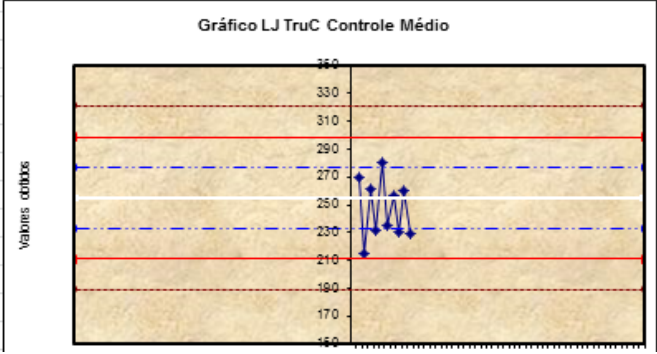
# Fase Pós-Analítica

Inserir o nome do laboratório						Ano: 2016
Dia	Mês	Número do Controle	Low (Baixo)	Mdium (Médio)	High (Alto)	Comentários
1	10	1	60	270		
2	10	2	38	215		
3	10	3	71	262		
4	10	4	42	232		
5	10	5	57	280		
6	10	6	39	235		
7	10	7	65	257		
8	10	8	53	230		
9	10	9	42	260		
10	10	10	58	229		
		11				
		12				
		13				
		14				
		15				
		16				
		17				
		18				
		19				
		20				
		21				
		22				
		23				
		24				



TruCount Control

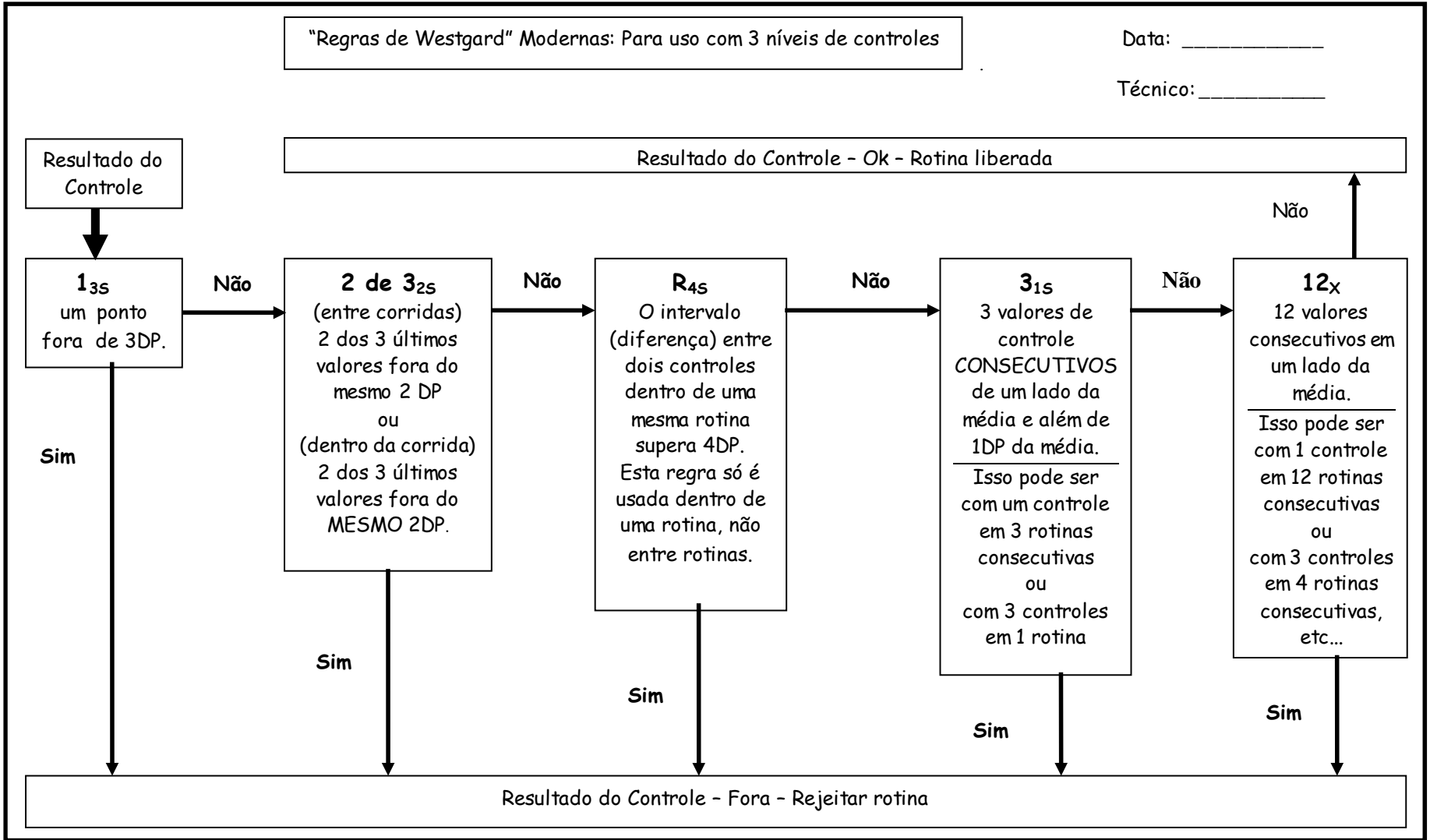
BAIXO - A		
	Lote #	DP
+3 DP	79,0	24,0
+2 DP	71,0	16,0
+1 DP	63,0	8,0
Média	55,0	
-1 DP	47,0	-8,0
-2 DP	39,0	-16,0
-3 DP	31,0	-24,0



MÉDIO - B		
	Lote #	DP
+3 DP	321,0	66,0
+2 DP	299,0	44,0
+1 DP	277,0	22,0
Média	255,0	
-1 DP	233,0	-22,0
-2 DP	211,0	-44,0
-3 DP	189,0	-66,0



# Fase Pós-Analítica

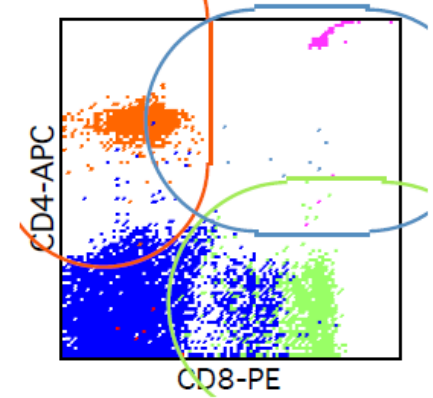
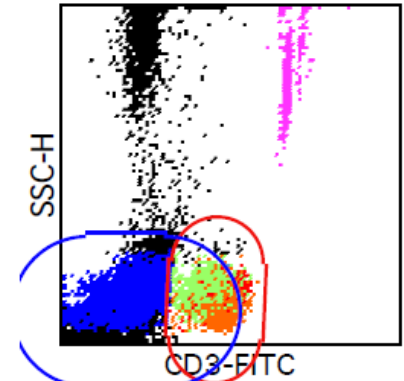
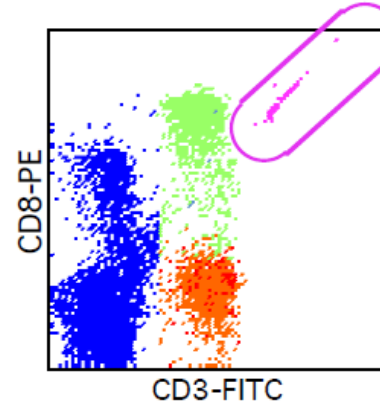
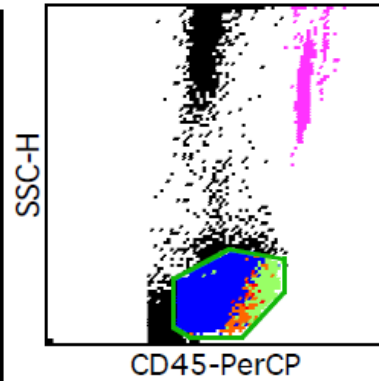




*AEQ*

***\*LAUDOS ENVIADOS PARA O EMAIL DO SUPORTE CIENTÍFICO***

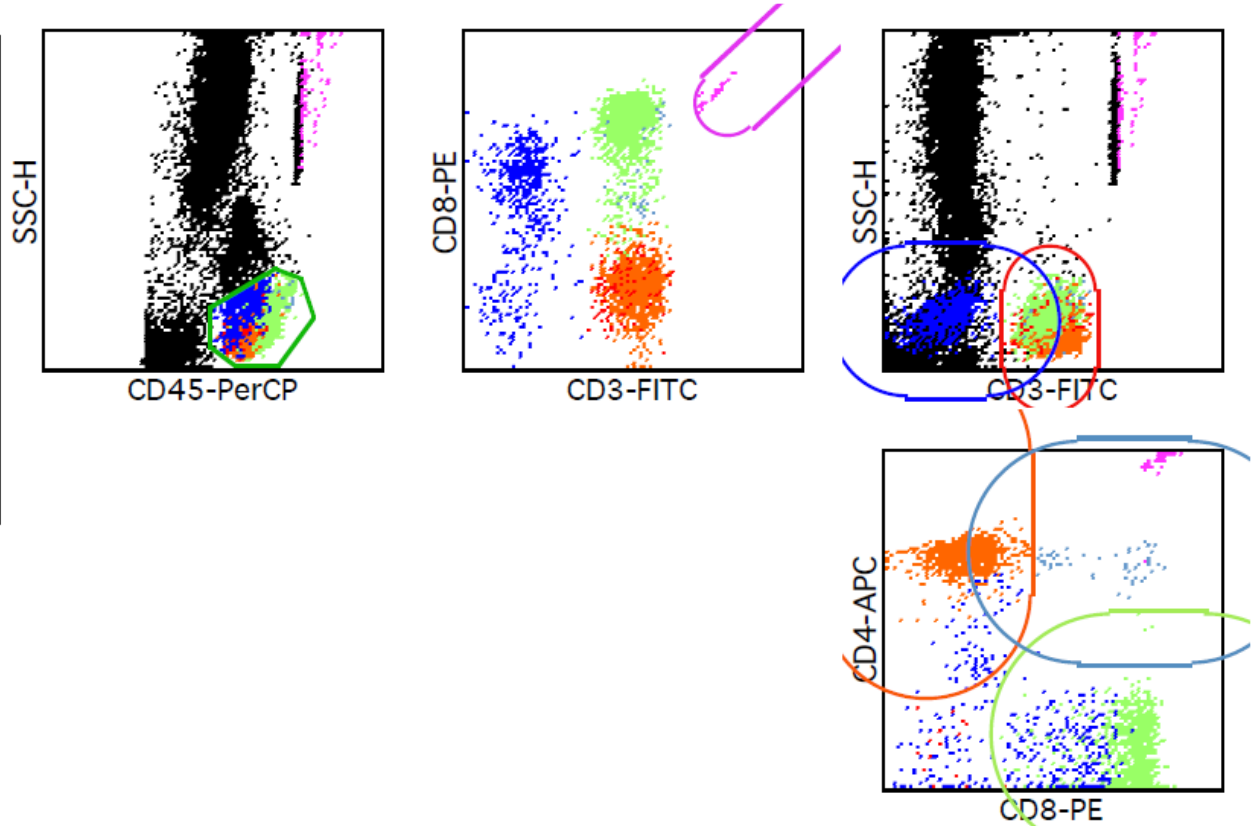
Lymph Events	80292	
Bead Events	2349	
CD3+ %Lymph	4	Lo
CD3+ Abs Cnt	1350	
CD3+CD8+ %Lymph	2	Lo
CD3+CD8+ Abs Cnt	759	
CD3+CD4+ %Lymph	2	Lo
CD3+CD4+ Abs Cnt	504	
CD3+CD4+CD8+ %Lymph	0	
CD3+CD4+CD8+ Abs Cnt	3	
CD45+ Abs Cnt	33464	
T H/S Ratio	0.66	Lo





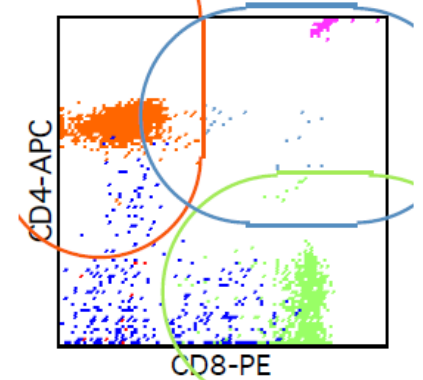
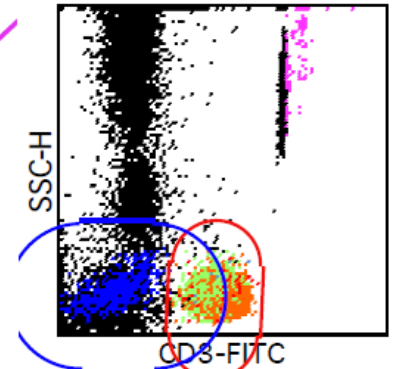
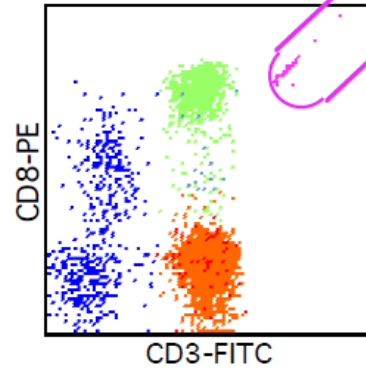
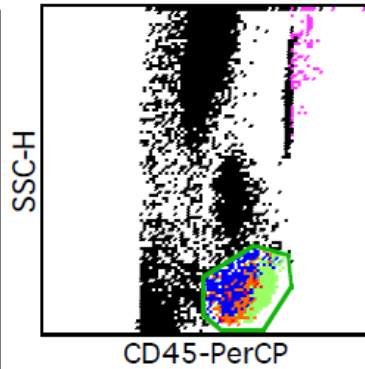
***\*LAUDOS ENVIADOS PARA O EMAIL DO SUPORTE CIENTÍFICO***

Lymph Events	5778	
Bead Events	828	
CD3+ %Lymph	71.82	
CD3+ Abs Cnt	5062	Hi
CD3+CD8+ %Lymph	44.74	Hi
CD3+CD8+ Abs Cnt	3153	Hi
CD3+CD4+ %Lymph	23.87	Lo
CD3+CD4+ Abs Cnt	1682	Hi
CD3+CD4+CD8+ %Lymph	0.90	
CD3+CD4+CD8+ Abs Cnt	63	
CD45+ Abs Cnt	7048	
T H/S Ratio	0.53	Lo



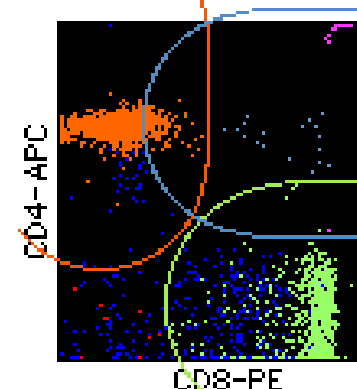
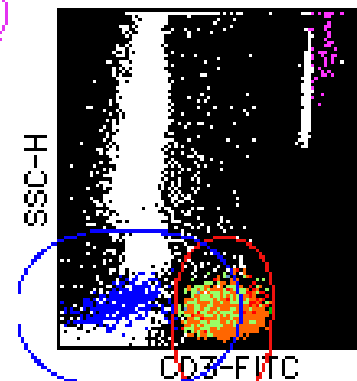
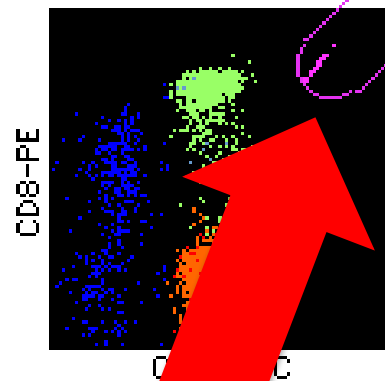
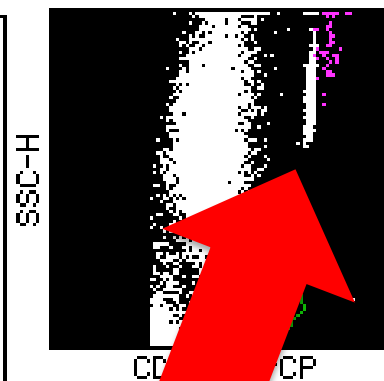
***\*LAUDOS ENVIADOS PARA O EMAIL DO SUPORTE CIENTÍFICO***

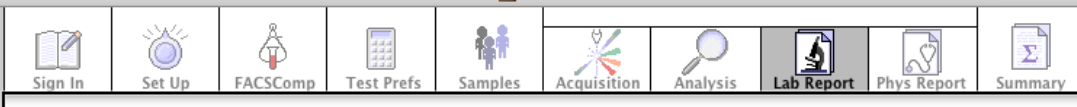
Lymph Events	6195
Bead Events	953
CD3+ %Lymph	66.10
CD3+ Abs Cnt	4340 Hi
CD3+CD8+ %Lymph	28.62
CD3+CD8+ Abs Cnt	1879 Hi
CD3+CD4+ %Lymph	35.66
CD3+CD4+ Abs Cnt	2341 Hi
CD3+CD4+CD8+ %Lymph	0.32
CD3+CD4+CD8+ Abs Cnt	21
CD45+ Abs Cnt	6566
T H/S Ratio	1.25



***\*LAUDOS ENVIADOS PARA O EMAIL DO SUPORTE CIENTÍFICO***

Lymph Events	4907
Bead Events	112
CD3+ %Lymph	80.05
CD3+ Abs Cnt	34370 Hi
CD3+CD8+ %Lymph	28.57
CD3+CD8+ Abs Cnt	12268 Hi
CD3+CD4+ %Lymph	49.05
CD3+CD4+ Abs Cnt	21061 Hi
CD3+CD4+CD8+ %Lymph	0.39
CD3+CD4+CD8+ Abs Cnt	166
CD45+ Abs Cnt	42936
T H/S Ratio	1.72





Message: After viewing the Lab Report for this sample, click the Continue button.

BD

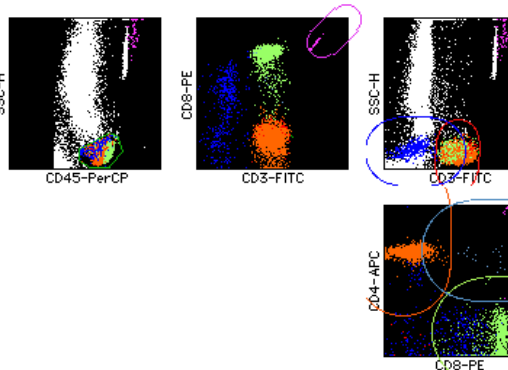
MultiSET™ Lab Report

Director: BD Software: MultiSET V2.2  
 Operator: BD Cytometer: (file-based analysis)

Sample Name: Amostra Date Acquired: 04-Apr-18 16:27:07  
 Sample ID: Amostra Date Analyzed: 07 de dez de 2018  
 Case Number: Ref. Range Type: BD  
 Panel Name: Rotina 4C

**CD3/CD8/CD45/CD4 TruC** Data Set [ 1 ] Data File: Amostra02.01  
 Reagent Lot ID: 72499 Events Acquired: 20000 Abs Cnt Bd Lot ID: 17142 Attr Def File: 3/8/4/5/4 MLT/TruC v2.0  
 File ID: 29BC8B38-383E-11E8-A518-00145116DCD2 Beads Per Pellet: 49000

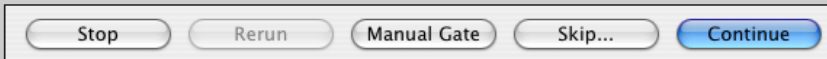
Lymph Events	4907
Bead Events	112
CD3+ %Lymph	80.05
CD3+ Abs Cnt	34370 HI
CD3+CD8+ %Lymph	28.57
CD3+CD8+ Abs Cnt	12268 HI
CD3+CD4+ %Lymph	49.05
CD3+CD4+ Abs Cnt	21061 HI
CD3+CD4+CD8+ %Lymph	0.39
CD3+CD4+CD8+ Abs Cnt	166
CD45+ Abs Cnt	42936
T H/S Ratio	1.72



**QC Messages:**  
 Code 2: Could not acquire the BDIS preferred 500 TruCount Bead events.  
 Code 4: The CD3+ Abs Cnt value lies outside the normal reference range.  
 Code 4: The CD3+CD8+ Abs Cnt value lies outside the normal reference range.  
 Code 4: The CD3+CD4+ Abs Cnt value lies outside the normal reference range.  
 Code 7: Manual Gate is in effect.

**Multi-tube QC**

T Helper/Suppressor Ratio: 1.72

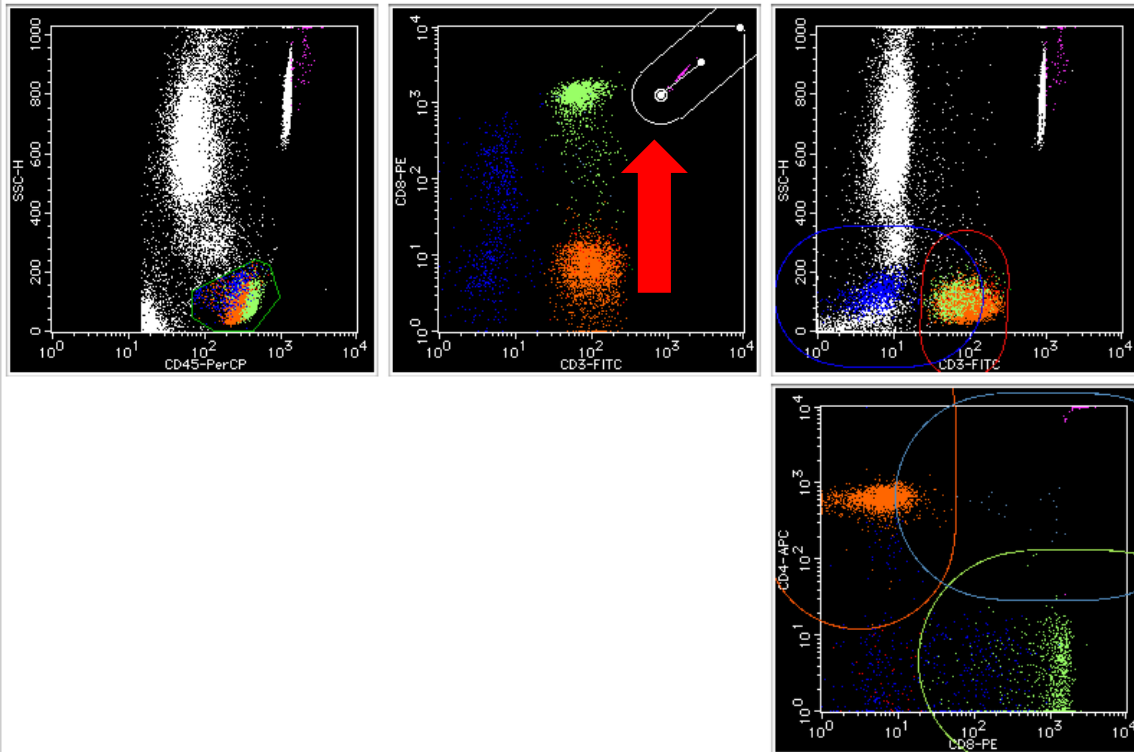




Lymph	
469	135
685	240
734	224
768	113
678	0
549	0
469	48

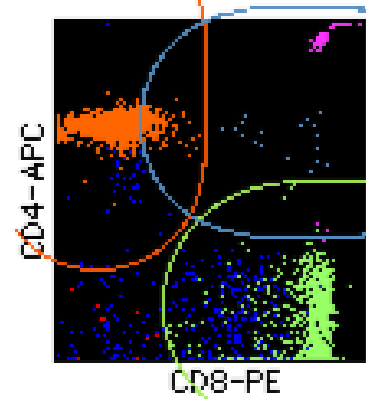
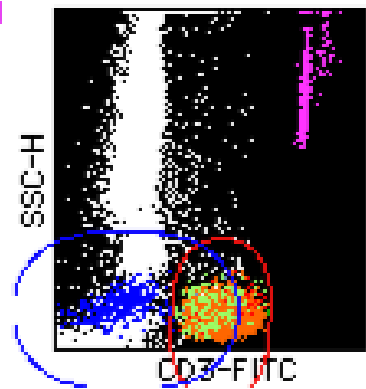
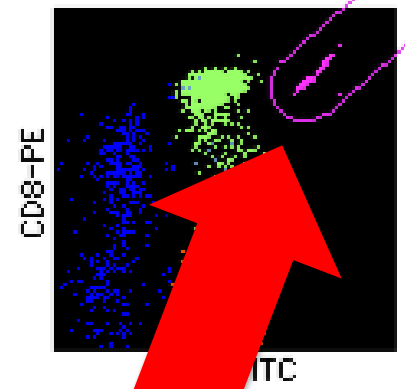
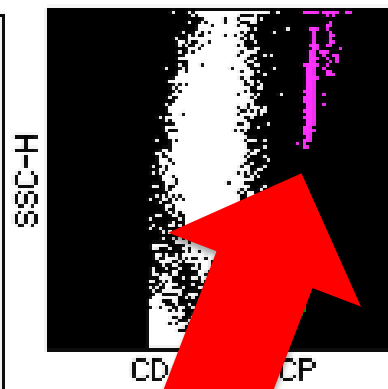
Mes

### Manual Gate



Show Attractors on Lab Report

Lymph Events	4907
Bead Events	2136
CD3+ %Lymph	80.05
CD3+ Abs Cnt	1802
CD3+CD8+ %Lymph	28.57
CD3+CD8+ Abs Cnt	643
CD3+CD4+ %Lymph	49.05
CD3+CD4+ Abs Cnt	1104
CD3+CD4+CD8+ %Lymph	0.39
CD3+CD4+CD8+ Abs Cnt	9
CD45+ Abs Cnt	2251
T H/S Ratio	1.72





*BD FACSVia*

FACSVia Instrument  
656874



23" Color LCD display  
655792



EU Computer Bundle  
660509



## BD™ CS&T beads QC beads



# Controle de Qualidade

Check Instrument Performance

QC History: Levey-Jennings | Instrument QC | HLA-B27 Setup

Cytometer is connected and ready.

BD CS&T Bead Lot: 217030104  
Expiration: DEC 31, 2013  
Operator:   
Lab Director: LJO

**Failed**  
MAY 14, 2015 09:47:01  
[11 QC Messages](#)

Undo Manual Adjustments | Save PDF | New QC | [View Previous QC](#)

Load CS&T Beads into A1  
**RUN** | Eject Rack

**Instrument Noise Measurement**

	Settings	Collected
Events in gate	7500	7500
Time	0 min 20 sec	0 min 0 sec
Events/sec		8896

**BD CS&T Beads**

	Settings	Collected
Events in gate	25000	20000
Time	5 min 0 sec	5 min 0 sec
Events/sec		

**BD CS&T Beads**

Gate: [No Gating]

Mid + Bright 53.7%  
Dim 31.7%

**BD CS&T Beads**

Gate: Dim and Mid + Bright

FL1 Bright 31.7%  
FL2 Bright 31.7%  
FL3 Bright 31.7%  
FL4 Bright 31.7%

Gráfico de identificação das esferas por tamanho e granulidade

Gráficos de identificação das esferas brilhantes

ABORT QC

Close



# Controle de Qualidade

## Results

### QC Report

Parameter	Bright Bead Median	MFI Range		% Bright Bead rCV	Instrument Sensitivity	Sensitivity Spec.	Parameter Pass/Fail
FSC	586853	477453	716179	2.5%	127	30	Pass
SSC	308774	240276	360414	11.3%	104	50	Pass
FL1	34828	27688	41532	2.8%	232	80	Pass
FL2	33543	26889	40334	3.3%	647	200	Pass
FL3	89522	68504	102755	3.3%	90	40	Pass
FL4	28973	24718	37077	5.4%	155	70	Pass



# Analise de resultados

**ID: AA107M8B6S6R2**

Report Date: FEB 26, 2016 22:28:06 PST

BD FACSVia Serial #: AC6568747046  
BD FACSVia Software Version: 2.0.216.1

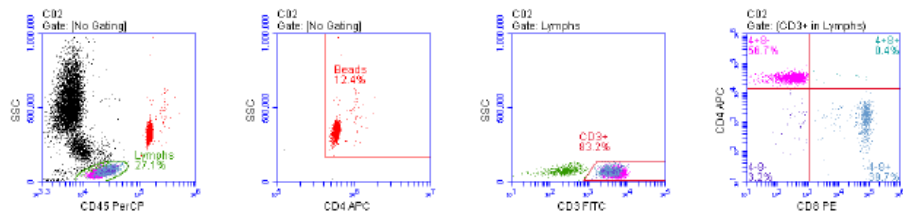
Name: 3\_8\_45\_4  
Case #: 7046

Date Acquired FEB 23, 2016 13:51:39 PST  
Trucount Lot # 15309  
Trucount Bead Count 48100  
Trucount Expiration Date NOV 30, 2017

Operator JN  
Prep JN  
Tube Rack N/A  
Lab Director NA  
Reviewed By JN FEB 26, 2016 22:27:53 PST

BD FACSVia File Name: C:\Users\Admin\Desktop\New folder\7046 AOBAS 022316 3\_JN.cs

**Multitest 3/8/45/4 + Trucount**

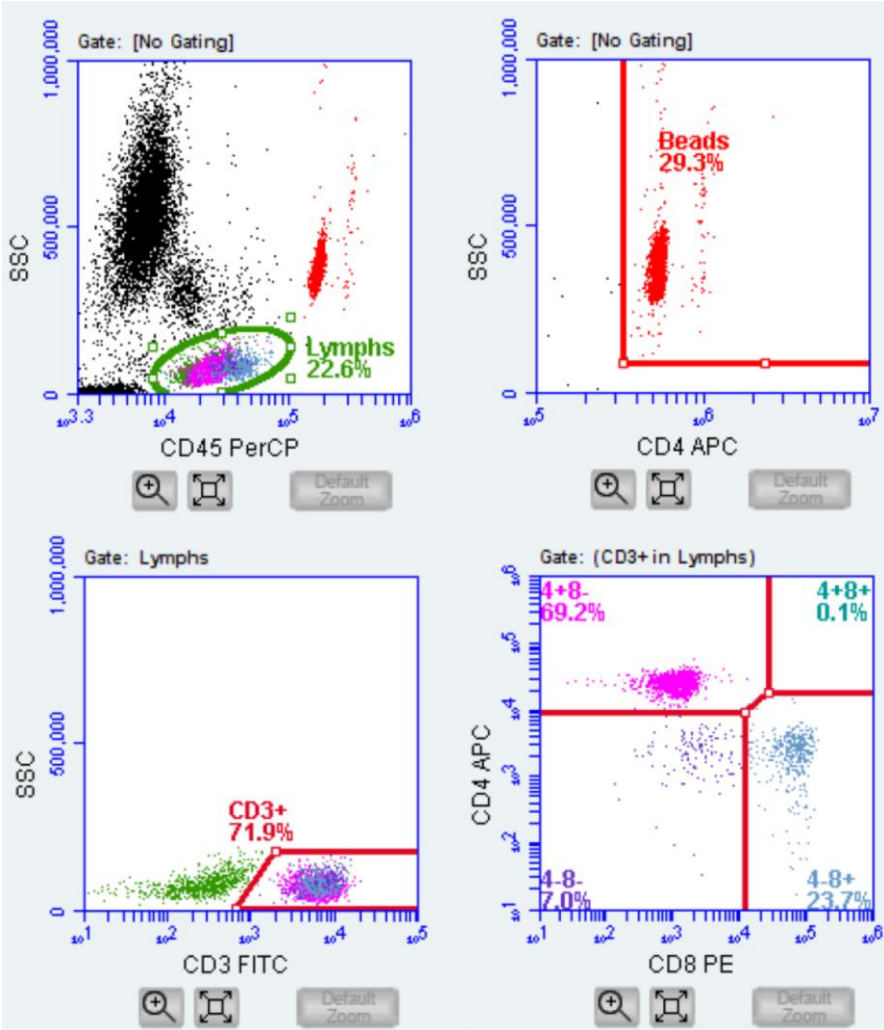


	Result
CD45+ Lymphs Events	2732
Beads Events	1247
CD45+ Lymphs Abs Count (cells/ $\mu$ L)	2108
CD3+ Percent of Lymphs	83.20%
CD3+ Abs Count (cells/ $\mu$ L)	1754
CD3+ CD4+ Percent of Lymphs	47.51%
CD3+ CD4+ Abs Count (cells/ $\mu$ L)	1001
CD3+ CD8+ Percent of Lymphs	33.38%
CD3+ CD8+ Abs Count (cells/ $\mu$ L)	704
CD3+ CD4+ CD8- Percent of Lymphs	47.14%
CD3+ CD4+ CD8- Abs Count (cells/ $\mu$ L)	994
CD3+ CD4- CD8+ Percent of Lymphs	33.02%
CD3+ CD4- CD8+ Abs Count (cells/ $\mu$ L)	696
CD3+ CD4+ CD8+ Percent of Lymphs	0.37%
CD3+ CD4+ CD8+ Abs Count (cells/ $\mu$ L)	8
CD3+ CD4- CD8- Percent of Lymphs	2.67%
CD3+ CD4- CD8- Abs Count (cells/ $\mu$ L)	58
4/8 Ratio	1.42
Percent T-Sum	3.2%

QC Messages



# Analise de resultados



	Result
CD45+ Lymphs Events	2705
Beads Events	1590
CD45+ Lymphs Abs Count (cells/ $\mu$ L)	1701
CD3+ Percent of Lymphs	71.90%
CD3+ Abs Count (cells/ $\mu$ L)	1223
CD3+ CD4+ Percent of Lymphs	49.83%
CD3+ CD4+ Abs Count (cells/ $\mu$ L)	847
CD3+ CD8+ Percent of Lymphs	17.12%
CD3+ CD8+ Abs Count (cells/ $\mu$ L)	291
CD3+ CD4+ CD8- Percent of Lymphs	49.76%
CD3+ CD4+ CD8- Abs Count (cells/ $\mu$ L)	846
CD3+ CD4- CD8+ Percent of Lymphs	17.04%
CD3+ CD4- CD8+ Abs Count (cells/ $\mu$ L)	290
CD3+ CD4+ CD8+ Percent of Lymphs	0.07%
CD3+ CD4+ CD8+ Abs Count (cells/ $\mu$ L)	1
CD3+ CD4- CD8- Percent of Lymphs	5.03%
CD3+ CD4- CD8- Abs Count (cells/ $\mu$ L)	86
4/8 Ratio	2.91
Percent T-Sum	7.0%



*Dúvidas??*

***CONTATO***

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***suporte.tecnico@bd.com***