



# curso de citometria de fluxo

MÓDULO BÁSICO

## *Avaliação de anticorpos antiplaquetários por citometria de fluxo*

*Beatriz M. Martinelli, BSc*

*Vagner de Castro - MD, PhD*

*Laboratório de Imunologia Plaquetária*

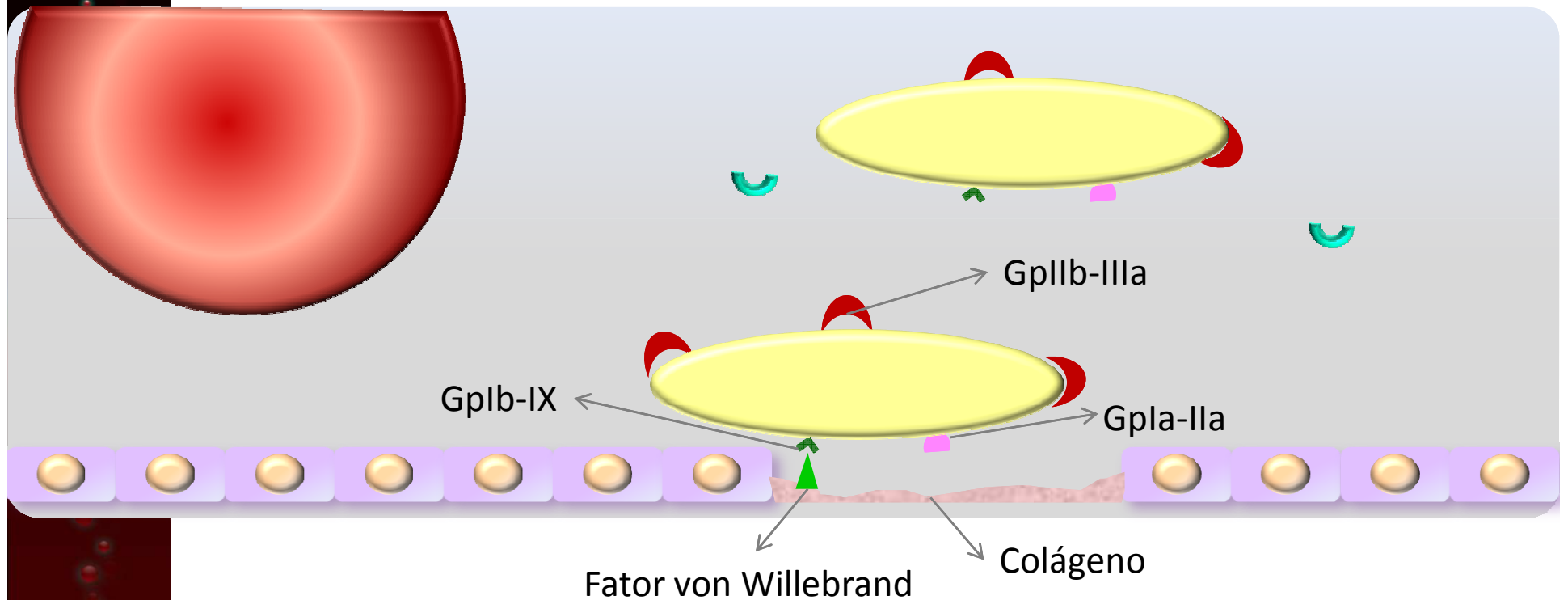
*Hemocentro da UNICAMP*



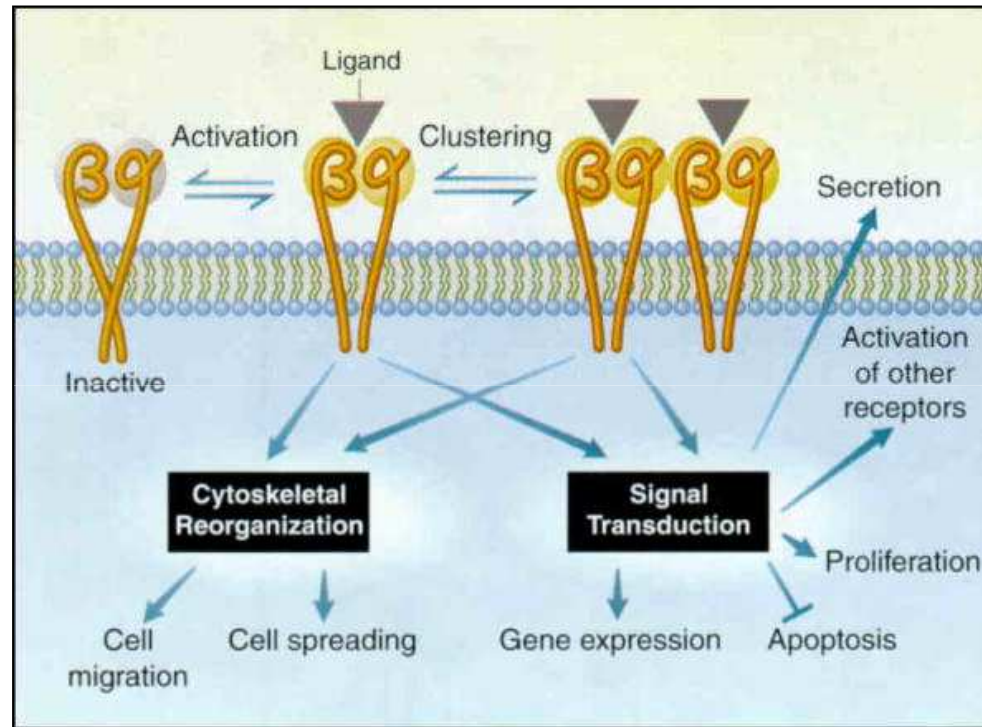
## *Linhas gerais*

- Plaqueta
- Função plaquetária
- Imunologia plaquetária
- Pesquisa de anticorpos antiplaquetários
  - utilização da CF
  - caso clínico
- Considerações finais

# *Função plaquetária*

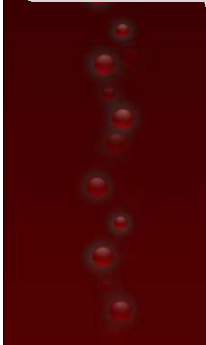


# *Função plaquetária*



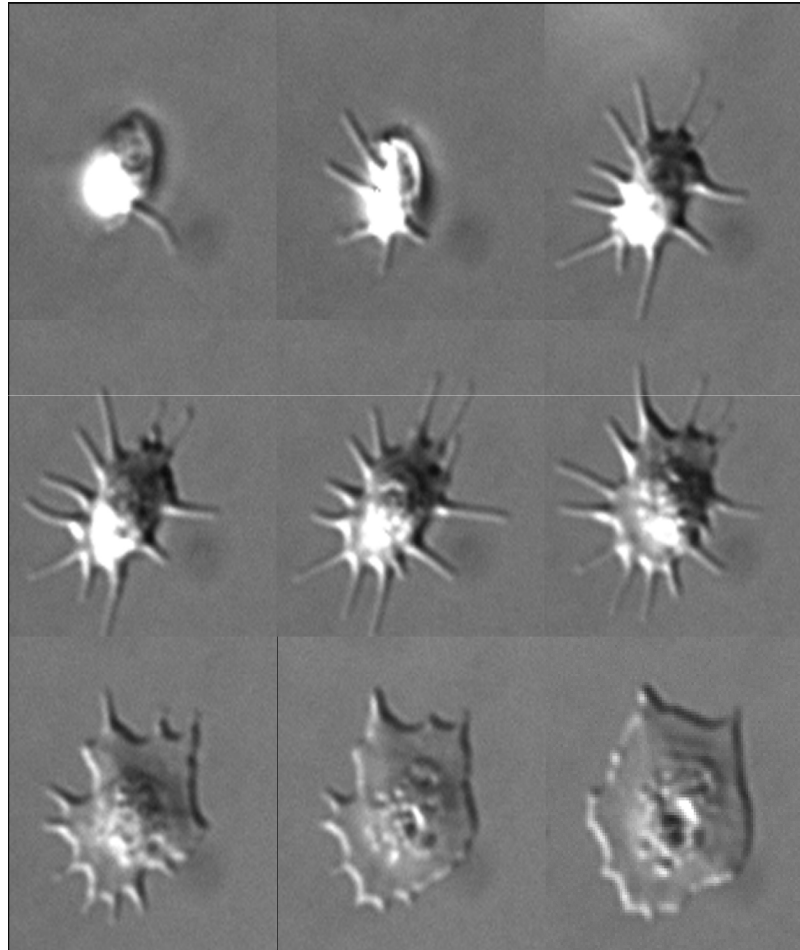
**Adhesion Molecules -- Part I.**  
Frenette, Paul; Wagner, Denisa

New England Journal of Medicine.  
334(23):1526-1529, June 6, 1996.

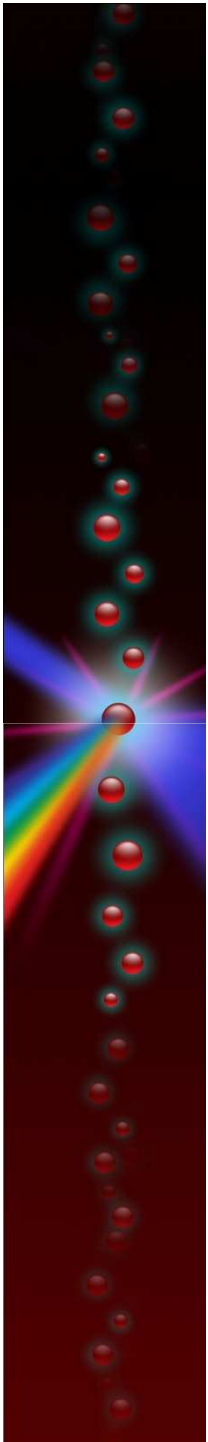




# *Função plaquetária*



<http://www.platelet.bham.ac.uk/>



# *Aloantígenos*

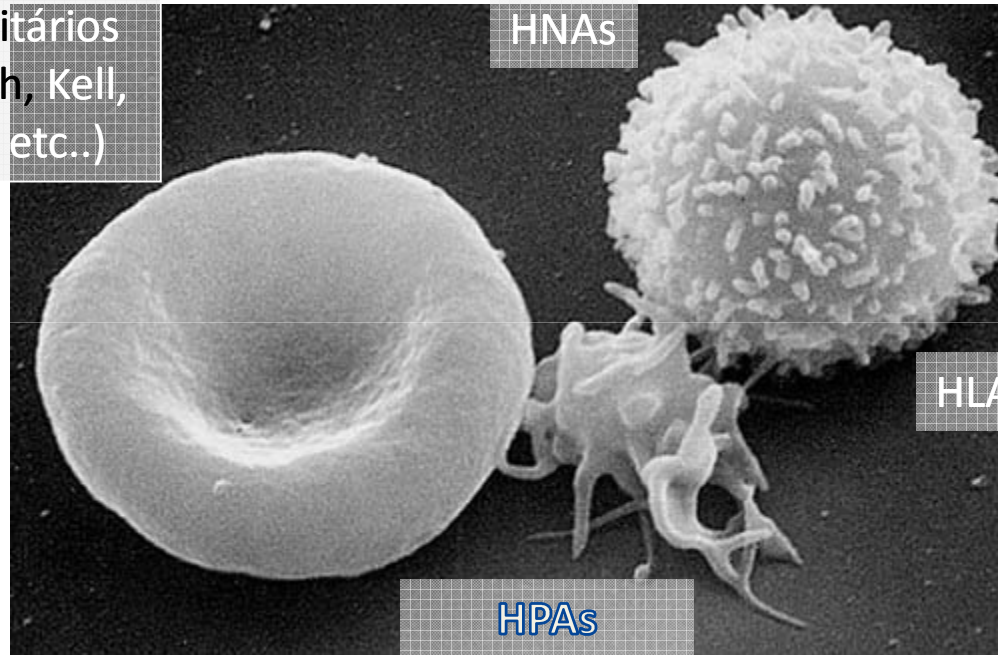
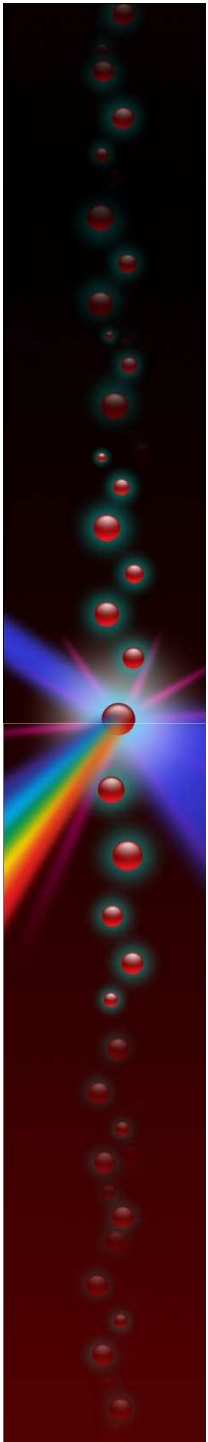
Antígenos  
eritrocitários  
(ABO, Rh, Kell,  
Duffy, etc..)

HNAs

HLAs

HPAs  
ABO  
HLA- Classe I

<http://home.comcast.net/~itpaware/platelets.html>





## *Cópias por célula*

- ABO – variação de até 20X
- HLA – 50-120.000
- GpIIb/IIIa – 50-80.000
- GpIa/IIa – 2-4.000
- GpIb/IX –  $\approx$  60.000

*Delaflor-Weiss & Mintz, 2000; Clemetson in PLATELETS, 2002*





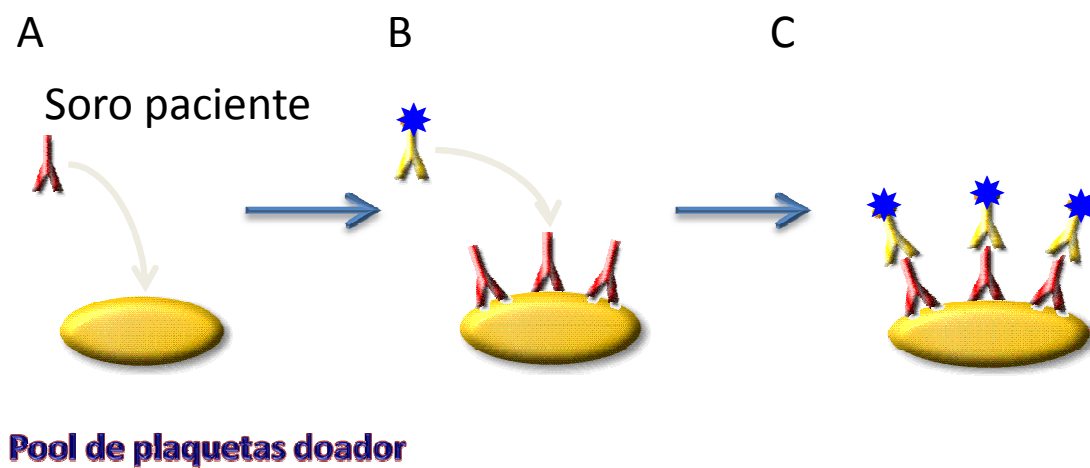
# *Imunologia plaquetária*

- Tipagem
  - Genotipagem ( biologia molecular)
  - Fenotipagem
- Pesquisa de anticorpos antiplaquetários - PAP
  - PIFT - microscopia fluorescência
  - PIFT - CF
  - ELISA
- Identificação de anticorpos antiplaquetários
  - ELISA - MAIPA

*Bray et al., 1994*

# PAP


## PIFT



*Kohler, M., J. Dittmann, et al. (1996) Transfusion 36(3): 250-5.*  
*McFarland, J. G. (2003) Transfus Apheresis Sci 28(3): 297-305.*



## *Definição positividade teste*

- Curva 50 indivíduos
  -  saudáveis
  - $\emptyset$  transfusão
  - calculada mediana
- Leitura
  - razão da leitura  $\geq 2DP \rightarrow$  teste +
  - razão da leitura 1-2DP  $\rightarrow$  teste ?



## *Definição positividade teste*

- Atualmente
  - $\geq 1,13 < 1,26 \rightarrow ?$
  - $\geq 1,26 \rightarrow +$
- Controle +
  - Pool
  - Soro Anti-HPA-1a

# Definição positividade teste

- Atualmente
  - $\geq 1,13 < 1,26 \rightarrow ?$
  - $\geq 1,26 \rightarrow +$
- Controle +
  - Pool
  - Soro Anti-HPA-1a

*European Journal of Immunogenetics* **26**, 355–360

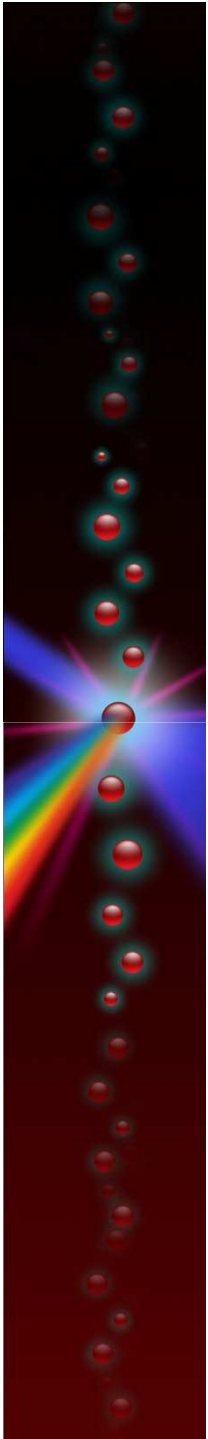
**Table 1.** Genotype and gene frequencies of HPA-1–5 systems in Amerindians, Brazilian Caucasians and Brazilian Blacks

HPA	Caucasians (n = 100)					Blacks (n = 150)					Parakanã Indians (n = 70)				
	Genotype (%)			Gene frequency		Genotype (%)			Gene frequency		Genotype (%)			Gene frequency	
	aa	ab	bb	a	b	aa	ab	bb	a	b	aa	ab	bb	a	b
1	86	13	1	0.925	0.075	82.6	15.4	2	0.903	0.097	100	—	—	1	0
2	73	24	3	0.850	0.150	65.4	31.3	3.3	0.810	0.190	71.4	21.4	7.2	0.821	0.179
3	37	46	17	0.600	0.400	45.4	42.6	12	0.666	0.334	58.5	34.3	7.2	0.757	0.243
4	100	0	0	1	0	100	0	0	1	0	100	0	0	1	0
5	85	14	1	0.920	0.080	76	23.3	0.7	0.876	0.124	100	—	—	1	0



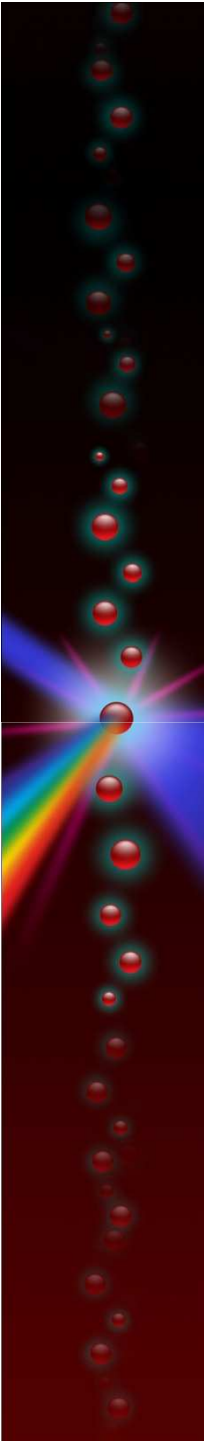
# PAP

- Limitações
  - Pool de doadores
    - doadores † regulares GS O
    - plaquetas frescas – genotipagem geral/e desconhecida
      - ausência do Ag → teste falso ⚠
    - pool → ↓ esse risco
  - Define presença Ac; não define especificidade
    - \* Identificação (especificidade) → MAIPA/MACE
- Vantagens
  - Reconhece anticorpos ligados à plaqueta (HPA, HLA,...)
  - Rapidez, versatilidade



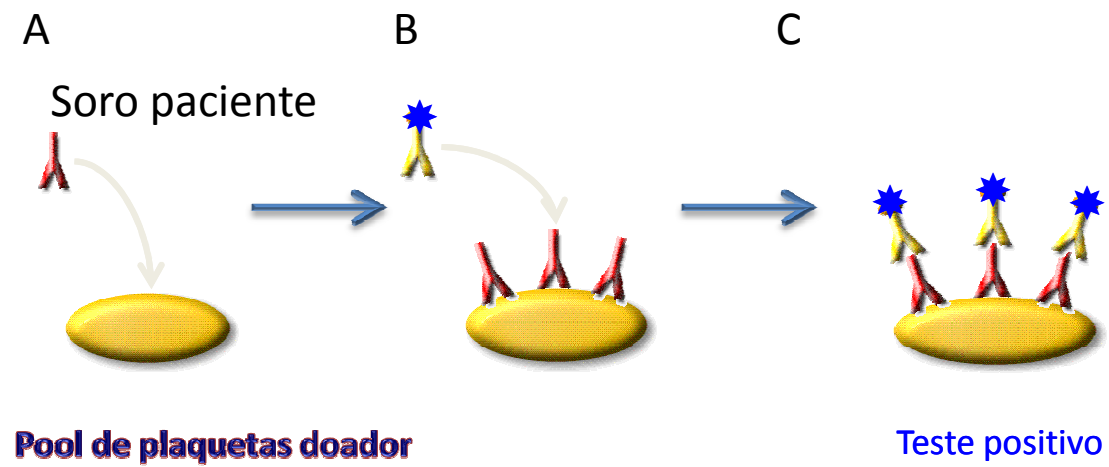
# PAP

- Utilizações
  - pesquisa de anticorpos antiplaquetários
    - teste direto (*análogo ao TAD/ "Coombs Direto" eritrocitário*)
    - teste indireto (*análogo ao PAI/ "Coombs Indireto" eritrocitário*)
    - anticorpos associados a drogas
  - prova de compatibilidade plaquetária



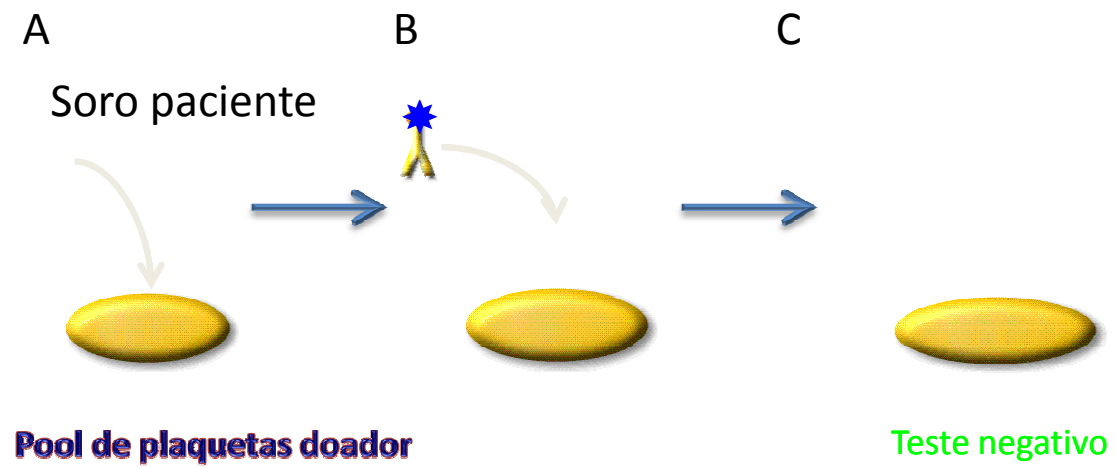
# *PAP*

## *Indireto*



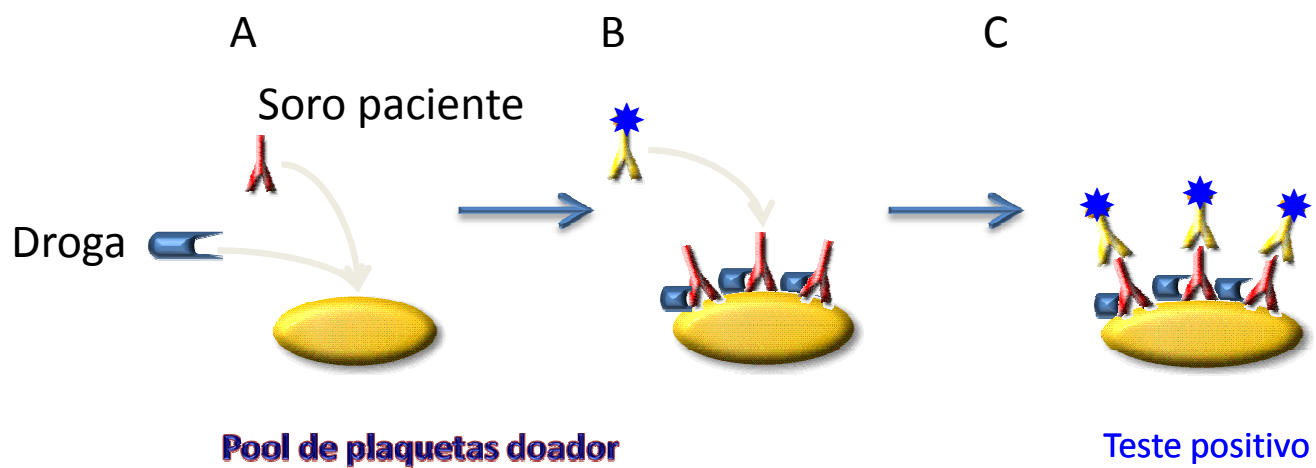
# *PAP*

## *Indireto*



# PAP

## Plaquetopenia por drogas

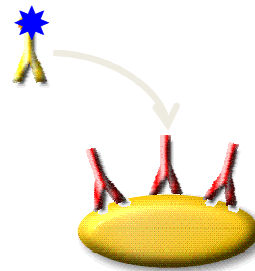




# *PAP*

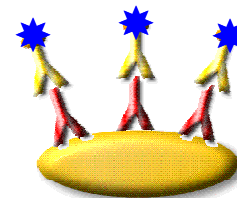
## *Direto*

A

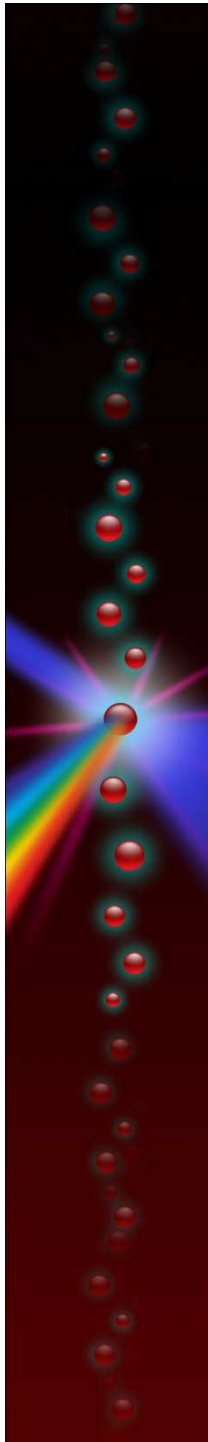


**Plaquetas paciente**

B

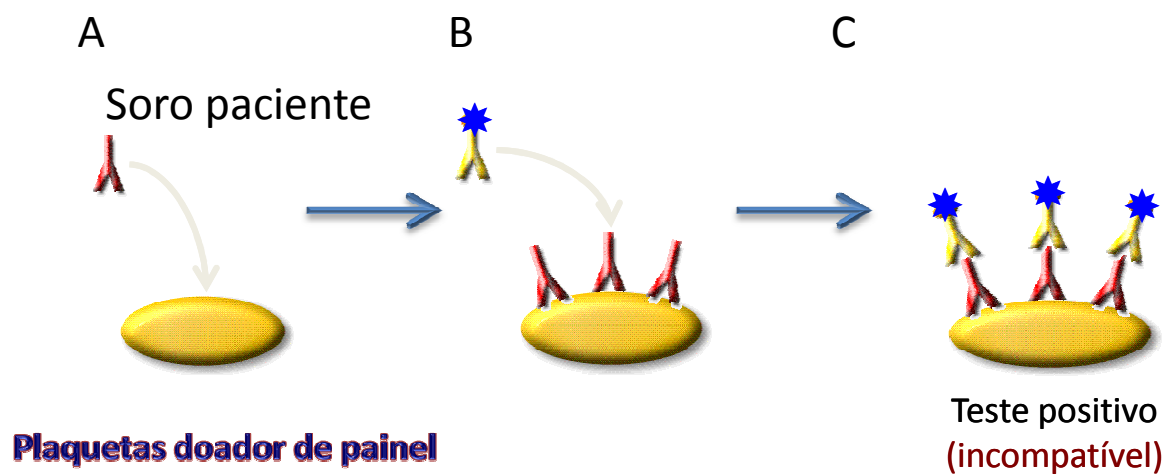


**Teste positivo**



# PAP

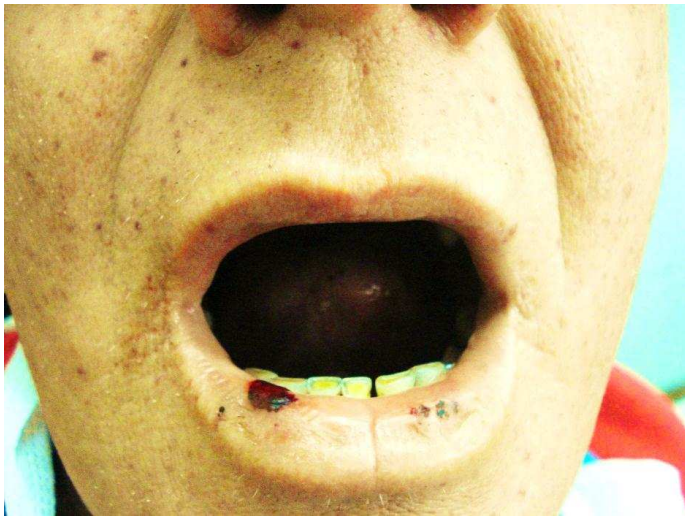
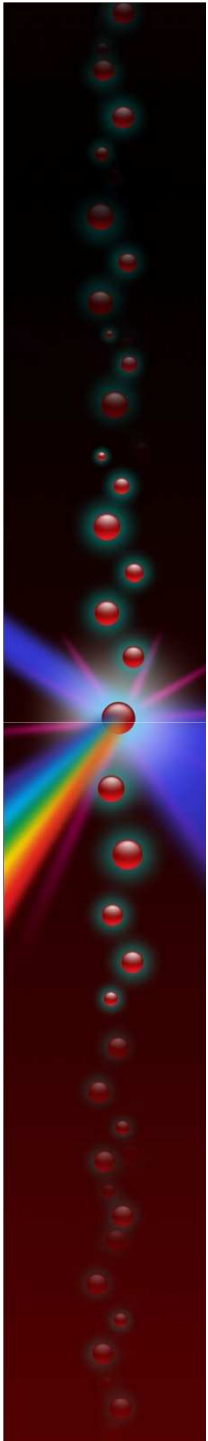
*x-match (compatibilidade)*





## *Caso clínico*

- JWG, ♂, 45a, LMA-M2
  - Diagnóstico RP set'09
  - História de tx prévias infância-18a (causa ???)
  - PIFT: +
  - PRA: + (reatividade 94%)
  - 2 doadores compatíveis (3match)
    - 1ddr: não localizável
  - Nov'09 → TMO





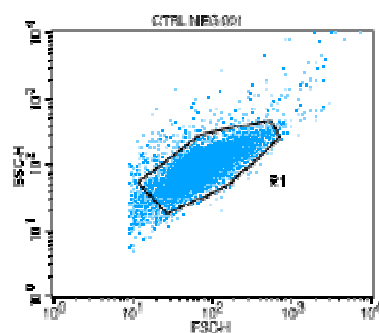
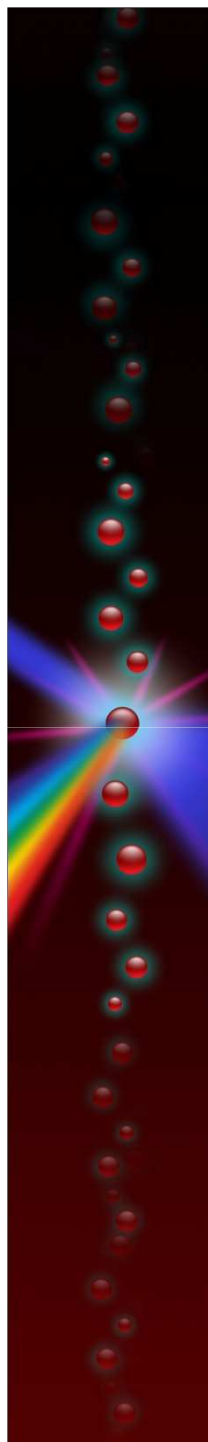
## **The Evaluation and Management of Platelet Refractoriness and Alloimmunization**

**Table 1. Grades of HLA Matching Between  
Recipient and Donor**

Grade	
A	All 4 antigens in the donor are identical to the recipient
B1U	Only 3 donor antigens are identified. All are present in the recipient.
B2U	Only 2 donor antigens identified. Both are present in the recipient.
B1X	Three donor antigens are identical to the recipient, fourth is a member of a cross-reactive group.
B2UX	Two of the 3 detectable donor antigens are present in the recipient. The third is a member of a cross-reactive group.
B2X	Two donor antigens are identical to the recipient, 2 are cross-reactive.
C	Three donor antigens are identical to the recipient, 1 antigen is mismatched.
D	Two donor antigens are identical to the recipient, 2 mismatched.

Adapted from McFarland.<sup>145</sup>



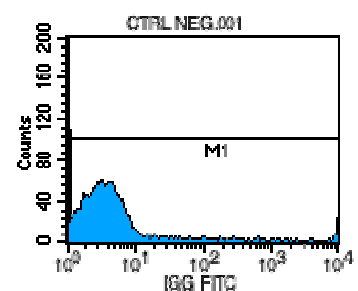


24/09/09

#### Region Statistics

File: CTRL NEG.001 Sample ID: 01  
 Acquisition Date: 24-Sep-09 Gate: No Gate  
 Gated Events: 10000 Total Events: 10000

Region	Events	% Gated	% Total
R1	9258	92.58	92.58

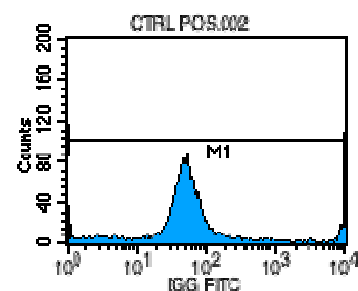


#### CTRL NEGATIVO

##### Histogram Statistics

File: CTRL NEG.001 Sample ID: 01  
 Acquisition Date: 24-Sep-09 Gate: G1  
 Gated Events: 9258 Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	CV	Median	Peak Ch
All	1, 9910	9258	100.00	92.58	847.22	3.13	1
M1	1, 9910	9258	100.00	92.58	847.22	3.13	1

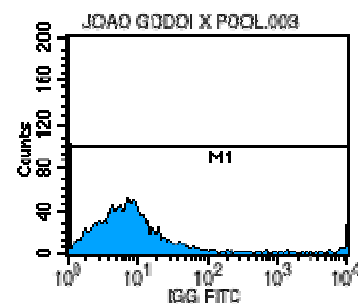


#### CTRL POSITVO

##### Histogram Statistics

File: CTRL POS.002 Sample ID: 01  
 Acquisition Date: 24-Sep-09 Gate: G1  
 Gated Events: 7507 Total Events: 10000

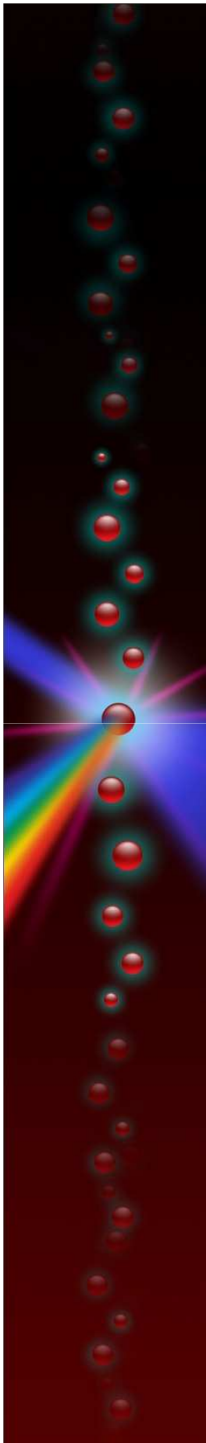
Marker	Left, Right	Events	% Gated	% Total	CV	Median	Peak Ch
All	1, 9910	7507	100.00	75.07	314.53	50.94	9910
M1	1, 9910	7507	100.00	75.07	314.53	50.94	9910



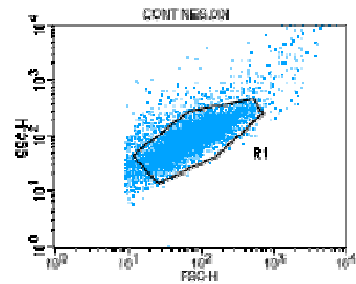
##### Histogram Statistics

File: JOAO GODOI X POOL.003 Sample ID: 01  
 Acquisition Date: 24-Sep-09 Gate: G1  
 Gated Events: 8793 Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	CV	Median	Peak Ch
All	1, 9910	8793	100.00	87.93	652.71	6.67	1
M1	1, 9910	8793	100.00	87.93	652.71	6.67	1



30/09/09

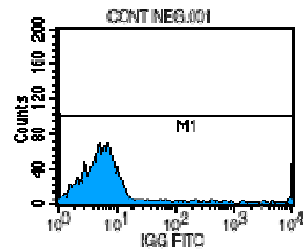


Region Statistics

File: CONT NEG.001	Sample ID: 01
Acquisition Date: 30-Sep-09	Gate: No Gate
Gated Events: 10000	Total Events: 10000

Region	Events	% Gated	% Total
R1	9993	99.93	99.93

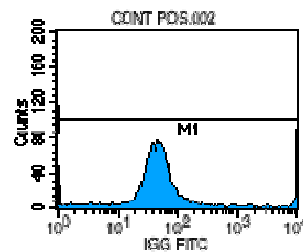


Histogram Statistics

File: CONT NEG.001	Sample ID: 01
Acquisition Date: 30-Sep-09	Gate: G1
Gated Events: 9993	Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	CV	Median	Peak Ch
All	1, 9999	9993	100.00	99.93	621.73	5.42	1
M1	1, 9999	9993	100.00	99.93	621.73	5.42	1



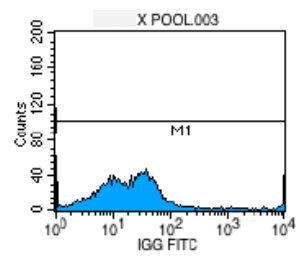
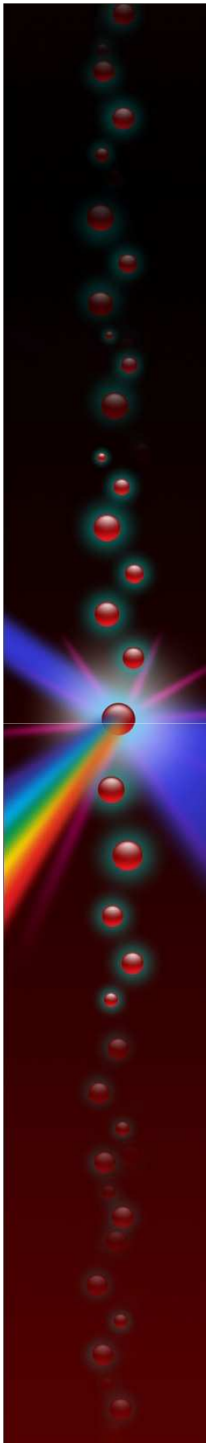
Histogram Statistics

File: CONT POS.002	Sample ID: 01
Acquisition Date: 30-Sep-09	Gate: G1
Gated Events: 7992	Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	CV	Median	Peak Ch
All	1, 9910	7992	100.00	79.92	382.58	45.73	9910
M1	1, 9910	7992	100.00	79.92	382.58	45.73	9910

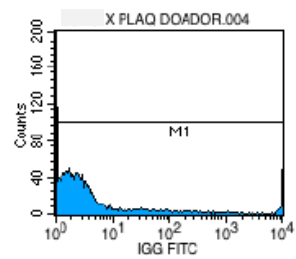
R=8,4



File: X POOL.003 Sample ID: 01  
Acquisition Date: 30-Sep-09 Gate: G1  
Gated Events: 9023 Total Events: 10000

Marker	Left	Right	Events	% Gated	% Total	CV	Median	Peak Ch
All	1	9910	9023	100.00	90.23	626.66	19.28	9910
M1	1	9910	9023	100.00	90.23	626.66	19.28	9910

R=3,6



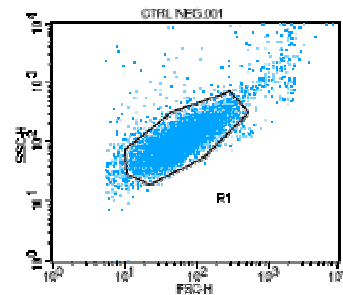
File: X PLAQ DOADOR.004 Sample ID: 01  
Acquisition Date: 30-Sep-09 Gate: G1  
Gated Events: 8108 Total Events: 10000

Marker	Left	Right	Events	% Gated	% Total	CV	Median	Peak Ch
All	1	9910	8108	100.00	81.08	582.49	1.95	1
M1	1	9910	8108	100.00	81.08	582.49	1.95	1

R=0,3

# Cuidado !!!

10 08 10

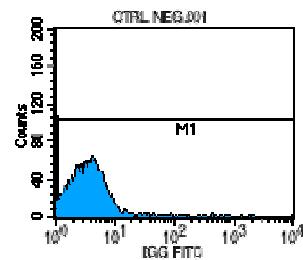


## Region Statistics

File: CTRL NEG.001 Sample ID:  
 Acquisition Date: 10-Aug-10 Gate: No Gate  
 Gated Events: 10000 Total Events: 10000

Region	Events	% Gated	% Total
R1	9341	93.41	93.41

## CTRL NEGATIVO

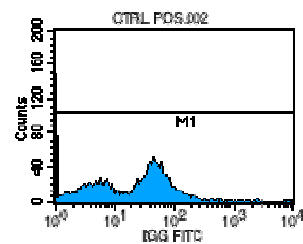


## Histogram Statistics

File: CTRL NEG.001 Sample ID:  
 Acquisition Date: 10-Aug-10 Gate: G1  
 Gated Events: 9341 Total Events: 10000

Marker	Left	Right	Events	% Gated	% Total	CV	Median	Peak Ch
All	1	9910	9341	100.00	93.41	1931.50	3.19	1
M1	1	9910	9341	100.00	93.41	1931.50	3.19	1

## CTRL POSITIVO



## Histogram Statistics

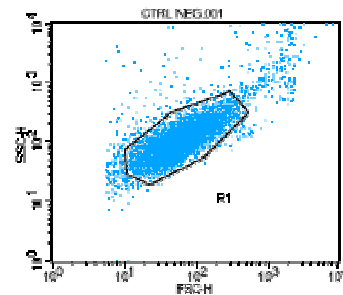
File: CTRL POS.002 Sample ID:  
 Acquisition Date: 10-Aug-10 Gate: G1  
 Gated Events: 8347 Total Events: 10000

Marker	Left	Right	Events	% Gated	% Total	CV	Median	Peak Ch
All	1	9910	8347	100.00	83.47	631.47	29.96	1
M1	1	9910	8347	100.00	83.47	631.47	29.96	1

R=8,6

# Cuidado !!!

10 08 10

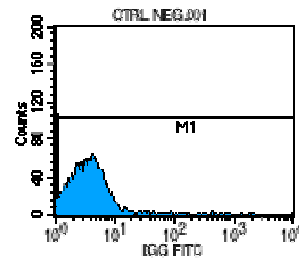


## Region Statistics

File: CTRL.NEG.001 Sample ID:  
 Acquisition Date: 10-Aug-10 Gate: No Gate  
 Gated Events: 10000 Total Events: 10000

Region	Events	% Gated	% Total
R1	9341	93.41	93.41

## CTRL NEGATIVO

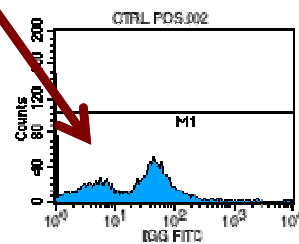


## Histogram Statistics

File: CTRL.NEG.001 Sample ID:  
 Acquisition Date: 10-Aug-10 Gate: G1  
 Gated Events: 9341 Total Events: 10000

Marker	Left	Right	Events	% Gated	% Total	CV	Median	Peak Ch
All	1.	9910	9341	100.00	93.41	1931.50	3.19	1
M1	1.	9910	9341	100.00	93.41	1931.50	3.19	1

## CTRL POSITIVO



## Histogram Statistics

File: CTRL.POS.002 Sample ID:  
 Acquisition Date: 10-Aug-10 Gate: G1  
 Gated Events: 8347 Total Events: 10000

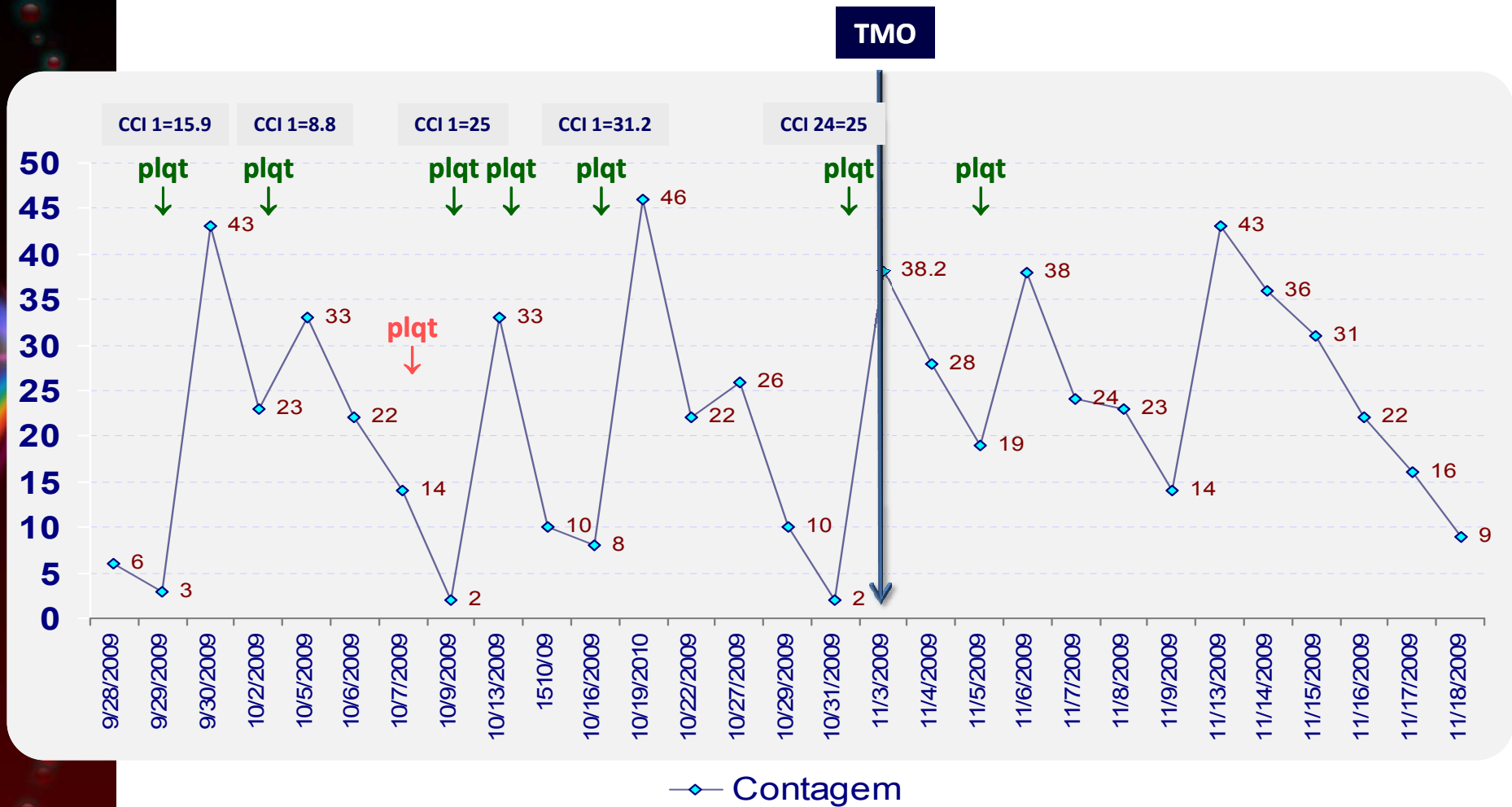
Marker	Left	Right	Events	% Gated	% Total	CV	Median	Peak Ch
All	1.	9910	8347	100.00	83.47	631.47	29.96	1
M1	1.	9910	8347	100.00	83.47	631.47	29.96	1

R=8,6



# Resultado tx BU

*paciente: JWG – LMA + TMO*



BU

All antigens identical, only three antigens detected

# *Caso clínico*





## *Considerações finais*

- CF → Ferramenta útil e importante na avaliação e suporte de pacientes hematológicos / imunologia plaquetária
- Laboratórios
  - CF → várias regiões do país
  - Imunologia plaquetária no Brasil → escassos
- **Necessidade de ampliação da rede de laboratórios especializados no país**

# *Laboratório de Imunologia Plaquetária - Giessen*






# ***Laboratório de Imunologia Plaquetária UNICAMP***



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  - Médico Hematologista e Hemoterapeuta  
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Obrigado !