

# Citometria de fluxo: pesquisa de refratariedade plaquetária

**Vagner de Castro - MD, PhD**

*Laboratório de Imunologia Plaquetária*

*Hemocentro da UNICAMP*

The banner features a large yellow wavy line on a black background. In the top right corner, it says "Evento com pontuação" and shows the "CNA" logo. The bottom left features the text "HEMO 2010" and "5 a 8 de novembro - BRASÍLIA - DF - BRASIL". The bottom right shows images of the Congresso Nacional de Assistência (CNA) building and the Congresso Nacional building. The center text reads "CONGRESSO BRASILEIRO DE HEMATOLOGIA E HEMOTERAPIA".

# Aloantígenos



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



HNAs



HLAs



HPAs  
*HLA-C I*  
*ABO*

# Cópias por célula

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

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

# Refratariedade

- Resposta inadequada à transfusão de plaquetas
  - Observado em 2 transfusões, preferencialmente consecutivas
  - dado objetivo!
    - CCI (1h - <5000; 24h - <2500)
    - % recuperação
    - Ø somente observação clínica...

*Hod & Schwartz, Br J Haem 2008, 142: 348*

# Refratariedade

- **Incidência: 5-15%**
  - 27% (143) de 528 pacientes politx.
  - **UNICAMP 2005-2010 (n=786 pctes com tx CP) : 3,7%**
    - 27,6% testes sorológicos  $\Theta$
- **Etiologia**
  - Não imune: presentes em >90% dos casos
    - Infecção/febre, esplenomegalia, CIVD, TMO
  - Imune:  $\approx$ 25% dos casos
    - incompat ABO
    - Ac HLA (3-90% - dependendo do tipo de hemocomponente: **leucoredução!!**)
    - Ac HPA (9-25%)

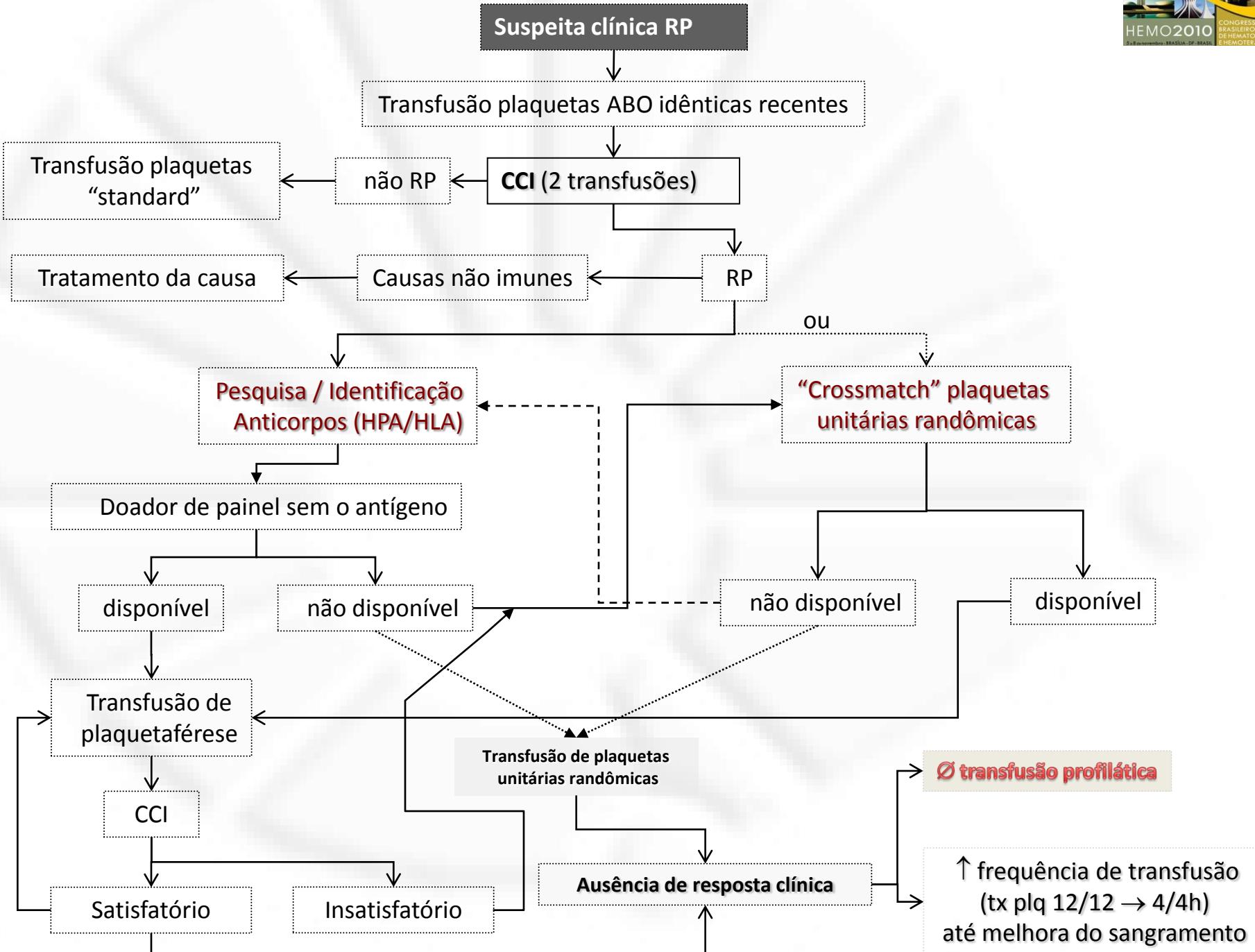
*Rebulla et al., 2004, Slichter et al., 2005, Vassallo, 2009*

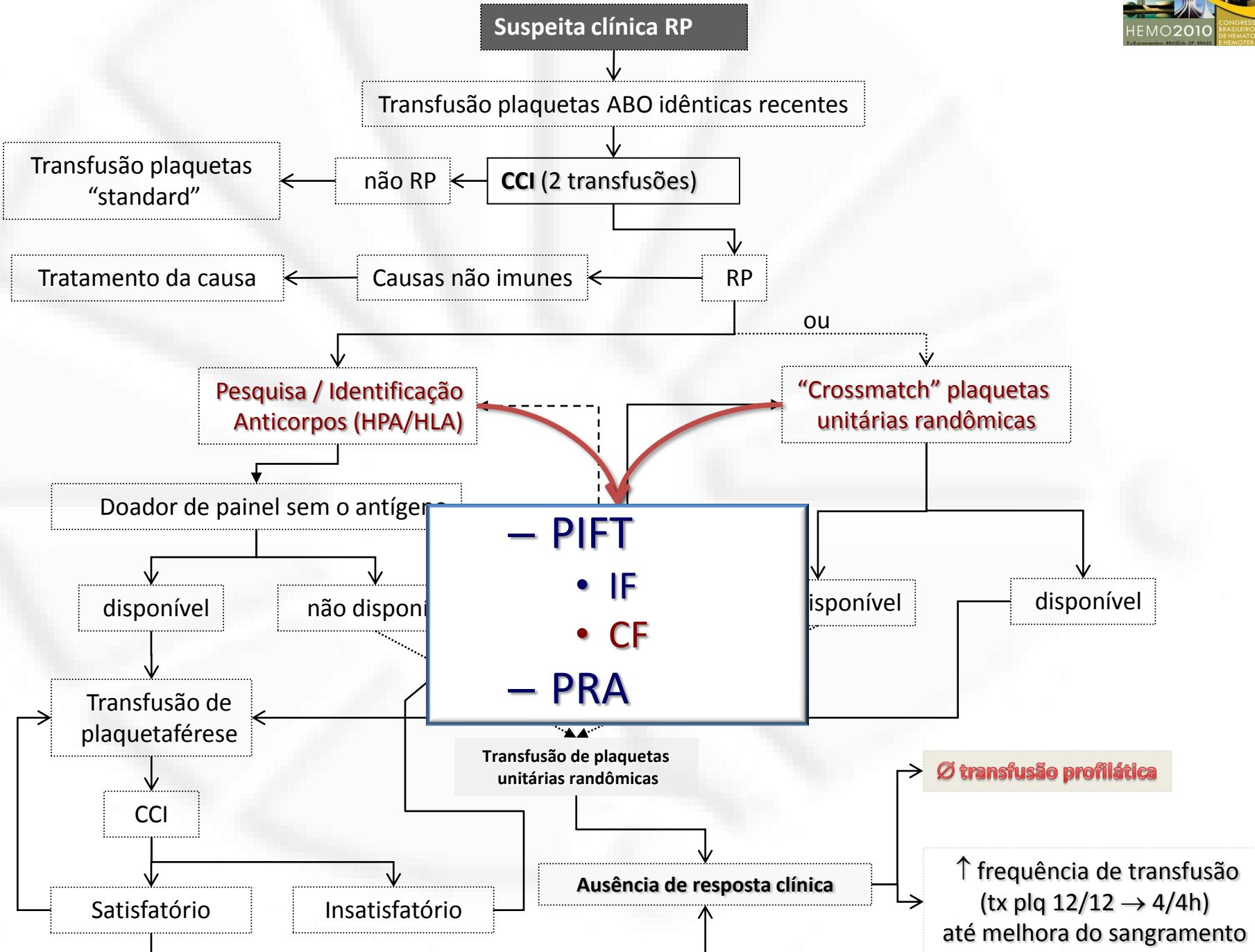
# Manuseio da RP

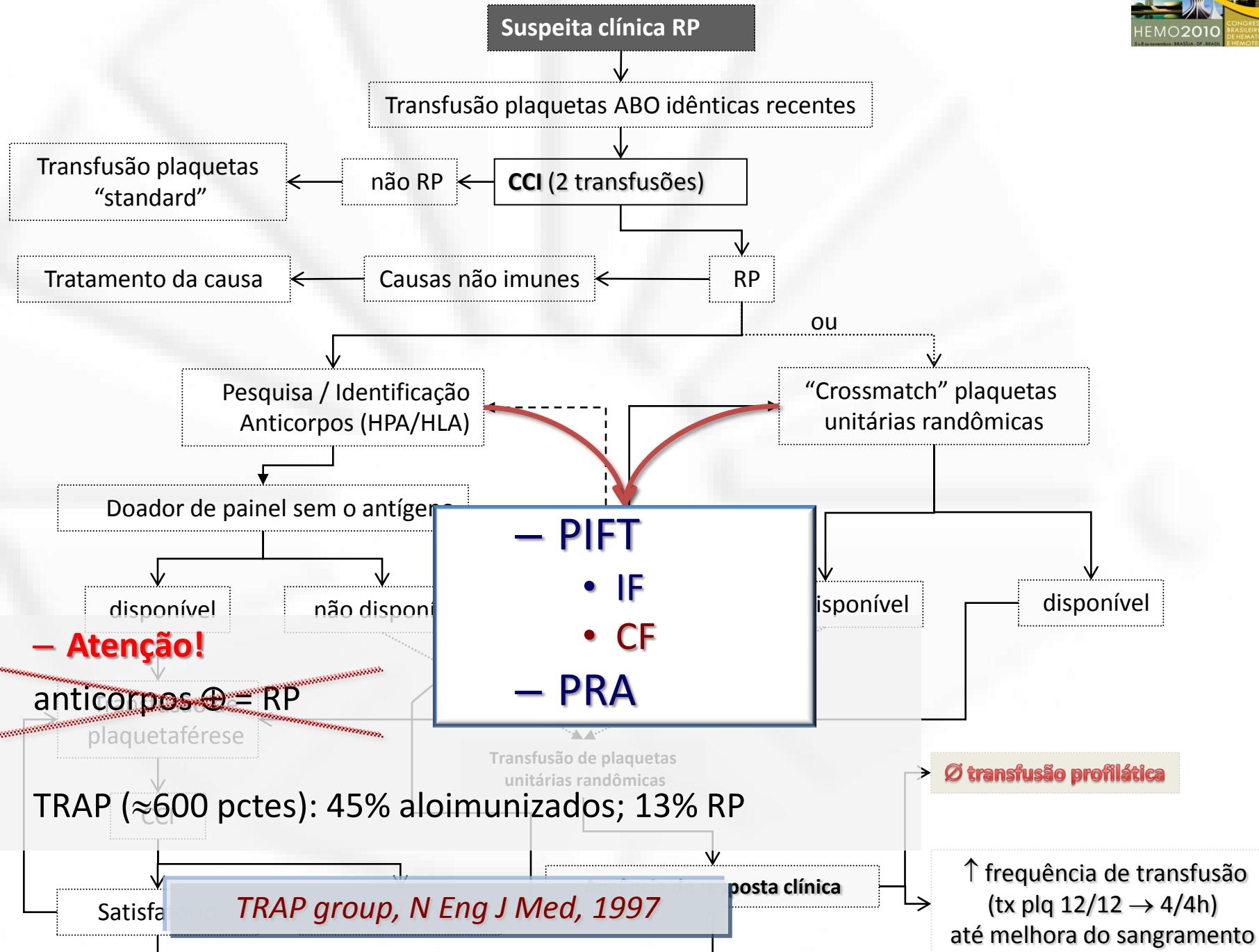
- Algorítmo
  - Suspeita clínica
  - Investigação
  - Suporte hemoterápico

# Manuseio da RP

- Algorítmo
    - Suspeita clínica
    - Investigação
    - Suporte hemoterápico
- 
- ```
graph TD; A[CF] --> B[Investigação]; A --> C[Suporte hemoterápico]
```



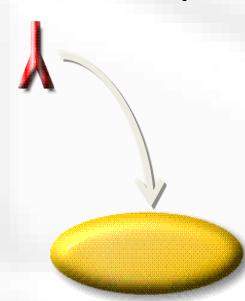




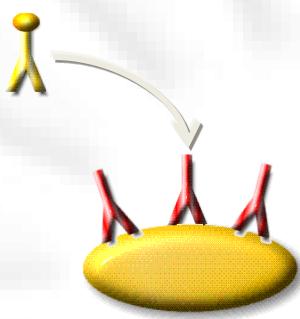
# PAP

A

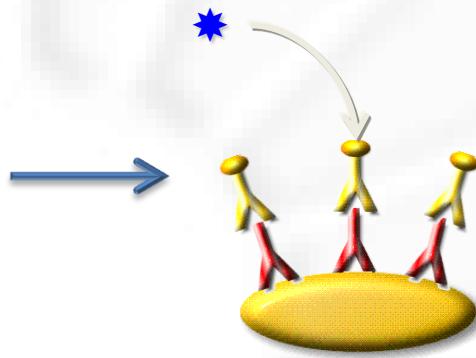
Soro paciente



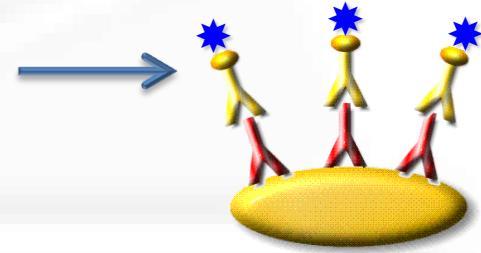
B



C

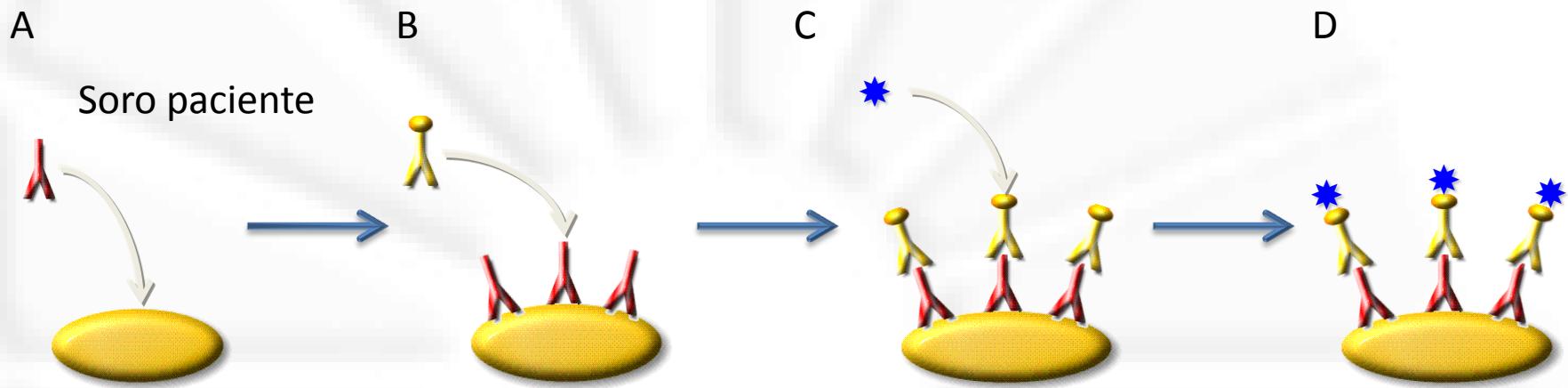


D



Pool de plaquetas doador

# Crossmatch



Plaquetas do doador para xmatch

# 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

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

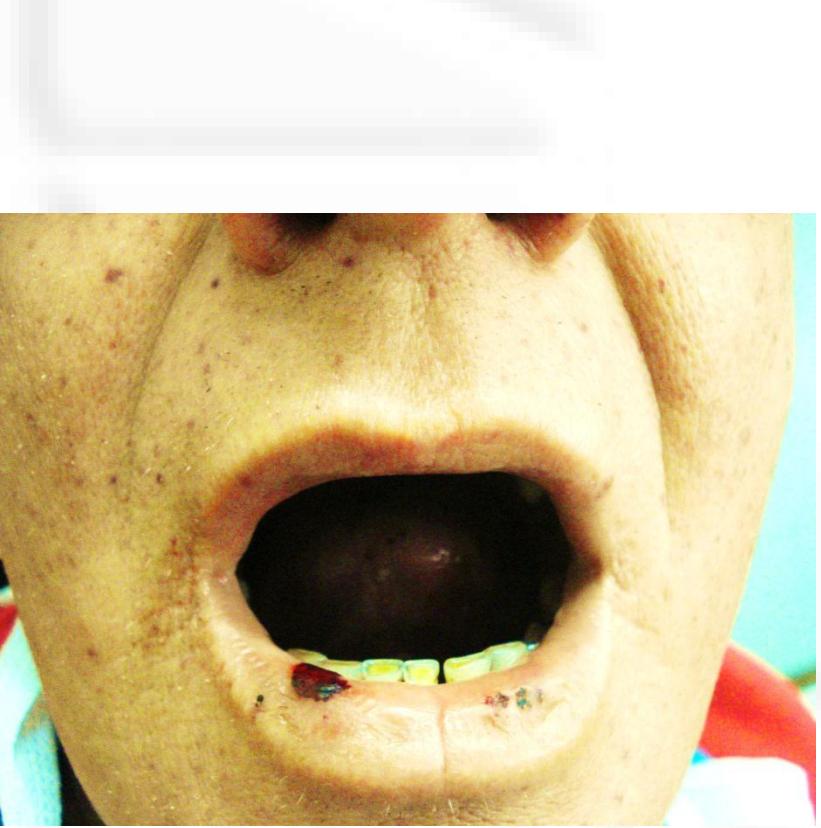
| Caucasians (n = 100) |              |    |    |                | Blacks (n = 150) |              |      |     |                | Parakanã Indians (n = 70) |              |      |     |                |       |
|----------------------|--------------|----|----|----------------|------------------|--------------|------|-----|----------------|---------------------------|--------------|------|-----|----------------|-------|
| HPA                  | 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     |

# PIFT

- Limitações
  - Pool de doadores
    - doadores  $\nexists$  regulares GS O
    - plaquetas frescas – genotipagem geral/e desconhecida
      - ausência do Ag  $\rightarrow$  teste falso  $\Theta$
    - pool  $\rightarrow \downarrow$  esse risco
  - Define presença Ac; não define especificidade
- Vantagens
  - Reconhece anticorpos ligados à plaqueta (HPA, HLA,...)
  - Rapidez, versatilidade

# 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



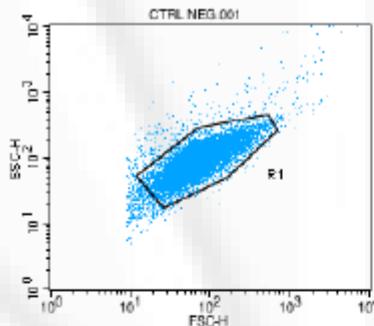
## The Evaluation and Management of Platelet Refractoriness and Alloimmunization

**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>

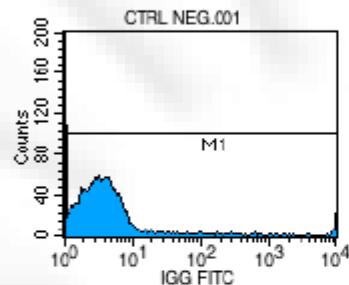
24/09/09



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   |

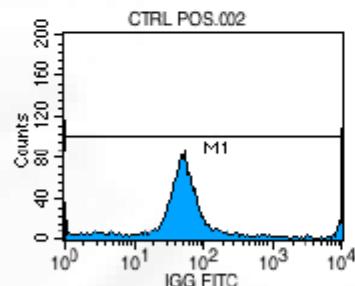


CTRL NEGATIVO

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       |



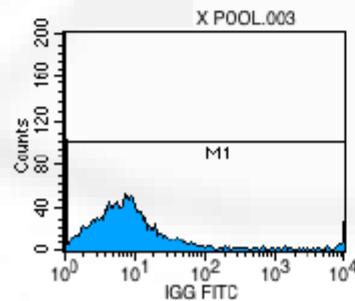
CTRL POSITIVO

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    |

R=16,3



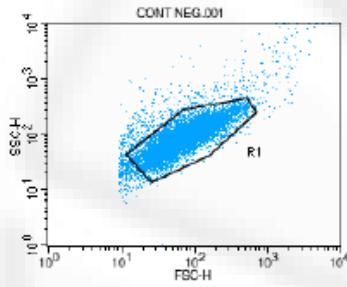
Histogram Statistics

File: JDAO GODDI X POOL003 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       |

R=2,1

30/09/09

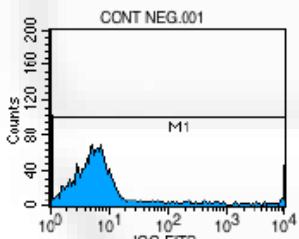


Region Statistics

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     | 9003   | 90.03   | 90.03   |

CTRL NEGATIVO

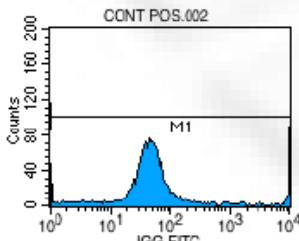


Histogram Statistics

File: CONT NEG.001 Sample ID: 01  
Acquisition Date: 30-Sep-09 Gate: G1  
Gated Events: 9003 Total Events: 10000

| Marker | Left, Right | Events | % Gated | % Total | CV     | Median | Peak Ch |
|--------|-------------|--------|---------|---------|--------|--------|---------|
| All    | 1, 9910     | 9003   | 100.00  | 90.03   | 621.73 | 5.42   | 1       |
| M1     | 1, 9910     | 9003   | 100.00  | 90.03   | 621.73 | 5.42   | 1       |

CTRL POSITIVO

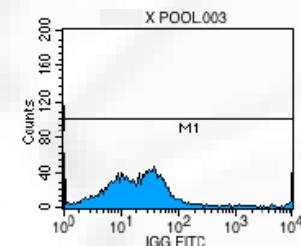


Histogram Statistics

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

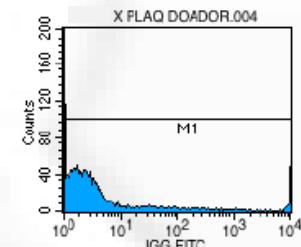


Histogram Statistics

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



Histogram Statistics

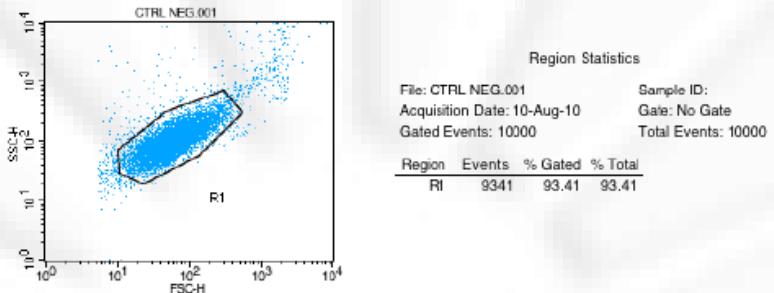
File: X PLAQ DOADOR.004 Sample ID: 01  
Acquisition Date: 30-Sep-09 Gate: G1  
Gated Events: 8108 Total Events: 10000

| 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       |

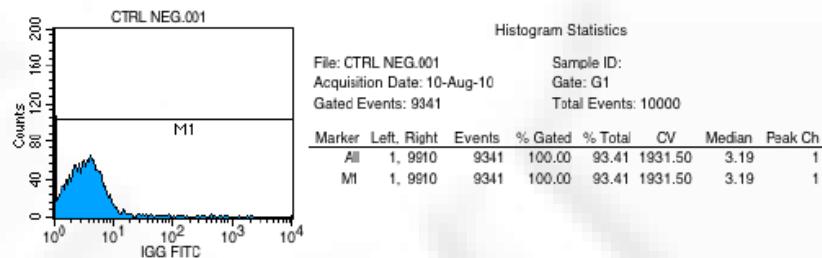
R=0,3

# Cuidado !

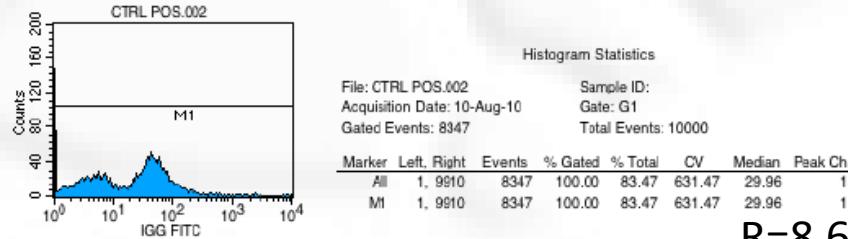
10 08 10



## CTRL NEGATIVO



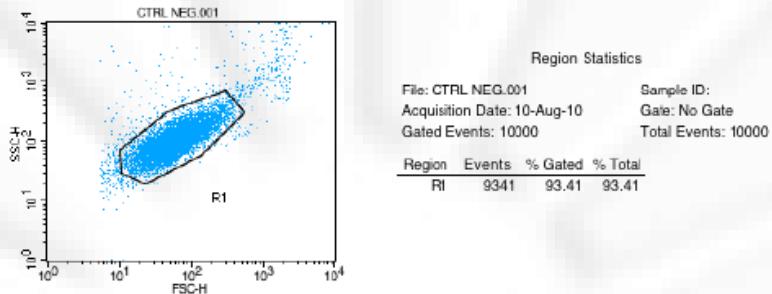
## CTRL POSITIVO



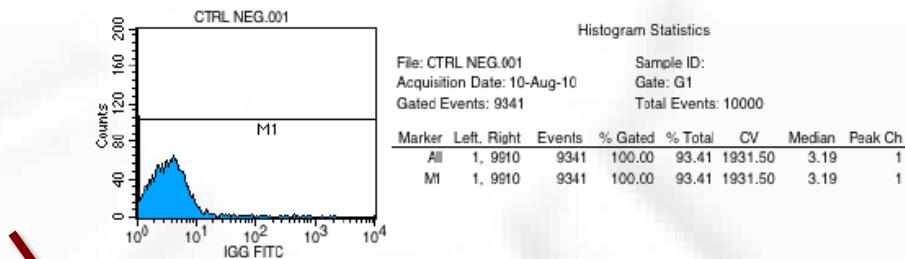
R=8,6

# Cuidado !

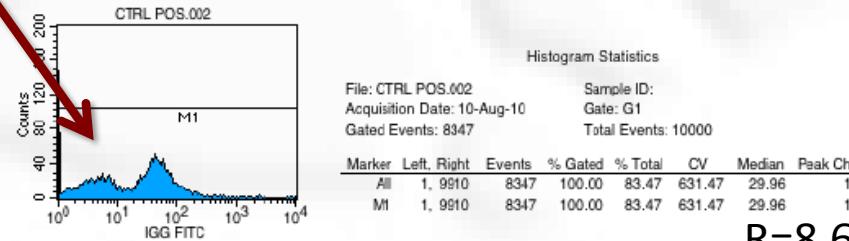
10 08 10



## CTRL NEGATIVO



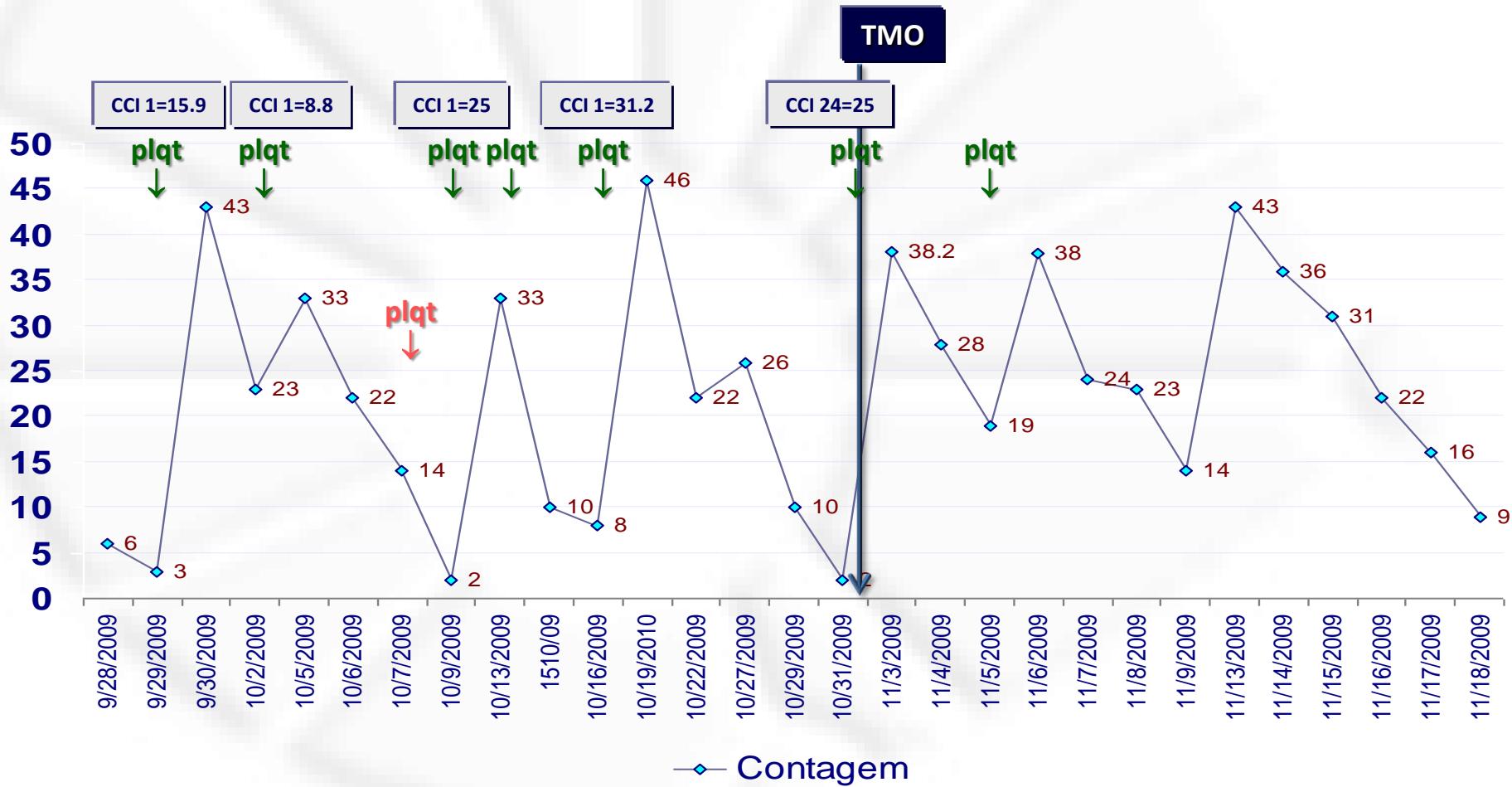
## CTRL POSITIVO



R=8,6

# Resultado tx BU

paciente: JWG – LMA + TMO



BU

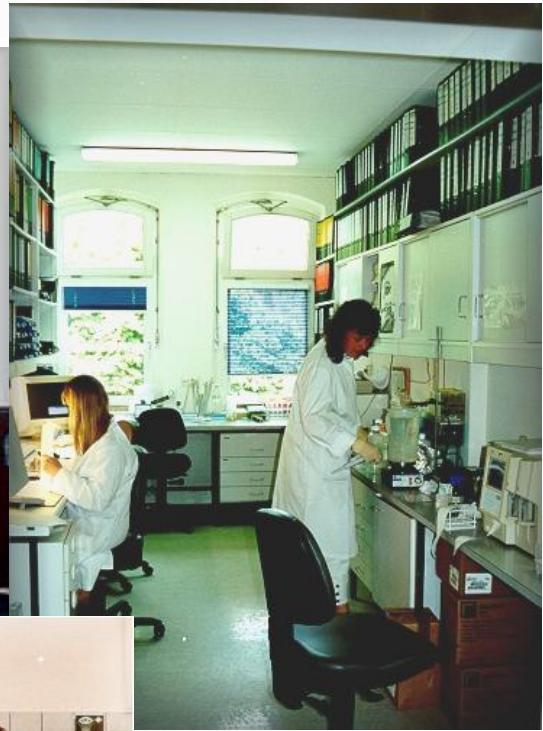
All antigens identical, only three antigens detected



# Considerações finais

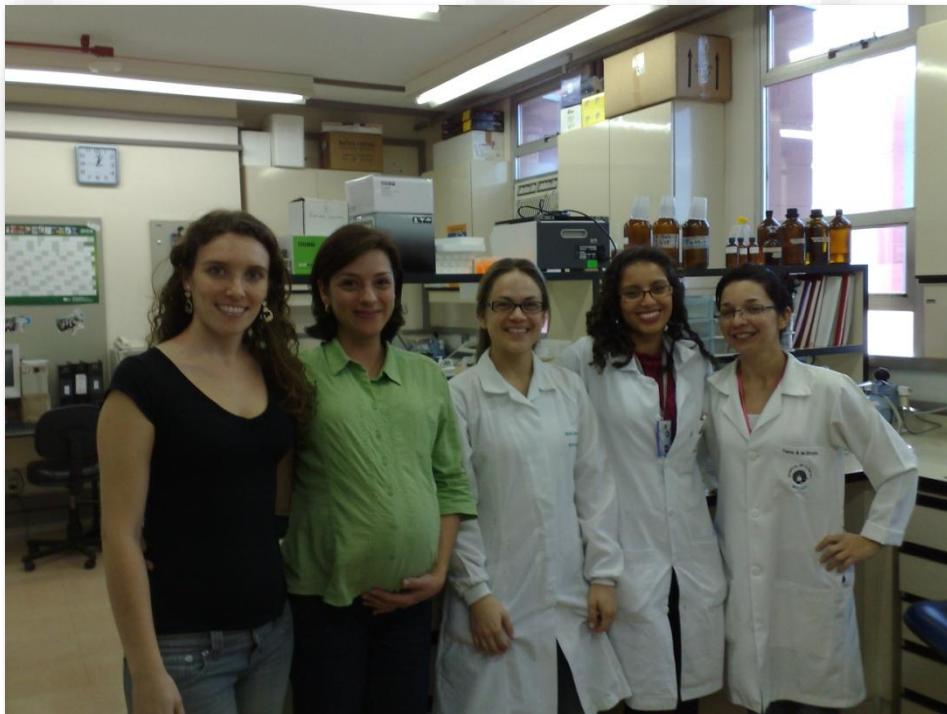
- CF → Ferramenta útil e importante na avaliação e suporte de pacientes com RP
- Laboratórios
  - CF → várias regiões do país
  - Imunologia plaquetária no Brasil → escassos
- **Necessidade de ampliação da rede de laboratórios especializados no país**

# Laboratório de Imunologia Plaquetária - Giessen



# Laboratório de Imunologia Plaquetária

## UNICAMP



- **Fernanda C Rossi**
  - Pós-graduanda
- **Beatriz M Martinelli**
  - Farmacêutica-Bioquímica
- **Tarsila M Camargo**
  - Biomédica
- **Milena P Vale**
  - Aluna Biologia
- **Telma Barbosa**
  - Pós-graduanda
- **Vagner Castro**
  - Médico Hematologista e Hemoterapêuta – Responsável  
*vagner@unicamp.br*

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Doe sangue.



[vagner@unicamp.br](mailto:vagner@unicamp.br)

