

Citometria de fluxo: pesquisa de refratariedade plaquetária

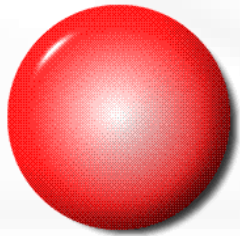
Vagner de Castro - MD, PhD

Laboratório de Imunologia Plaquetária

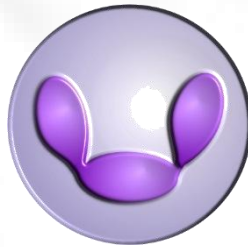
Hemocentro da UNICAMP



Aloantígenos



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



HNA's



HLA's



HPA's
HLA-C /
ABO

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

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 ☺
- **Etiologia**
 - Não imune: presentes em >90% dos casos
 - Infecção/febre, esplenomegalia, CIVD, TMO
 - Imune: ≈25% dos casos
 - incompat ABO
 - Ac HLA (3-90% - dependendo do tipo de hemocomponente: **leucoredução!!**)
 - Ac HPA (9-25%)

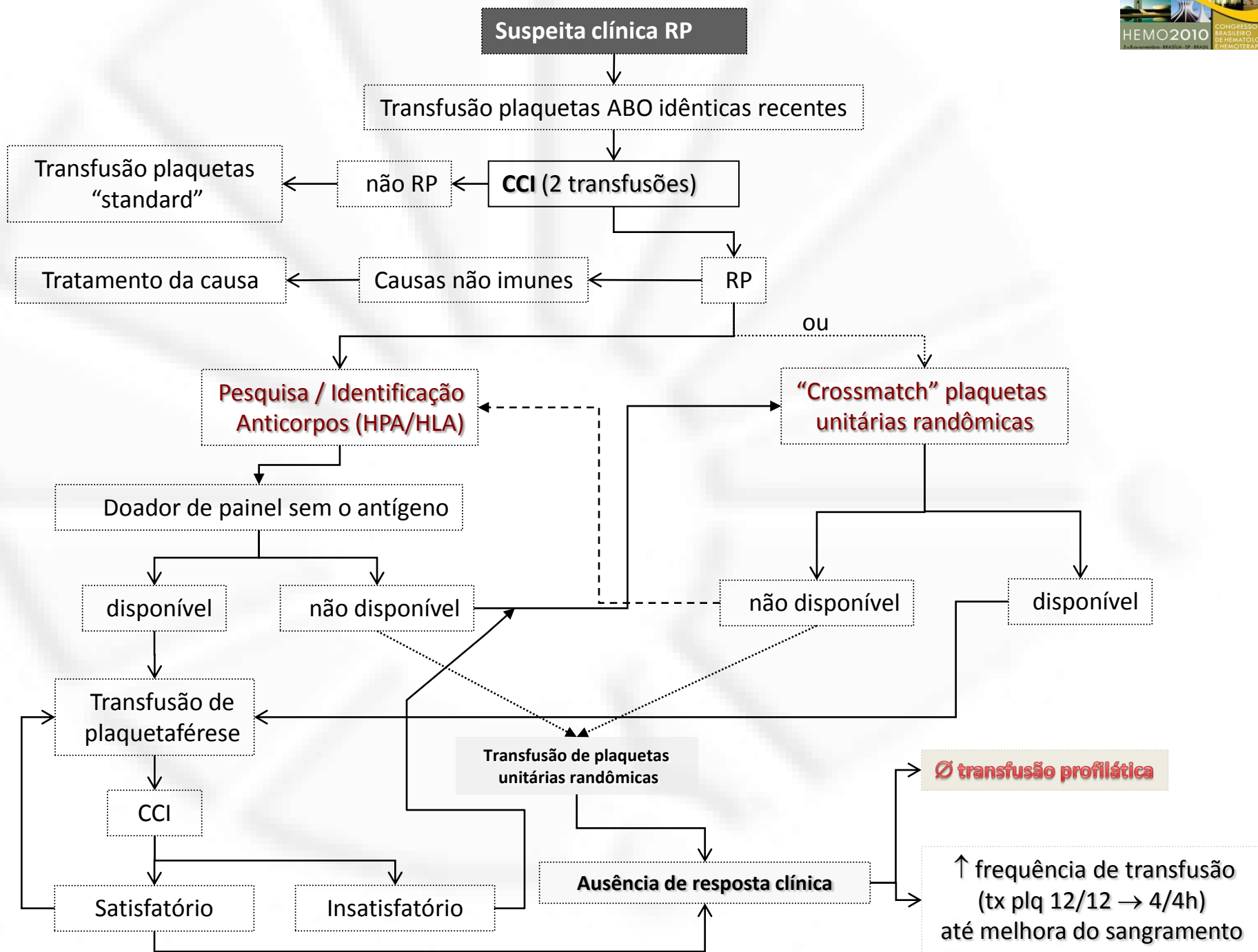
Rebulla et al., 2004, Slichter et al., 2005, Vassallo, 2009

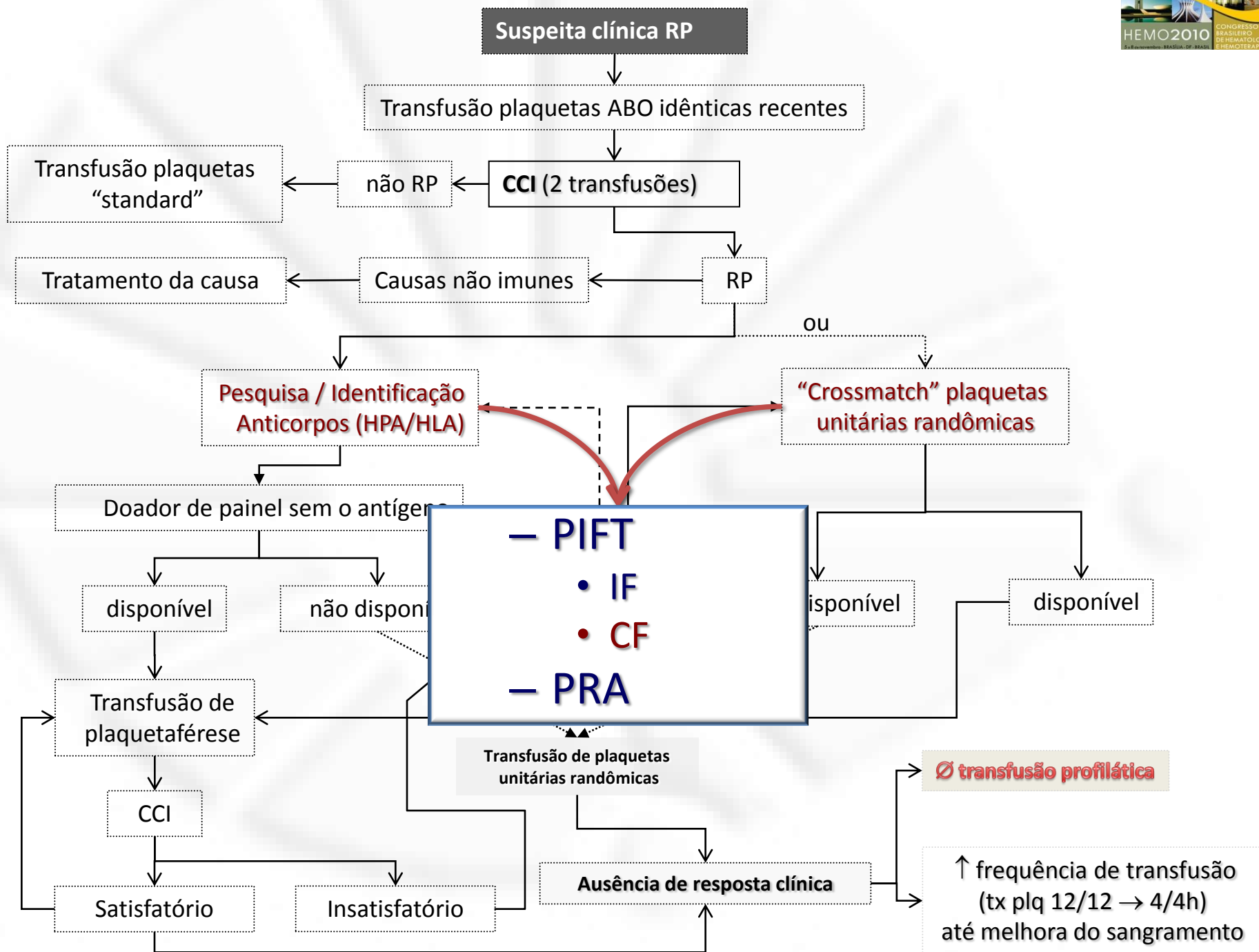
Manuseio da RP

- Algoritmo
 - Suspeita clínica
 - Investigação
 - Suporte hemoterápico

Manuseio da RP

- Algoritmo
 - Suspeita clínica
 - Investigação
 - Suporte hemoterápico
- 
- ```
graph LR; A[Investigação] --- B[]; B[] --- C[]; C[] --- D[Suporte hemoterápico]; D[] --- E[]; E[] --> F[CF];
```
- The diagram shows a flowchart where 'Investigação' and 'Suporte hemoterápico' are connected by a horizontal line. From the midpoint of this line, a vertical line descends, and from its bottom, a horizontal line points to the right, ending in an arrowhead pointing to the text 'CF'.





**Suspeita clínica RP**

Transfusão plaquetas ABO idênticas recentes

Transfusão plaquetas  
"standard"

não RP

**CCI (2 transfusões)**

Tratamento da causa

Causas não imunes

RP

ou

Pesquisa / Identificação  
Anticorpos (HPA/HLA)

"Crossmatch" plaquetas  
unitárias randômicas

Doador de painel sem o antígeno

disponível

não disponível

**– Atenção!**

~~anticorpos  $\oplus$  = RP~~

~~plaquetaférese~~

**– PIFT**  
• IF  
• CF  
**– PRA**

disponível

disponível

Transfusão de plaquetas  
unitárias randômicas

**Ø transfusão profilática**

TRAP ( $\approx 600$  pctes): 45% aloimunizados; 13% RP

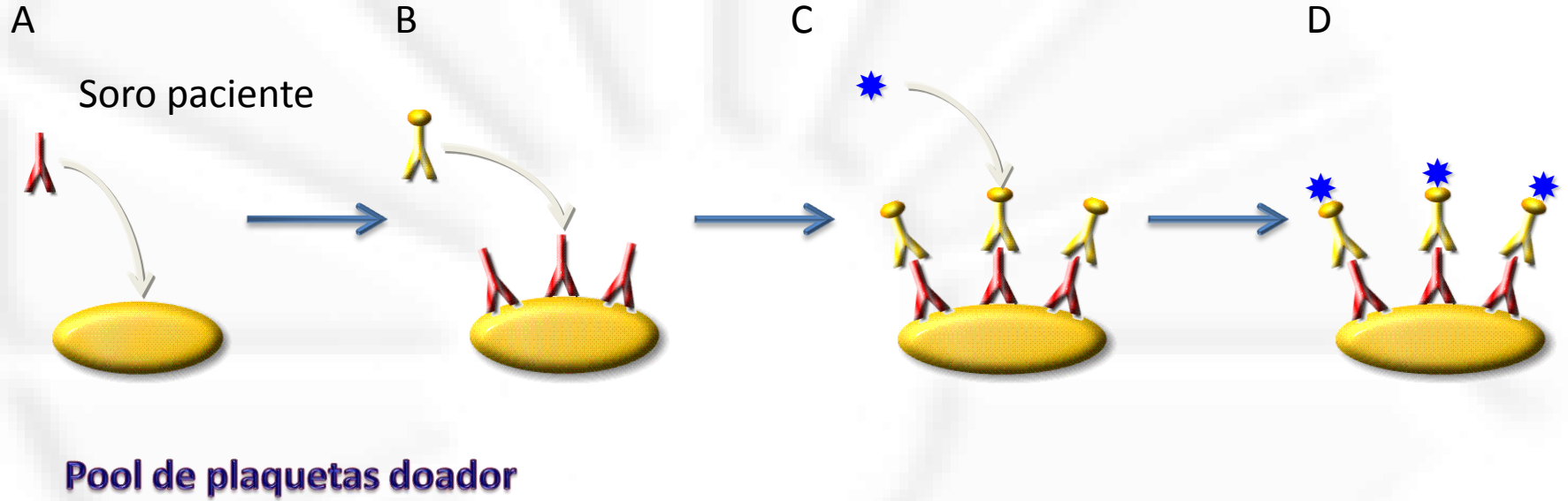
Satisfa

**TRAP group, N Eng J Med, 1997**

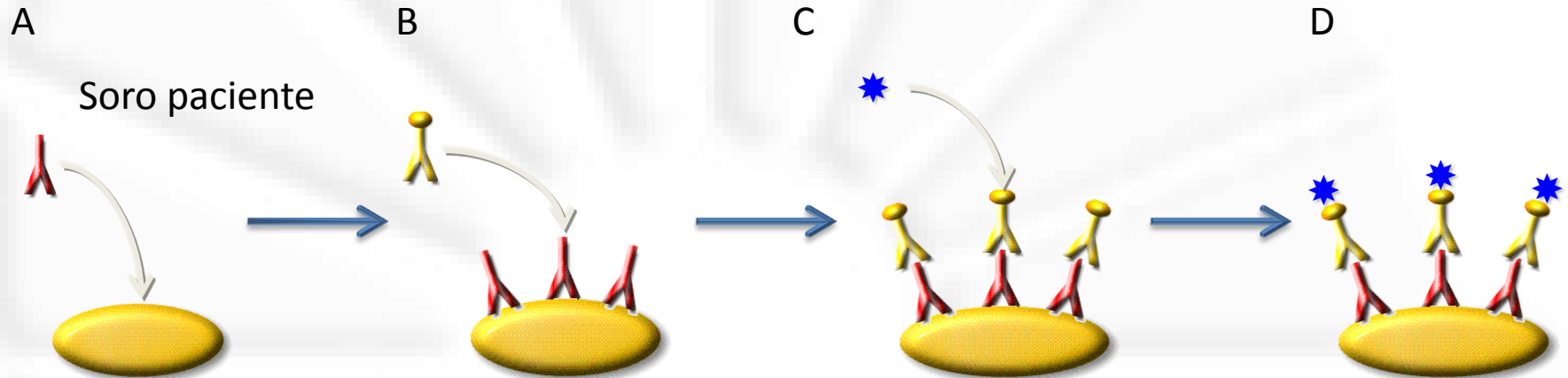
postea clínica

↑ frequência de transfusão  
(tx plq 12/12  $\rightarrow$  4/4h)  
até melhora do sangramento

# PAP




# 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

| 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     |

# PIFT

- 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

- 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



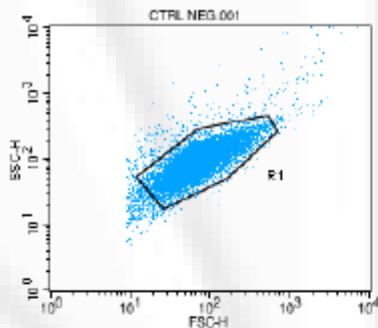
## **The Evaluation and Management of Platelet Refractoriness and Alloimmunization**

**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

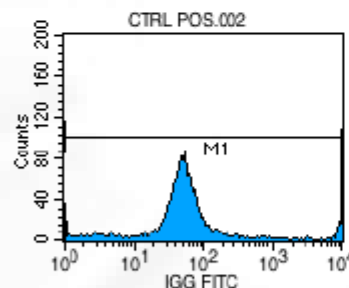
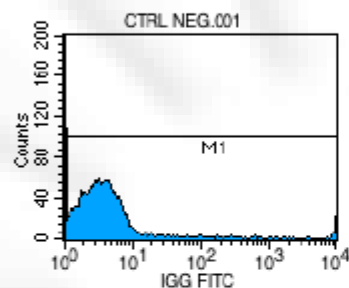
| Region | Events | % Gated | % Total |
|--------|--------|---------|---------|
| R1     | 9258   | 92.58   | 92.58   |

#### 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       |
| MI     | 1, 9910     | 9258   | 100.00  | 92.58   | 847.22 | 3.13   | 1       |



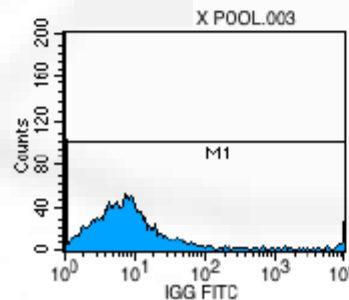
#### CTRL POSITVO

##### 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    |
| MI     | 1, 9910     | 7507   | 100.00  | 75.07   | 314.53 | 50.94  | 9910    |

R=16,3



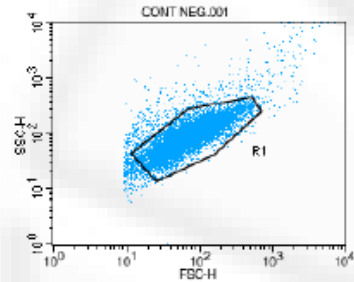
##### Histogram Statistics

File: JOAO GODDI 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       |
| MI     | 1, 9910     | 8793   | 100.00  | 87.93   | 652.71 | 6.67   | 1       |

R=2,1

30/09/09

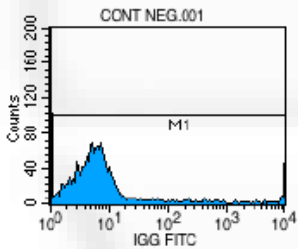


#### Region Statistics

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   |

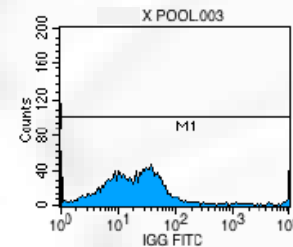
#### CTRL NEGATIVO



#### 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       |



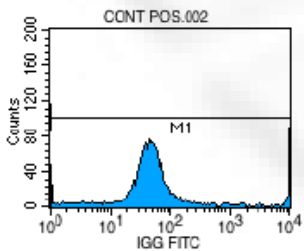
#### Histogram Statistics

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

#### CTRL POSITIVO

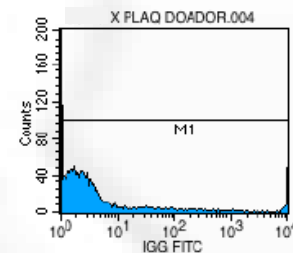


#### Histogram Statistics

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

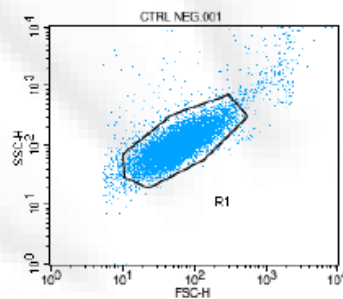
File: X PLAQ DOADOR.004 Sample ID: 01  
 Acquisition Date: 30-Sep-09 Gate: G1  
 Gated Events: 8108 Total Events: 10000

| 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

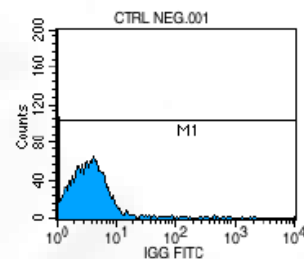


## Region Statistics

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

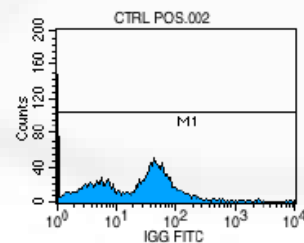


## Histogram Statistics

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

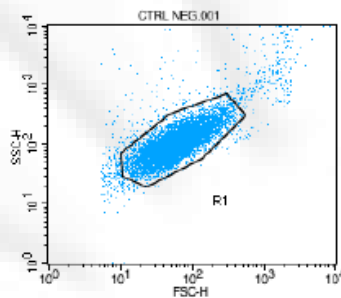
File: CTRL POS.002 Sample ID:  
 Acquisition Date: 10-Aug-10 Gate: G1  
 Gated Events: 8347 Total Events: 10000

| 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

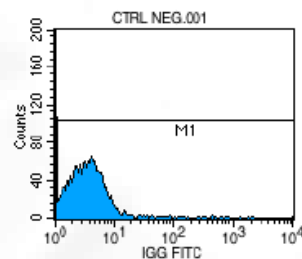


## Region Statistics

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

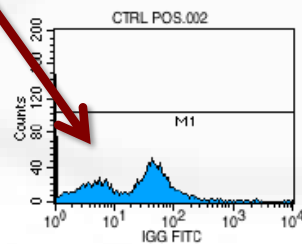


## Histogram Statistics

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

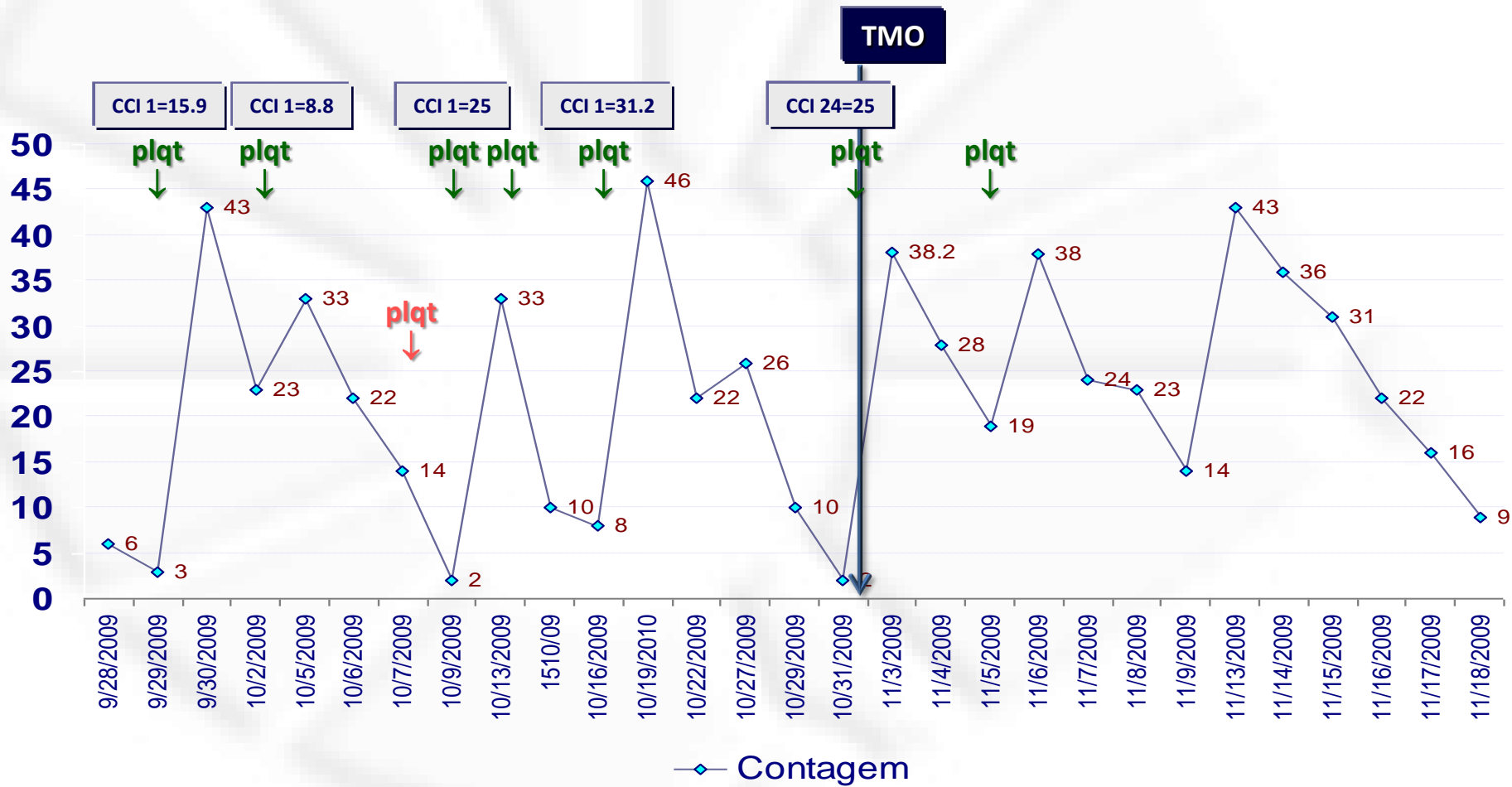
File: CTRL POS.002 Sample ID:  
 Acquisition Date: 10-Aug-10 Gate: G1  
 Gated Events: 8347 Total Events: 10000

| 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



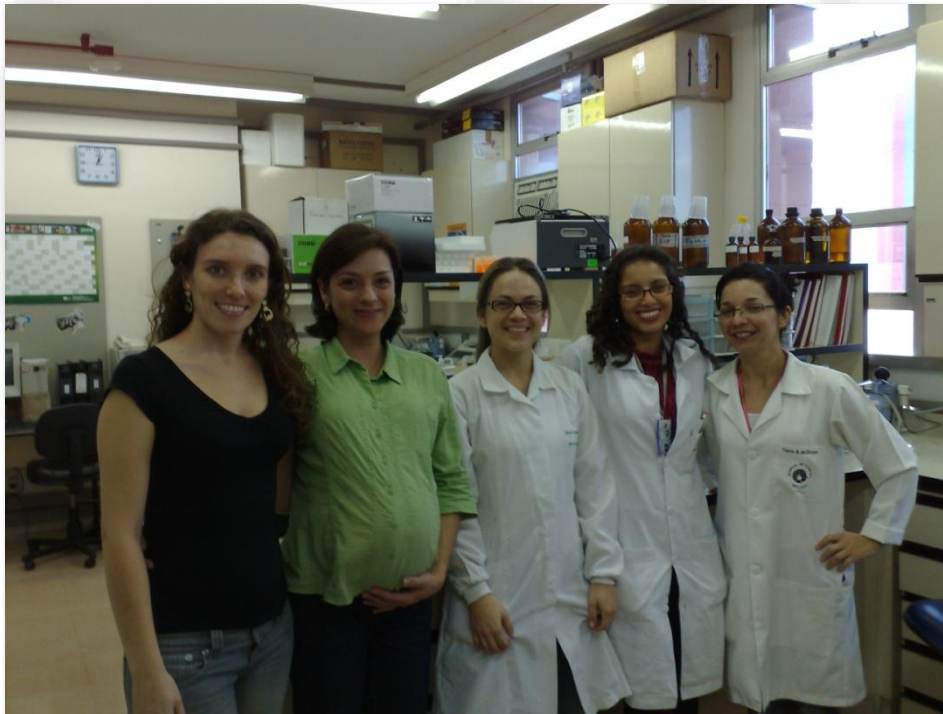
# Considerações finais

- 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



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Doe sangue.



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

