The efficient use of RhIg

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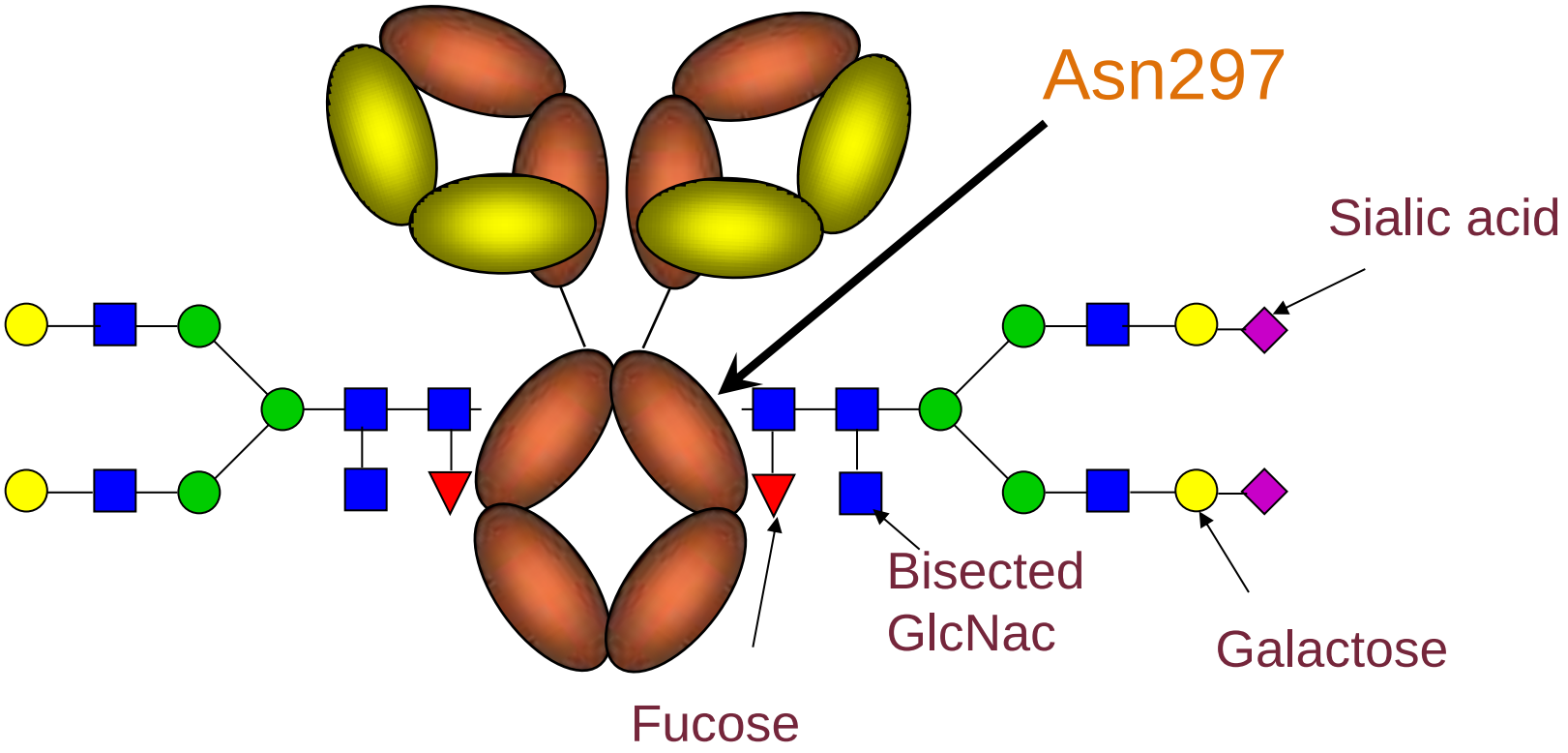
Increasing the efficiency of RhIg

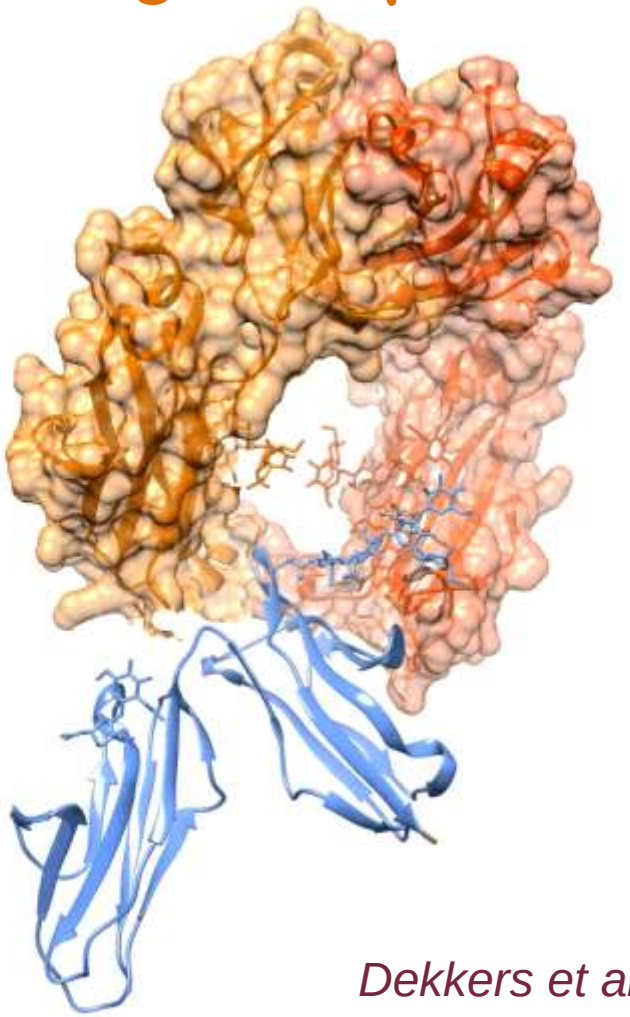
1. Increasing the biological activity of RhIg
   - Effect of Fc-core glycosylation of anti-D immunoglobulin

2. Decreasing the unnecessary use of RhIg
   - Guiding of immunoprophylaxis by fetal RHD typing

Fc-glycans



Glycans affect binding to FcγR

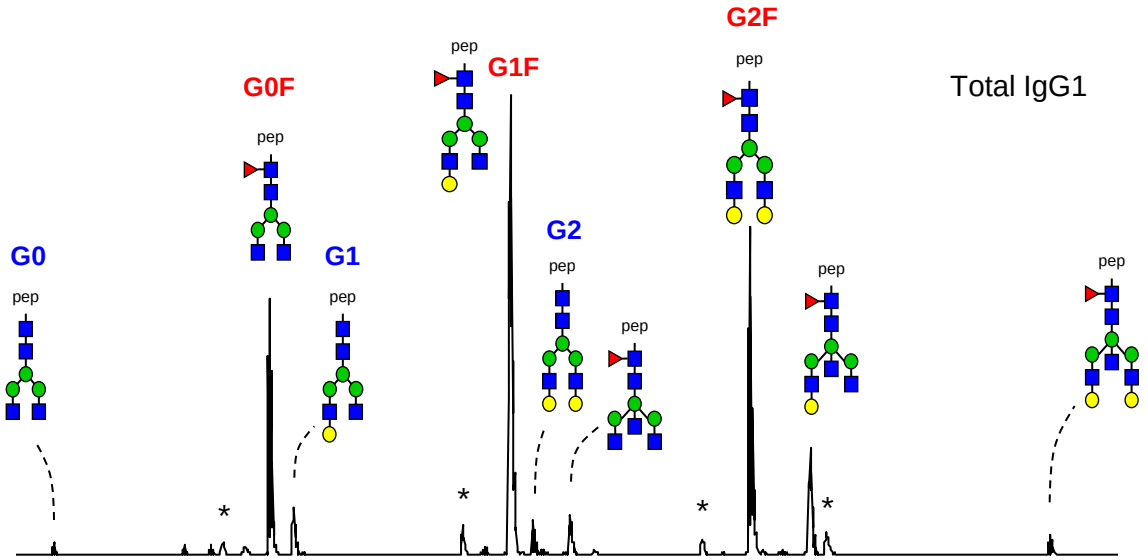


Low Fc-fucosylation results in **50x** enhanced binding To FcγRIIIa

Dekkers et al. Front Immun 2017

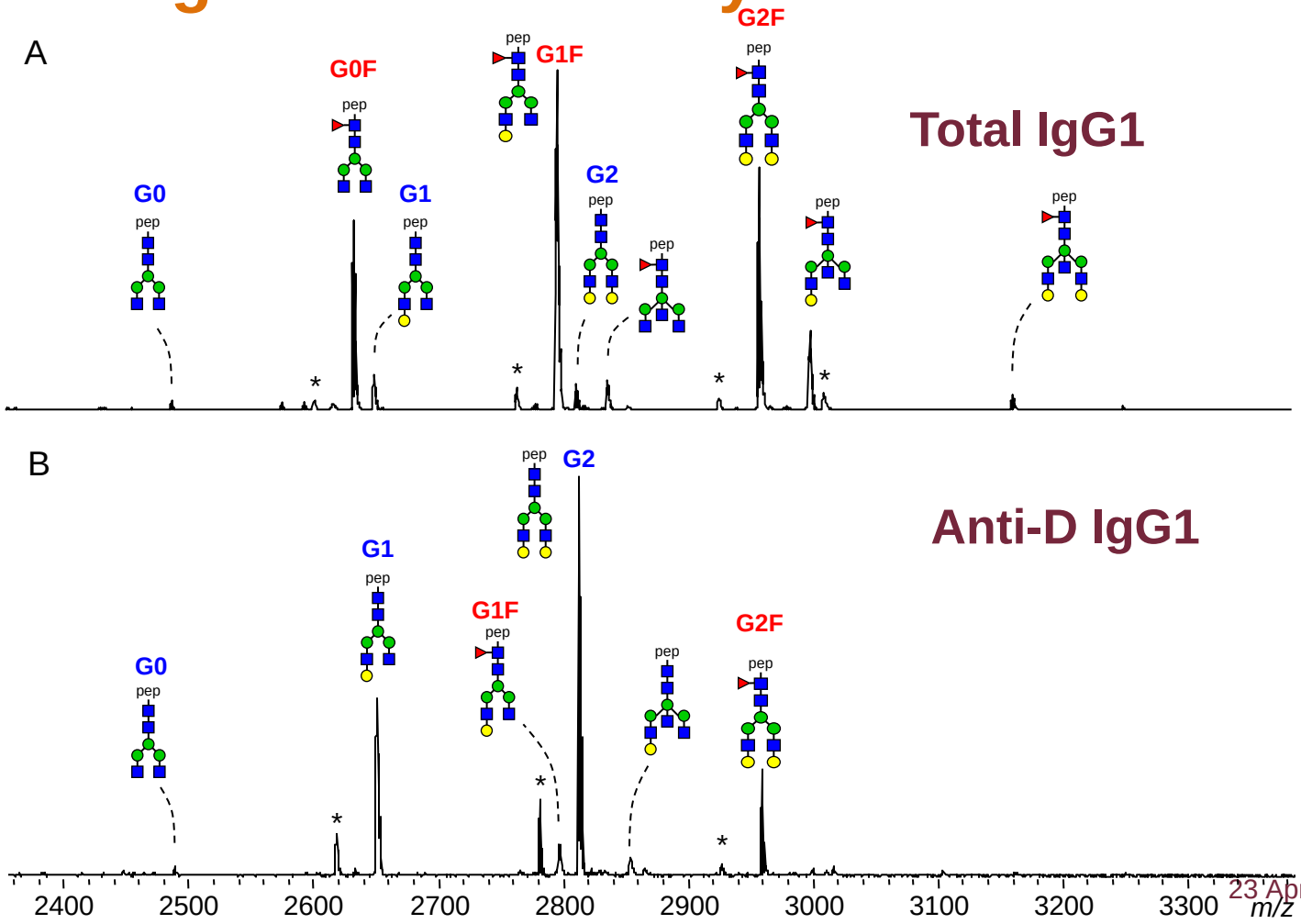
Fc glycopeptides: Mass spectrometric analysis

Glycan species	Glycan structure	IgG1 $E_{293}EQYNSTYR_{301}$[a] P01857[b] $[M+H]^+$
No glycan	-	1190.5198
G0		2487.9880
G0F		2634.0459
G1		2(A
G1F		2796.0987
G0N		2691.0674
G0FN		2837.1253
G2		2812.0936
G2F		2958.1515
G1N		2853.1202
G1FN		2999.1781
G2N		3015.1730
G2FN		3161.2309



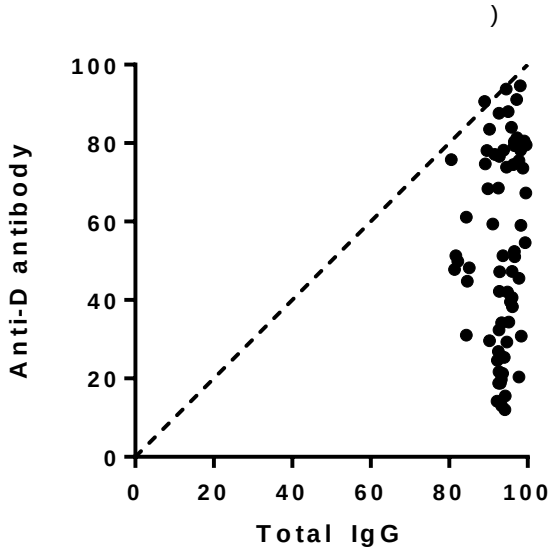
Majority of IgG1 in plasma is fucosylated

Anti-D IgG is low - fucosylated

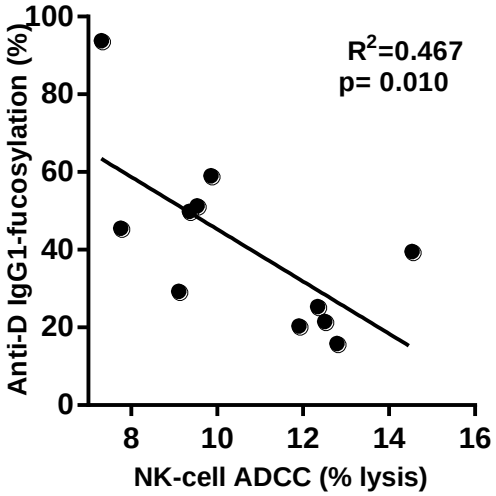


Fc-fucosylation of anti-D IgG is variable and related to pathogenicity

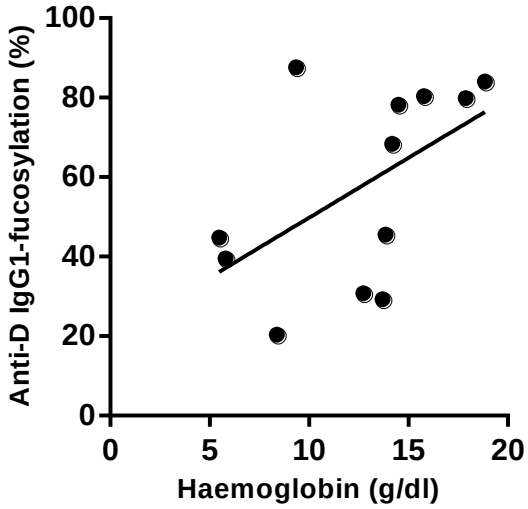
Fucosylation(%)
p<0.0001



NK-cell ADCC
R^2=0.467,p=0.010

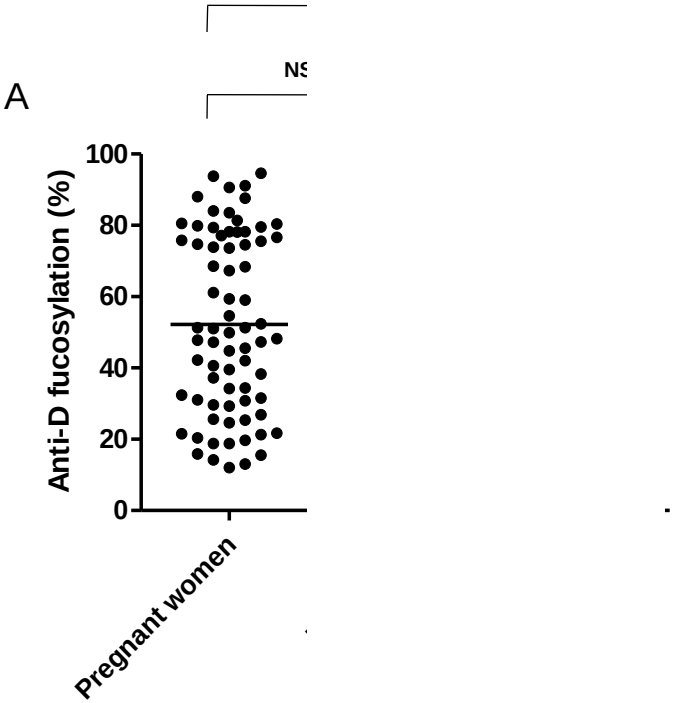


Neonatal Hb
R^2=0.289, p=0.038

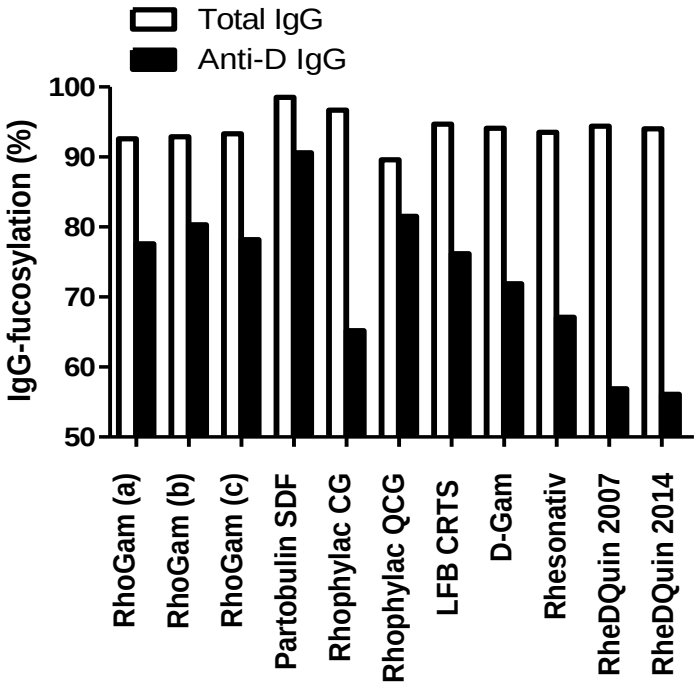


Kapur ,Della Valle , Br J Haemat 2016

Fc-fucosylation of anti-D IgG from hyperimmune donors is variably low



Anti-D preparations display variable decreases in Fc-fucosylation



Efficacy of anti-D appears to be related to Fc-fucosylation

- *Human polyclonal anti-D immunoglobulin*:
  - **Low** level of fucosylation:
  - high ADCC, very rapid RBC clearance, **prevented** D-immunization.

- *Moab anti-D produced in human B cell lines* (BRAD3, BRAD5)
  - **Medium** level of fucosylation
  - medium ADCC, fast clearance, prevented D-immunization **in 93%** subjects
  -

- *Moab anti-D produced in human-mouse hetero hybridoma:* (Fog1, AD1, G7, G12)
  - **High** level of fucosylation
  - low ADCC, variable and slow clearance, **stimulated** D-immunization.

Kumpel et al. submitted

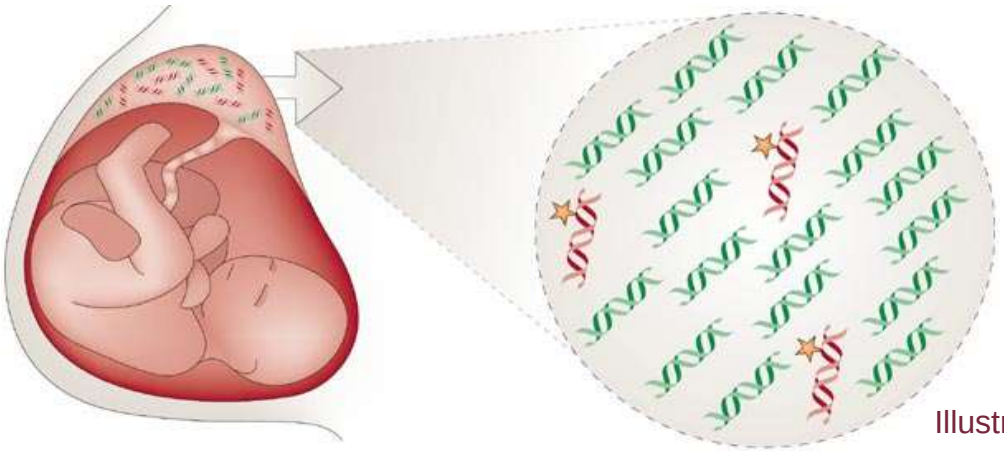
Conclusion (1)

Production of RhIg from hyperimmune donors with low fucosylated anti-D Ig might result in more potent RhIg

Guiding Rh immunoprophylaxis by fetal *RHD* typing

- 40% of D-neg women carry a D-neg child and do not need Rh-Ig
- Fetal DNA is present in low concentration in maternal plasma from week 5

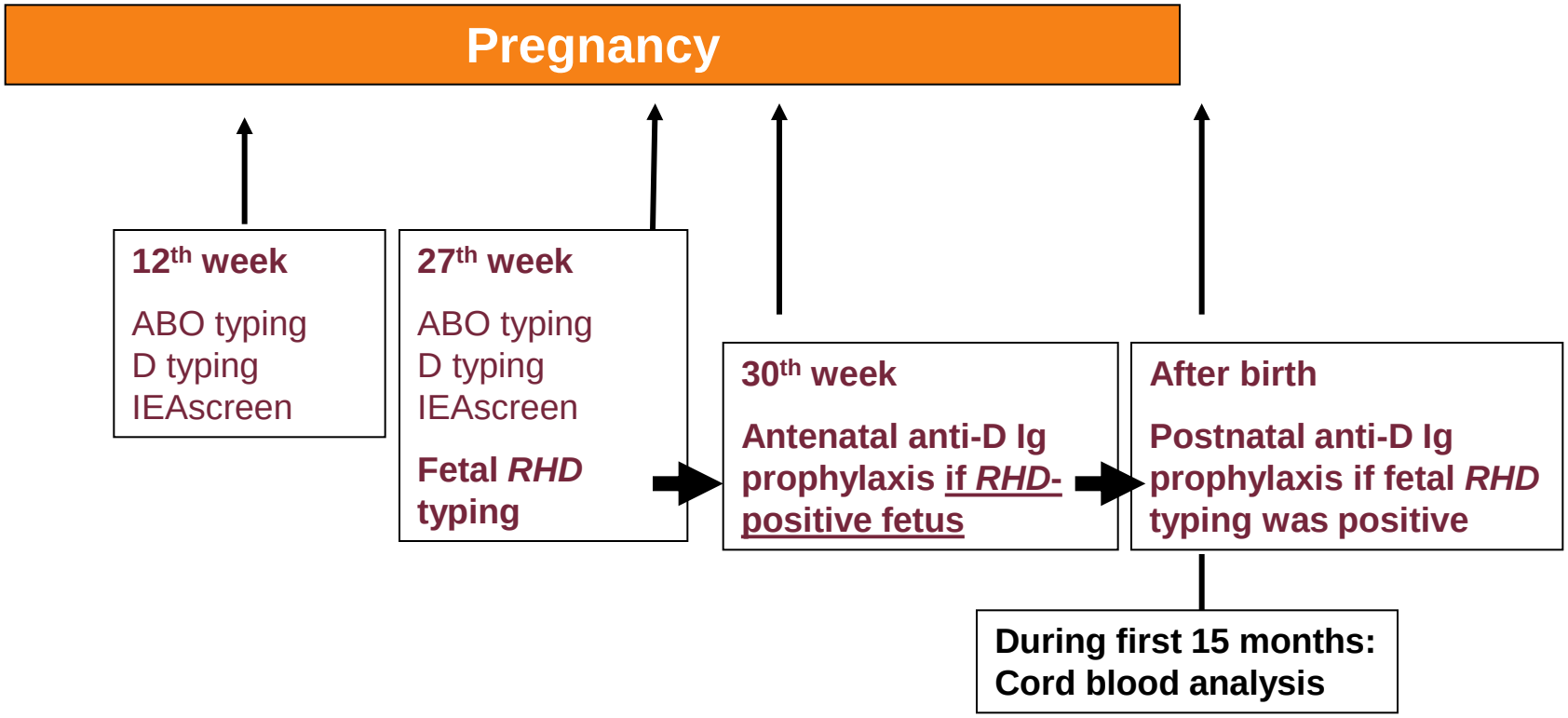
placenta → plasma of pregnant woman



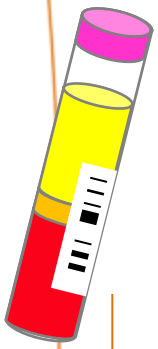
Mixture of DNA from mother and DNA from fetus

Illustration: Lo, 2007

Dutch screening program



Technical approach



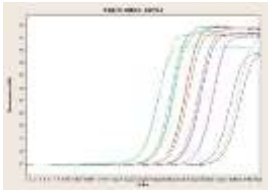
- Centralized at one laboratory (Sanquin, Amsterdam)
- DNA isolation from 1 ml of EDTA plasma in **27th week** of pregnancy
- First 15 months comparison with cord blood

Electronic Result

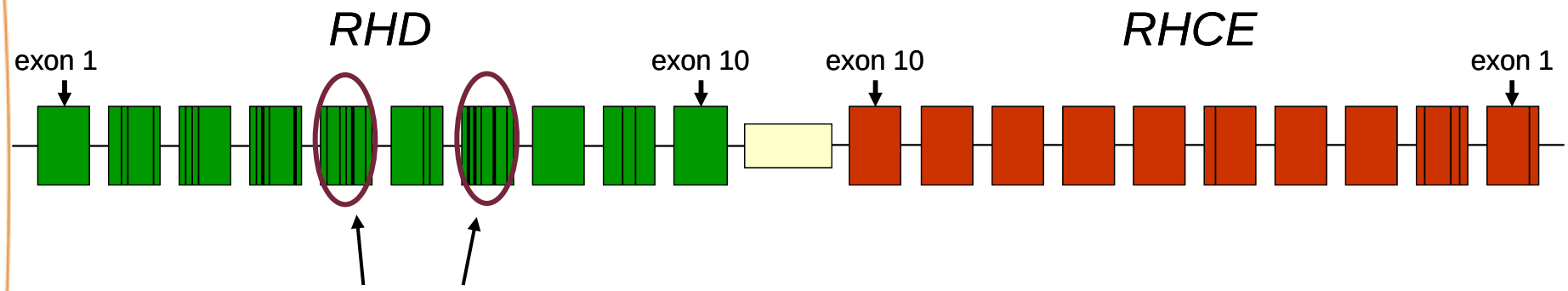
Plasma Pipetting → **DNA Purification** → **PCR Setup** → **Amplification Detection** → **Report**







Design fetal *RHD* typing



***RHD*-PCR Multiplex**

exon 5: not amplified in majority of *RHD* variants

in Caucasians: RHD*DVI

in Blacks: RHD*Ψ; RHD*01N.06 and RHD*03N.01 (r's)

exon 7: present in most *RHD* variants

First 25.500 samples:
Diagnostic accuracy of at least 99.1%

CB-serology / Plasma-PCR	Pos	Neg
Pos	15,826	225 (0.87%)
Neg	9 (0.03%)	9,740

de Haas et al. BMJ 2016

Systemic analysis of false results

- **False negative fetal RHD typing:**
  - No antenatal prophylaxis : 0.61% risk of alloimmunization
  - No postnatal prophylaxis: 17% risk of alloimmunization
- **False positive fetal RHD typing:**
  - Unneccessary RhIg is given, no clinical consequences

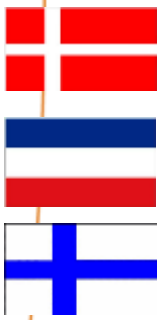
Thurik et al. Prenat Diagn 2016
Stegman et al. Brit J Haematol 2016

Sanquin

Potential effect of PCR guided ante- and postnatal immunoprophylaxis

	Unnecessary antenatal anti-D	No antenatal anti-D, while at risk	No postnatal anti-D, while at risk
Old program (no PCR, only cord blood)	38,3%	0%	0,09%
New program (only PCR, no cord blood)	0,43%	0,03%	0,03%

Accuracy of implemented fetal RHD typing (Denmark, the Netherlands, Finland)



	Samples	TRUE Pos	False Pos	TRUE Neg	FALSE Neg	**sensitivity %**	specificity %
Clausen et al., 2014	12688	7636	41	4706	11	**99.86**	99.14
de Haas et al., 2016	25789	15816	225	9739	9	**99.94**	97.74
Haimila et al., 2017	10814	7080	7	3640	1	**99.99**	99.81
Total	**49291**				**21**	**99.93**	

van der Schoot et al. Curr Opin Hem 2017

Conclusion (2)

Implementation of fetal *RHD* typing has resulted in decreased unnecessary use of antenatal RhIg, without loss of efficiency of the program

Acknowledgements

- Fc-glycosylation
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