Maastricht UMC+

Positieve 12de week screening
Rol van de foetale rhesus D typering

Joyce van Beers
Laboratoriumspecialist Medische Immunologie i.o.
Laboratoriumspecialist Klinische Chemie i.o.
Centraal Diagnostisch Laboratorium

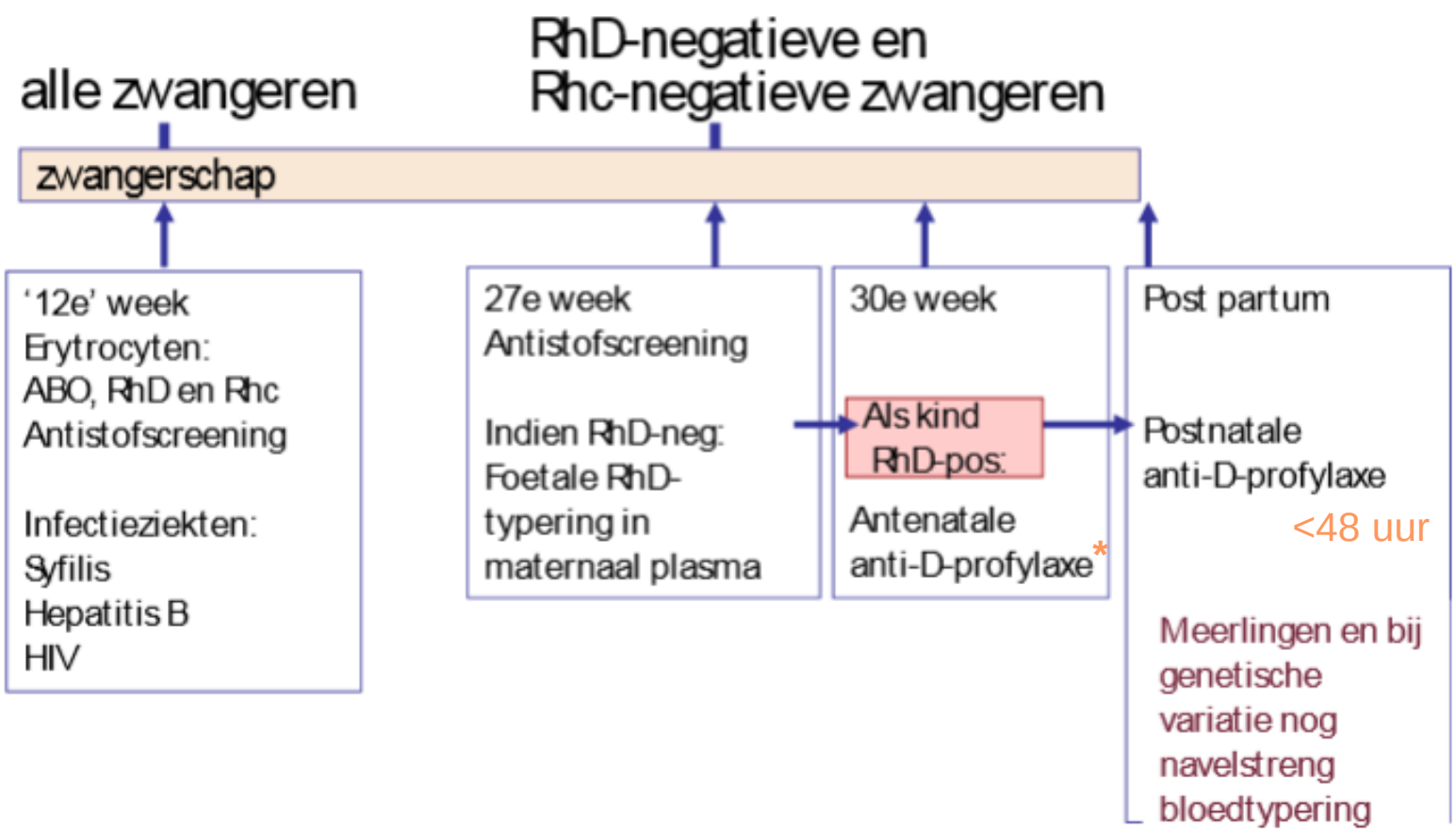
Maastricht UMC+

Joyce.van.beers@mumc.nl

Casus

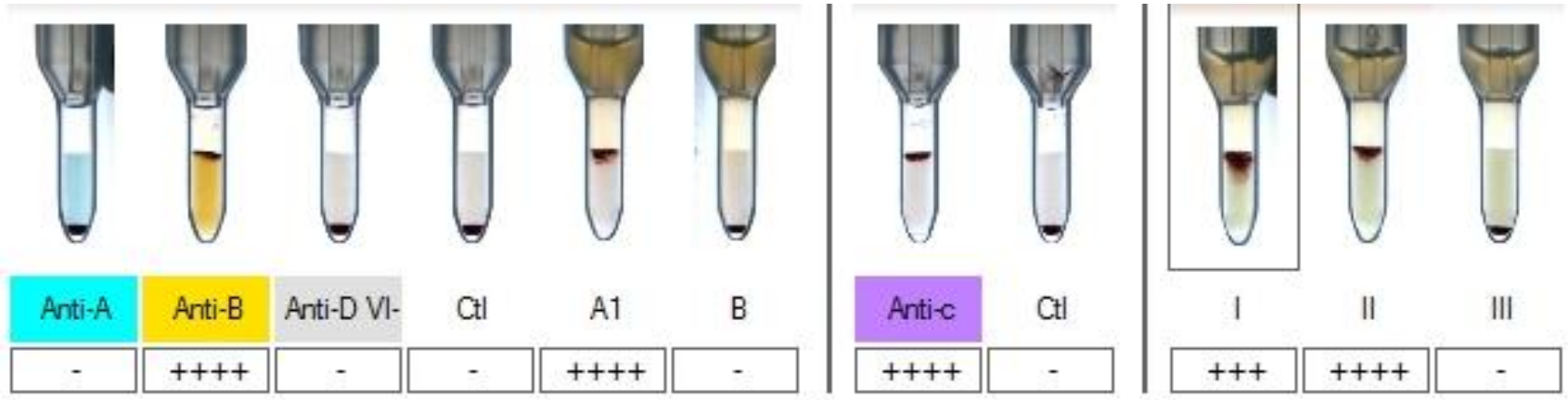
- Vrouw, 1998, afkomstig uit Democratische Republiek Congo
- VG: blanco
- Zwanger; geen klachten
  - G2P1
    - Spontane partus 13-7-2013 in Congo: gezond kind (jongen), niet in NL
    - Nu: meerling
- Januari 2018: twaalfde week screening

12de week screening



*vanaf week 30+0 tot uiterlijk 31+6

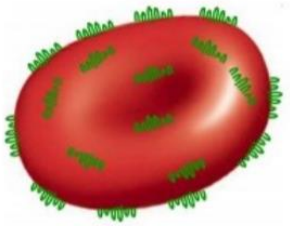
Casus: 12de week screening



- Bloedgroep: B neg
- c-antigeen: pos
- Screening (IRA): pos
- Virusserologie: neg

- IRA positief: opgestuurd naar Sanquin

Rhesus systeem

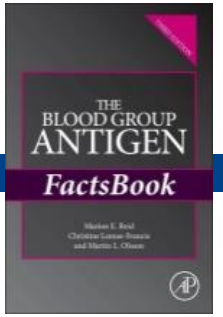


10.000 – 33.000

- Rhesus genen gelegen op chr. 1
  - RHD gen → RhD antigen
  - RHCE gen → RhCc en RhEe antigenen

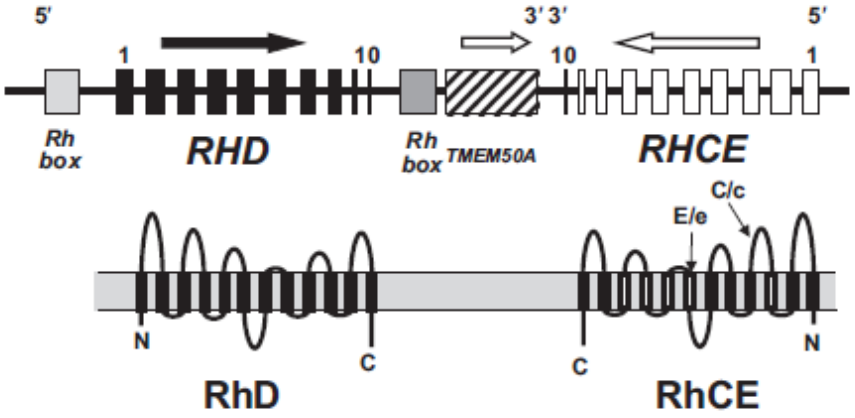
93,8% homologie

- Totaal: >45 antigenen
  - Splice varianten, polymorfisme
- Transmembraan eiwitten
- Expressie afhankelijk van RHAG gen (chr. 6)
- Expressie wisselt per etniciteit

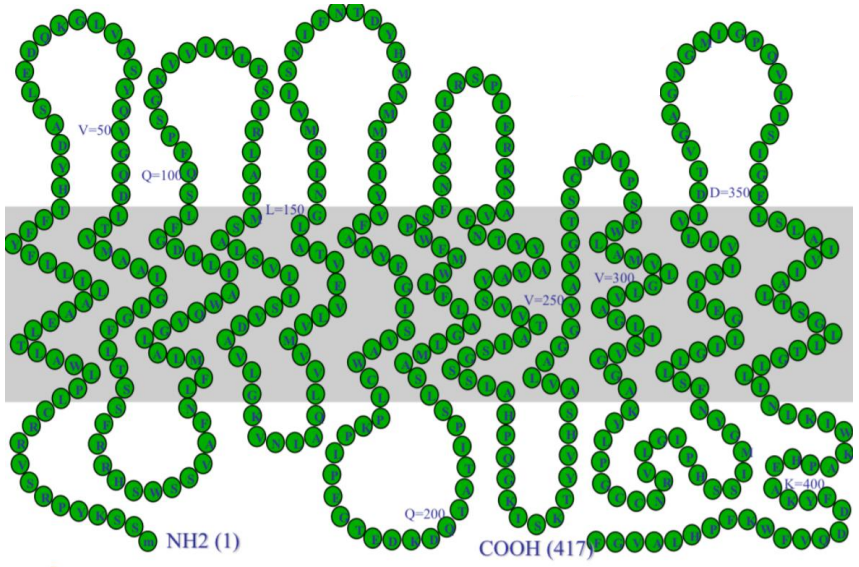


Rhesus D gen versus eiwit

Rhesus D gen

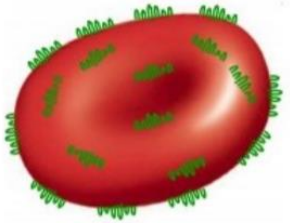


Rhesus D eiwit



British Journal of Haematology, 2013, 161, 461–470

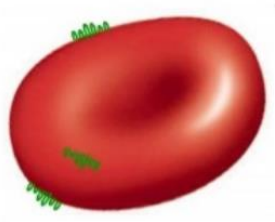
Kwantitatieve versus kwalitatieve variatie



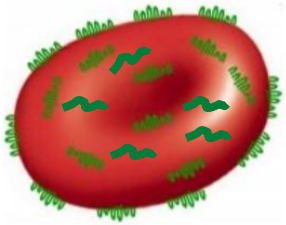
10.000 – 33.000

Kwantitatieve variatie

Volledig D antigeen



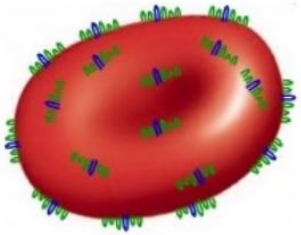
<100 – 33.000

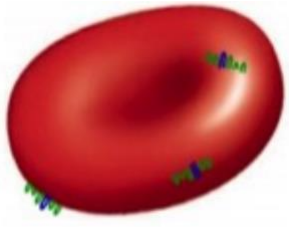


75.000 - 200.000 (-D-)

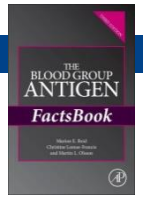
Kwalitatieve variatie

Partieel D antigeen

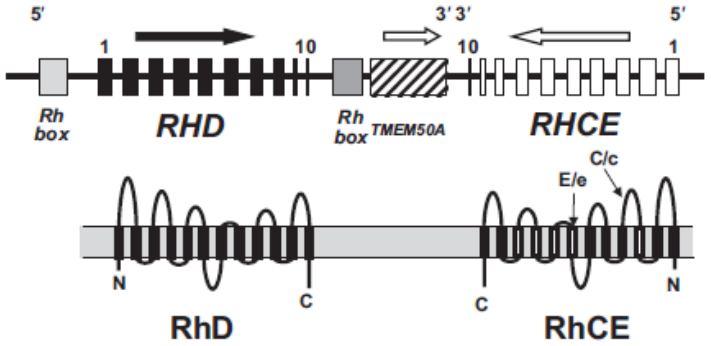




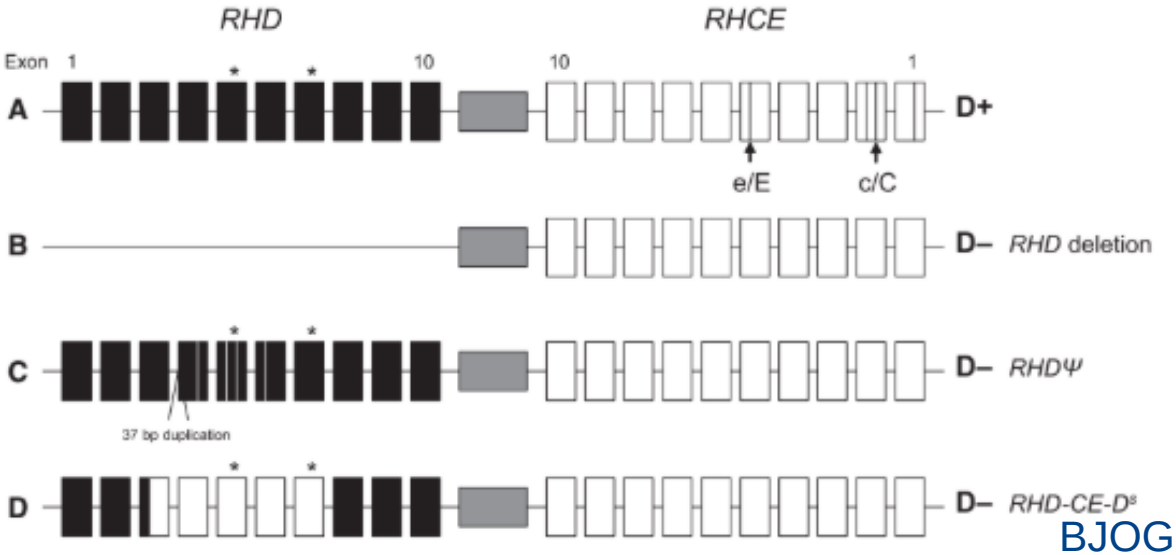
Beschouwd als D neg (donoren: D pos!)



Rhesus D negatief

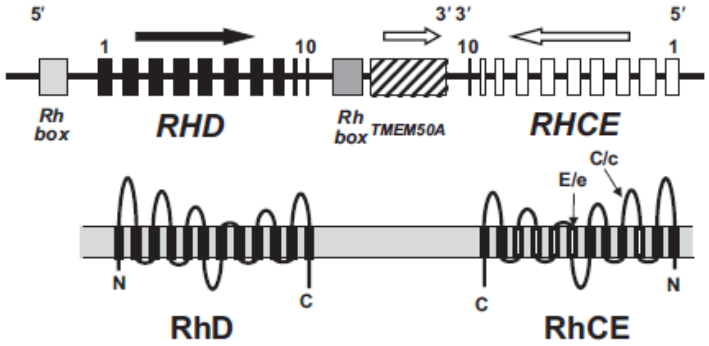


1. Deletie RhD gen (B)
2. Inactiverende mutaties in RhD gen (C)
   - Rhesus D pseudogen
   - Afro-Amerikanen (66% van RhD negatieve personen)
3. Hybride RHD-CE-D gen (D)
   - Afrikaanse achtergrond (19% van RhD negatieve personen)



BJOG 2011;118:1340–1348

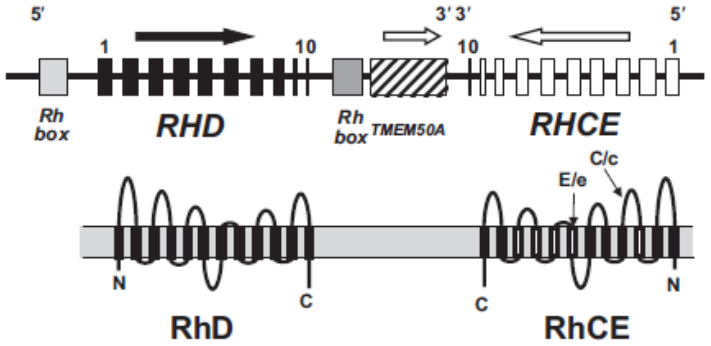
Rhesus D negatief



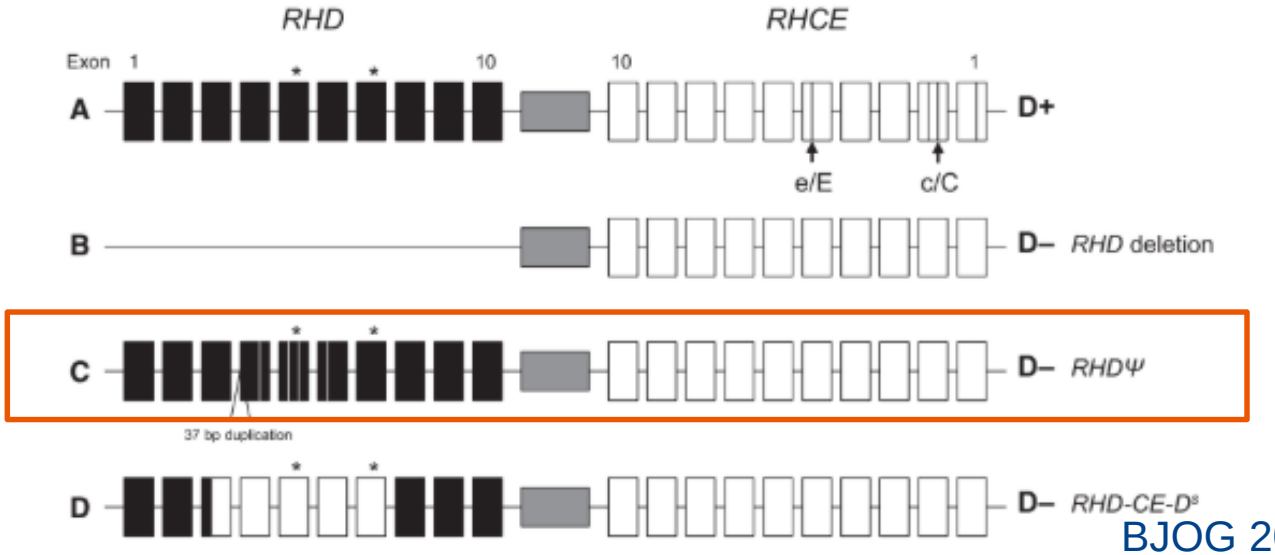
1. Deletie RhD gen (B)
2. Inactiverende mutaties in RhD gen (C)
   - Rhesus D pseudogen
   - Afro-Amerikanen (66% van RhD negatieve personen)
3. Hybride RHD-CE-D gen (D)
   - Afrikaanse achtergrond (19% van RhD negatieve personen)

BJOG 2011;118:1340–1348

Rhesus D negatief

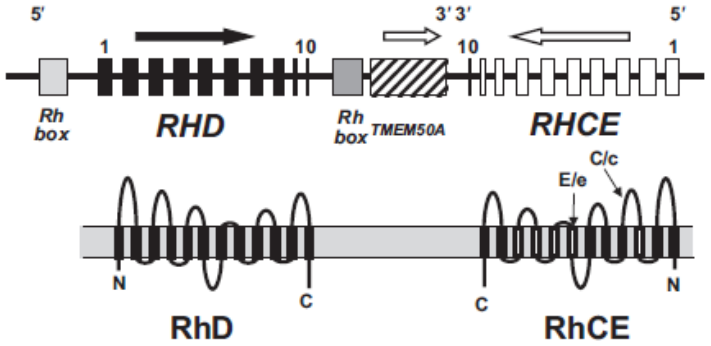


1. Deletie RhD gen (B)
2. Inactiverende mutaties in RhD gen (C)
   - Rhesus D pseudogen
   - Afro-Amerikanen (66% van RhD negatieve personen)
3. Hybride RHD-CE-D gen (D)
   - Afrikaanse achtergrond (19% van RhD negatieve personen)

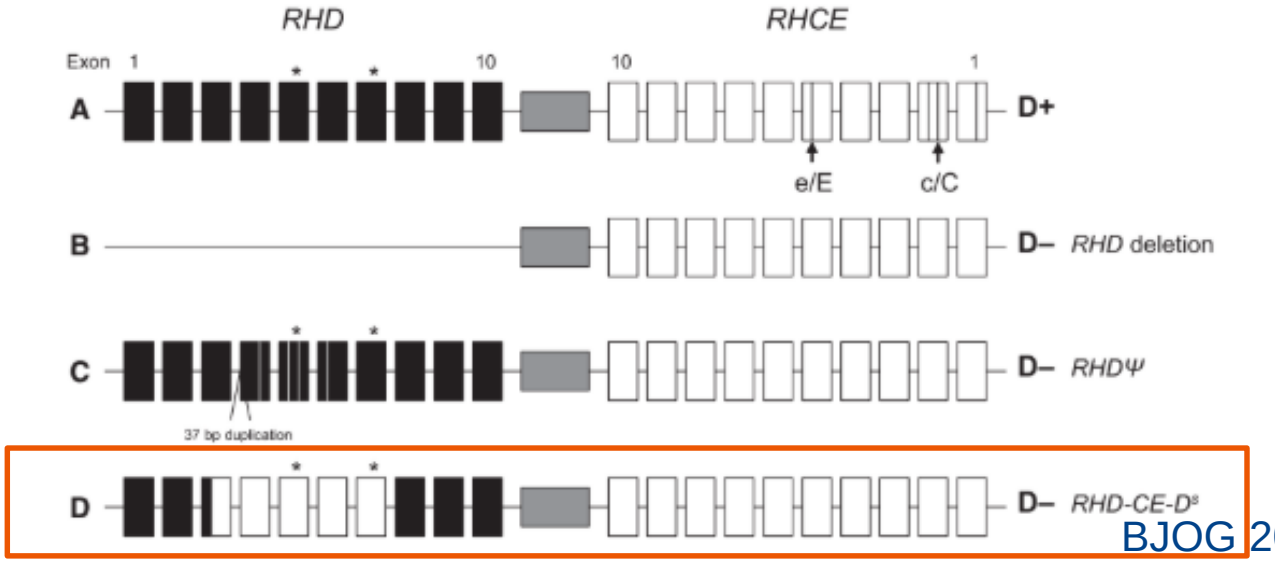


BJOG 2011;118:1340–1348

Rhesus D negatief



1. Deletie RhD gen (B)
2. Inactiverende mutaties in RhD gen (C)
   - Rhesus D pseudogen
   - Afro-Amerikanen (66% van RhD negatieve personen)
3. Hybride RHD-CE-D gen (D)
   - Afrikaanse achtergrond (19% van RhD negatieve personen)



BJOG 2011;118:1340–1348

Casus: uitslag screening

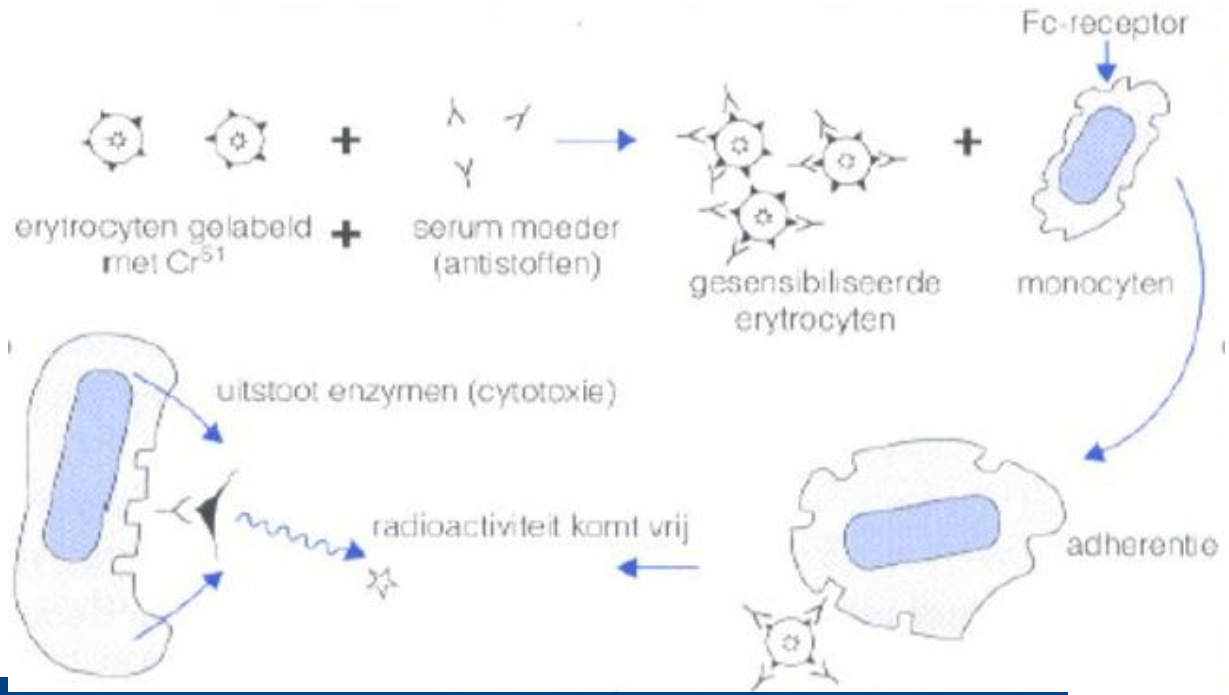
- ABO bloedgroep: B
- Rhesus D antigeen: neg
- Rhesus fenotype: ccdee
- K: neg

- Vrije erytrocyten antistoffen: anti-D
- Titer: 1:32

- ADCC test is ook ingezet

ADCC (antibody dependent cell-mediated cytotoxicity) test

- Vaststellen van activiteit van antistof ‘in vivo’(schatting)
  - Correlatie met ernst van de HZP



Casus:

- ADCC test: 20%

Advies: over 4 weken herhalen titer en ADCC

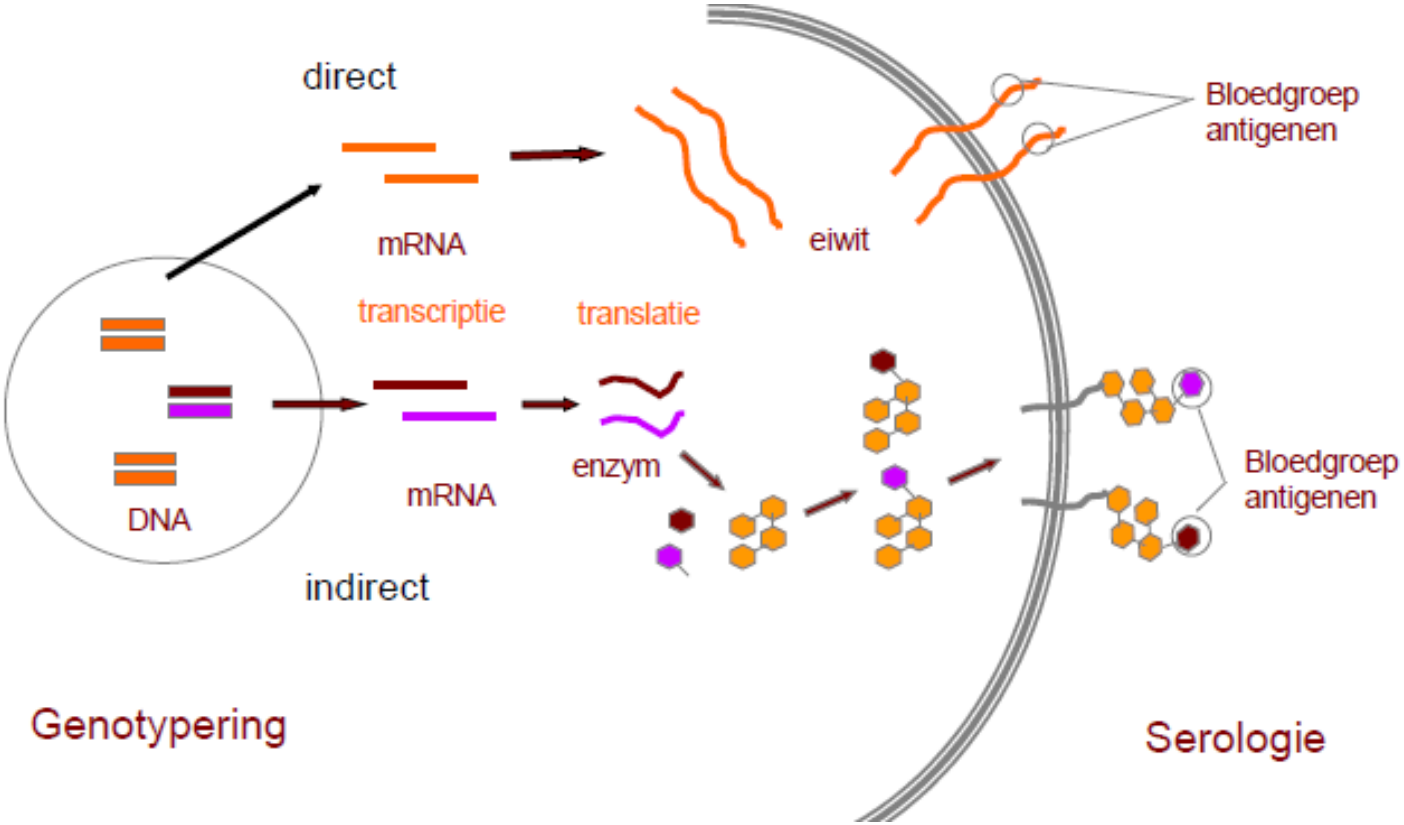
- ADCC uitslag:
  - < 10% geen hemolyse te verwachten
  - 10-30% zeer milde hemolyse
  - 30-50% milde – matige hemolyse
  - > 50% ernstig-zeer ernstige hemolyse → intra-uteriene transfusie!

4 weken later….

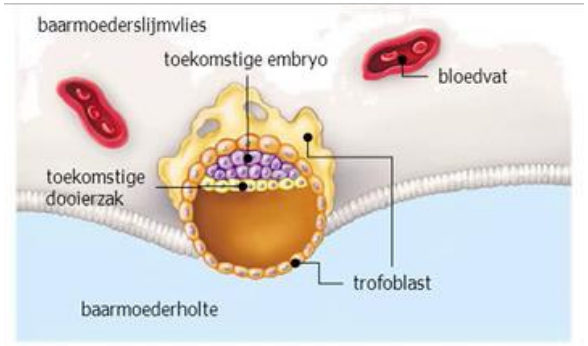
- Uitslag:
  - Vrije erytrocyten antistoffen: anti-D
  - Titer: **1:64**
  - ADCC test: **30%**

- Overige antistoffen zijn uitgesloten
- Advies: over 2 weken herhalen titer en ADCC + foetale genotypering

Genotypering versus fenotypering

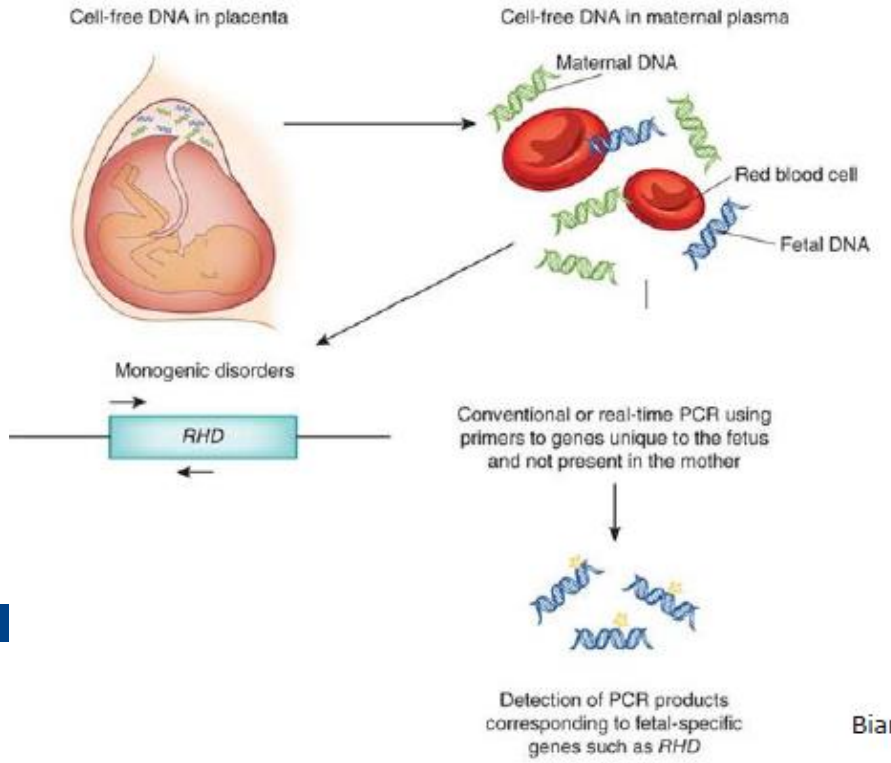


Foetale genotypering



Tijdens de innesteling (ongeveer 7 dagen na bevruchting)

- Sanquin: foetale Rhesus D, C, c, E of K genotypering in maternaal plasma
  - DNA van foetus in maternaal bloed
  - Foetaal DNA aanwezig voor aanleg foetoplacentaire circulatie
  - Herkomst: trophoblast
- Niet invasief
- Vanaf 12 weken

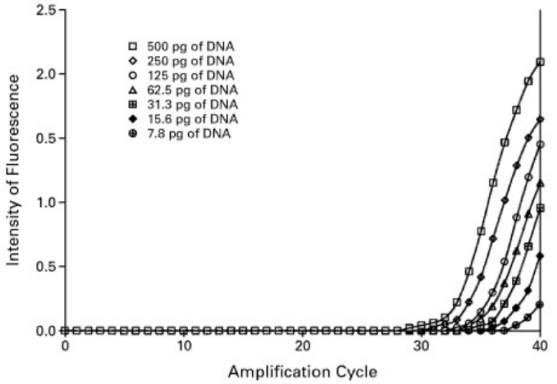


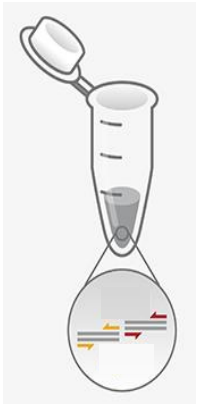
Bianchi et al, Nature Medicine, 2012, 18:1041–1051

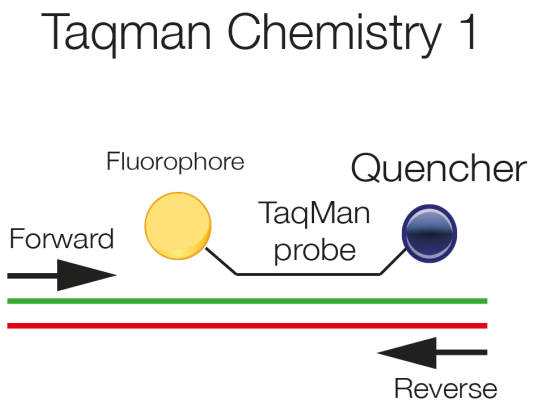
Maastricht UMC+

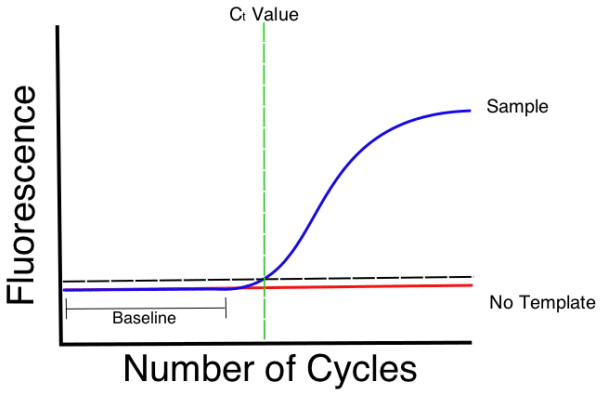
Foetale genotypering: de analyse

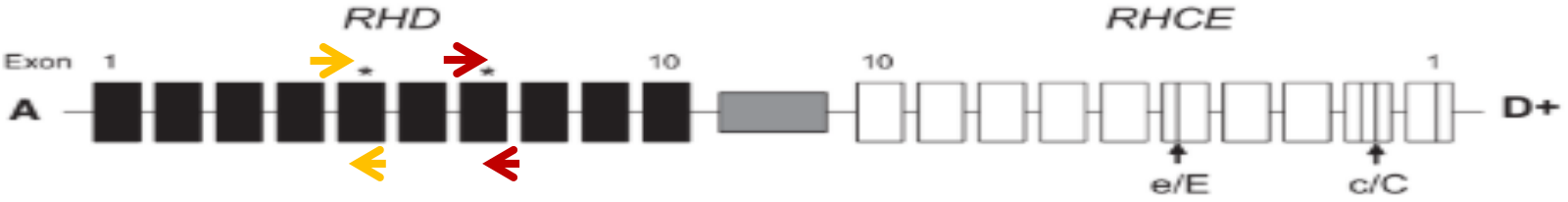
- Sequenties afwezig van maternaal genoom
- RhD: primers exon 5 en 7 (duplex PCR)
- Controle op aanwezigheid foetaal DNA
  - SRY gen, indien negatief: andere marker











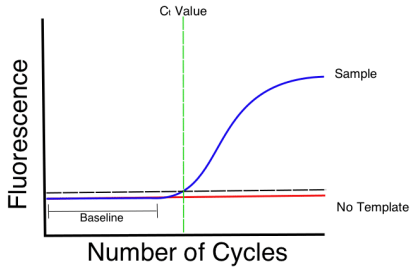
Maastricht UMC+

BJOG 2011;118:1340–1348

NJEM 1998; Volume 339 Number 24

Foetale genotypering: de interpretatie

PCR exon 5	PCR exon 7	uitslag
Positief*	Positief*	RhD kind aanwezig
Negatief	Negatief	RhD kind afwezig



**Verwachte Ct waardes voor foetaal DNA: 34 - 39*

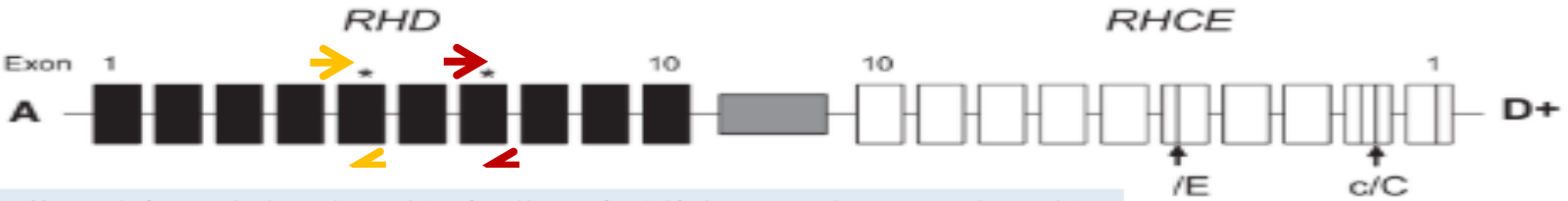


Table 2. Results for maternal and paternal *RHD* analysis performed because of atypical fetal *RHD* exon 5 and/or *RHD* exon 7 polymerase chain reaction results

Case	*RHD* PCR plasma		Maternal *RHD* analysis (genotype)	Paternal *RHD* analysis (genotype)	Conclusion fetal RhD status
	Exon 5 Ct	Exon 7 Ct			
1	37	32	*RHDΨ/d*	N/A	D positive
2	39	29	*RHDΨ/d*	N/A	D positive
3	35	30	*RHDΨ/d*	*RHD/DAU*	D positive
4	38	31	*RHDΨ/d*	N/A	D positive
5	38	32	*RHDΨ/d*	*RHD/RHD*	D positive
6	37	30	*RHDΨ/RHD-CE-D*^{5}	*RHD/DIII type 5*	D positive
7	38	Und	*d/d*	*DIVa/d*	D positive
8	Und	36	*d/d*	*RHD/DAU5*	D positive
9	35	39	*d/d*	*RHD/DNU*	D positive
10	Und	31	*RHDΨ/d*	*RHDΨ/DAU*	D negative
11	Und	31	*RHDΨ/RHD-CE-D*^{5}	N/A	D negative
12	30	30	*RHD(343delC)/d*	N/A	inconclusive
13	31	31	*RHD(IVS1+1G>A)/d*	*d/d*	inconclusive

Ct, cycle threshold value; *d*, deletion/complete absence of *RHD* gene; N/A, not available; *RHD*, normal *RHD* gene; *RHDΨ*, *RHD* pseudogene; Und, undetermined (no amplification).
RHDΨ, *RHD-CE-D*^{5}, *RHD(343delC)* and *RHD(IVS1+1G>A)*: *RHD* variant genes leading to a D-negative phenotype.
DAU, *DIII type 5*, *DIVa*, *DAU5 and DNU*: *RHD* variant genes leading to a D-positive phenotype.

BJOG 2011;118:1340–1348

2 weken later….

- Uitslag:
  - Foetale genotypering: RhD pos
    - Tenminste 1 kind is RhD pos
    - PCR uitslagen exon 7 passen bij variant bij moeder
  - Titer anti-D: **1:128**
  - ADCC test: **30%**
- Advies: over 4 weken herhalen titer en ADCC
- Rhesus genotypering vader (?)

Uitslag rhesus typering vader

- Rhesusfenotypering: RhD positief

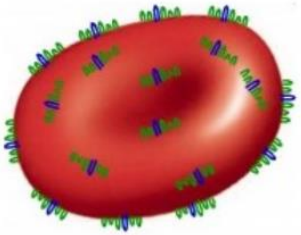
- Rhesus (D) genotypering vader (in eerste instantie niet gedaan):
  - Als homozygoot: beide kinderen RhD positief
  - Als heterozygoot: geen extra informatie

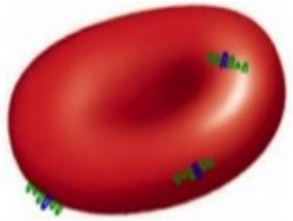
- Uitslag: 2 rhesus D allelen (homozygoot) MAAR
  - 1 normale en 1 met variant
  - Variant: DAU-3

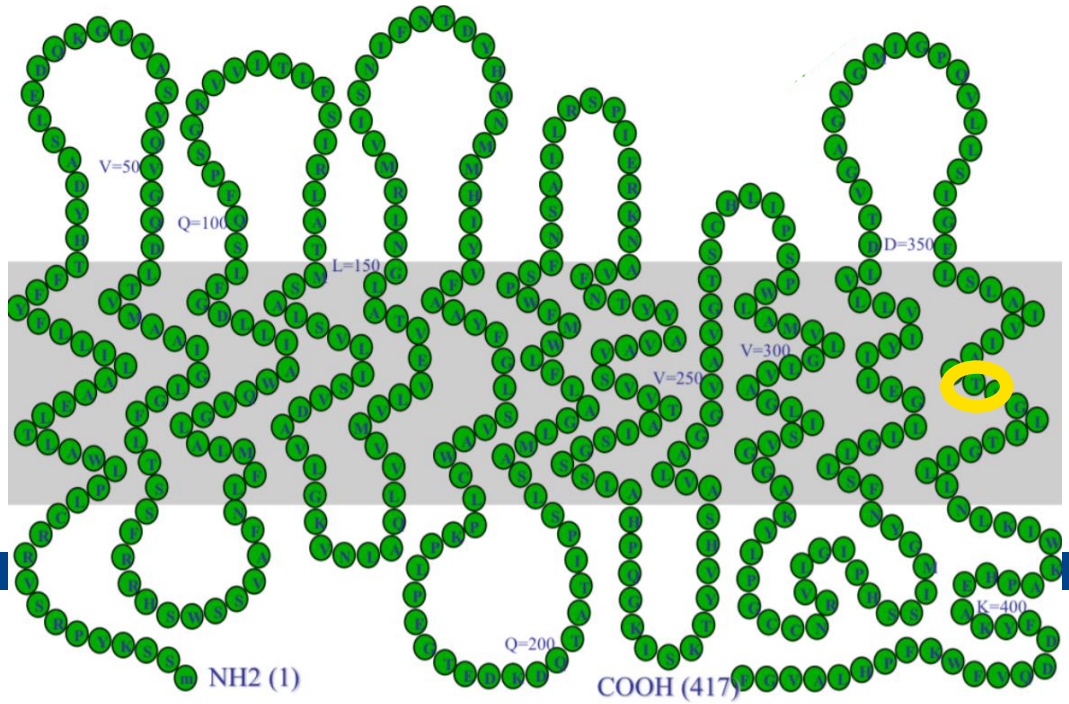
- Sanquin:
  - Advies om beide kinderen als hoog risico te behandelen
  - VERZOEK: graag navelstrengbloed bij geboorte verzamelen om dit nader te bekijken

DAU

- Deel van DAU allel cluster (“Der Afrikanischen Ursprungs”)
  - Rhesus D gen: 1136 C → T single nucleotide polymorphism (SNP)
    - Exon 8
  - Oorzaak van meeste RhD polymorfisme in Afrika
  - DAU0-DAU14
    - Partiele Rhesus D
    - Expressie rhesus D: zwak tot normaal
    - Rhesus D fenotypering problematiek of alloimmunisatie







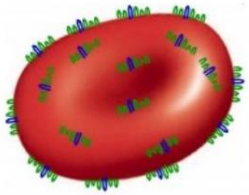
Alle DAU fenotypen: T379M

BLOOD, 1 JULY 2002 VOLUME 100, NUMBER 1

DAU cluster

Designation	ISBT name	Haplotype	Phenotype	Mechanism	Alterations	First mention	Definitive publication
DAU-13	RHD*10.13 RHD*DAU13	cDe		Multiple missense mutations	1136C>T (T379M) 48G>C (W16C)	2013	
DAU-0	RHD*10.00 RHD*DAU0	cDe	D positive (apparently normal) Partial D	Single missense mutation	1136C>T (T379M)	2002	2002
DAU-0.1	RHD*10.00.01 RHD*DAU0.01	cDe		Multiple missense mutations	1136C>T (T379M) 579G>A	2003	2003
DAU-1	RHD*10.01 RHD*DAU1	cDe	Partial D	Multiple missense mutations	1136C>T (T379M) 689G>T (S230I)	2002	2002
DAU-2	RHD*10.02 RHD*DAU2	cDe	Partial D weakened D expression	Multiple missense mutations	1136C>T (T379M) 209G>A (R70Q) 998G>A (S333N)	2002	2002
DAU-3	RHD*10.03 RHD*DAU3	cDe	Partial D	Multiple missense mutations	1136C>T (T379M) 835G>A (V279M)	2002	2002
DAU-4	RHD*10.04 RHD*DAU4	cDe	Partial D weakened D expression	Multiple missense mutations	1136C>T (T379M) 697G>A (E233K)	2002	2002
DAU-5	RHD*10.05 RHD*DAU5	cDe	Partial D	Multiple missense mutations	1136C>T (T379M) 667T>G (F223V) 697G>C (E233Q)	2005	2005
DAU-6	RHD*10.06 RHD*DAU6	cDe	Partial D	Multiple missense mutations	1136C>T (T379M) 998G>A (S333N)	2005	2005
DAU-7	RHD*10.07 RHD*DAU7	cDe	Partial D	Single missense mutation	1136C>T (T379M) 835G>A (V279M) 998G>A (S333N)	2009	2009
RHD(201A,203A,1136T)		not reported		Multiple missense mutations	1136C>T (T379M) 201G>A 203G>A (S68N)	2014	
DAU-11	RHD*10.11 RHD*DAU11	cDe	weakened D expression	Multiple missense mutations	1136C>T (T379M) 254C>T (A85V) 835G>A (V279M)	2012	2016
DAU-9	RHD*10.09 RHD*DAU9	not reported	D positive (no further data)	Multiple missense mutations	1136C>T (T379M) 535T>C (F179L)	2012	2012
DAU-5.1	RHD*10.05.01 RHD*DAU5.01	cDe	weakened D expression	Multiple missense mutations	1122C>T (I374I) 1136C>T (T379M) 667T>G (F223V) 697G>C (E233Q)	2014	2016
RHD(G263L,T379M)		not reported		Multiple missense mutations	1136C>T (T379M) 787GG>TT (G263L)	2017	
RHD(IVS4+1G>T,1136C>T)	RHD*01N.69	not reported	D negative	Splice site mutation	1136C>T (T379M) IVS4+1G>T		
DAU-12	RHD*10.12 RHD*DAU12	cDe		Multiple missense mutations	1136C>T (T379M) 542T>C (L181P)	2012	
RHD(M1V,T379M)	RHD*10.15 RHD*DAU15	not reported		Complex changes	1136C>T (T379M) 1A>G (M1V)	2008	
DAU-8	RHD*10.08 RHD*DAU8	not reported	D positive (no further data)	Multiple missense mutations	1136C>T (T379M) 340C>T (R114W) 579G>A	2012	2012
RHD(S254L,T379M)		not reported		Multiple missense mutations	1136C>T (T379M) 761C>T (S254L)	2017	
DAU-14	RHD*10.14 RHD*DAU14	cDe	D positive (no further data)	Multiple missense mutations	1136C>T (T379M) 201G>A 203G>A (S68N)	2014	2014
DAU-10	RHD*10.10 RHD*DAU10	not reported	D positive (no further data)	Multiple missense mutations	1136C>T (T379M) 579G>A 739G>C (V247L)	2012	2012

DAU-3

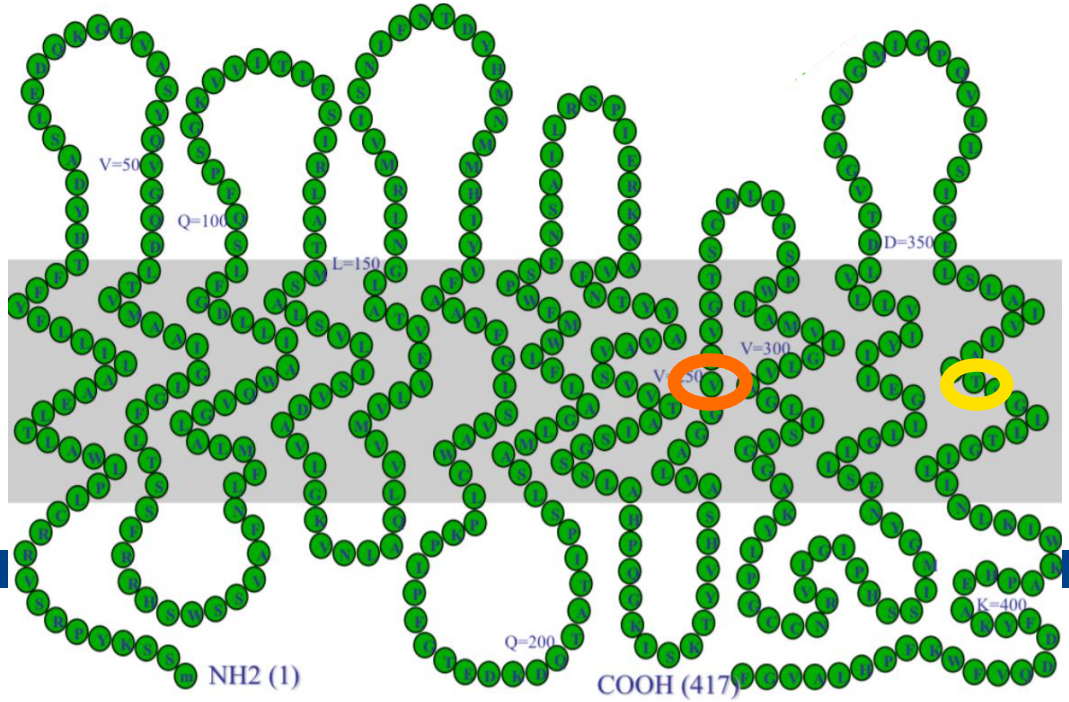


- Partiele D fenotype
- Geassocieerd haplotype: ce
- Aantal D antigenen op cel: ~10.000
- Anti-D vorming is mogelijk

Exon	2	4	5				6	7	8
Nucleotide	209 G>A	579 G>A	667 T>G	689 G>T	697 G>C	697 G>A	835 G>A	998 G>A	1136 C>T
*RHD*DAU0*									
*RHD*DAU0.1*									
*RHD*DAU1*									
*RHD*DAU2*									
*RHD*DAU3*									
*RHD*DAU4*									
*RHD*DAU5*									
*RHD*DAU6*									
*RHD*DAU7*									

Fig. 1: Nucleotide changes characteristic for all various *RHD*DAU* alleles. Note that all alleles share one common nucleotide change in exon 8 (1136C>T) suitable for identification of *RHD*DAU* in general by routine PCR-SSP genotyping of donors and patients.

Meyer et al., *Swisstransfusion Jahreskongress 2014, Luzern, Switzerland*



Alle DAU fenotypen: T379M

DAU-3: V279M

BLOOD, 1 JULY 2002 VOLUME 100, NUMBER 1

4 weken later: anti-D titer en ADCC

- Uitslag:
  - ABO bloedgroep: B
  - Rhesus D antigeen: neg
  - Foetale genotypering: RhD pos
    - Tenminste 1 kind is RhD pos

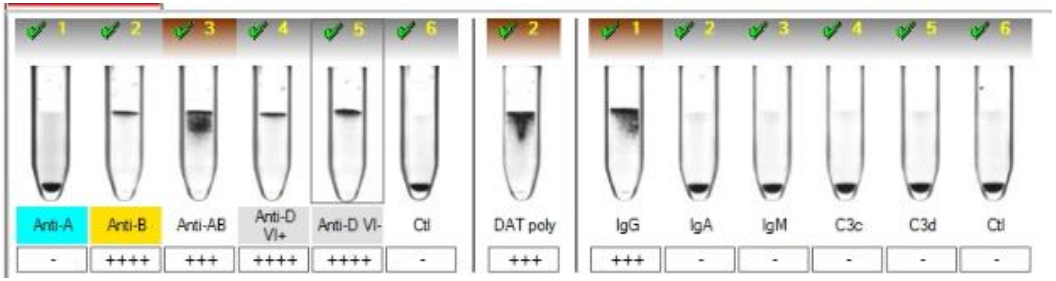
  - Titer anti-D: **1:128**
  - ADCC test: **30%**

- Laatste ADDC 25-5-2018: 30%; titer anti-D 1:64

Verder beloop casus

- Bij amenorroeduur van 36 + 3: opname i.v.m. contracties
  - Primaire sectio was reeds gepland bij 37 wkn i.v.m. Rhesus D antagonisme
- Ongecompliceerde sectio caesarea
  - stuitligging van kind 1 en dwarsligging van kind 2
- 2 zonen

Beloop jongens



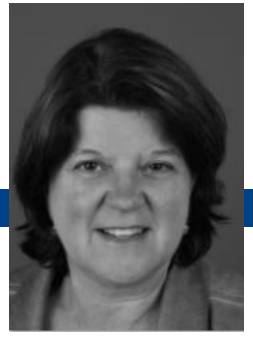
- Geboren op 05.06.2018
- Bloedgroep: Bpos
- Goede start
- Hyperbilirubinemie o.b.v. Rh-immunisatie wv fototherapie
- Arteriele hypertensie e.c.i. wv propranolol

- Er is geen navelstrengbloed verzameld om Rhesus D nader te bekijken

Conclusie

- In het geval van alloimmunisatie bij zwangeren:
  - foetale bloedgroeptypering biedt een non-invasieve manier om gemakkelijk en veilig het risico op HZFP in te schatten

- Andere etniciteiten: andere rhesus D ‘varianten’!



Maastricht UMC+