Irish Blood Transfusion Service
Seirbhís Fuilaistriúcháin na hÉireann

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# Fetal RhD Genotyping in Ireland

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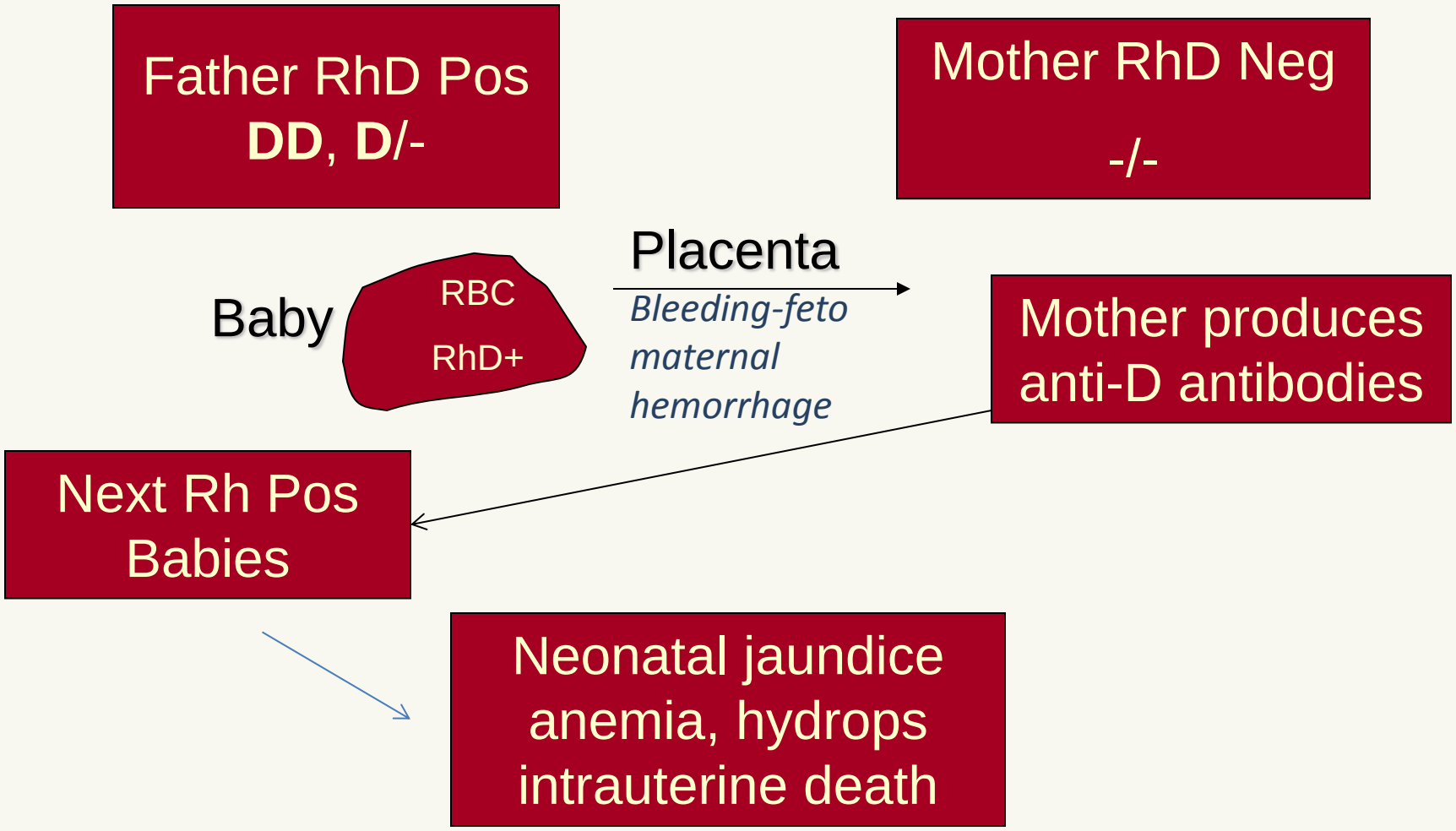
# Fetal RhD Genotyping in Ireland

- Hemolytic disease of the newborn
- Molecular genetics of the RHD gene
- Fetal RhD testing service

# HEMOLYTIC DISEASE OF THE NEWBORN

# Rh Haemolytic Disease



# Treatment & Prevention of Rh Hemolytic Disease

- Treatment of Affected Child
  - Exchange Transfusion
  - Monitoring in utero
  - Ultrasound Amniocentesis
  - Intrauterine Transfusion

- Prevention: Anti-D Immunoglobulin post partum, sensitising events
- Incidence reduced from 16% to 2%

# What is Routine Antenatal Prophylaxis (RAADP)?

- Routine antenatal anti-D prophylaxis (RAADP)
  - Administration to RhD negative women in the **third trimester** as prophylaxis against small amounts of feto-maternal hemorrhage that **can occur in the absence of observable sensitising events**

- A further reduction in the sensitization rate ranging from 0·17 to 0·28%

# RAADP international availability

- Throughout Europe, N. America and Australia
- 2002, UK National institute of health and Care Excellence (NICE), now almost 100% coverage

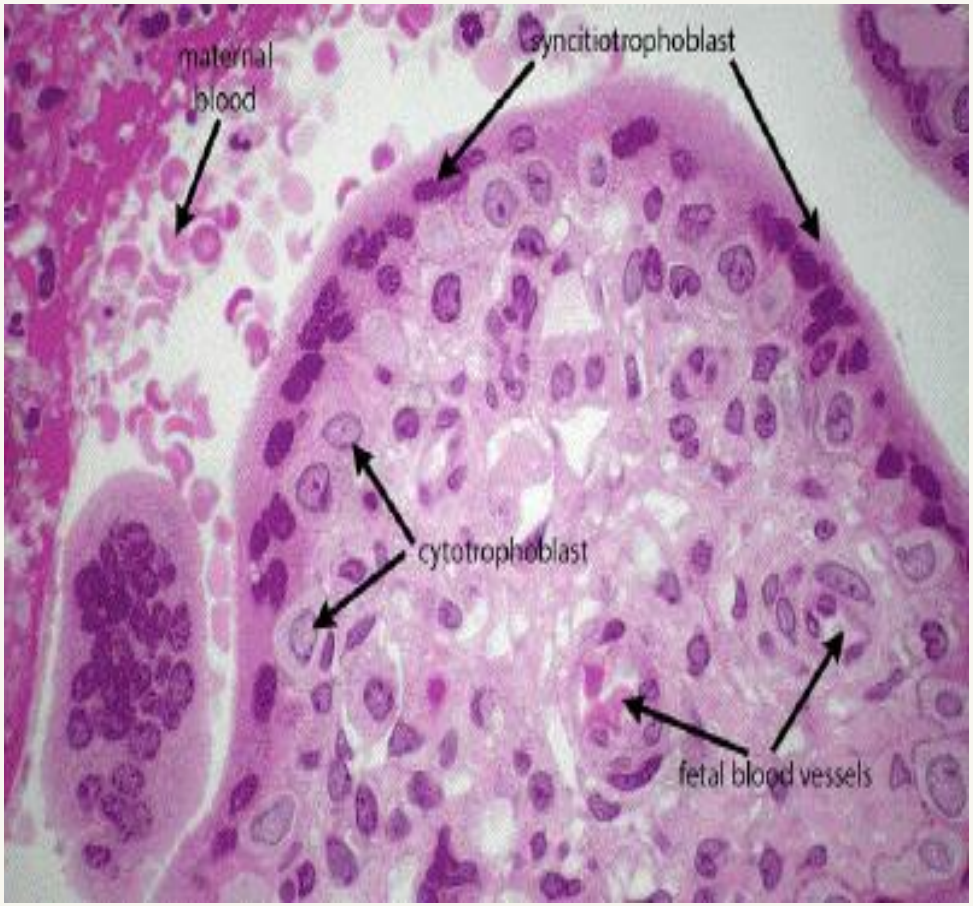
- 2012, Irish Clinical Practice guideline recommended introduction (HSE)

# What is targeted Prophylaxis?

- **Targeted- tRAADP**
- Reduce unnecessary use of anti-D in RhD negative women
- Potential cost savings
- Product conservation
- TTI risk

# What is cell free fetal DNA?

- Discovered in 1997
- DNA Size <350bp.
- Detectable from 6 weeks
- Apoptotic microvesicles from the syncitiothrophoblast
- Increases with gestation
- Pregnancy specific and rapidly cleared after birth
- 1-1000 copies per mL
- 10-30% fetal fraction
- ↑ ~1% per week



# Targeted RAADP by cell free fetal DNA (cffDNA) testing

- Fetal RhD status can be determined early in pregnancy
- 40% of RhD negative women of European descent carry a RhD negative fetus
- Denmark (2010), Netherlands (2011) introduced RhD genotyping with targeted RAADP
- UK, Nov 2016, NICE recommendation to screen

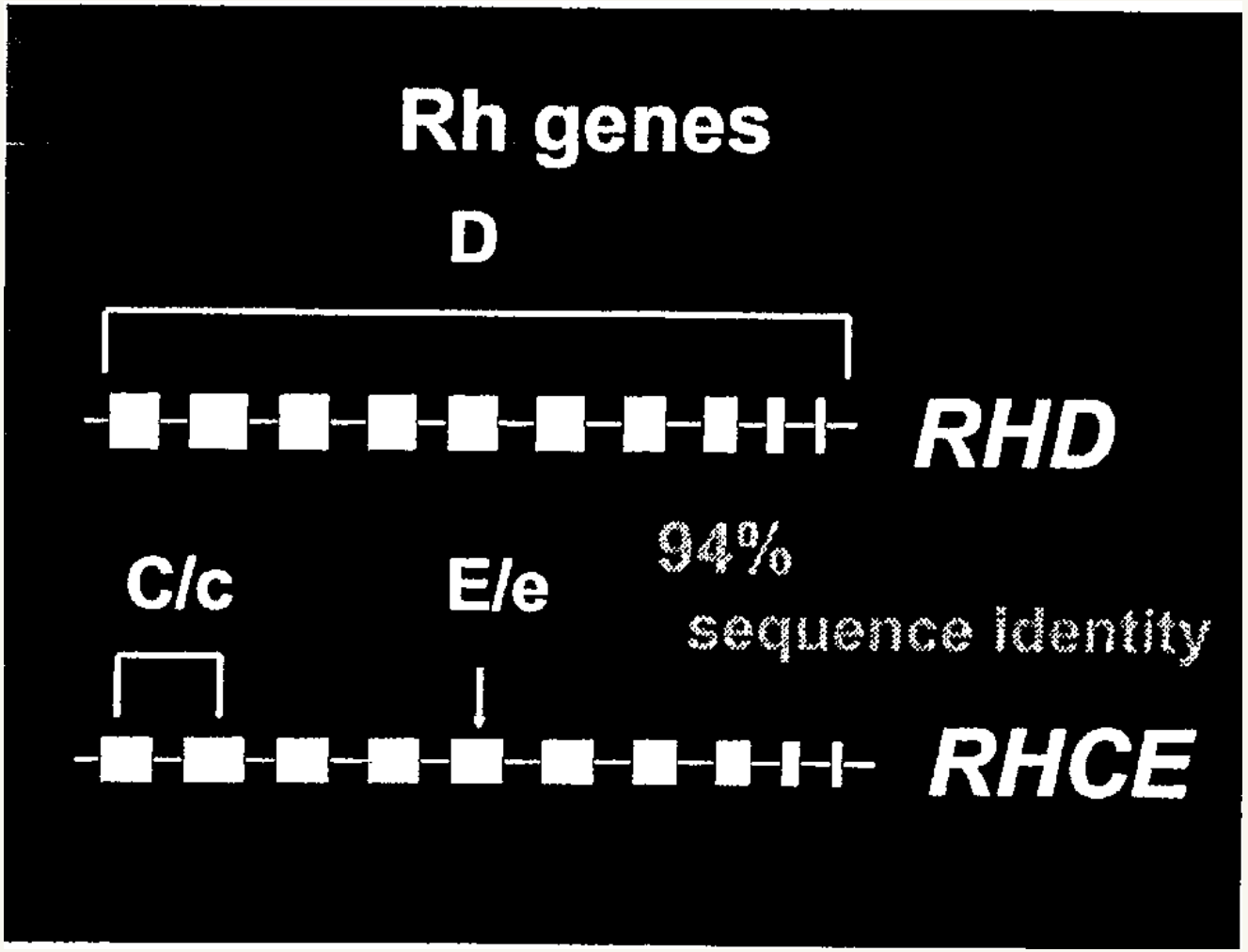
# Irish cffDNA RHD testing service

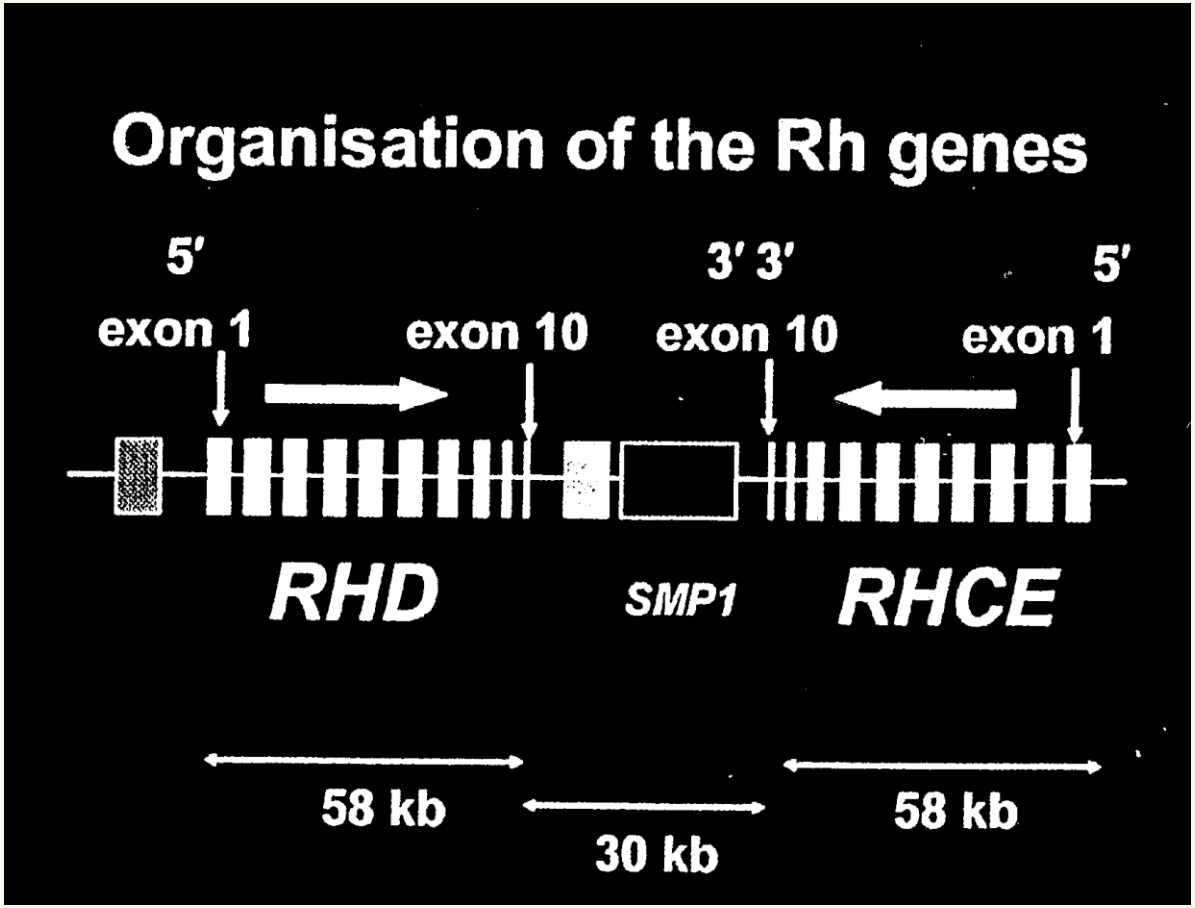
- 2012, Irish Clinical practice guideline for RAADP, also recommended development of a national testing capability
- In collaboration with the Irish Blood Transfusion Service
- Laboratory Developed Test
- 2018, service went live
- RhD negative, non alloimmunised patients

# How does the test work?

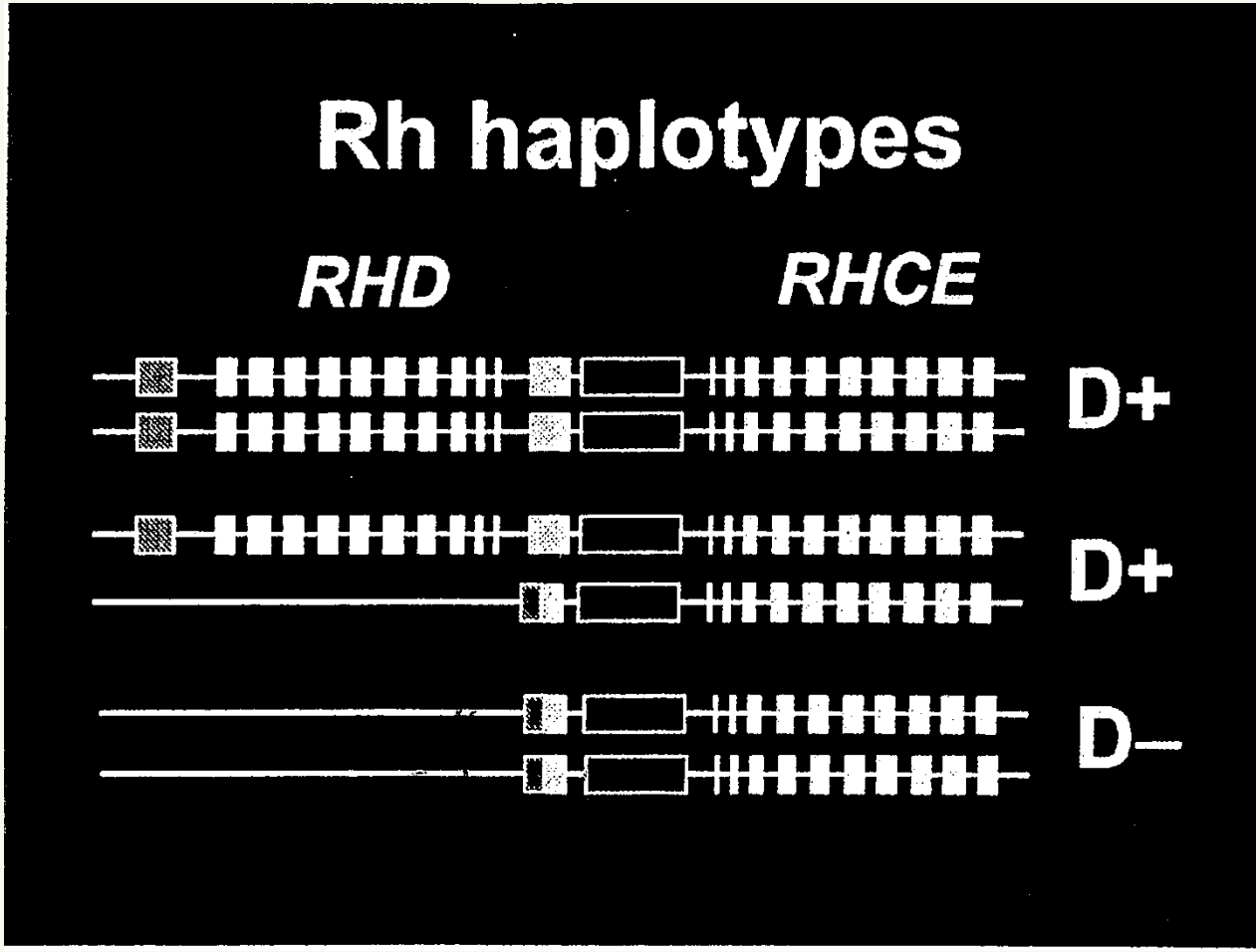
- **Rh blood group system**
- Most complex of blood groups!
- Multiple antigens (50+)
- 2 closely linked genes on chromosome 1
- Encode D, Cc, Ee antigens
- RHCE the ancestral RH gene
- RHD a duplicated gene

# Rh

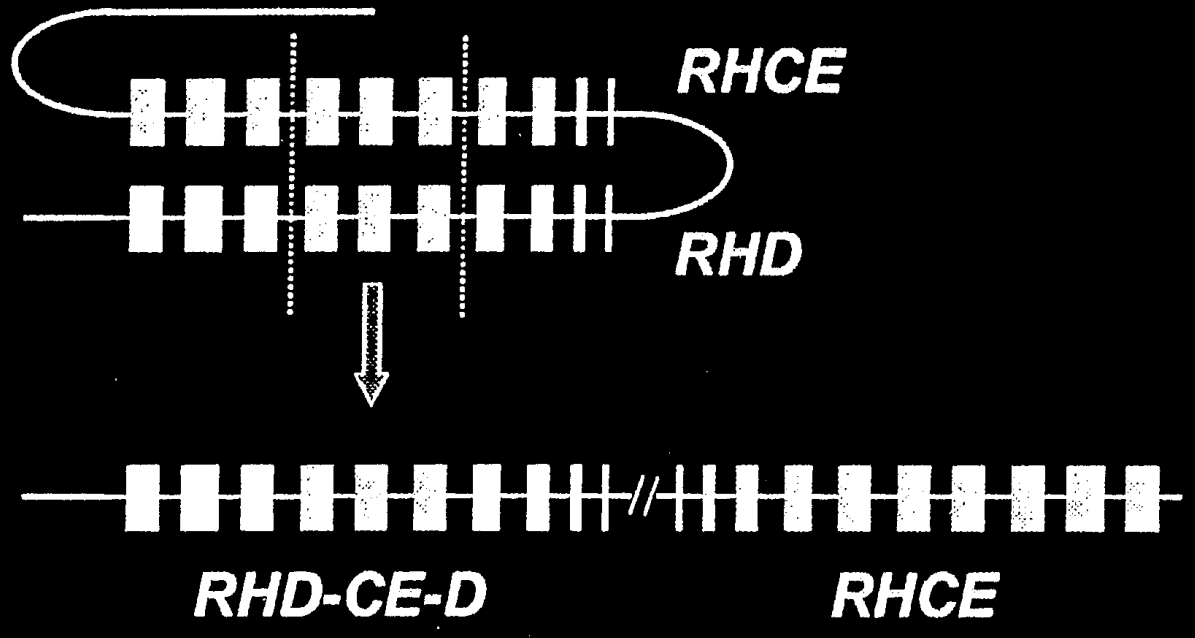



Organisation of the Rh genes
5′
exon 1
exon 10
3′ 3′
exon 10
5′
exon 1
RHD
SMP1
RHCE
58 kb
30 kb
58 kb

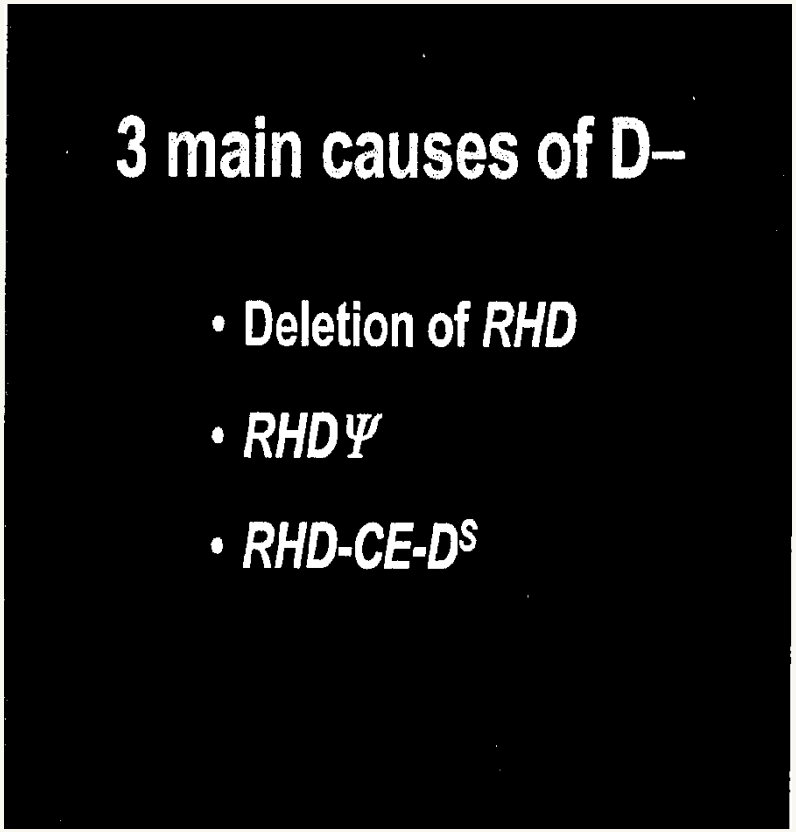
# Caucasian populations



# Chromosomal misalignment and recombination

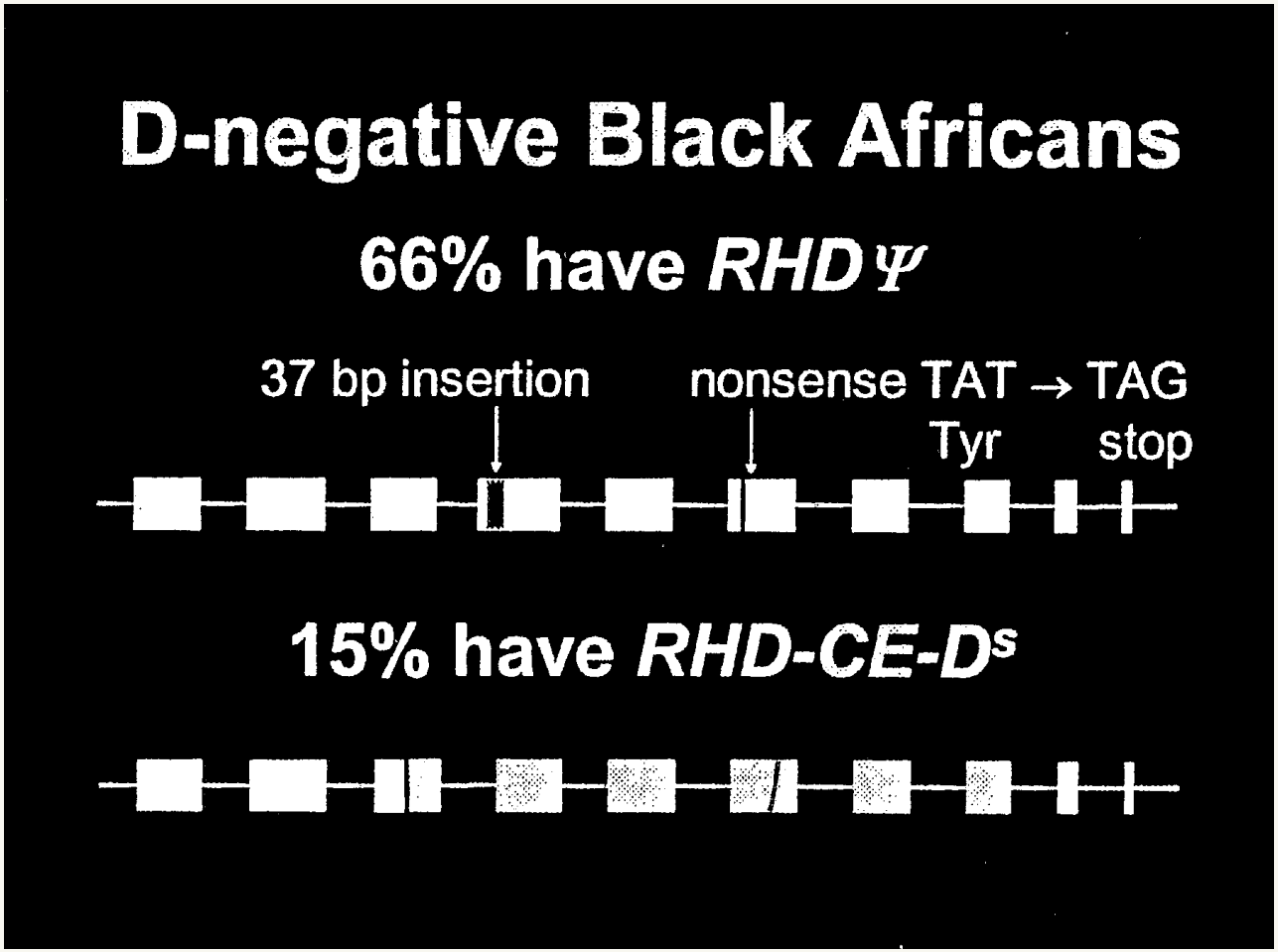


# RhD negative phenotype causes




- *Caucasian ancestry*
  - *RHD* gene deletion
- African ancestry
  - Intact but dysfunctional *RHD* gene-pseudogene
  - Rearranged *RHCE-RHD* genes-hybrid gene

# African ancestry



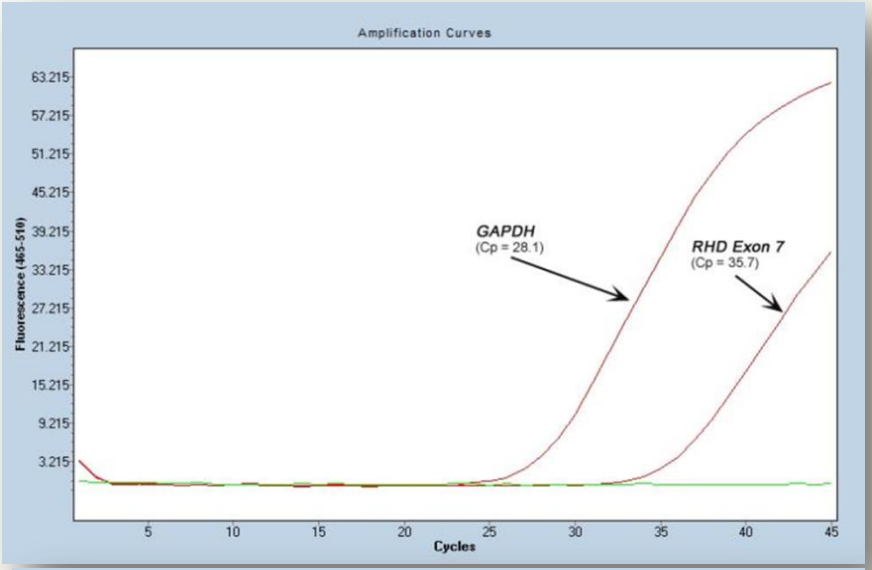

Just 5% are D negative

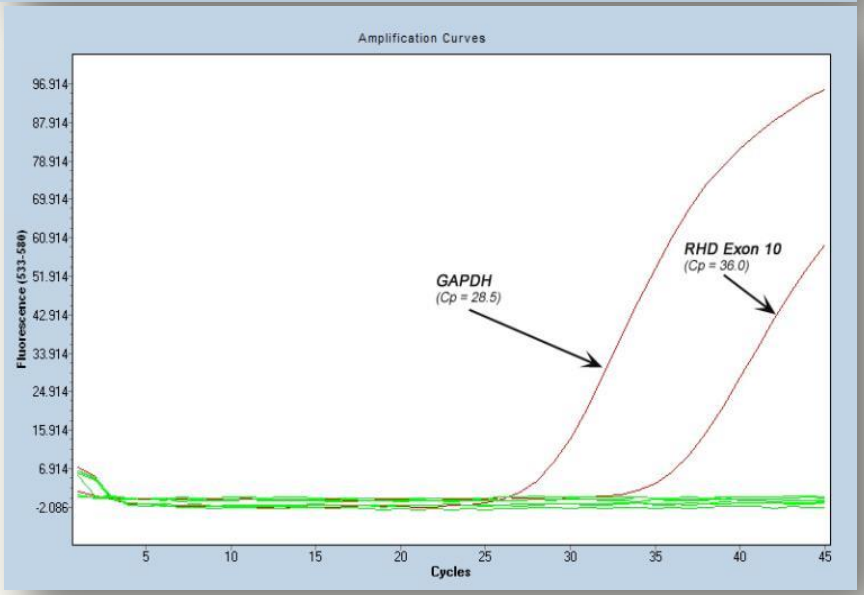
# RhD detection method in Ireland

- Exons 7 and 10 are tested.
- Combination is suitable non alloimmunised Caucasian populations
- Demonstrates deletion of D gene in Caucasian, when neither detected
- *Does detect presence of the RhDΨ pseudo gene which is also a RhD negative phenotype however (would require additional time, cost and analysis paternal samples)*

# Fetal RHD Genotyping (cffDNA)

- Assay clinical evaluation:
- Samples taken > 11 and 28 weeks gestation
- Quantitative PCR
- RhD triplicate (exons 7&10), GAPDH



# cffDNA validation study

| Genotypes vs Cord RhD to Date | |
|---|---|
| True Positives | 384 |
| True Negatives | 220 |
| False Negatives | 0 |
| False Positives | 6 |
| Inconclusives | 25 |
| Maternal cffDNA RhD Variants | 14 |
| Genotype/phenotype cord check | 649 |

| FINAL | | 95%CI Lower | 95% CI Upper |
|---|---|---|---|
| Sensitivity | 100.00% | 99.24% | **100%** |
| Specificity | 97.35% | 95.25% | 99.44% |
| PPV | 98.46% | 97.24% | 99.68% |
| NPV | 100.00% | 98.66% | 100% |

**98.46% Probability the fetus is RhD positive**

# Fetal RHD Genotyping Result Actions

| Results | Action |
| --- | --- |
| **RHD-negative fetus** | **No anti-D** |
| RHD-positive fetus | Give anti-D |
| Maternal RHD | Give anti-D |
| Inconclusive | Give anti-D |

# False negative and false positive

- **Definition of ‘false negative result’**
  - test has predicted an antigen negative fetus when the baby is found to be antigen positive at birth.

- **Definition of ‘false positive result’**
  - the test has predicted an antigen positive fetus when the baby is found to be antigen negative at birth

# False negative and false positive

- **‘false negative result’**
  - test has predicted an antigen negative fetus when the baby is found to be antigen positive at birth.
  - Anti-D given at after birth, but not antepartum

- **‘false positive result’**
  - the test has predicted an antigen positive fetus when the baby is found to be antigen negative at birth
  - Antenatal prophylaxis given unnecessarily
  - Would have occurred in RAADP programs

# What will the IBTS do if they are notified of an incorrect result?

- This a **screening test**, to predict a phenotype
- False positives are not investigated. Retest the archive plasma sample (if available) to confirm genotype.
- Errors (other than false positive cases) will be investigated as a quality incident in the IBTS quality management system and if error in procedure is identified, corrective and preventative actions will be put into place.
- The user will be notified of the findings and any recommended further action.

# False positive

- Wrong-blood-in-tube
- Rare RhD alleles that are detected by assay but are non functional and don’t express RhD
- Laboratory error
- Sample contamination
- Vanishing twin

# False negatives

- Insufficient fetal DNA
- Wrong-blood-in-tube
- Laboratory error

# Program to date:

- 3 years, 10 months
- 5435 referrals
- 2018 – 114 samples
- 2019 – 1388 samples
- 2020 – 1751 samples
- 2021 (up to 20/09/2021) – 2182 samples

# Program to date:

- Predicted RHD-negative fetus: 2038 (38%)
- Predicted RHD-positive fetus: 3186 (59%)
- Inconclusive/Not Tested: (3%)

- **Over 2000 avoided unnecessary anti-D**

# False positives and False negatives to date

- False Positives: 12 (0.2%)
- False Negatives: 2 (0.03%)
  - Laboratory process change
- Within range of European peers (https://www.bmj.com/content/355/bmj.i5789)

# RhD Alloimmunised women

- Test unsuitable for patients already alloimmunized and have anti-D
  - Additional exon requirement
  - Cost
  - Sample number
- Need IBGRL referral

# Results transmission

- Electronic Result transmission via *medibridge*
  - *Rotunda*
  - *Coombe*
- Would be available to all sites upon request
  - Subject to funding
  - Faster result transfer
  - Transcription errors eliminated

# Challenges in Fetal RHD Genotyping (cffDNA)introduction

- Irish hospitals can/need to purchase services with public money
- Blood service income blood products and testing
- Some testing already performed in UK
- Cost competitiveness

# Service uptake, thank you!

| Hospital Code | Number of Samples |
|---|---|
| DN0017 (Rotunda) | 843 |
| DN0016 (NMH, Holles St) | 773 |
| CN0001 (Cavan) | 70 |
| G00003 (Portiuncula) | 67 |
| DN0015 (Coombe) | 54 |
| WX0001 (Wexford) | 12 |
| SO0001 (Sligo) | 2 |
| LK0001 (Limerick) | 6 |
| TOTAL | 1827 |

# Further details

- IBTS website, Laboratory User guide
- Samples: 8 mL EDTA, 5 day testing window
- Cost: 40 euro
- TAT: 2 weeks
- Samples from 11 weeks

- Cord blood RhD result, discrepancies please, and to assist in future publication

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