Fetal genotyping from maternal blood

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D-negative pregnant woman with anti-D

Valuable to know D type of fetus

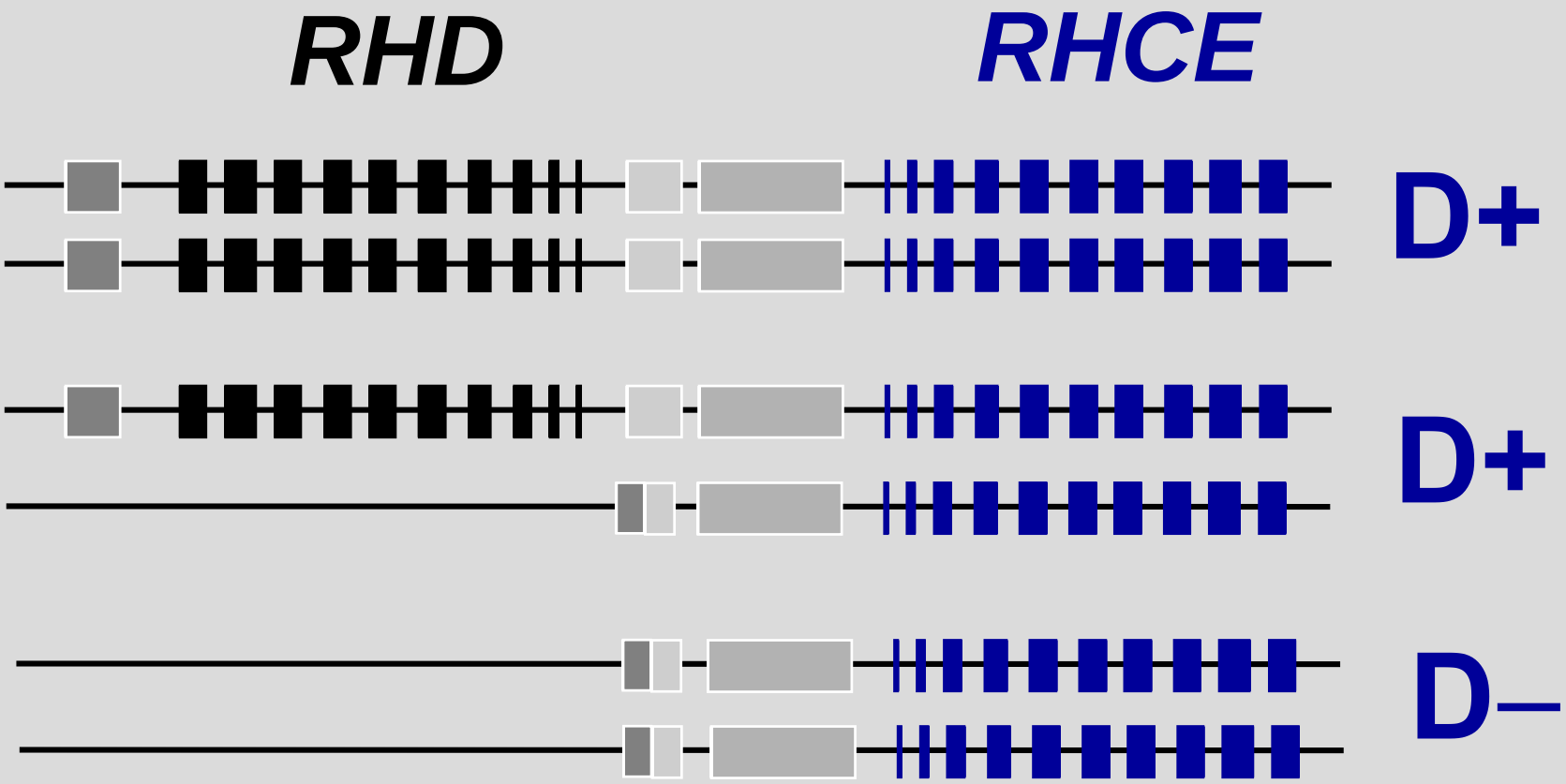
Fetus D-positive: at risk

- **pregnancy should be managed appropriately**

Fetus D-negative: not at risk

- **no need for intervention**

Rh haplotypes



RhD genotyping tests detect presence or absence of *RHD*

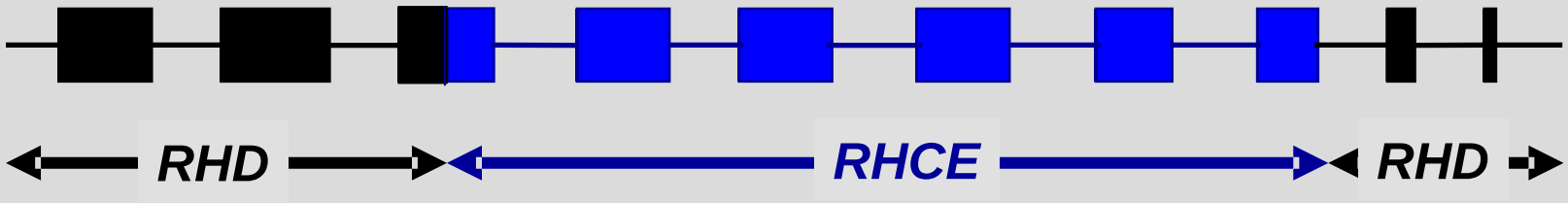
Complicated by variants, but these can be taken into account in when developing the tests

Variant D genes

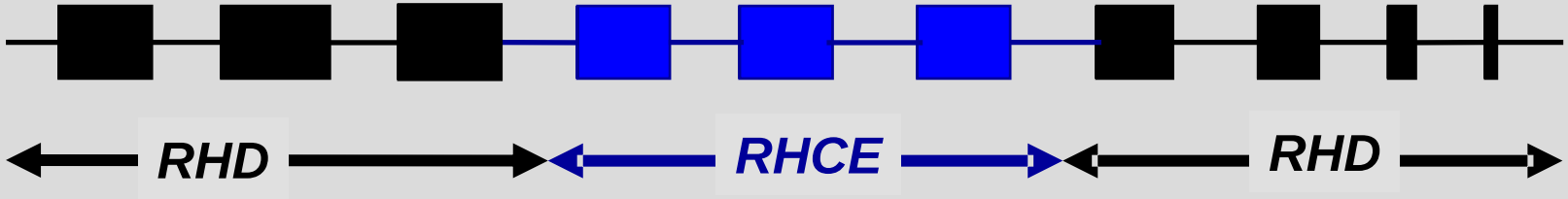
RHD*Ψ **D–**

37 bp duplication nonsense

RHD-CE-D^{s} **D–**



RHD*DVI **D+**



Source of fetal DNA

Before 2001

DNA from amniocytes or chorionic villi

Amniocentesis:

0.5-1.0% risk of spontaneous abortion
20% risk of transplacental haemorrhage

CVS: similar risks

Cell-free fetal DNA in maternal plasma

10–20 weeks: 10–15% cell-free DNA = fetal

Range: 3–30%

>21 weeks: increases by ~1% per week

Excellent source of fetal DNA for fetal *RHD* testing in D-negative pregnant women

Fetal DNA in maternal plasma

DNA isolated from maternal plasma

85-90% maternal DNA

No *RHD* (mother D-neg)

10-15% fetal DNA (fetal fraction)

***RHD* present if fetus D-pos**

No *RHD* if fetus D-neg

Real-time quantitative PCR

Taqman chemistry

Measures quantity of product at every cycle

Advantages of real-time quantitative PCR

- **Highly sensitive**
- **Quantitative**

  ensures testing fetal, not maternal, DNA
- **Closed system**

  reducing risk of contamination

IBGRL, Bristol

University of BRISTOL

NHS Blood and Transplant

1994: Fetal blood group genotyping

2002: Fetal D typing on cff-DNA
Later extended to K, C, c, E

Service provided for D– women
with anti-D (>4 IU/ml) or
with history of fetal/neonatal haemolysis

Standard of care in England

University of BRISTOL

IBGRL, Bristol

NHS Blood and Transplant

RHD exons 4, 5, 7, 10

Real-time QPCR

Only exons 7 & 10 amplify

*RHD*Ψ, RHD-CE-D^{s}, RHD*DVI*

Other tests on fetal DNA in maternal plasma

K	***KEL***	**T698**	**exon 6**
Rh c	***RHCE***	**C307**	**exon 2**
Rh E	***RHCE***	**C676**	**exon 5**
Rh C	***RHCE***	**insert**	**intron 2**

RQ-PCR with an allele-specific primer

University of BRISTOL

IBGRL, Bristol

NHS
Blood and Transplant

No. fetal samples tested for blood groups 2012/13

RhD	205
K	148
Rhc	72
RhE	65
RhC	10
Total	500

IBGRL, Bristol

University of BRISTOL

NHS Blood and Transplant

~500 pregnancies per year

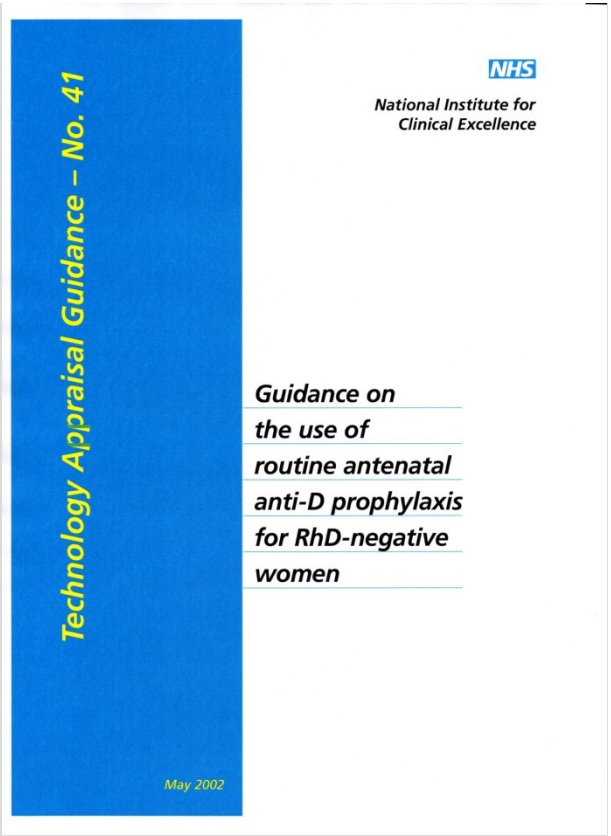
Charge: £260

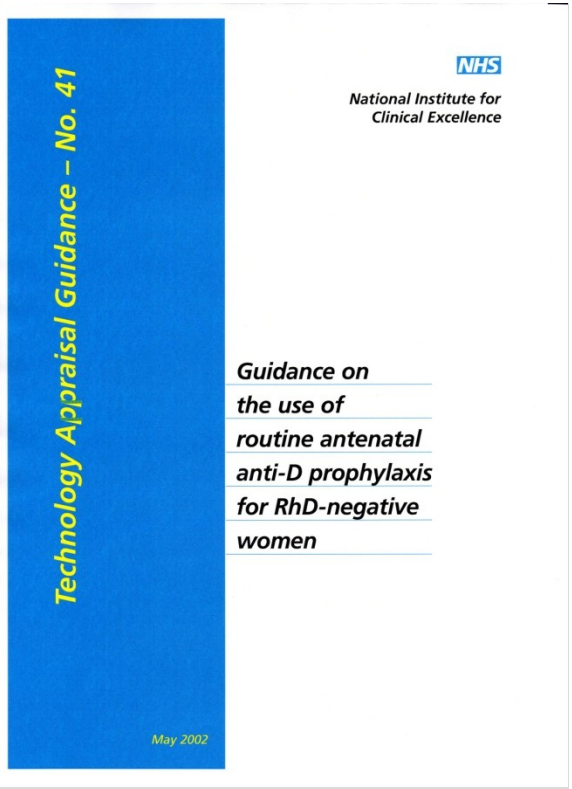
Rh: test at 16 weeks gestation

K: after 20 weeks gestation

Recommendation

All RhD-negative pregnant women should be offered 500 IU anti-D Ig at 28 & 34 weeks





Endorsed studies into the feasibility of mass testing antenatally for fetal blood group by analysis of fetal DNA in maternal plasma

Routine antenatal anti-D prophylaxis

1 or 2 doses at ~ 28–34 weeks

All D– pregnant women treated

37–40% have D– fetus

All D– pregnant women receive anti-D after sensitising event, after 12 weeks

High-throughput test developed at IBGRL, Bristol

Finning *et al*. BMJ 2008;336:816

DNA isolated robotically

Real time PCR –

RHD **exons 5 (*RHD*Ψ–) & 7 (*RHD*Ψ+)**

1869 pregnancies

High level of accuracy

~28 weeks gestation

7 hospitals

Newcastle

South Tyneside

Sunderland

Birmingham women's

UCLH, London

Southmead, Bristol

St Michael's, Bristol

~5000 samples from 1769 women

Approved by National Research Ethics Service

Taken at

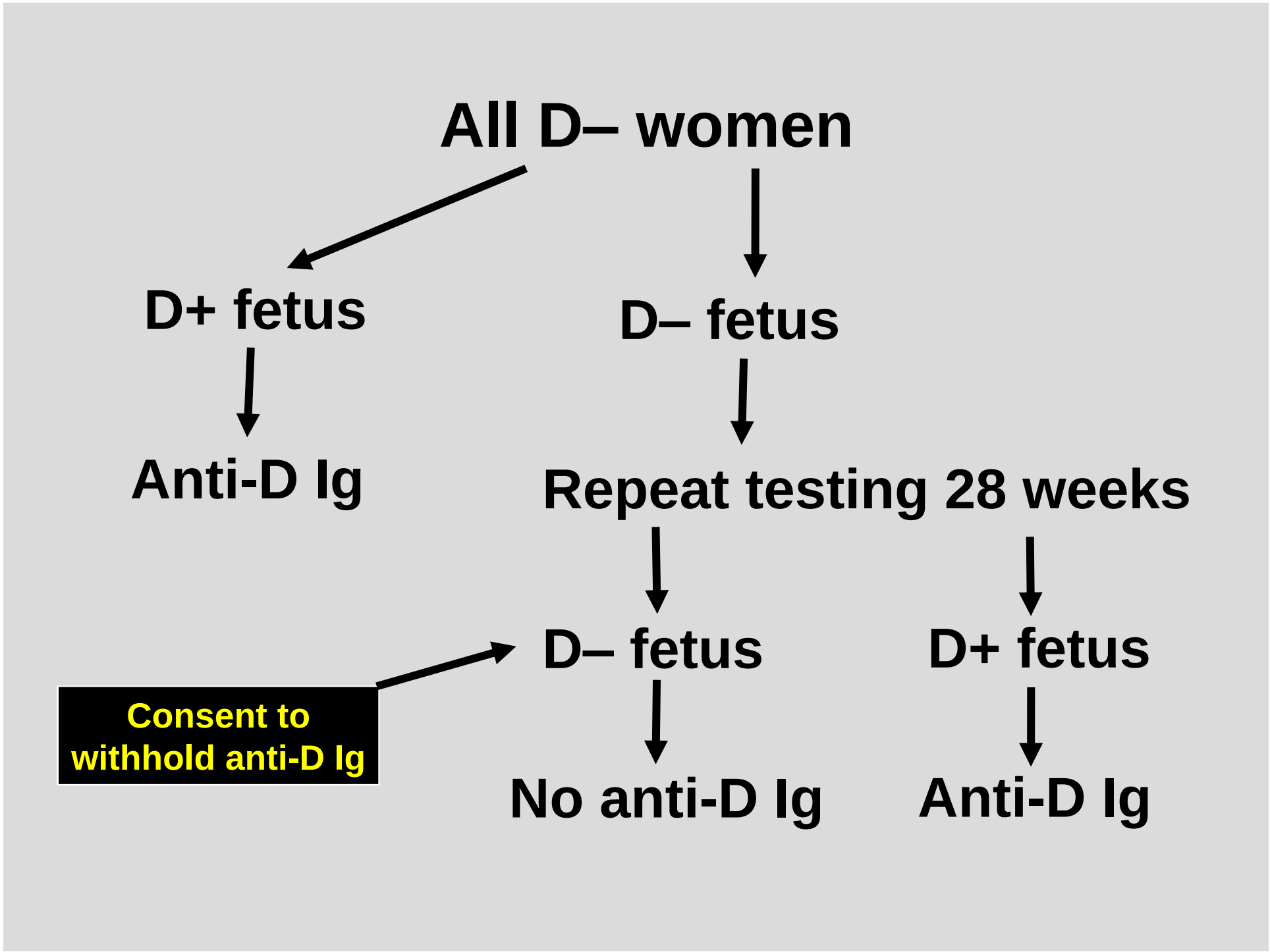
Booking	7-10 weeks
Down's screening	11-17 weeks
Routine anomaly scan	18-23 weeks
Antibody screen	28 weeks

Cord blood tested serologically

4913 fetal genotyping results
up to 4 analyses per woman

78% Caucasian
6% Asian
4% Black or mixed race
12% unknown

Gestational age: 5–35 weeks
(mean & median 19 weeks)


All D– women
D+ fetus
D– fetus
Anti-D Ig
Repeat testing 28 weeks
D– fetus
D+ fetus
Consent to withhold anti-D Ig
No anti-D Ig
Anti-D Ig

Results

	Gestation (weeks)				
	<11	11-13	14-17	18-23	>24
Correct	737	876	497	813	1525
False D–	16 (1.8%)	1 (0.10%)	1 (0.18%)	1 (0.11%)	0
False D+	1	4	1	5	7
Inconclusive	111	75	43	69	93
Total	865	956	542	888	1625
Specificity D–	96.2	99.8	99.5	99.8	100

½ inconclusive variant *RHD* in mother or fetus

& ½ result below threshold

Testing is accurate after 11 weeks

Economic analysis

Based on audit of anti-D usage
by D– women
booking in a maternity unit
over a 2-year period
with costs modelled

What are the economic savings that can be made from this testing?

Antenatal anti-D Ig:

- **routinely at around 28 weeks**
- **after potential sensitising events**

Kleihauer test following events associated with feto-maternal haemorrhage

Serological testing following delivery.

Economic analysis

Testing at >11 weeks, but <25 weeks would generate modest additional costs

if 20,000–80,000 samples per year tested

Costs estimated at between £1.3 & £14

Earlier the typing & larger no. tested

– the lower the costs

Lowest costs arise from early fetal testing, but test must coincide with routine early pregnancy appointments

Conclusion

Fetal D typing could be introduced at a small additional cost to the NHS

If delivered at the time of

routine clinic visit or

routine midwife visit

at or before 16 weeks gestation

Questionnaire, interview, & focus group-based study

(Oxenford K, *et al*. *Prenat Diagn* 2013;33:688-694)

D– pregnant women & health professionals would welcome the introduction of routine fetal D typing

Both groups are keen to avoid unnecessary administration of anti-D

Any implementation must be preceded by education of midwives delivering the service

Advantages

- **Cost effective?**
- **Anti-D Ig in short supply**
- **Eliminates unnecessary treatment of pregnant women with blood products**
- **Bristol study:**
  Negative results in 36% women
  England & Wales:
  ~40,000 mothers per year spared anti-D Ig

Fetal RhD testing in all D– pregnant women

Provided as service in:

Denmark	**26 weeks'**
Netherlands	**28 weeks'**

How are we going to implement fetal testing for all D-negative pregnant women in England?

Region covered by NHSBT,
~100,000 pregnancies per year

Save ~38,000 women from receiving anti-D Ig unnecessarily

Setting up such a project is a big task

With many the hospitals extremely short on funding, it is possible that they would not pay

Bristol pilot

Initiated on April 1 2013 for 1 year

Involves 3 hospital trusts

Initial plan – samples to be taken at 12 weeks, Down's syndrome test

Community midwives did not want discussions on Down's testing confused by obtaining consent for *RHD* testing

Agreed to blood samples taken at 15–16 weeks by community midwives at routine visit

Bristol pilot

- Significance of the test explained to mothers
- When result obtained from lab, mothers with a D– fetus advised not to receive Ig
- If they choose it, then it will be given
- Mothers with D+ fetuses and those with inconclusive results recommended to receive Ig
- Too early to provide any results
- Expect to test about 1,500 pregnancies in duration of the pilot

Bristol pilot

Audit to assess:

Pathway efficiency

Accuracy of the test

Adherence to protocol by analysis of case notes from 100 consecutive D– women

Overall change in anti-D Ig & Kleihauer test usage following introduction of the service

Bristol pilot

- Charging hospitals £12 for test
- After 1 year, will hospitals pay a more realistic charge?
- Hope to expand to the rest of England

What's around the corner?

Next generation sequencing?

Next generation sequencing
or
massively parallel sequencing

One sequencing procedure and one operator can generate as much data as several hundred Sanger-type capillary sequencers

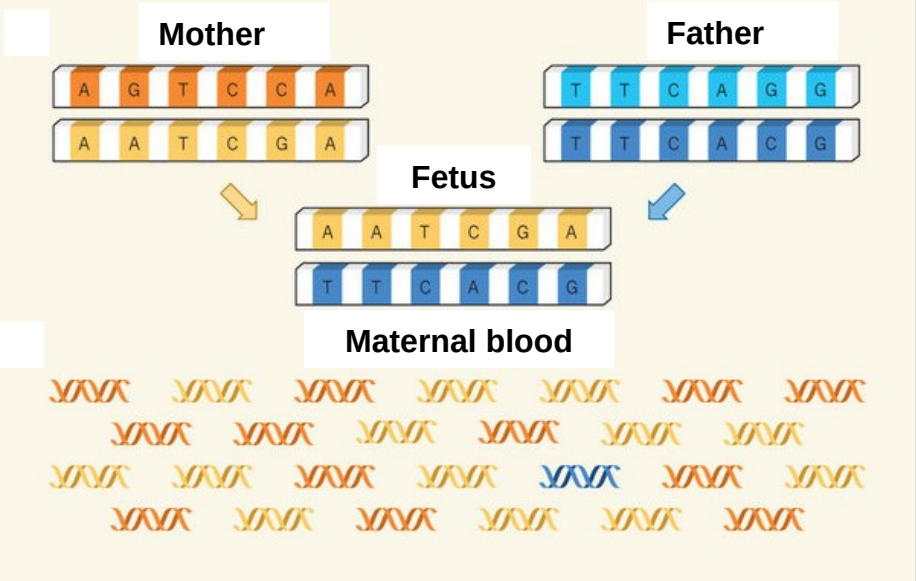
Next generation sequencing

Sanger sequencing

Next generation sequencing

- Capacity to sequence the whole genome of 10 people in one run
- Capacity to sequence limited regions of genome of many individuals in one run

Next generation sequencing can be applied to fetal genotyping



Now possible to determine sequence of fetal genome from 5 ml of maternal blood

Henry T Greely

“Regulators, doctors and patients need to prepare for the ethical, legal and practical effects of sequencing fetal genomes from mothers’ blood”

Transfusion, ahead of print

ORIGINAL ARTICLE

Next-generation sequencing: proof of concept for antenatal prediction of the fetal Kell blood group phenotype from cell-free fetal DNA in maternal plasma

Klaus Rieneck, Mads Bak, Lars Jønson, Frederik Banch Clausen, Grethe Risum Krog, Niels Tommerup, Leif Kofoed Nielsen, Morten Hedegaard, and Morten Hanefeld Dziegiel

The future

Down's syndrome screening will probably be done by next generation sequencing

Fetal *RHD* genotyping could be incorporated and then would be cost saving

Thanks

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