



West Middlesex University Hospital

Blood Grouping in Pregnancy, Guidelines and Foetal Genotyping




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NHS Foundation Trust



Guideline for blood grouping and red cell antibody testing in pregnancy

White, J¹ Qureshi, H² Massey, E³ Needs, M⁴ Byrne, G⁵ Daniels, G⁶ Allard S⁷ & British Committee for Standards in Haematology

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The purpose of the guideline is to make evidence-based recommendations for the application of blood grouping and red cell antibody testing in pregnancy and if possible to prevent, haemolytic disease of the foetus and newborn (HDFN).

The blood group and antibody status of a pregnant woman should be tested at booking and at 28 weeks gestation to identify the ABO group and D status and to detect red cell antibodies that have the potential to be clinically significant.



ABO/D typing

ABO and D grouping should be performed in accordance with the guidelines for compatibility procedures in blood transfusion laboratories (BCSH, 2012b)

- All not clear positive of RhD typing, should be classified as D negative until the D status is confirmed.
- All pregnant women found to be D negative should be given written information about their D-negative status and the importance of anti-D Ig prophylaxis. The D status should be clearly recorded in the notes to inform those responsible for their care of the need to offer prophylactic anti-D Ig.

Screening for red cell antibodies

Maternal antibody screening is undertaken to detect clinically significant antibodies, which might affect the foetus and/or newborn, and to detect antibodies that may cause problems with the provision of compatible blood components for the woman and for the foetus/newborn.

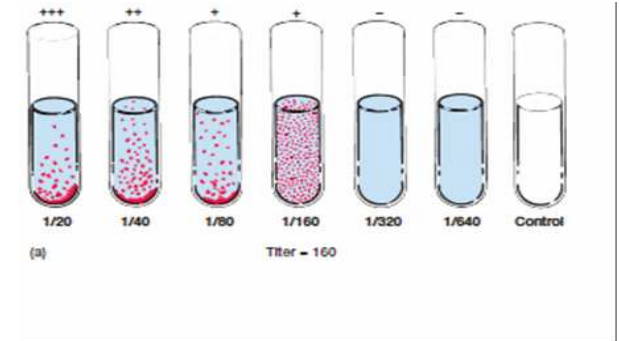
Approximately 1% of pregnant women are found to have clinically significant red cell antibodies (Howard et al., 1998; Koelwijn et al., 2008; Smith et al., 2013).

The concentration of each antibody capable of causing HDFN should be assessed independently. For specificities where there is a national standard preparation, quantification should be undertaken. Other antibody specificities should be measured using titration.

The ABO Blood System				
Blood Type (genotype)	Type A (AA, AO)	Type B (BB, BO)	Type AB (AB)	Type O (OO)
Red Blood Cell Surface Proteins (phenotype)	 A agglutinogens only	 B agglutinogens only	 A and B agglutinogens	 No agglutinogens
Plasma Antibodies (phenotype)	 b agglutinin only	 a agglutinin only	NONE No agglutinin	 a and b agglutinin



Antibody testing



Antibody quantification.

Quantification requires specific equipment and measures antibody concentration against a national standard [National Institute for Biological Standards and Control (NIBSC)].

Anti-D and anti-c are the only antibodies that are currently quantified, and they are reported as IU per millilitre.

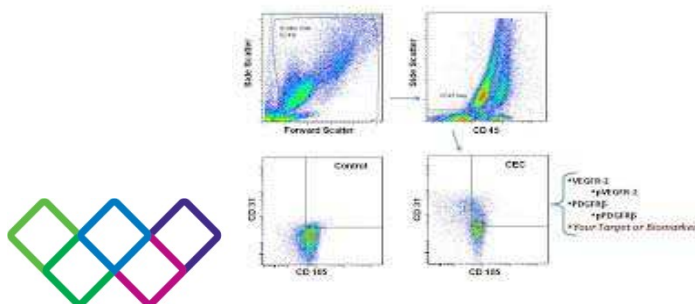
Where possible, each sample should be tested in parallel with the previous sample and the results compared to identify significant change in antibody concentration.

Antibody titration.

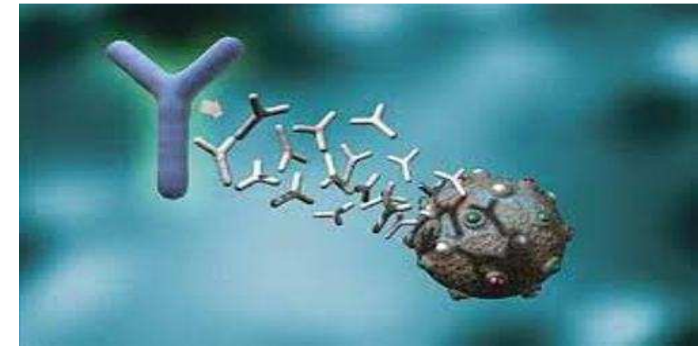
Titration is used to assess the concentration of clinically significant red cell antibodies other than anti-D and anti-c.

Doubling dilutions (1 in 2, 1 in 4, etc) of plasma prepared in phosphate-buffered saline are tested by IAT using reagent red cells, where possible, showing heterozygous expression of the corresponding antigen(s).

Sometimes there are more than one antibody specificity is present (including prophylactic anti-D). The concentration of each specificity should be assessed independently, e.g. where anti-K+Fya are present titrate against K-, Fy(a+b-) and K+k+, Fy(a-) cells.



Protocol for patients with antibodies



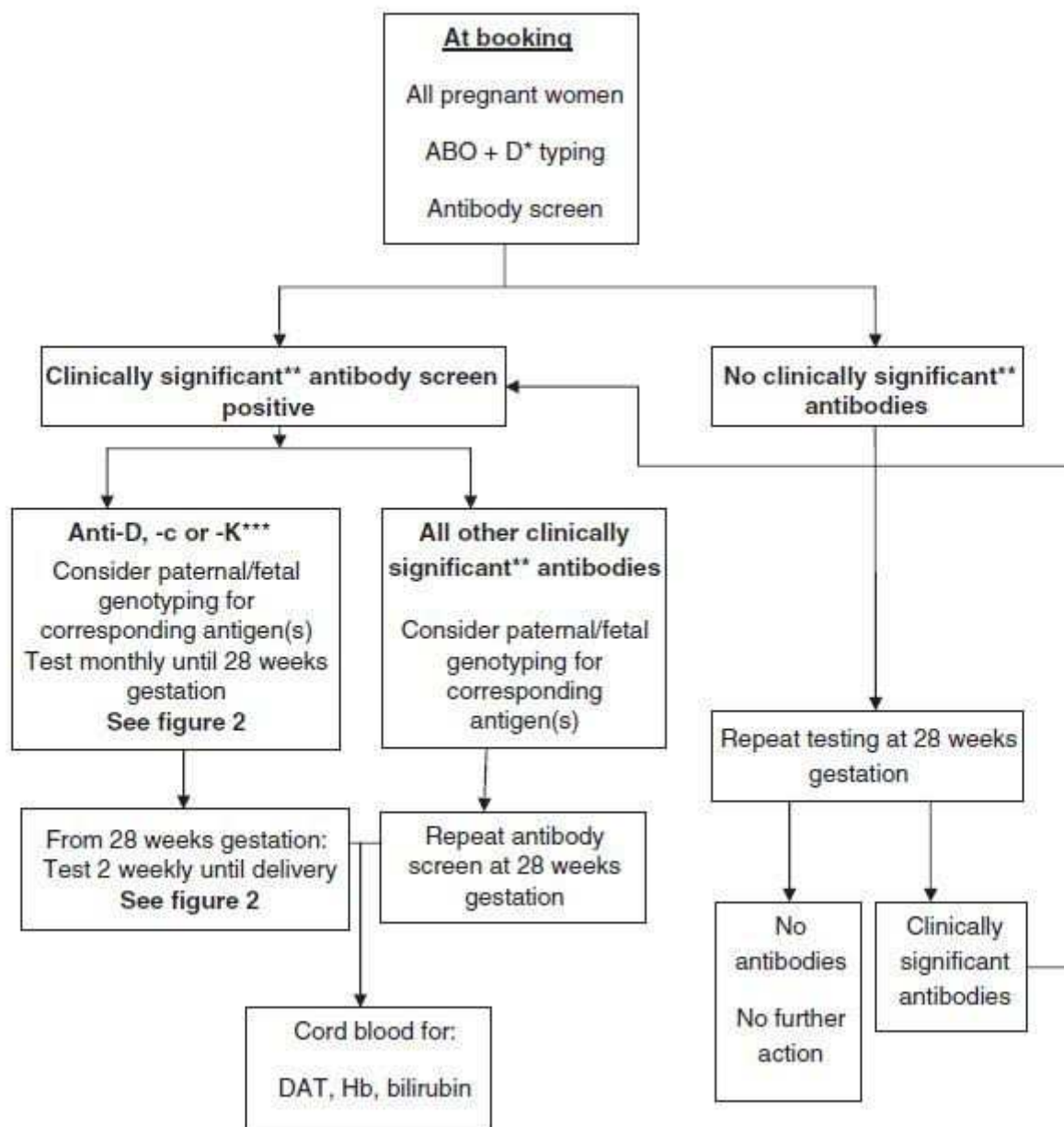
- Samples from pregnant women with immune anti-D or anti-c should be assessed serologically at 4 weekly intervals to 28 weeks gestation and at fortnightly intervals thereafter until delivery. Such cases should be referred to a foetal medicine specialist if the antibody reaches the critical level and/or the level is rising significantly, where assessment of the need for further monitoring will be made.
- Pregnant women with anti-K or other Kell system antibodies (unless the father is confirmed to be negative for the corresponding antigen) should be assessed serologically at monthly intervals to 28 weeks gestation and at fortnightly intervals thereafter until delivery and referred to a foetal medicine specialist when the antibody is first identified

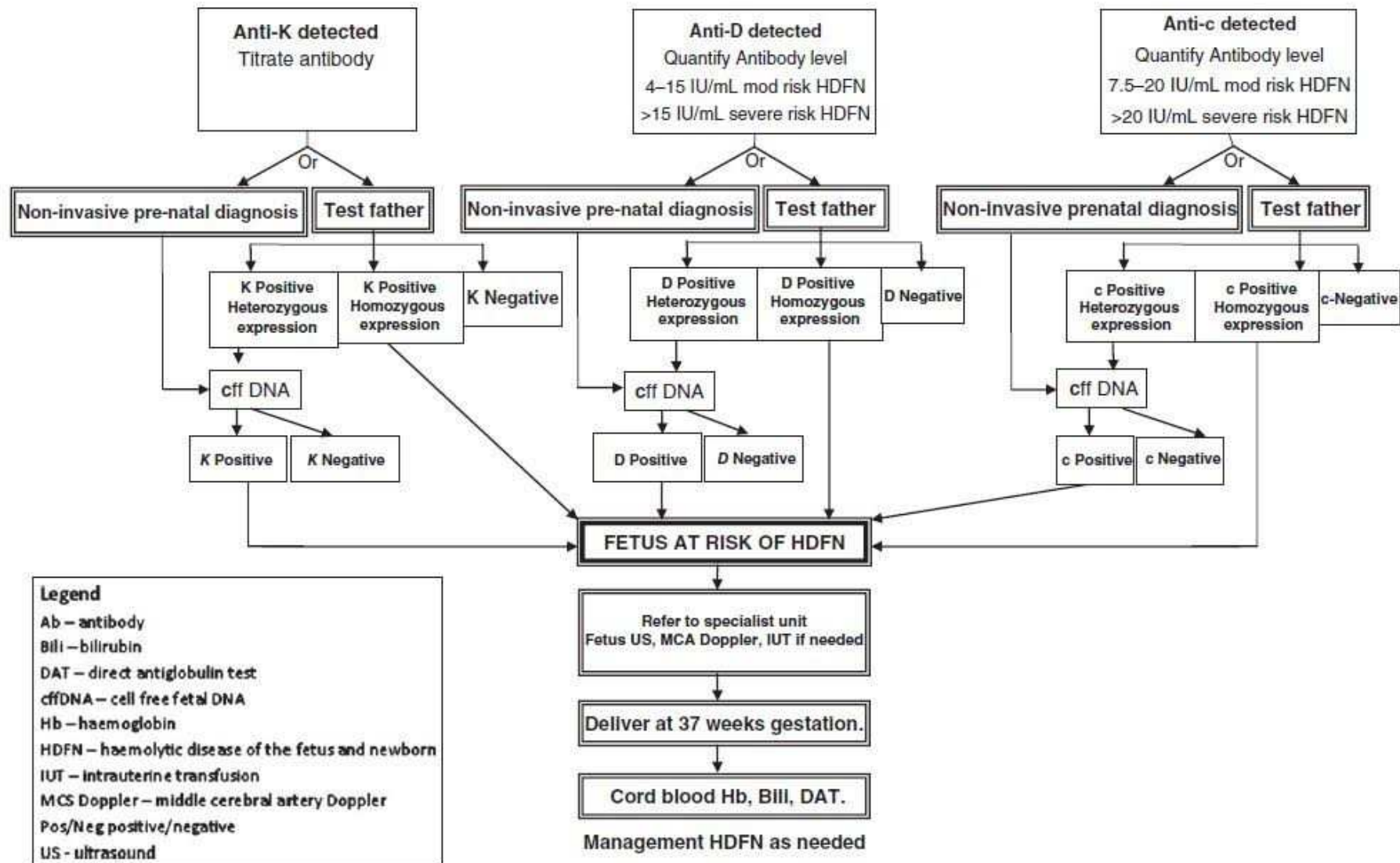
Clinically significant antibodies, other than anti-D, -c or -K, should be excluded or, if present, assessed by titration at the booking appointment and at 28 weeks gestation. If deemed necessary based on a high titre (>32) and/or a past history of HDFN, referral to a specialist in foetal medicine should be made for further assessment.

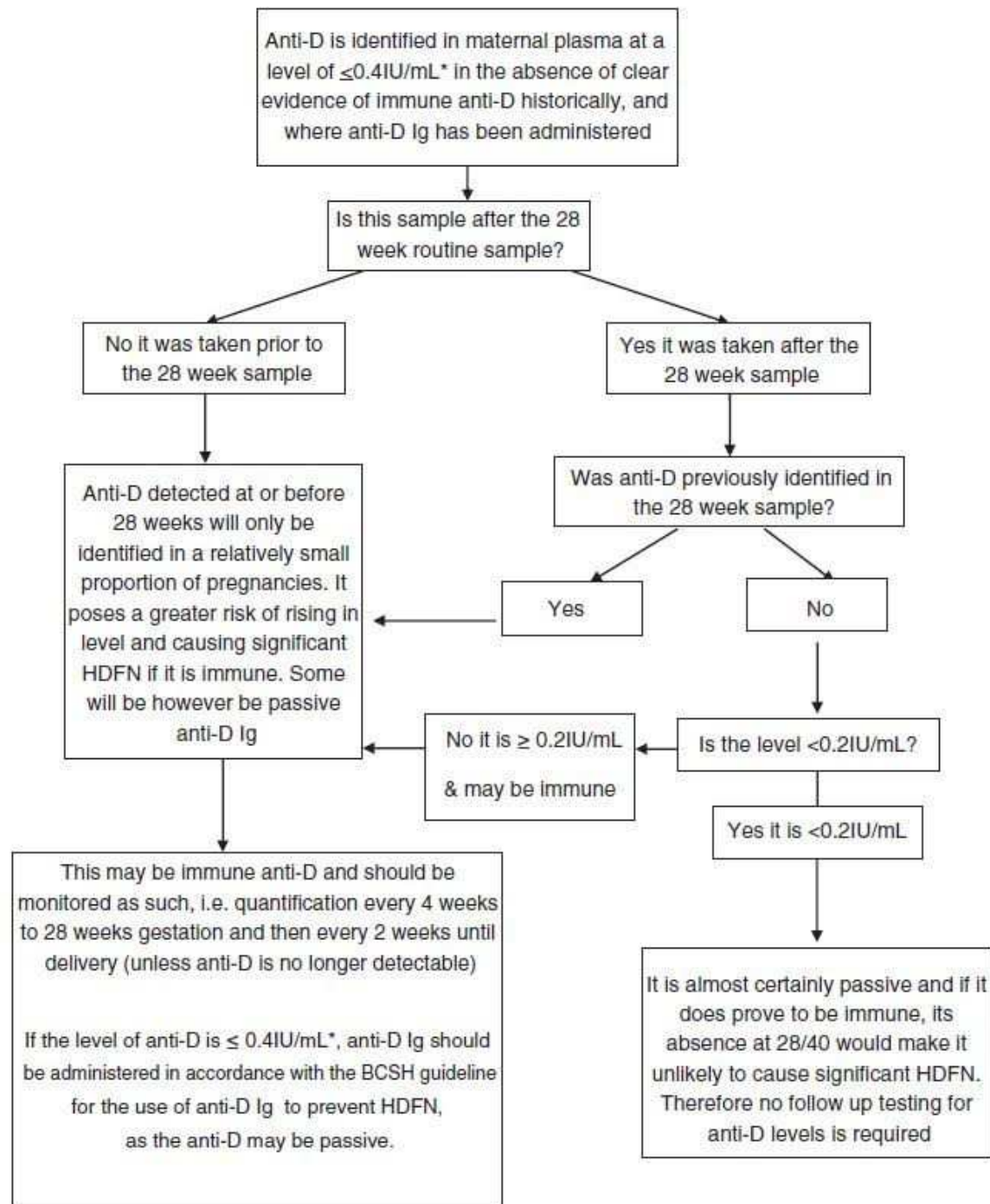
All babies born to women who have clinically significant antibodies should be closely observed for evidence of HDFN. A DAT should be performed on a cord blood sample, and haemoglobin and bilirubin concentrations should be measured.

A positive DAT is not, in itself, diagnostic of HDFN. Where the DAT is positive and the baby shows signs of HDFN, a red cell eluate may be helpful to confirm the red cell antibody specificity. IgG ABO antibodies occasionally cause severe HDFN, and so, if the baby has a major ABO mismatch with the woman, the eluate should also be tested with A1 and/or B cells, negative for any other antigen against which the woman has made IgG alloantibodies. Regular assessment of bilirubin and haemoglobin concentrations is necessary, and hence, early discharge is not advisable









Anti-D immune and prophylaxis

Recommendation

- Blood transfusion laboratories should keep a record of anti-D Ig administration to provide a basis for distinguishing between immune anti-D and prophylaxis anti-D Ig.
- If anti-D is detected in an antenatal maternal sample (except for that taken immediately prior to delivery), testing should include a measurement of antibody concentration by CFA or similar technique that gives a result that is expressed in or can easily be converted to IU per millilitre of anti-D.
- When there is doubt as to the passive or immune nature of anti-D, the level should be monitored as if it could be immune. In this situation, anti-D Ig prophylaxis should continue to be offered, until the nature of the anti-D is established.

Table 1. The significance of levels of anti-D

Anti-D concentration	Predicted clinical outcome
Less than 4 IU mL ⁻¹	HDFN unlikely, continue to monitor
4–15 IU mL ⁻¹	Moderate risk of HDFN, requiring referral to a fetal medicine specialist
More than 15 IU mL ⁻¹	High risk of HDFN requiring referral, as above

Table 2. The significance of levels of anti-c

Anti-c concentration	Predicted clinical outcome
Less than 7.5 IU mL ⁻¹	HDFN unlikely, continue to monitor
7.5–20 IU mL ⁻¹	Moderate risk of HDFN, requiring referral to a fetal medicine specialist
More than 20 IU mL ⁻¹	High risk of HDFN requiring referral, as above

Transfusion Medicine, 2016, **26**, 246–263



Paternal testing

Paternal testing

When a clinically significant antibody capable of causing HDFN is present in a maternal sample, determining the father's phenotype can provide useful information to predict the likelihood of the foetus expressing the relevant red cell antigen and for counselling the couple regarding future pregnancies.

It should be recognised that in any pregnancy, the partner may not be the biological father (assisted conception with sperm donation from a donor panel, the pregnant woman's partner will not be the biological father).

It is reasonable to avoid paternal testing and proceed directly to foetal genotyping using cffDNA.

Recommendation

If potentially clinically significant maternal antibodies have been identified, paternal testing should be considered to predict the risk to current and future pregnancies. This may be particularly relevant if non-invasive foetal genotyping is not available for the corresponding red cell antigen.

Foetal genotyping in alloimmunised pregnancies. Foetal genotyping is a useful diagnostic tool when:

- a) A pregnant woman has a clinically significant antibody;
- b) A pregnant woman has a history of HDFN;
- c) The father's antigen status is unknown or he expresses the corresponding antigen.



Taking part in the NHS cffDNA testing

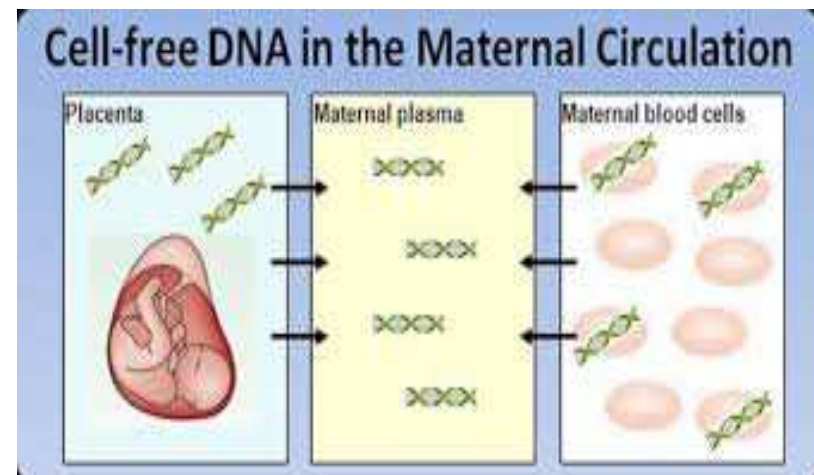
Administer anti-D selectively, only to RhD Negative women with a RhD Positive baby

Improve patient pathway and satisfaction

Focus midwifery / obstetrician time on clinically indicated care

Cost neutral

Potentially save laboratory time



WHY Change the system??

Routine Antenatal Anti-D Prophylaxis (RAADP) has been hugely successful in UK:

1930's RhD antigens identified

1969 First use of anti-D as postnatal prophylaxis

1999 RAADP introduced

Maternal isoimmunisation reduced from 16% to 0.2%

Perinatal mortality 46 to 1.6 / 100000 births



Potentially Sensitising events during pregnancy

12 weeks	12–20 weeks	20+ weeks
<p>At least 250 IU anti-D Ig if:</p> <ul style="list-style-type: none"> •surgical intervention •termination of pregnancy (surgical or medical) •unusually heavy bleeding •unusually severe pain •unsure of gestation <p>Kleihauer test not required</p>	<p>At least 250 IU anti-D Ig</p> <p>Kleihauer test not required</p>	<p>At least 500 IU anti-D Ig</p> <p>Take maternal blood for Kleihauer test</p> <p>Further anti-D Ig if indicated by Kleihauer</p>

**TRANSFUSION
MEDICINE**

Official Journal of
the British Blood Transfusion Society

Transfusion Medicine | GUIDELINES



BCSH guideline for the use of anti-D immunoglobulin for the prevention of haemolytic disease of the fetus and newborn

H. Qureshi,¹ E. Massey,² D. Kirwan,³ T. Davies,⁴ S. Robson,⁵ J. White,⁶ J. Jones⁷ & S. Allard⁸



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Facts

16% UK population RhD negative [lower in non Caucasian populations]

~40% of their babies will be RhD Negative = 40,000 women / year receive unnecessary anti D in UK

Introducing cffDNA testing potentially could reduce issuing of proph-D by 30%.

Considering implications:

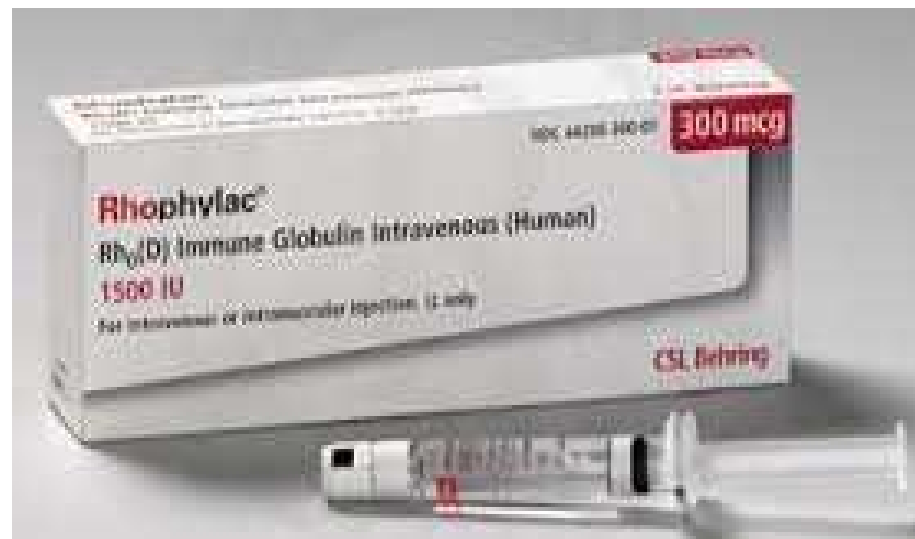
-financial

-ethical

-safety



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2002- Recommended further research into foetal RhD typing

Guidance on
the use of
routine antenatal
anti-D prophylaxis
for RhD-negative
women

May 2002



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1994: Fetal blood group
genotyping introduced
2001: Fetal D typing on cffDNA
Extended to K, C, c, E

2006-11 High throughput foetal RhD
testing trials at different gestations:

Highly accurate from 11⁺² weeks
gestation

2013/14 Foetal RhD service pilot
- North Bristol
- UHs Bristol
- Weston Area



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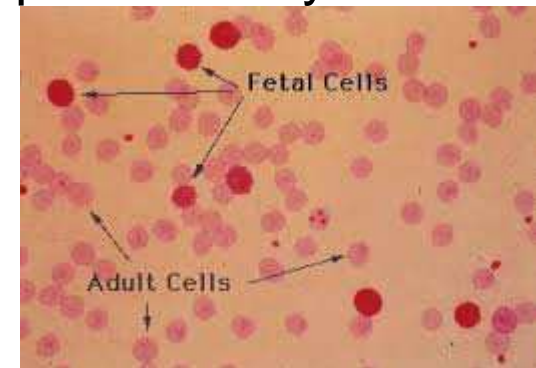
BMJ 2014;349:g5243 doi: 10.1136/bmj.g5243
DOI: 10.1111/1471-0528.13055 www.bjog.org

Clinical implications: Antenatal

RhD Negative pregnant women tested after 12 weeks' gestation.

Possible results:

1. Baby Predicted RhD Positive – continue usual RAADP
2. Baby Predicted RhD Negative – no RAADP required;
- **no Kleihauer** or anti-D for sensitising events and post delivery
3. Inconclusive – treat as if RhD Positive
4. Not tested ('Rejected')



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Clinical implications: Postnatal



Options include:

1. Continue current policy of cord blood on all babies born to RhD Negative women, with anti-D dose determined by Kleihauer
2. Cord blood only on babies predicted to be RhD Negative

Women delivering babies predicted to be RhD Positive given anti-D, without cord blood group testing.



Setting up the service

‘Self appointed’ multidisciplinary working party:

Obstetrician

Haematologist

Blood Transfusion Manager

IT expert

Experienced Midwife



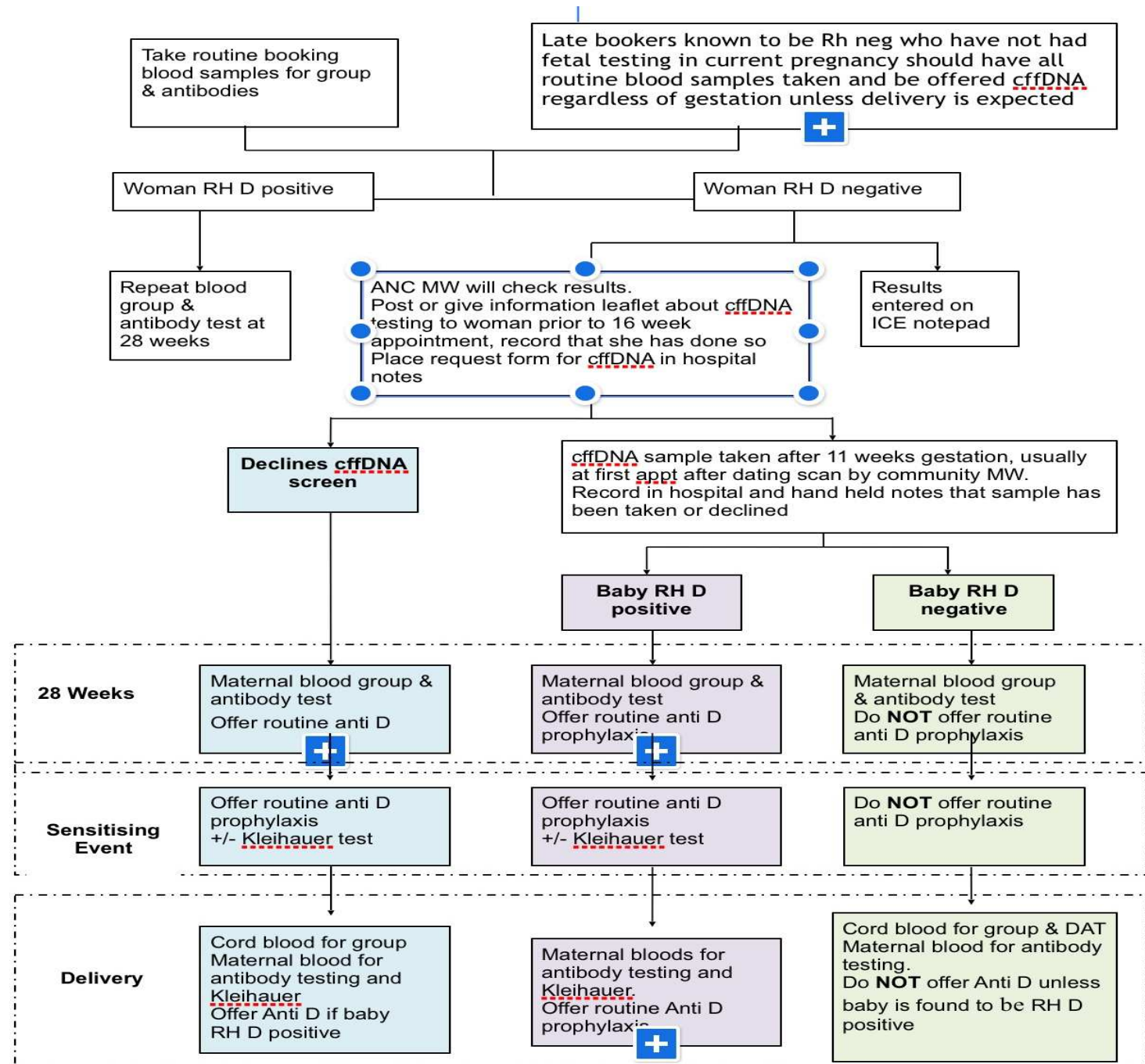
Guideline and pathway writing; midwife champions identified; patient involvement; business case



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Developing Protocol



Concerns to make decisions relating to:

- Identifying the relevant women
- Ensuring sample taken, correctly
- Handling the samples on receipt
- Handling the results
- Availability of results to the clinical teams
- How to audit pathway and get patient feedback



How to identify relevant women... and what to do about 'catch up'

Multipronged approach, all based around current ways of working

1.cffDNA test described in 'Safe In Our Hands' pregnancy information booklet given to all pregnant women at booking

2.All print outs of booking bloods received by ANC sisters and reviewed

3.Notes of RhD Negative women given **PINK cover**, information leaflet posted to her, name entered on secure spreadsheet

4.Target to take during '16 week' midwife appointment, or at any other meeting if delivery 'not imminent'



Taking the samples, correctly

Departmental education for all staff via monthly Maternity Forum,
Newsletters, Team Leaders meetings etc

Midwife champions

HCA's working in ANCs 'empowered'

Single full PINK top sample

at any clinical point from 14weeks

Form ST1871

Request for cell free fetal DNA (cffDNA) Screen **Blood and Transplant**
RhD Fetal Genotyping Service

This form is only to be used for RhD negative pregnant women.
Please **DO NOT USE** this form for samples from women who have anti-D antibodies. For those cases, please speak to the Fetal Maternal Unit first (a different form and sample volume are required).
At least three points of matching identification must be used on form and sample tubes

Mother's Details:

NHS No. _____ or* Hospital No. _____
*If NHS No. is not known. Please ensure that the numbers are the same on this form and the sample tube i.e. NHS No. on both form and sample and/or Hospital No. on both form and sample

Surname _____
First name _____
Address _____

DOB _____ EDD from scan* _____
*If scan has not been done, then one should be arranged before taking sample

Please provide 6ml EDTA blood sample from the mother

Date of sample taken _____ Name of person taking sample _____

Hospital and Requester Details:

Full Hospital Trust Name _____ Hospital NHS Code* _____
*OQS code (Formerly NACS code)

Midwife code _____ Practice code _____

Sender's name and address	For Hospital Laboratory use
Telephone:	Date received:
Email:	For NHSBT use

SEND SAMPLE WITH THIS FORM TO THE PATHOLOGY LABORATORY



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Handling samples in lab

Received in laboratory via standard pathways from all clinics; sample stable at room temperature

Entered into Laboratory IT system [Winpath]

The new genetic RhD (cffDNA) is now active in Winpath.

Code = GRHD

Please inform Manny if you need the test to be available in ICE as well.



Sent to NHSBT Colindale on standard daily transport

Transported from Colindale to Bristol on standard NHSBT transport



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Handling Results

Results are expected within 2 weeks from receiving sample in Bristol:

- available immediately after authorisation on Specialist Services electronic reporting using the Sunquest ICE web browser (Sp-ICE) and can be seen by all Sp-ICE users, important for shared care patients
Paper copy is sent in batches
- BMS must enter this manually to the WinPath system, and checked by second person before authorised on system, unable to use electronic transfer from Sp-ICE
- Results from WinPath are sent to ICE (hospital IT system).

Original report is send to ANC/Maternity



From 1 April NHSBT implementing additional charge £1 for every paper copy, therefore, we going to stop receiving paper copy and get thee results from Sp-ICE. This may be additional stretch for lab staff




- Problem: ?MHRA requirement for using electronic entry.

Authorised Winpath report

Results Entry - C000055			
Hospital Number: 1111111		NHS No.	Verification:
Sex: F	Age: 33	DoB: 20/08/1982	Fasting: A Urgent: N On-Call: N
Source: ANCENT		Clinician: HOLLS	
Status: Complete		Demographics: Verified	
Edit All View All			
Tests	Results		
r A Genetic RhD test (cffDNA)	NEGATIVE		
r A cffDNA Report Date	20/08/2016		
r A EDD	02/02/2016		
r A	This result applies to pregnancy with EDD above.		
r A cffDNA Comment	Fetal RhD typing predicts that this fetus is RhD		
r A	NEGATIVE		
p A Special Requirements	K-		

Working process to build the result page on LIMs and Hospital IT systems

Report as displayed in ICE

Reported	Specialty	Location	Clinician	Status
 11 Feb 2016 10:50	Blood Bank	ANTENATAL CLINIC	Mrs S Hollamby (Obstetrics & Gynaecology)	F

Sample 0000C000055 (TYPE UNSPECIFIED) Collected 11 Feb 2016 10:30 Received 11 Feb 2016 10:39

GENETIC RHD TESTING

Genetic RhD test (cffDNA) NEGATIVE

cffDNA Report Date 20/08/2016

EDD 02/02/2016

This result applies to pregnancy with EDD above.

cffDNA Comment

Fetal RhD typing predicts that this fetus is RhD

NEGATIVE

End of report

Old setup

Results Entry - C335235					
Sex: F	Age: .	DoB:	Fasting: A	Urgent: N	On-Call: N
Source: ANCWM		Clinician: NP			
Tests: GRHD					
Status: Incomplete			Demographics: Verified		
Edit All View All					
		Tests	Results		
e H		Genetic RhD test (cffDNA)	{ to send BRI }		
e H		cffDNA Report Date			
n H		EDD			
n H		cffDNA Comment			

New setup

Results Entry - C000301					
Sex: F	Age: 87	DoB: 13/05/1929	Fasting: A	Urgent: N	On-Call: N
Source: GYN&EOPD		Clinician: AJAJ			
Tests: GRHD					
Status: Incomplete			Demographics: Unverified		
Edit All View All					
		Tests	Results		
e H		Genetic RhD test (cffDNA)	{ to send BRI }		
e H		cffDNA Report Date			
n H		NHSBT No			
n H		NHS No			
n H		Sample No			
n H		EDD			
n H		cffDNA Comment			
p A		Special Requirements	None		



Newrequest - 'GRHD'

Results Entry - C000055			
Hospital Number: 1111111	NHS No.	Verification:	Name: TESTPATIENT,TESTPATIENT,
Sex: F Age: 33	DoB: 20/08/1982	Fasting: A Urgent: N	On-Call: N
Source: ANC101	Clinician: HOLLS		
Status: Incomplete		Demographics: Verified	
Edit All View All			
Tests	Results		
a A Genetic RhD test (cHfDNA)	NEGATIVE		
a A cHfDNA Report Date	POSITIVE		
n A EDD	INCONCLUSIVE		
n A cHfDNA Comment	NOT TESTED		
p A Special Requirements	K-		

Resulting entered from referral report

Results Entry - C000055			
Hospital Number: 1111111	NHS No.	Verification:	Name: TESTPATIENT,TESTPATIENT,
Sex: F Age: 33	DoB: 20/08/1982	Fasting: A Urgent: N	On-Call: N
Source: ANC101	Clinician: HOLLS		
Status: Complete		Demographics: Unverified	
Edit All View All			
Tests	Results		
a A Genetic RhD test (cHfDNA)	NEGATIVE		
a A cHfDNA Report Date	20/08/2016		
a A EDD	02/02/2016		
p A cHfDNA Comment	Fetal RhD typing predicts that this fetus is RhD		
p A	NEGATIVE		
p A Special Requirements	K-		

Once saved – Comment auto-added below EDD

Results Entry - C000055			
Hospital Number: 1111111	NHS No.	Verification:	Name: TESTPATIENT,TESTPATIENT,
Sex: F Age: 33	DoB: 20/08/1982	Fasting: A Urgent: N	On-Call: N
Source: ANC101	Clinician: HOLLS		
Status: Complete		Demographics: Verified	
Edit All View All			
Tests	Results		
a A Genetic RhD test (cHfDNA)	NEGATIVE		
a A cHfDNA Report Date	20/08/2016		
a A EDD	02/02/2016		
a A	This result applies to pregnancy with EDD above.		
a A cHfDNA Comment	Fetal RhD typing predicts that this fetus is RhD		
a A	NEGATIVE		
p A Special Requirements	K-		

Example request

WinPath : WP 5.32 BT TEST - Ashar Ettienne-Chen

File Edit View Patient Utilities Codes Tools Help

Patient

Results Lookup - C000062

Hospital Number: NHS No. Verification: Name: TEST,TEST
 Sex: M Age: : DoB: Fasting: A Urgent: N On-Call: N
 Source: AEYM Clinician: MIRZ

Status: Incomplete Demographics: Unverified

eH Genetic RhD test (cffDNA) Result expected in 14 days
 eH cffDNA Report Date
 nH NHSBT No
 nH NHS No
 nH Sample No
 nH EDD
 aH cffDNA Comment Foetal RhD typing predicts that this foetus is
 aH RhD

Screenshot below confirms the comment was auto-added by the 'SYSTEM'

Results Entry - C000062

Sex: M Age: DoB: Fasting: A Urgent: N
 Source: AEYM Clinician: MIRZ
 Tests: GRHD

Status: Incomplete Demographics: Ur

Edit All View All

Tests	Results	User	Authorisation
e H Genetic RhD test (cffDNA)	{ to send BRI }		
e H cffDNA Report Date			
n H NHSBT No			
n H NHS No			
n H Sample No			
n H EDD			
a H cffDNA Comment	Foetal RhD typing :	SYSTEM	
a H	RhD	SYSTEM	



Creating comments

Comments				
File Edit				
	Code	comment	Section	Subsection
	for crossmatching. Please send a fresh sample if		
	blood is required. Thankyou.		
	GP1	Sample haemolysed so not suitable for antibody		
	screen or crossmatch. Please send repeat.		
	ABO & Rh group acceptable for first sample		
	GP2	Sample not acceptable for crossmatch due to		
	Please send repeat.		
	ABO & Rh group acceptable for first sample		
	WAB	Weak Anti-B		
	WAA	Weak Anti-A		
	C-K-	Please select C-, K- red cell units		
	EDDR	This result applies to pregnancy with EDD above.		

Result Rules				
File Edit				
	Rule	criteria	Test	W'list
	7400	[IF TLC] GRHD		
		[IF TEST STATUS] EDDR (a)		
		EDDR <\$\$> @EDDR		
		[THEN] ADD COMMENT EDDR below tlc EDDR		



Ongoing audit and patient questionnaire

Started 1st June 2016, in 1st 4 months:

- ☐ 375 Letters sent to RhD Negative women re testing
- ☐ 235 Results received back
- ☐ 87 Babies predicted to be RhD Negative [37%]



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Service Problems

Relatively few...



- Occasional wrong labelling and wrong sample size
- Occasional missed opportunity to take sample
- Likely some cord samples taken unnecessarily
- IT issues were complex to set up, but have been trouble free in clinical practice
- Typing results rather receiving electronically directly from Bristol
- From 1 April 2017, no paper copy (additional charge), could be more challenging for hospital laboratory staff



Costs

Estimated that 15% RhD Neg women have 1 or more sensitising event, mostly >20wks

	£	£
ffDNA	19.58	
Anti D		35 / 22.95
Kleihauer		25
Cord blood group		11



Accuracy of the cffDNA results?

Predicted RhD Negative – <1 in 1000 inaccurate

ie actually RhD Positive

check cord blood group for confirmation

1 in 86,000 chance of isoimmunisation

Predicted RhD Positive - 2% inaccurate

ie given anti D unnecessarily

Indeterminate - ~8% may fall as technology improves

80-90% actually RhD Positive

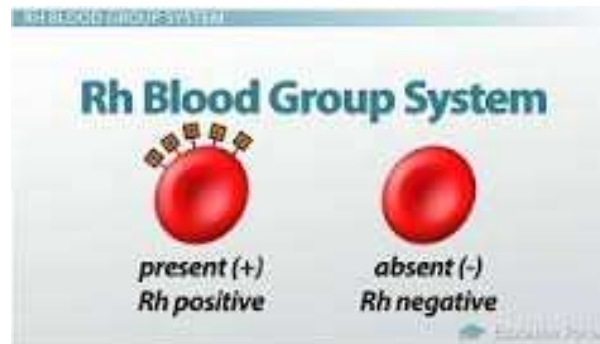


Laboratory findings re: discrepant results

- **Predicted RhD Positive** - confirmed after birth RhD Negative - October 2016

Note: unable to get more discrepant results as the protocol was changed and there is no Cord blood taken for Predicted RhD Positive babies

- **Predicted RhD Negative** - confirmed after birth RhD Positive - January 2017, investigation in process, sample was sent to Bristol



Summary

Implementation of cffDNA RhD testing is:

- Relatively straightforward
- Cost effective/neutral
- Saving time in midwifery area (less patients in clinic and less worrying if patients has PVB)
- Could save time on KL tests and issuing the anti-D, but BMS spend more time on booking sample for testing, packing, and sending to Bristol
- Also BMS spend more time typing and checking results
- Well received by Maternity staff and patients



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Thank You
for
Listening...
any
questions?