NHS
Blood and Transplant

Fetal *RHD* screening test: questions & answers

New questions
This Q&A sheet is updated monthly. New questions will appear each month, and the following month will be moved into the relevant topic area. If your question is not covered here, please send your questions to erika.rutherford@nhsbt.nhs.uk

Questions from September 2018

Q: How will the results be sent to the hospital?
A: The fetal *RHD* screening results will be **only** available via our online reporting system Sp-ICE from 1st April 2019 for NHS Trusts. IBGRL will not print paper reports to be compliant with the government requirement that the public sector should be paperless. The other reasons are to keep the cost down for this test as well as having a low impact on the environment.

Q: How much does the test cost?
A: Please contact our Business Development Manager via email: erika.rutherford@nhsbt.nhs.uk

Topics

Please note: All topics and questions are hyperlinked to the answers within the document

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Who is eligible and why

Q: Who can have the fetal *RHD* screening test?
A: The test is for D-negative pregnant women who do not have anti-D or G antibodies at booking. Please see User Guide INF1259 – and the question below that Women who have been confirmed to be weak D or D variant are unlikely to benefit from the fetal RHD screening test

Q: Is the test suitable for women who have week D or D variant
A: IBGRL cannot differentiate between maternal and fetal DNA. Please see User Guide INF1259 - *Women who have been confirmed to be weak D or D variant are unlikely to benefit from the fetal RHD screening test because the maternal RHD gene will prevent prediction of fetal D phenotype and an inconclusive test result will be issued. Women who are confirmed weak D should be treated as D-positive and prophylactic anti-D is not required. Women who are confirmed D variant should be given anti-D prophylaxis in line with local policy*.

Q: Can the test be used for women who expect multiple births?
A: Yes, we can test women pregnant with twins / triplets, but the report will predict the (single) fetus as being positive or negative. A positive result in this case means **at least one** of the babies is D-positive. A negative result would mean that all the babies are D-negative.

Q: What if the husband/partner claims to be D-negative?
A: There are two reasons why the fetal *RHD* screening test should be considered:

- The husband/partner might not be the biological father of the baby.
- Although the paternal phenotype might be D-negative (test done in hospital laboratories) the fetal genotype (test done at Molecular Diagnostics) might reveal a weak D gene and be D-positive

Q: What is the percentage of D-negative women who will have a D-negative baby?
A: The prevalence of D-negative women in a Caucasian population is 15% of which 38% to 40% will have D-negative babies. However, these figures will differ depending on the ethnic diversity of the local population, which may make a difference when calculating cost savings for implementation of the test.

Q: What is the percentage of women who are D-negative and have a D-positive baby who produce anti-D when they do not receive anti-D Ig?
A: The usual figures quoted are 1% of women become sensitised if they receive post-natal anti-D but do not receive antenatal anti-D at 28 weeks. Sensitisation is reduced to 0.35% with the addition of routine antenatal anti-D prophylaxis (RAADP). – see also Anti-D prophylaxis sections

Q: What is the official number for potentially sensitising events occurring during pregnancy?
A: The number of potentially sensitising events was taken from the UK audit on anti-D immunoglobulin use National comparative audit of blood transfusion: 2013 audit of anti-D immunoglobulin prophylaxis. The probability of women having at least 1 (reported) potentially sensitising event was estimated as15.5%. Of these, 69.3% were estimated to have had a feto-maternal haemorrhage (FMH) test and 95.8% were estimated to have had anti-D immunoglobulin after the event. It was estimated that about 80% of these events happened after 20 weeks' gestation, and it was assumed that these events were treated with the minimum required dose of 500 IU anti-D immunoglobulin. For the remaining 20% of events (before 20 weeks' gestation), it was assumed that women had the minimum required dose of 250 IU anti-D immunoglobulin.

Pregnant women with antibodies

Q: Can we use the fetal *RHD* screening test for mothers who have anti-D or anti-G antibodies?
A: No, the tests for fetal *RHD* screening and the diagnostic test are different. (please see User Guides: INF1259 – page 1, 3rd paragraph – for screening test and INF1135 page 2, second paragraph for diagnostic test)

The diagnostic test for mothers with antibodies requires manual DNA extraction whereas the screening test uses automated DNA extraction in large batches. Both tests have a similar level of sensitivity (99.9%) but the diagnostic test has a higher specificity owing to the increased coverage of the *RHD* gene and the algorithm applied to predict D status.

For women with anti-D or anti-G the consequences of both false negative **and** false positive results are significant. The diagnostic test can often identify D variant genes (in either mother or fetus) and reports can be tailored to give a recommendation for treating the fetus in that pregnancy as D-positive or D-negative. This is **not** the case for the screening test which will instead produce a report stating the test result is inconclusive and the pregnancy should be treated as if the fetus is D-positive.

The screening test is not validated for women with anti-D or anti-G alloantibodies, for this reason the User Guide states: *This guide concerns fetal D blood group testing for pregnant women **who have not made anti-D (or -G)** and who require the test to determine their need for antenatal anti-D prophylaxis.*

Comparing gestation and sample requirements of these tests:

1. The fetal *RHD* screening test
    a. From 11^{+2} weeks gestation

   b. Sample volume is 6mL EDTA and must reach IBGRL within 7days
2. The diagnostic test
   a. From 16 weeks gestation
   b. Sample volume 16mL EDTA and must reach IBGRL within 3 days of sample taken

Q: Can samples be tested when pregnant women have alloantibodies other than anti-D or anti-G?
A: Yes, samples can be tested for fetal *RHD* in the screening test which establishes the predicted D status of the fetus.

Q: Can samples be tested when pregnant women have already received anti-D Ig?
A: Yes, samples can be tested for the fetal *RHD*. The anti-D Ig does not interfere with this test. However, the serological antibody test at booking and prior to fetal *RHD* screening **must** be negative for D and G-antibodies.

Q: Can a sample be tested from a woman who had anti-D Ig due to termination/miscarriage within the last 6 months and is pregnant again?
A: Yes and No,

- Yes – we can accept a sample if the antibody screen at booking is negative for D or G antibodies
- No – we cannot accept a sample, if the serological investigation **at booking** identifies an anti-D antibody because an alloantibody cannot be excluded.
  Please follow the BSH guidelines for **Blood grouping and antibody testing in pregnancy** and treat this pregnancy as if an allo-immunisation has taken place. This is due to the potential failure of anti-D Ig (0.37%) although this is a very rare occurrence. See also: anti-D failure rate and related questions.
  You can send a sample for fetal genotype (mothers with antibodies) see our website for further information

Anti-D prophylaxis

Q: Do other European countries use the fetal *RHD* screening test or are there countries that don't use prophylactic anti-D during pregnancy?
A: Administration of post-natal anti-D prophylaxis is widespread in Europe and many countries have also implemented antenatal anti-D prophylaxis to prevent immunisation during pregnancy. Some countries, such as Denmark and Finland, have introduced national fetal D screening and antenatal prophylaxis concurrently. In the Netherlands, in common with England, an antenatal prophylaxis regime was already established before the introduction of fetal *RHD* screening**.**

Q: What are the official statistics regarding adherence to routine antenatal anti-D prophylaxis (RAADP)?
A: The National comparative audit of blood transfusion: 2013 audit of anti-D immunoglobulin prophylaxis was used to provide estimates of adherence to RAADP. It reported that, out of all eligible women: 99% had at least 1 RAADP injection; full adherence (that is the correct dose at the correct time) was better with the single-dose regimen (90%) compared with the 2-dose regimen (59%); 98.4% had postpartum anti-D prophylaxis; and 96% had anti-D immunoglobulin for documented potentially sensitising events. Within the economic model, it was assumed that adherence to RAADP was 99.0% and that adherence to postpartum anti-D prophylaxis was 98.4%. There was limited evidence on adherence to NIPT for fetal D genotype, so it was assumed that using NIPT has no additional effect on adherence to anti-D prophylaxis.

Q: What is the official quoted figure for the risk of transmission of infection with anti-D?
A: There has never been a transmission of infection from anti-D in the United Kingdom from any anti-D Ig. There have been two episodes of transmission of Hepatitis C decades ago, in the Republic of Ireland and Germany; both were prior to the introduction of modern modes of testing and pathogen inactivation which would have prevented them. Transmission is effectively zero with the current products. However, unknown agents (like vCJD) should be considered in any risk assessment.

Q: What is the risk of acute adverse [anaphylactic] reaction?
A: You would need to contact the individual manufacturers of each specific anti-D Ig product, as it will vary depending on the preparation of the anti-D Ig. However, the risk should be considered as very low.

Q: Can anti-D Ig be given in the community i.e. home birth?
A: I am not aware of any advice stating that anti-D Ig can't be given in the community i.e. at home. Please consider the risk of anaphylaxis and follow your Trust policy.

However, when delivering a baby at home, prior to the implementation of the **fetal *RHD* screening test,** the midwife will **not** know the D group of the baby and will **not** know if anti-D Ig should be given. Neither would she/he know the correct dose because the cord blood group and fetal maternal haemorrhage (FMH) estimation has **not** been done.

This will be different when the **fetal *RHD* screening test** has been introduced. The midwife will now know the baby's D group and can give anti-D Ig immediately. Although the midwife will have to take an FMH sample (or cord blood sample if baby is predicted to be D-negative) when the baby is predicted to be D-positive and will have to give additional anti-D Ig if a large bleed has been established.

Please also note that anti-D Ig can cause anaphylactic reactions, although very rarely. Precautions must be taken when administering anti-D Ig in a home setting. Please refer to your Trust policy.

Q: What is the failure rate of anti-D Ig?
A: Apart from the failure to administer a correct dose at the correct time, the failure rate is quoted as 0.37% NICE Health technology assessment 2003. See Table below taken from the NICE assessment for implementation of the Fetal *RHD* Screening test.

Table 4 Clinical effectiveness of RAADP and postpartum anti-D prophylaxis

	Odds ratio: sensitisation with RAADP[1] (95% CI)	Odds ratio: sensitisation at birth, follow-up up to 6 months, with postpartum anti-D prophylaxis[2] (95% CI)	Sensitisation rate without RAADP[3] (95% CI)	Sensitisation rate with RAADP (95% CI)	Sensitisation rate without RAADP and without postpartum anti-D prophylaxis (95% CI)
NICE TA156 (2009)	0.37 (0.21 to 0.65)	–	0.95 (0.18 to 1.71)	0.35 (0.29 to 0.40)	–
Crowther et al. (1997)[4]	–	0.08 (0.06 to 0.11)	0.95[5] (0.18 to 1.71)	–	10.7 (8.0 to 13.8)
1 Versus no RAADP, conditional on having postpartum anti-D prophylaxis. 2 Versus no postpartum anti-D prophylaxis, conditional on no RAADP. 3 Conditional on having postpartum anti-D prophylaxis. 4 Sensitisation 6 months after delivery. 5 Baseline-sensitisation rate of no RAADP assumed the same.					
Abbreviations: CI, confidence interval; RAADP, routine antenatal anti-D prophylaxis.					

Leaflets and request form ordering

Q: How do we order the patient information leaflet?
A: The patient information leaflet is now available to order via our Hospitals and Science website
Leaflets can be ordered using the distribution hub (on your first visit to the hub you will need to create an account, please read our **guidance document** for instructions).

Q: I need more referral forms / patient information leaflets, how do I obtain them?
A: You should contact your local NHS Trust midwifery lead, your Transfusion Practitioner or your Transfusion/Pathology Laboratory Manager who hold a regional stock of leaflets and referral forms.

Documentation and sample requirements

Q: What happens when the Hospital name and NHS code information is incomplete or missing on the request form?
A: See User Guide INF1259 Due to the increase in referrals for this test, it is no longer sustainable for IBGRL staff to investigate origin of requests. IBGRL is **rejecting samples which have incomplete mandatory data.** We are unable to notify you when these samples are rejected, because we will not know the origin of the sample.

Please note that:

1. The **hospital name and NHS code are mandatory fields on the request form**
2. The **NHS code** will determine the hospital where the **report and the invoice** for this test will be allocated to.

Action:

1. Please ensure that your:
    a. **full hospital name, with no abbreviation,** is on the request form
    b. correct **NHS code** is on the request form to guarantee that you receive the report and invoice at the correct hospital.

Q: Can we use our hospital specimen numbers on the sample tube and request form?
A: Yes, hospitals can use their hospital laboratory number, ensuring not to obstruct any patient identifiers, dates or signatures. The hospital laboratory number, if used, must be placed on the request form, bottom right hand above NHSBT information and sample tube if you would like this to be included in the report. We will scan your laboratory number into Hematos and it will appear on the results document (not bar-coded).
See User Guide:

Use of the hospital laboratory / pathology sample number

IBGRL will only include the hospital laboratory / pathology sample number on the report if the following conditions are met:

i. *The hospital laboratory / pathology sample number must be on both the sample tube and the request form. The number must be in a labelled, designated area of the request form so that it is clear this is the hospital laboratory / pathology sample number.*

- ***If the hospital / pathology sample number on tube and request form differ, the sample will be rejected.***

- *If the hospital / pathology sample number is only on the tube OR the request form, the number will not be recorded.*
- *If the hospital / pathology sample number is not in the designated area of the request form, the number will not be recorded.*

ii. *For hospitals that use their own approved electronically generated request form and the form does not have a labelled, designated area for the hospital / pathology sample number, a hand-written label "Hospital / pathology sample number" can be written next to the number, otherwise the number will not be included on the report.*

Q: Could hospitals continue to use their electronically generated request form for the Fetal *RHD* screening test instead of the NHSBT request form?
A: Yes, hospitals can use their electronically generated request form, if it contains all the mandatory information outlined in the NHSBT Fetal Genotype Screening request form guidance. Please note that this will have to be agreed with IBGRL prior to implementation.

Q: Would IBGRL accept 'Demand Printed Labels' on the samples?
A: This practice must be approved by NHSBT Quality for these sample tubes to be acceptable. However, if this has been approved for RCI and H&I samples IBGRL will accept your demand printed labels.

Q: Which blood tube do I send, pink or purple top?
A: For the fetal *RHD* screening test use a 6mL EDTA tube; this can be either a pink or a purple top. Try to fill the sample tube correctly because samples will be rejected if there is less than 4mL inside the tube.

Q: Would IBGRL ever ask for repeat samples, if yes in which circumstances would they do this?
A: If the sample could not be tested due to one of the following:

1. No hospital name or abbreviation of hospital name or NHS code (note that we would not be able to send the result to your hospital Trust because we wouldn't know who you are)
2. Insufficient patient identifiers on tube and referral form,
3. Insufficient sample <4mL
4. Sample more than 7 days old,
5. Sample damage e.g. broken tube,
6. Sample haemolysed
7. Sample tube opened or used for another test
8. Samples not labelled, dated and signed by the person taking the sample
9. EDD not confirmed by scan or sample taken before 11 weeks gestation.
10. Where a failure occurred during processing or testing so that a result cannot be issued

Q: Would IBGRL report reasons for rejection?
A: Yes, we do give some generic reasons but owing to volume of samples processed we are unable to give detailed reasons for each sample. Examples include, but are not limited to:

- 'Insufficient blood in tube' if there was insufficient plasma
- Sample grossly haemolysed therefore unable to test
- EDD not supplied
- Inadequate labelling of sample or sample not dated / signed
- There was a discrepancy between the sample and request form

Q: What would IBGRL consider as 'short sample'? Or in other words what is IBGRL's minimum sample size?
A: 4mL is the minimum sample requirement. However, if you only have 4mL sample tubes available, please send 2 of these per patient. However, the minimum blood volume will not always equate to the minimum volume of plasma required for testing.

Q: Why do samples for fetal genotyping have to be stored at room temperature?
A: The reason the blood sample need to be stored at room temperature, for all fetal genotyping tests, is that the white blood cells (platelets, granulocytes, lymphocytes) deteriorate when exposed to temperature changes. This results in high levels of cell free DNA from the mother, which interferes with the test i.e. mum's DNA compared with the cell free **fetal** DNA, the ratio is not correct and can give incorrect results.

For the same reason Platelet and Granulocyte donations must be stored at room temperature

Q: How many samples does IBGRL reject?
A: If the hospital sends required sample from a woman eligible for the test in the specified time (7 days), there would not be any rejection as long as patient identifiers, EDD and all mandatory fields are correct on form and tube.

Please note that the full hospital name (no abbreviations) and NHS code (5-digit code) **must** be on the referral form otherwise IBGRL will reject the sample as they cannot identify the referring hospital! Your Trust will **not be notified** because IBGRL does not know who to contact.

See User Guide page 4, last paragraph for full explanation

IT - LIMS

Q: How do other hospitals set up the fetal *RHD* screening test on their LIMS?
A: Hospitals usually set up a simple test in their LIMS linking it to the Trust's ward reporting system. This should be subject to change control and validation.

Mandatory data sets for this test are:
1. The test name – fetal *RHD* screen or cffDNA screen. Please note that the word '*screen'* should be included so that this test is not confused with the fetal genotyping diagnostic test for mothers with antibodies.
2. The pregnant women's three identifiers
3. The EDD by dating scan - this will give the information to which pregnancy/baby the test result belongs to.
4. The date the sample was taken

Results section:
1. Tests results are: Fetal RhD typing predicts that this fetus is RhD-positive, D-negative, inconclusive or not tested
2. Test comments: This result applies to the pregnancy with EDD above, or advice on giving anti-D Ig for inconclusive results, or reason for not testing the sample

Results and reports

Q: How do IBGRL report results for fetal *RHD* screen?
A: Please request sample results from Erika Rutherford for more details

1) Fetal RhD typing predicts that this fetus is RhD-positive
2) Fetal RhD typing predicts that this fetus is RhD-negative
3) Fetal RhD typing was Inconclusive. Manage this pregnancy as if this fetus is RhD-positive
4) Rejected sample, please send a repeat sample before 25 weeks gestation
5) Rejected sample, inappropriate test request (reason will be given)

Q: Would IBGRL report reasons for rejection?
A: Yes, we do give some generic reasons but owing to volume of samples processed we are unable to give detailed reasons for each sample. Examples include, but are not limited to:

- 'Insufficient blood in tube' if there was insufficient plasma
- Sample grossly haemolysed therefore unable to test
- EDD not supplied
- Inadequate labelling of sample or sample not dated / signed
- There was a discrepancy between the sample and request form

Q: It has been 14 days since a sample was sent but results do not appear to be available – who do I contact?
A: You should contact your local hospital 'Send Away' laboratory (usually within the departments of Pathology or Blood Transfusion). NHSBT are not able to give fetal *RHD* screening results directly to clinical staff.

Q: Could Midwives look up results on Sp-ICE?
A: Yes, midwifes can be trained and added as users by the Sp-ICE Laboratory Administrator for this system and can look up their patient results on Sp-ICE.

Q: Is it possible that there is insufficient fetal DNA so that IBGRL could not give a result?
A: This should not be the case if the sample is taken after the 11^{+2} week based on the dating scan. Although, samples could fall in the 'inconclusive' category.
IBGRL has, according to the latest statistics, an approximate 4% inconclusive rate (recommendation is to treat as D-positive). Follow up has shown that 70-80% of these cases do result in a D-positive fetus and therefore recommendation to receive anti-D Ig is correct.
However, less frequently the result could also be falsely called D-negative. The rate for false negatives is approximately 0.1%, as stated on the patient information leaflet. The causes include insufficient fetal DNA and wrong blood in tube

Q: Can hospitals receive email alerts when reports are verified?
A: The email alert functionality is not activated on Sp-ICE. Hospitals can view their latest reports either in "View Latest Reports" or by using a date filter on the "View Reports by Location" folder.

Q: Would it be possible to receive reports via email?
A: No, we only report via Sp-ICE.

Q: Is Sp-ICE the only way to receive reports?
A: Yes, we only report electronically via Sp-ICE which is the safest way to deliver patient results and to save the environment. We are developing electronic data interchange (EDI) between the hospital's LIMS and our Hematos system but that will take some time.

Q: Is NHSBT developing EDI – electronic requesting (order comms) and reporting for this test?
A: Yes, we are considering this, but it is a long-term development and will not be in place within the next couple of years.

Q: Could hospital sample numbers be bar coded and included on the results sheet?
A: Hospital barcoded sample numbers, which are unique to the sample (not to the patient) can be included on the referral form and will be included on the result report. However, we cannot currently insert a barcode into our result reports. Please note that the hospital barcode sample number is not acceptable as a point of identification.

Q: Could the results be barcoded?
A: NHSBT are not currently able to add a barcode to the reported results.

Cord blood testing

Q: Can hospitals drop the testing of cord blood for D at delivery when the result of the test states the baby will be D-positive/negative?
A: NHSBT are not currently recommending that hospitals discontinue cord blood testing at this time, although the cord blood test for predicted D-positive babies could be suspended.

Other European countries have continued cord blood testing for several years post introduction of the national screening test. (See above questions on sensitisation and anti-D Ig failure rates). However, Denmark and the Netherlands have discontinued cord blood testing now.

Q: Do hospitals need to continue to test all cord blood samples?
A: Your Trust would need, as a minimum, to test cord blood samples for the D-negative predicted babies or continue to test all cord bloods. At the NICE assessment, the reason for continuing to test all was heavily influenced by concerns on whether it was possible to implement differential testing for busy midwives. If it is thought by the organisation that is implementing this that it is safe and practical to only test the blood of mothers predicted to have a D-negative fetus and not those predicted to be D-positive, then the modelling suggested that there would be further cost savings.

The rationale behind this is explained below:
An alternative postpartum-testing strategy to those included in the NICE scope was assessed. *The strategy separated women in whom NIPT identified a D-positive fetus from women in whom NIPT gave an inconclusive result (and were therefore treated as if the fetus was D-positive). Cord blood typing was done for women identified as having either a D-negative fetus by NIPT or who had an inconclusive NIPT result, but not done for women in whom NIPT indicated a D-positive fetus and resulted in total costs of £15,230,372 and £2,433,756 QALYs per 100,000 pregnancies. This postpartum approach dominated all other NIPT strategies, and the ICER for this strategy compared with current practice was £1,638,356 saved per QALY lost.*

NICE would like to reassess, after a couple of years, if the UK could discontinue with the cord blood testing in line with the Netherlands and Denmark.

Please report any discrepancies, between the predicted fetal group and laboratory findings at birth, to IBGRL.

Wrong blood in tube

Q: Should two samples be tested to avoid wrong blood in tube (WBIT)?
A: This subject was discussed at the NICE assessment. A decision had been made (due to cost pressures) that IBGRL will only test one sample, the same as RCI or H&I, and we will not require or test a second sample. This is a routine test and samples will be taken in a controlled environment by trained staff to avoid wrong blood in tube incidents.

Q: Has the mitigation of Wrong Blood in Tube (WBIT) been considered in any Trust who has implemented fetal *RHD* screening?
A: The answer is- I don't know. None of the Trusts, which implemented, have told me that they have a process in place to mitigate WBIT for this test, but this does not mean they haven't.

This could be detected at birth only when the cord blood testing is done, and an additional genetic test is performed to confirm WBIT.

I discussed false D-negative predictions with SHOT. They would appreciate if this is reported to SHOT and the cord blood and mothers blood samples send to IBGRL so that we can compare the genotype results and establish WBIT, incorrect result or other causes. IBGRL would report back to the Trust with corrective and preventative actions.

The only time this would affect the pregnant women would be in a D-negative prediction of the fetus, who would only get anti-D Ig at birth. The mothers with D-positive predictions would receive anti-D Ig, although unnecessarily, but they would have received it under the existing pathway anyway.

NICE have considered false negative predictions in their scenarios – see sensitisation rates table copied from the full NICE guidance.

The false D-negative rate, which would include WBIT, for fetal *RHD* screening is 0.1% according to the literature. At present IBGRL has a false negative prediction rate of 0.08%. This is comparable to the findings

from Denmark and the Netherlands, which reported a rate of 0.03%. Both countries have stopped cord blood testing after one year of national implementation.

Discrepant results

Q: Do we have to report discrepancies between the predicted fetal group and laboratory findings at birth?
A: Yes, you should report all discrepancies to IBGRL.

1. If IBGRL have reported a **fetal D-positive result** and the cord blood phenotype result is D-negative we would appreciate if you inform us, but we will **not** investigate cord and mothers blood samples. The rationale behind this is that although the mother had received anti-D Ig unnecessarily at 28 weeks there has been no harm done as procedures had been followed according to the advice/result given. It is not SHOT reportable either.
2. If IBGRL have reported a **fetal D-negative result** and the cord blood phenotype result is D-positive you should send us some cord and mum's blood. We will investigate for wrong blood in tube. You should report to SHOT, if the IBGRL result is found to be a false D-negative prediction.

NICE and SHOT indicated that they would like our findings for the following reasons:

1. NICE would like to reassess, after a couple of years, if the UK could discontinue with the cord blood testing in line with the Netherlands and Denmark
2. SHOT would like to know failure rates where **anti-D was not given to mums at 28 weeks** when their fetus was predicted to be **D-negative and was confirmed D-positive** by IBGRL. The investigation might give the root cause as:
    a. wrong blood in tube (mum or cord)
    b. genuine incorrect results
    c. lab errors
    d. other – to be specified when root cause has been established
3. Please contact SHOT if you have any questions regarding reporting.

Explanation regarding discrepancies:

1. How do you define a discrepancy between fetal *RHD* screening result and cord blood?
   This is a screening test and gives predicted results – therefore there is no discrepancy with the actual cord blood result. You could view it like a microbiology reactive screen and the actual result confirmatory result which might be negative.
2. What happened when fetal *RHD* screening test predicted RhD-positive and the cord blood result is RhD-negative?
   The fetal *RHD* screen has been set up to be highly sensitive for detection of fetal *RHD*, thus avoiding false negative results as in such cases anti-D Ig will not be given. The false negative rate in this case is 0.1% i.e. highly accurate.
   The false positive prediction is up to 2% although this is dependent on the ethnicity of the population.

   Please see our User Guide
   During pregnancy a small amount of cell-free fetal DNA is present in maternal blood. This DNA can be analysed for RHD exons 5 and 7 using real-time polymerase chain reaction to predict the baby's D blood group to see if it differs from that of the mother. The test is highly accurate and can be performed from 11+2 weeks gestation (crown rump length > 45mm). However, owing to the sensitivity of the test, there is a small chance (0.1%) that a fetus predicted to be D-negative will be D-positive at birth. Please inform the Molecular Diagnostics department as soon as possible if this occurs and send a sample of cord and mother's blood if available. Owing to the presence of rare variant RHD genes, up to 2% of fetuses predicted to be D-positive will in fact be D-negative at birth.
   and the Patient information leaflet
   How will the results affect my treatment? Unborn baby is D-positive If your blood test report shows that your unborn baby is D-positive, or the result is inconclusive, you will be offered an anti-D injection. However, 2.0% of these babies may in fact be D-negative. This is of no concern as anti-D prophylaxis would have been offered in all cases if DNA testing had not taken place and the injection will not harm your baby

False positive results may occur owing to the following reasons:

- Rare silent or variant Rh genes or weak D alleles
- Vanishing twin
- Extraneous low-level DNA contamination of the sample (every effort is made to avoid this during testing).

3. How do other hospitals manage their false positive results?
    a. One Trust detected weak D cases and acted accordingly, other hospitals either do not test cord blood from D-positive predictions or do not test for weak D and continue to use the results of cord blood testing to guide postnatal prophylaxis.
    b. Since 2013, when fetal *RHD* screening was introduced as a pilot service, IBGRL have been made aware that the fetal *RHD-positive* genotype was correct and the cord blood D-negative result was incorrect owing to wrong cord blood in tube.
4. Should the cord blood be tested at RCI for weak D investigation?
    You could investigate the cord blood for weak D, however, current guidelines do not recommend this course of action.
5. Uncomfortable with NOT issuing anti-D Ig when there is a discrepancy, having given anti-D Ig unnecessarily at 28 weeks?
    The women will have been informed about this in the Patient information leaflet see above under point 2.
6. Although not SHOT reportable, would a discrepancy casts doubt over our results and how to manage these patients?
    As I outlined above this does not cast doubt over your results post-delivery as it is expected that 2% of the D-positive predictions will be D-negative as described above under point 2.
7. Has NICE done a risk assessment, do we need to do one?
    I searched the NICE documentation on the website – apart from the copied sections NICE do not analyse false D-positive results, although anti-D Ig had been given unnecessarily the cord blood group will determine the anti-D Ig use at birth.

Implementation process

Q: How do we set up the fetal *RHD* screen in our Trust?
A: The most important step is to identify a project lead. This could be someone who needs project management experience either for personal development or their portfolio. Other options would be the Transfusion Practitioner or an enthusiastic member of staff who can drive the project forward and liaise with the pathology and maternity teams, ensuring actions are completed on time.

The overall implementation plan could look like this (please note that the outlined steps can be started simultaneously):

1. **Project lead:** Contact the NHSBT Business Development Manager (BDM) email: erika.rutherford@nhsbt.nhs.uk for the latest documentation, calculation template, maternity pathways etc.
2. **Project lead:** Write a business plan using the calculation template and documents our BDM can email you
    - Sign off by CEO and Finance Director
3. **Maternity team:** Agree a maternity pathway, using the maternity pathway examples from other hospitals
    - Sign off by relevant committees
    - Train all staff
4. **Pathology team:** Set up changes in Pathology
    - Set up test in IT systems – using the sample reports provided by BDM
    - Agree booking in, send away and resulting of reports – using the sample SOP
5. **Project lead:** Inform NHSBT to send contract for sign off
    - Ensure CEO has signed contract

6. **Project lead:** Complete and sign communications document which our BDM will send you when you are closer to your start date
7. Start!

Suggestions for key performance indicators

Q: Do other hospitals measure success and key performance indicators for this test?
A: Yes, Royal Berkshire and Southampton have published posters. Please contact Erika.rutherford@nhsbt.nhs.uk if you would like a copy.
I have summarised some suggestions below:

1. Take up rate
    a. Measure increase in take up over a set period – ½ year or 1 year
    b. Record reasons for not taking the test
    c. Record reason for refusing to have anti-D Ig with or without the fetal *RHD* screen
2. Anti-D Ig compliance – to demonstrate improvement to compliance pre and post implementation of fetal *RHD* screen
    a. RAADP
    b. Post-natal
    c. Potential sensitising event
    d. Failure to give anti-D Ig or given when not required
3. FMH test compliance
    a. Potential sensitising event
    b. Post-natal
4. Cord blood testing
    a. Compliance – cord blood tested, maternal sample sent – not sent
5. Test failure rate for fetal D-negative prediction versus Cord blood group result D-positive
6. Cost saving
    a. Anti-D Ig prior to implementation
    b. Anti-D Ig post implementation
    c. Other savings or cost incurred
        i. Quantifications – compliance with BSH guidelines
        ii. Bed occupation – turnaround time as midwives know the group of the baby and can give anti-D Ig immediately
        iii. Clinic time reduction or increase due to additional samples take or less anti-D Ig given
7. Turnaround time
    a. Sample sent versus result received on Sp-ICE – in days
8. Population distribution
    a. % of inconclusive results versus D-positive/D-negative cord blood groups
    b. % of D-positive and D-negative predictions
    c. % of not tested samples
9. Other measures
    a. Lost samples
    b. Near misses – i.e. samples **not** sent by Pathology to IBGRL because of labelling errors, missing EDD etc. – record reason and improve outcome
    c. Samples send by hospital Pathology to incorrect NHSBT lab – this might result in rejected tests due to time expiry for testing i.e. older than 7 days