Management of Rhesus Negative Women in Pregnancy

Guideline information

Guideline number: 644
Classification: Clinical
Supersedes: Previous Version
Local Safety Standard for Invasive Procedures (LOCSSIP) reference: N/A

National Safety Standards for Invasive Procedures (NatSSIPs) standards: N/A
Version number: 6.0
Date of Equality Impact Assessment: 08/10/2024

Approval information

Approved by: Obstetric Written Control Documentation Group
Date of approval: 12/12/2024
Amendment discussed and agreed 11.6.2025 ASW
Date made active: 18/06/2025
Review date: 12/12/2027

Summary of document:
Management of Rhesus D Negative women in pregnancy.

The guidance below uses the term 'woman' (pronouns she or her) to describe individuals whose sex assigned at birth was female, whether they identify as female, male or non-binary. It is important to acknowledge it is not only people who identify as women for whom it is necessary to access women's health and reproductive services. Therefore, this should include people who do not identify themselves as women but who are pregnant or have recently given birth. Obstetric and Midwifery services and delivery of care must therefore be appropriate, inclusive, and sensitive to the needs of those individuals whose gender identity does not align with the sex that they were assigned at birth.

Scope: For health professionals manage caring for women who have a Rh D Negative group.

To be read in conjunction with:
http://onlinelibrary.wiley.com/doi/10.1111/tme.12091/abstract (opens in a new tab)
(BSH)

Routine antenatal anti D prophylaxis for women who are rhesus D negative
https://www.nice.org.uk/Guidance/TA156 (opens in a new tab)

Owning group:
Maternity Guidelines, Audit and Research Group
12/12/2024

Executive Director job title:
Interim Executive Director of Nursing, Quality and Patient Experience

Reviews and updates:
1.0 – New Guideline
2.0 – Three yearly review
3.0 – Three yearly review
4.0 – Update following discussions with Antenatal Screening Wales re: Rh Negative women who have had cffDNA (page 12/13)
5.0 – Clarification of pathway relating to dealing with discrepant results.
Clarification that in the situation where woman has a fetal loss and has had a cffDNA screening result predicting that baby is Rhesus D Negative, a Kleihauer Test is NOT to be requested. (ASW 2025).
6.0 - Discussed at ASW 11.6.2025 – guideline updated to reflect "A Kleihauer (irrespective of group) over 20 weeks gestation would be requested in order to assess for, and quantify, feto-maternal haemorrhage if clinical suspicion e.g. history of trauma to abdomen,. antepartum haemorrhage, known vasa praevia ."

Keywords
Rhesus Disease, Pregnancy, Anti D Immunoglobulin, Prophylaxis, Cell Free Fetal DNA

Glossary of terms
ADAU - Antenatal Day Assessment Unit
CMW - Community Midwife
HDN - Haemolytic Disease of the Newborn
Ig – Immunoglobulin

IM - Intra Muscular
FMH - Feto Maternal Haemorrhage
MLC - Midwife Led Care
Rh D - Rhesus Disease
PV - Per Vagina
CffDNA - Cell Free Fetal DNA
RAADP - Routine antenatal anti D Prophylaxis

Key points:
This guideline has been updated and reflects changes in management following the introduction of antenatal cell free fetal DNA (cffDNA) screening offered to Rh D negative in Wales and provides guidance of the management of all Rh Negative women in Pregnancy.

Contents

Guideline information 1
Approval information 1
Introduction 5
Scope 5
Management of Rhesus Negative women who are predicted as carrying a Rh D positive baby and Rhesus Negative women where fetal Rh D status is unknown 6
Cell Free Fetal DNA (cffDNA) Screening 10
Fetal Loss and cffDNA screening results 13
Discrepant Results 13
References 14
Pathway 1 Flowchart for Anti-D administration following a potentially sensitising event 15
Pathway 2 Flowchart for Routine Antenatal Anti-D Prophylaxis 16
Pathway 3 Blood group and antibody screening cell free fetal (cff) DNA Genotyping 17
Appendix 1 – Fetal RhD Sample Collection 18
Appendix 2 – Antenatal Blood Group Serology Request 19
Pathway 4 – Management pathway for discrepant results following cffDNA screening 20
Appendix 3 – ASW Information for Professionals cffDNA 21
Links to Antenatal Screening Wales Information for Professionals 26
Links to Antenatal Screening Wales Patient Information and Leaflet 26

Introduction

Rhesus disease is caused due to incompatibility between a rhesus negative woman and a rhesus positive baby. Severity can range from mild fetal anaemia to hydrops fetalis in utero, which can ultimately be fatal.

Sensitisation usually occurs due to small fetomaternal haemorrhages occurring during pregnancy and which may occur undetected (“silent bleeds”) . It may also occur if insufficient anti D is given after Birth. Sensitisation may lead to Haemolytic Disease of the Newborn (HDN) in the current or future pregnancies.

All pregnant women, irrespective of maternal or predicted fetal Rh D status, should be offered their blood grouping and antibody screening performed at booking (at 8 to 12 weeks of pregnancy) and once again at 28 weeks. If antibodies are detected, they should be identified and if necessary quantified to assess the likelihood of HDN. (NICE NG201)

All pregnant women should be offered blood group and antibody testing at booking.

Cell Free fetal DNA (cffDNA) for RhD Typing

Note cffDNA is fully discussed in Section 3 of this guideline but for the purpose of introduction a brief summary is as follows:

Women who are RhD negative, singleton or twin pregnancy, and are non-sensitised to anti-D or anti-G antibodies (i.e.no maternal antibodies) are offered a screening test called Cell Free fetal DNA (cffDNA) to predict fetal Rh D group.

On the normal antenatal screening pathway cffDNA screening is offered at 16 weeks but can be done up to 26 weeks gestation. Women who are Rh D negative and present for care at ≥26+1 weeks of pregnancy or greater, will be offered anti-D immunoglobulin. If the unborn baby Rh D group is predicted to be Rh D negative, then administration of Anti D to the woman will not be required either following a sensitising event or prophylactically at 28 weeks' gestation.

Scope

For health professionals manage caring for women who have a Rh D Negative group.

Management of Rhesus Negative women who are predicted as carrying a Rh D positive baby and Rhesus Negative women where fetal Rh D status is unknown

Consent

All pregnant women who are found to be rhesus negative should receive appropriate written and verbal information about anti-D immunoglobulin and the offer of cffDNA screening to inform their choice and be given time to consider their options.

As a human derived blood product, the administration of anti-D Immunoglobulin requires consent.

The difference between RAADP (i.e. routine prophylaxis at 28 weeks) and prophylactic anti-D Ig given following potentially sensitising events should be clearly explained to the woman (NICE, 2008).

Who to offer Anti D immunoglobulin?

Anti D prophylaxis is offered to non-sensitised Rh-D negative women:

- Who have declined cffDNA screening.
- Where cffDNA screening predicts a RhD positive fetus
- The woman is not suitable for cffDNA (e.g. booked later than 26 weeks)

When to offer Anti D immunoglobulin

- Pregnant women who are non-sensitised Rh D negative and report a potentially sensitising events (see table 1 below).

- All non-sensitised Rh D negative women are offered a routine antenatal anti-D immunoglobulin prophylactically around 28 weeks.

- Post-Birth where the baby is Rh D positive, confirmed from a cord blood sample.
- Note: Eligibility for further doses of anti-D is not affected by any routine antenatal Anti-D prophylaxis given in the 3rd trimester.

Table 1. Potential Sensitising Events

Potential Sensitising Events
• Surgical management of Ectopic pregnancy • Surgical Management of Molar pregnancy • Miscarriage / threatened miscarriage/vaginal bleeding **from** 12 weeks gestation • Any invasive fetal Medicine Procedure e.g.amniocentesis, Chorionic villus biopsy, and cordocentisis In-utero therapeutic interventions (transfusion, surgery, insertion of shunts, laser treatment) • Therapeutic termination of pregnancy • External Cephalic Version. • **Abdominal** Trauma (Sharp, blunt, open/closed) • Intrauterine death and stillbirth • Birth: normal, instrumental or caesarean • Intra-operative cell salvage

Kleihauer Test from 20 weeks

From 20 weeks gestation a Kleihauer screening film should be offered to Rh D neg women following every sensitising event to determine extent of transplacental bleeds.

If significant Fetal maternal Haemorrhage is determined by kleihauer to be more than/ > 4mls then additional dose of Anti D and repeat maternal bloods samples will be required when advised by local blood bank.

Administration of Anti D after sensitising event

(See Pathway 1)

Anti D should be given as soon as possible after **every** sensitising event but **always within 72 hours.** If it is not given within 72 hours, every effort should still be made to administer Anti D as a dose given within 9 – 10 days may provide some protection.

Intramuscular (IM) Anti D is best given into the deltoid muscle (upper arm) as injections into the gluteal region often only reach the subcutaneous tissues and absorption may be delayed.

Verbal consent must be obtained prior to administering Anti D.

Anti D given for any sensitising event must be prescribed by doctor on drug chart.

Record administration on drug chart, patient's notes and on blood bank administration form.

Dosage of Anti D Immunoglobulin

Anti D 500 units < 20 weeks (usually given on the gynaecological ward) Anti D 500 units > 20 weeks

Routine Antenetal Anti D Prophylaxis

(See Pathway 2)

It is recommended that routine Anti D prophylaxis is offered to all non-sensitised pregnant women who are Rh-D negative (NICE 2002) unless the fetus has been predicted to be Rh D negative as a result of cffDNA screening. However, women can opt out if they:

- Are to be sterilised after childbirth.
- In a stable relationship with the baby's parent and they are known to be Rh D negative or
- Is certain not to have any further children.

All eligible Rh-D negative people will receive verbal and written information to enable them to make an informed choice.

Anti D declined

If routine or prophylactic Anti D is declined it must be clearly documented in the woman's All Wales hand held Records.

Process for routine antenatal Anti D Prophylaxis

- Appointments will be sent by ADAU or ANC to attend for routine Anti D between 28 – 30 weeks of pregnancy.
- Group and antibody screen sample must be taken no more than 72 hours before administration of Anti D. A repeat sample will be requested if over 72hours]
- If anti D is identified in the booking sample, then further investigations should be undertaken to determine whether this is immune or passive (i.e. previous administration of Anti D). If no clear conclusion as to the origin of the anti D detected then the woman should continue to be offered anti D Ig prophylaxis and should continue to be monitored monthly until 28 weeks gestation and fortnightly thereafter.
- Single dose 1500 units (Rhophylac 300) given IM in the deltoid muscle. The woman should remain in ADAU for 20 minutes to ensure no adverse reaction.
- Use of routine antenatal anti D Ig prophylaxis should not be affected by previous anti D Ig prophylaxis administered for a sensitising event earlier in the same pregnancy.

Note: If a woman **declines** anti D, still take the 28-30 week group and antibody screen sample and send to the lab. Document reasons for declining in maternal handheld notes and on blood bank sample slip before returning anti D to blood bank.

Sensitising Events after Routine Anti D

- Anti D must be given after **any event**, even if routine prophylaxis has been given.
- Kleihauer to be taken and requested if over 20 weeks gestation.

Recurrent Vaginal Bleeding

Gestation between 12-20 weeks

Anti D 500 units to be given at a minimum of 6 weekly intervals.

Gestation over 20 weeks

After 20 weeks gestation Anti D 500 units should be given at a minimum of 6 weekly intervals. Kleihauer to be carried out at 2 weekly intervals.

Post-natal Prophylaxis

A maternal blood sample for Kleihauer to be taken within 2 hours post-Birth accompanied by a cord blood sample for all Rh D negative women regardless of cffDNA prediction. This will act as a safety net to ensure the cffDNA screening result was correct.

If the baby blood group is confirmed as Rhesus positive, non-sensitised women who are RhD negative should be offered postnatal Anti D immunoglobulin within 72 hours of Birth. (RCOG 2011).

Homebirths and early discharges from the postnatal ward:

- Birth suites should review early discharge procedures to avoid omission or late administration of anti D immunoglobulin if required.
- In cases of early discharge, consideration should be given to administration of anti D, if required, no later than day two of Birth in the community (SHOT 2020).

Documentation after Anti D administration

When Anti D immunoglobulin is administered during pregnancy ensure that the accompanying serology form is completed with time, date and signature and is returned to blood bank. Document in the handheld record/ drug chart as appropriate.

Management of Blood Group Immunisation

- On detection of any abnormal blood antibodies refer to next Consultant Clinic
- If partners' genotype unknown – blood sample to be obtained (currently not offered under All wales screening)
- Paediatric referral form to be sent.
- USS to assess fetal wellbeing and evidence of hydrops • If any evidence of hydrops refer immediately to Cardiff FMU.

- Give steroids if evidence of fetal compromise > 24/40 gestation
- If low level of Anti D detected follow Anti D protocol for serial quantitative assay
- Refer to UHW if rising titre
- Non Rhesus D – refer to UHW for further assessment

Decision for birth or when in labour

(In the presence of unusual / high antibody titre levels)

Inform: -

1. SCBU
2. Paediatric Department
3. Blood Bank

As much notice as possible needs to be given in order to ensure availability of blood for both parent and baby from Blood Transfusion Service.

Cell Free Fetal DNA (cffDNA) Screening

All pregnant women should be offered blood group and antibody testing at booking (NICE NG201). Women must be informed of the maternal blood Rh group by 16 weeks with results documented, with a printed copy of Blood bank result filed, in maternal hand held notes.

Screening for cffDNA commences as part of the normal ASW antenatal screening pathways at 16 weeks gestation and offered to singleton and twin pregnancies of women who are Rh negative and do not have anti D or anti-G antibodies .

The test is optional.

The cffDNA test can be performed from 11 weeks and 2 days therefore screening will be available to some women who are referred to /attending the Fetal Medicine Unit at an earlier gestation.

The latest gestation that cffDNA screening can be offered is ≤ 26 weeks gestation

Further information from ASW about offering cffDNA can be accessed via this link. (opens in a new tab)

Results and appointments

If Rh D Negative women will receive from the Hywel Dda Antenatal clinic:

- The woman is contacted via letter to confirm Maternal Rh D Negative blood group result. Maternal RhD Negative Result letter sent:
    - Confirming Maternal Blood Group as Rh D negative and offering option to have cffDNA screening test to predict fetal Rh D status. ○ ASW Information leaflet “Information if you are pregnant and D negative blood group” given (paper or via QR code on letter)
    - Details of an appointment date for antenatal clinic to have cffDNA screening test

Sampling Procedure for cffDNA

(See Appendix 1)

- 10ml maternal blood collected in EDTA tube from Rh D negative pregnant women who have not made anti-D antibodies. Note :Samples will be rejected if less than 9mls in bottle
- Once sample taken, invert bottle gently 8-10 times.
- The sample tubes must not be opened following blood collection.
- The sample tube should be stored at room temperature. DO NOT place in fridge or freezer
- The sample tube must be handwritten by the person taking sample (**Note**: sample will be rejected if an addressograph is used on bottle)
- Samples and request card must be labelled with identification, including name, date of birth and NHS number.
- Labels pre-printed prior to phlebotomy e.g. addressograph labels are not acceptable on samples. The laboratory however, will accept addressograph labels on **reques**t forms providing they do not obscure other vital details.
- Hand written alterations on the either the sample or request form may make the sample invalid. Any minor alterations must be initialled by the person taking the sample to be acceptable for testing
- Request form MUST be correctly completed. (See Appendix 2)
- Document in handheld maternity notes that cffDNA screening has been performed
- Document patient information in Hywel Dda antenatal cffDNA audit book record.
- Samples should be taken to blood bank who will then send to Welsh Blood Service Molecular Genetics Laboratory, preferably within 24 hours of blood draw.

Rejected samples

- If a sample is rejected it will need be repeated before 26 weeks gestation.
- Examples of reasons for rejection: ○ ” Insufficient blood in tube” if there was insufficient plasma ○ EDD not supplied ○ Inadequate labelling of sample/ not signed or dated ○ Sample grossly haemolysed therefore unable to test ○ Discrepancy between sample and request form

Follow up and documentation of results

- Results will be available 10 working days from receipt of sample in.

- Currently cffDNA results are sent to the requesting antenatal clinics generic email by Welsh Blood Service Molecular Genetics Laboratory and Local Blood bank are not informed of fetal cffDNA screening results.
- Antenatal clinic:
  - manually add the cffDNA result to Welsh PAS as Maternal alert information
  - Enter results into Hywel Dda antenatal cffDNA audit book
  - All antenatal clinics to ensure that monthly data is sent to Antenatal screening Coordinator.

Results and Management

There are three possible results and management should be as follows:

Women with:

Inconclusive results will be managed as if the fetus is Rh positive and the mother will be offered routine prophylactic Anti-D at 28 weeks and after any sensitising event. The test will not be repeated.

Positive results will be managed as if the fetus has Rh positive status and patient will be offered routine prophylactic Anti-D at 28 weeks and after a sensitising event.

Negative results where the status of the fetus is Rh negative should not be offered Anti-D but the patient can still request to receive anti-D if she desires.

Communication of results to patient

A copy of the results and a letter explaining the cffDNA results and subsequent management during pregnancy will be sent to the woman.

Appointments for women who require Anti A Prophylaxis

When ccfDNA result predicts fetus as Rh D Positive or when result is inconclusive and will be treated as Rh D positive an appointment for the Routine Antenatal Anti D Prophylaxis will be offered. The appointment will be organised by the antenatal clinic midwife.

Management at Birth

For failsafe purposes, cord bloods and Maternal bloods for Kleihauer are to be performed at Birth on **all** Rh D negative women **regardless of cffDNA results**.

It is currently the Midwife responsibility to confirm that all postnatal cord results correlate with the antenatal cffDNA results (if preformed) to ensure that no discrepant results.

Fetal Loss and cffDNA screening results

In the event of fetal loss where the woman is RhD negative and has had cffDNA screening where the result has predicted that baby has RhD negative blood then Anti- D is **not** required.

A Kleihauer (irrespective of group) over 20 weeks gestation would be requested in order to assess for, and quantify, feto-maternal haemorrhage if clinical suspicion e.g history of trauma to abdomen,. antepartum haemorrhage, known vasa praevia .

If the baby is predicted to be RhD positive Anti D is recommended (in line with guideline).

Discrepant Results

(See Pathway 4)

1. False Negative Result.

Where screening predicted fetal Rh D group to be negative but cord bloods results show baby to be Rh D positive

- Inform mother of the result
- Inform local blood bank laboratory of discrepancy
- Midwife must complete a datix linking in blood bank
- Obtain a repeat blood group from the infant and send to local blood bank to confirm the result.
- Advised the woman that anti-D is recommended and should be administered (if accepted) without delay within 72 hours of birth.

NOTE: Women who may wish to wait for results of baby's Rh D group to confirm that baby is RhD Positive before accepting Anti D must be informed of recommendations that anti D is given within 72 hours to avoid sensitisation.

Local Blood Bank laboratory will:

- Request a repeat blood group from the infant to confirm the result.
- Notify Welsh Blood Service.
- Initiate a local investigation

2. False Positive Result

Where screening predicted fetal Rh D group to be positive but cord bloods results show baby to be Rh D Negative

- Inform woman result
- Advise that anti-D is not recommended.

- Inform blood bank of discrepancy
- Obtain repeat blood group from the infant and sent to local blood bank to confirm the result
- Midwife must complete a datix linking in blood bank

The local Blood bank laboratory will

- Request a repeat blood group from the infant to confirm the result.
- Notify Welsh Blood Service.
- Initiate a local investigation

All discrepancies must be reported to the local blood bank who will inform the Welsh Blood Service.

All discrepant results will be reported to the Serious Hazards of Transfusion (SHOT) haemovigilance scheme either by the local blood bank or Welsh Blood Service.

References

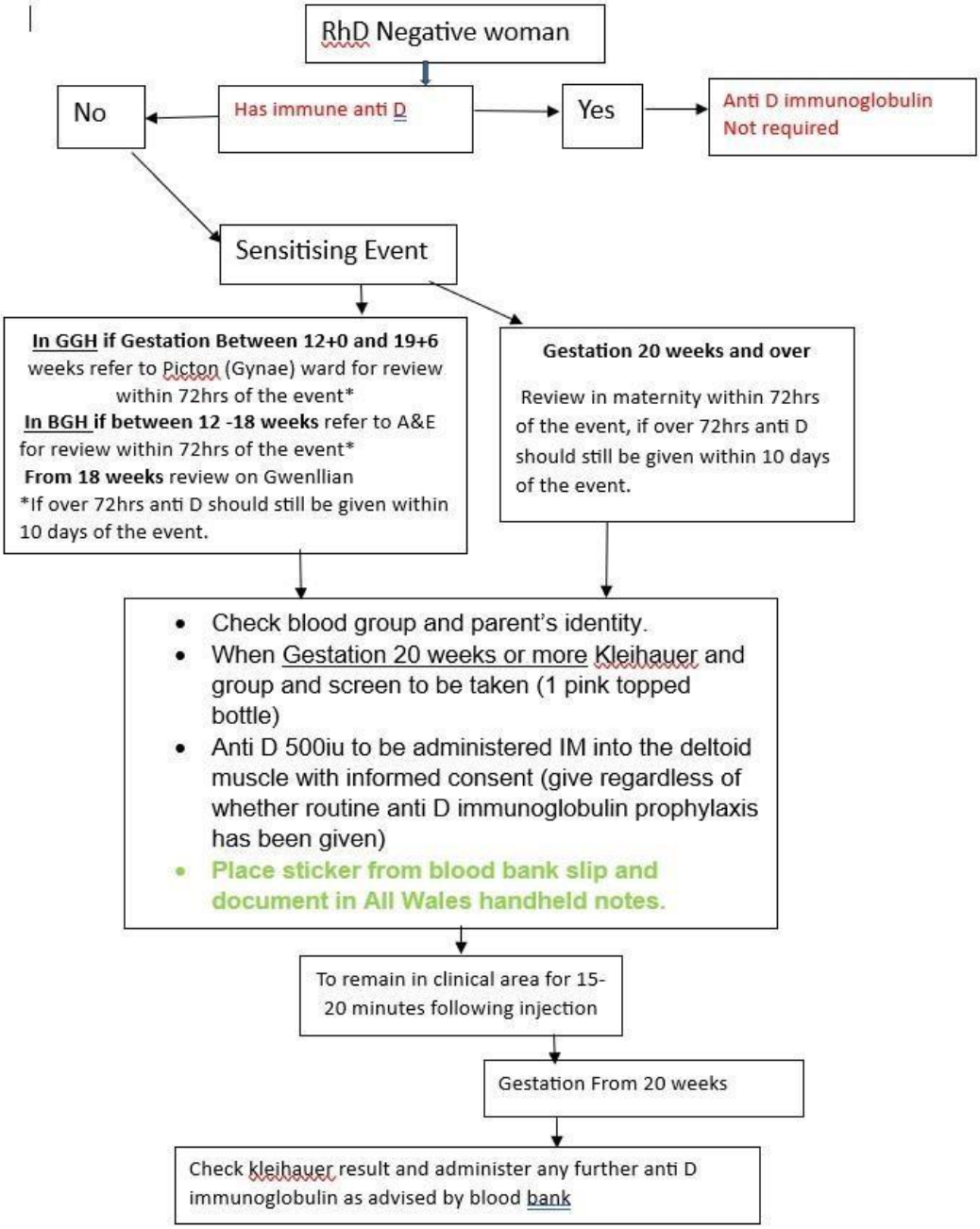
British Committee for Standards in Haematology (BCSH) (2011) guideline on anti D administration in pregnancy. H Quereshi et al. 92014) BCSH guideline for the use of anti D immunoglobulin for the prevention of haemolytic disease of the fetus and newborn. January 2014.

National Institute for Clinical Excellence (2008) *National Institute for Clinical Excellence* Guidance on the use of Routine Antenatal Anti D Prophylaxis for Rh D Negative Women. August 2008

Serious Hazzards of transfusions 9SHOT) (2020) Summary of Recommendations. Annual report 2019.

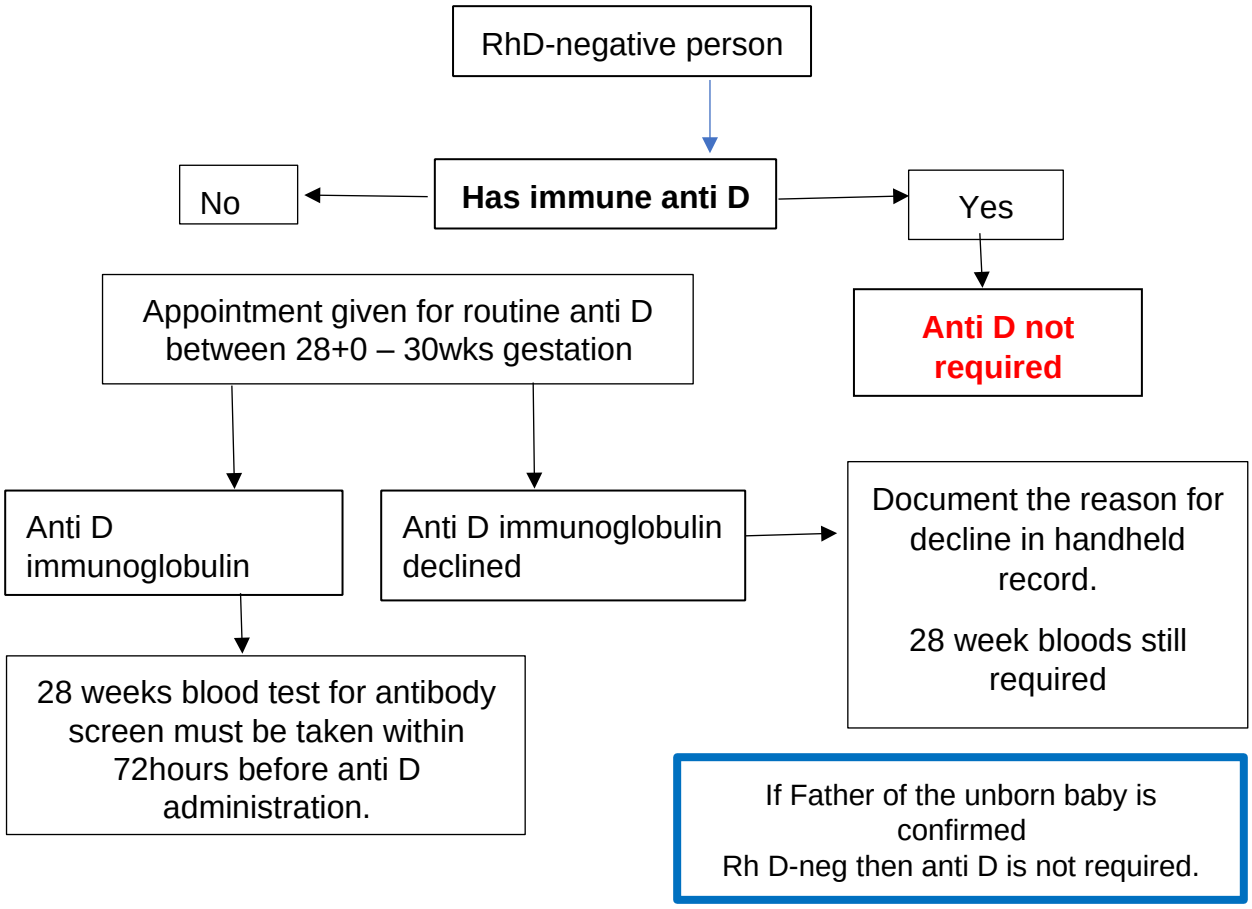
Royal College of Obstetricians and Gynaecology (2011) Royal College of Obstetricians and Gynaecology Green Top Guidelines Use of Anti D Immunoglobulin for Rh Prophylaxis (22) available from https://www.rcog.org.uk/en/guidelines-research-services/guidelines/gtg22/

Pathway 1 Flowchart for Anti-D administration following a potentially sensitising event



Routine antenatal anti D prophylaxis (RAADP) at 28 weeks must be administered regardless of anti D that may have been given for potentially sensitising event

Pathway 2 Flowchart for Routine Antenatal Anti-D Prophylaxis

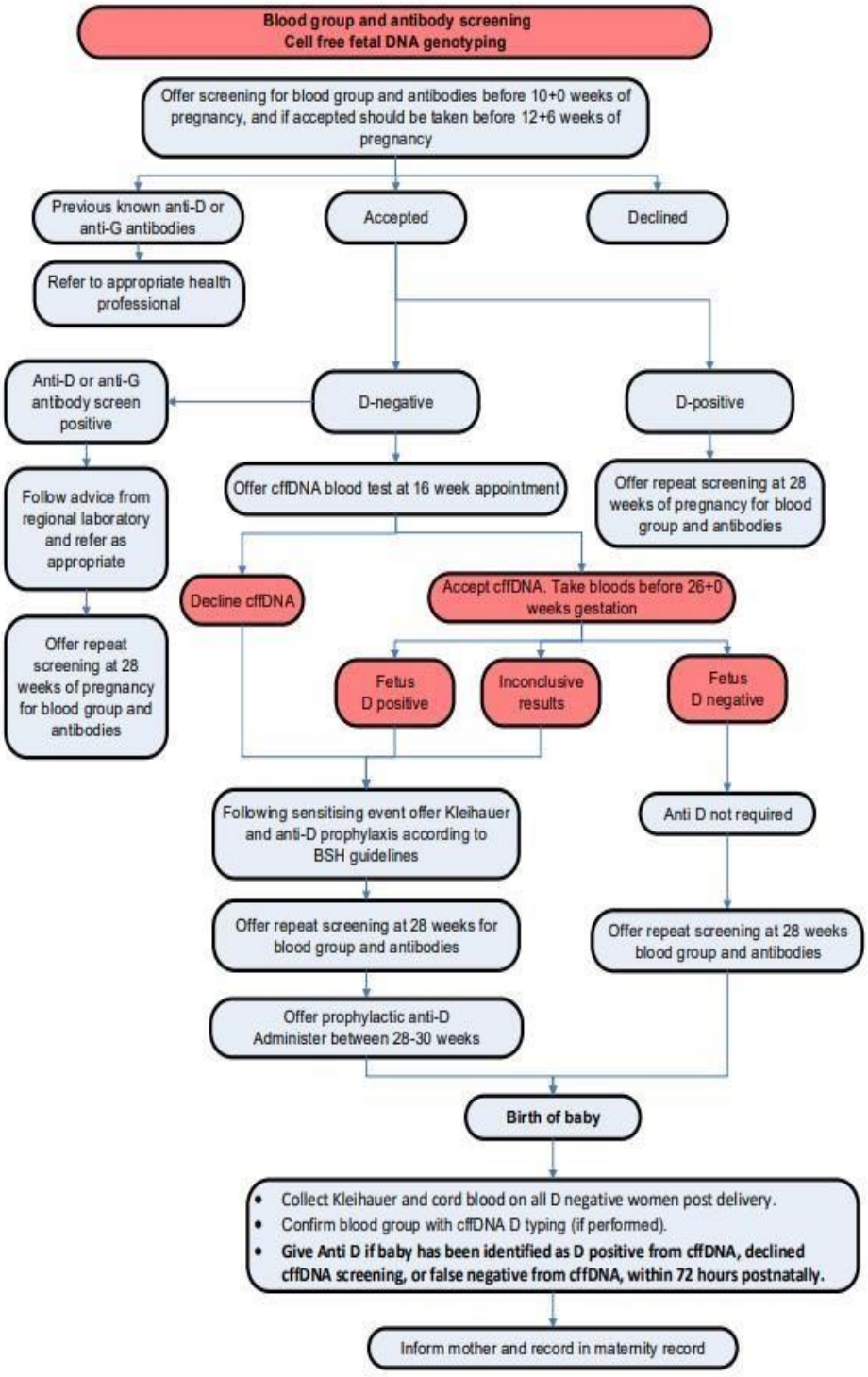


- Check gestation is correct.
- Check Blood group.
- Verbal consent obtained.
- Anti D 1500 units to be administered into the deltoid muscle.
- Place sticker from blood bank slip and document in handheld notes.
- Rhesus Negative sticker on handheld notes
- Complete blood bank slip and return to blood bank.

To remain in DAU for 15-20 minutes following injection.

Observe for any reactions.

Pathway 3 Blood group and antibody screening cell free fetal (cff) DNA Genotyping



Appendix 1 – Fetal RhD Sample Collection

Gwasanaeth Gwaed Cymru
Welsh Blood Service

Cyfarwyddwr | Director - Mr Alan Prosser
Heol Cwm Elái | Ely Valley Road
Tonysguboriau | Talbot Green
Pontyclun, CF72 9WB

FETAL RhD SAMPLE COLLECTION

IMPORTANT SAMPLE HANDLING REQUIREMENTS

- FETAL RhD samples MUST be stored at room temperature. They must NOT be placed in a fridge or freezer.
- FETAL RhD samples must NOT be centrifuged.

1. Complete the Fetal RhD request card.

Complete all sections in full and ensure all details are legible. Please note that failure to do so may result in sample rejection. **Positive patient identification procedures must be followed**.

2. Blood samples must be taken in a **single 10ml EDTA tube**:
   - Draw 10mls of blood into a single EDTA tube using a Vacutainer blood draw system.
   - Try to fill the sample tube correctly because samples will be rejected if there is less than 9mL inside the tube.
   - After blood is drawn into the tube, it is mandatory to mix the tube by gentle inversion 8-10 times.
   - Label the tube with the sample identification and draw date.
   - The samples and request card must each be labelled with identification, including the woman's name, date of birth and NHS number. A minimum of three points of identification on the sample and request card must match
     - The label on the sample **must be handwritten**. Addressograph labels will not be accepted.
     - Identification must include the woman's name, date of birth and NHS number.

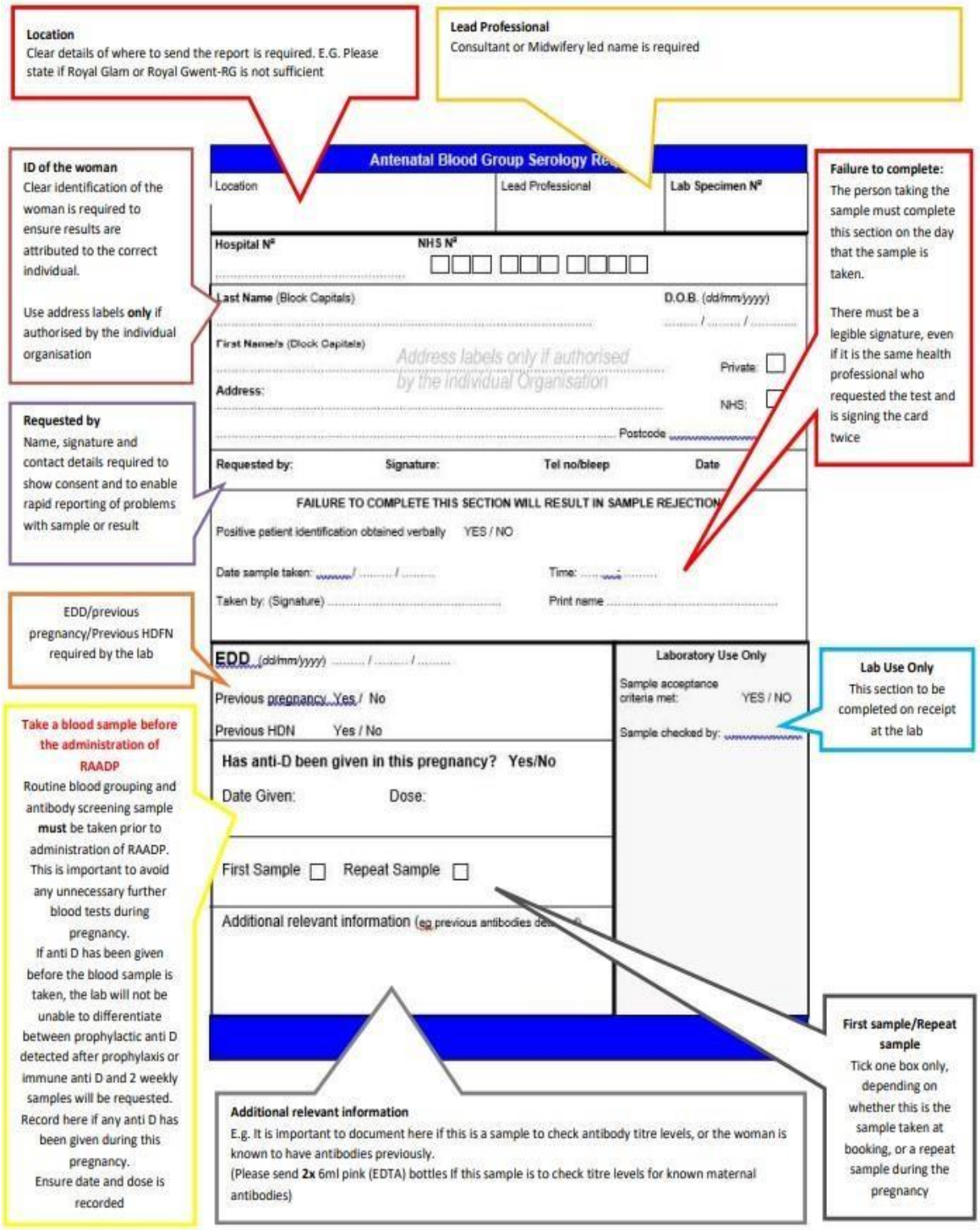
3. Samples must be sent to the Welsh Blood Service Molecular Genetics laboratory in a timely manner, preferably within 24 hours of blood draw.
   - Samples should be stored/transported at ambient temperature (approximately 22°C)
   - Samples must reach the laboratory in time to be processed during laboratory working hours **within 5 days** post venepuncture (where day of collection is day 1).
   - The normal laboratory working hours are Monday to Friday, 09:00 - 17:00 excluding Bank holidays

Please contact the laboratory if you have any queries relating to sample collection:

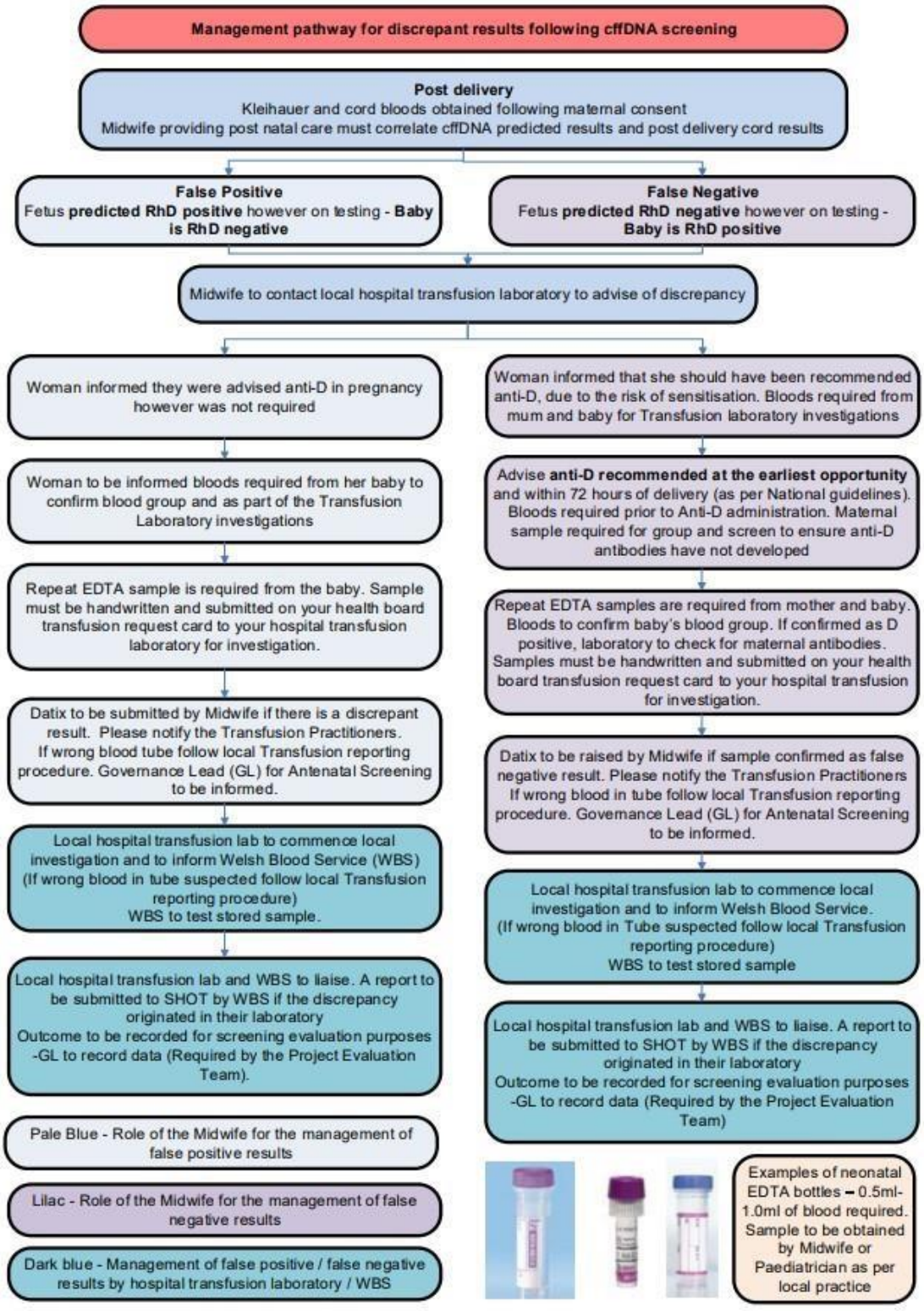
Molecular Genetics, Welsh Blood Service, Ely Valley Road, Pontyclun, CF72 9WB

Email: molecular.genetics@wales.nhs.uk. Telephone: 01443 622186 (09.00-17:00)

Appendix 2 – Antenatal Blood Group Serology Request



Pathway 4 – Management pathway for discrepant results following cffDNA screening



Appendix 3 – ASW Information for Professionals cffDNA

Information for Professionals

Cell Free Fetal DNA (cffDNA) screening test: A Guide for Health Professionals

Summary

cffDNA screening test is a highly accurate test. We know that there will however be a small percentage of false positive and false negative results reported, which is a risk of any screening programme.

Who can have the fetal RhD screening test?

The test is for D-negative pregnant women who do not have anti-D or anti-G antibodies at booking.

The normal antenatal screening pathway will enable women whose pregnancies have progressed to 16^{+0} weeks gestation (samples must not be collected prior to 11+2 weeks gestation).

The cffDNA test can be performed from 11^{+2} weeks gestation, therefore screening will be available to some women who are attending Fetal Medicine Unit at an earlier gestation. If a woman requires referral to FMU, she can have cffDNA screening, however the turnaround time will be the same as the current/ normal pathway.

If the woman has had confirmation that her baby is RhD positive, or, the RhD status of the baby is inconclusive, then we should consider the baby as being RhD positive and recommend anti-D immunoglobulin.

Welsh Blood Service (WBS) should be notified if a sample is performed in these circumstances. You can contact WBS on - 01443 622186 or email: molecular.genetics@wales.nhs.uk

The latest gestation the test can be performed as per the normal antenatal screening pathway will be $\leq 26^{+0}$ weeks gestation. This will allow time for the sample to be processed and for the routine 28/40 anti-D appointment to be arranged if appropriate.

Women who are expecting twins.

We can offer the test to women pregnant with twins. A positive result in this case means at least one of the fetuses is D-positive. A negative result would mean that both fetuses are D-negative. **The test cannot be offered to women who are pregnant with higher multiple pregnancies.**

Women who have weak D or D variant.

It is difficult to differentiate between maternal and fetal DNA. 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.

Pregnant women with antibodies

We do not offer the cffDNA screening test to pregnant women who have anti-D or anti-G antibodies **due to the complexities** of these antibodies.

We **can** offer the cffDNA screening test to pregnant women who have alloantibodies other than anti-D or anti-G.

Potential Sensitising Events (PSE)

Women who have a potential sensitising event and have anti-D Immunoglobulin administered prior to the offer of cffDNA screening can still have screening performed as the anti-D Ig does not interfere with this test. However, the serological antibody test at booking, prior to the cffDNA screening must be negative for D and G antibodies.

Previous termination/miscarriage within the last 6 months

Women who are RhD negative and have had a termination of pregnancy or miscarriage within the last six months can be offered screening providing the antibody screen at booking is negative for D or G antibodies.

If the serological investigation at booking identifies an anti-D antibody (or G antibodies) -. please follow the BSH guidelines for Blood grouping and antibody testing in pregnancy Blood Grouping and Antibody Testing in Pregnancy (b-s-h.org.uk).

Vanished Twin

CffDNA screening can be offered to a woman who has had a twin pregnancy initially, but now has a vanishing or vanished twin. However, in this group of women there is a higher chance of a false positive result if circulating cffDNA from the vanished/demised twin is still present.

Test Performance

Sensitivity of cffDNA screening

- The tests' ability to correctly identify the proportion of true positive results is ≥99.9%.
- A true positive (TP) is defined as a clinical sample with detected fetal RhD DNA that is confirmed as RhD Positive.

Specificity of cffDNA screening

- The tests' ability to correctly identify the proportion of true negative results is ≥99.8%.
- A true negative (TN) is defined as a clinical sample with no detected fetal RhD DNA that is confirmed as RhD negative.

False positive (FP) rate of cffDNA screening

- The false positive rate is 0.14%.
- For fetal blood group genotyping tests a false positive result means that cffDNA screening test has predicted a fetus to be RhD positive when the baby is found to be RhD negative at birth.

False negative (FN) rate of cffDNA screening

- The false negative rate is 0.09%.
- For fetal blood group genotyping tests, a false negative result means that cffDNA screening test has predicted a fetus to be RhD negative when the baby is found to be RhD positive at birth.

Results

The fetal RhD screening results will only be available via the Welsh Results Reporting System (WRRS). This allows health care professionals across Wales to access and view results across all health boards in Wales. The turnaround time will be 10 working days from receipt of sample.

If a result is not available 10 working days since a sample was taken, then you should contact WBS on 01443 622186 or email: molecular.genetics@wales.nhs.uk

Reports

Test Reporting (Singleton pregnancy).

The report will state if the fetus is predicted to be RhD Positive, RhD Negative or if the test was inconclusive. Examples for each scenario are shown below.

- Fetal RhD typing predicts that this fetus is RhD POSITIVE. This result only applies to the pregnancy with EDD above.
- Fetal RhD typing predicts that this fetus is RhD NEGATIVE. This result only applies to the pregnancy with EDD above.
- Fetal RhD typing was INCONCLUSIVE. Manage this pregnancy as if this fetus is RhD POSITIVE. This result only applies to the pregnancy with EDD above.

Test Reporting (Twin pregnancy).

In a twin pregnancy, a positive result means at least one of the fetuses is D-positive. A negative result would mean that both fetuses are D-negative.

- Fetal RhD typing predicts that one or both fetuses are RhD POSITIVE. This result only applies to the pregnancy with EDD above.
- Fetal RhD typing predicts that both fetuses are RhD NEGATIVE. This result only applies to the pregnancy with EDD above.
- Fetal RhD typing was INCONCLUSIVE. Manage this pregnancy as if one or both fetuses are RhD POSITIVE. This result only applies to the pregnancy with EDD above.

If a sample has been rejected this will also be noted on the report. Reasons for sample rejection will 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 card

Samples will need to be repeated before 26 weeks gestation.

Documentation and sample requirements

Positive patient identification procedures must be followed.

There is clear guidance for sample requirements. The NHS number is mandatory as it will be the unique identifier, there must be a minimum of three points of identification that match between the sample and request card. In some cases where a woman may not initially have an NHS number, the sample may be accepted providing that there is a minimum of three points of identification. WBS will not accept samples which have incomplete mandatory data. Rejected samples will have a report explaining why the sample has not been accepted. CffDNA Sample Taker SOP.

It is acceptable to have addressograph labels on the request card providing they do not obscure other vital details. However, the sample must be handwritten, addressograph labels are not acceptable on the samples. Any minor alterations must be initialled by the person taking the sample to be acceptable for testing.

For the fetal RhD screening test use a 10mL EDTA tube with a purple top. Fill the sample tube correctly because samples will be rejected if there is less than 9mL inside the tube.

Postnatal – maternal and cord blood sampling

Test Reporting (Singleton pregnancy).

A maternal blood sample is required from all RhD negative women who have had cffDNA screening regardless of whether the fetus is predicted to be RhD negative or RhD positive. This is to assess feto-maternal haemorrhage in RhD negative women who have delivered a RhD positive baby to establish whether the woman requires additional anti-D prophylaxis. It will also be needed if a false negative result from cffDNA screening is discovered.

A cord blood sample is required from all RhD negative women who have had cffDNA screening regardless of whether the fetus is predicted to be RhD negative or RhD positive. This is to test for the fetal RhD group and confirm the antenatal cffDNA result, if applicable.

- WBS and ASW are not currently recommending that hospitals discontinue cord blood testing.

All postnatal cord blood samples should be correlated with the antenatal cffDNA result to ensure there are no discrepant results.

Discrepant results

You must report all discrepancies to the local blood bank who will inform the WBS. A pathway for discrepant results can be located here; Management pathway for discrepant results.

False negative result

If a false negative result is discovered, the woman should be informed of the result and advised that anti-D is recommended. A maternal antibody screen should be taken prior to the administration of anti-D and the women should be offered and recommended anti-D. This should be administered (if accepted) without delay and within 72 hours of birth.

A repeat blood group from the infant to confirm the result should be obtained.

The local laboratory should be informed and an incident should be raised as per local practices e.g. Datix and the relevant teams (maternity risk & governance team midwives, local laboratory, WBS) informed so that appropriate actions can be taken.

The local laboratory will –

- Request a repeat blood group from the infant to confirm the result.
- Notify Welsh Blood Service.
- Initiate a local investigation by submitting a Datix.

NB – A false negative result is where screening has predicted the RhD group of the fetus to be negative, but the cord blood results show the infant to be RhD positive. We anticipate approximately 5 false negative results annually throughout Wales. This has been risk assesses and agreed as acceptable.

False positive result

If a false positive result is discovered, the woman should be informed of the result and advised that anti-D is not recommended.

A repeat blood group from the infant to confirm the result should be obtained.

The local laboratory should be informed. The local laboratory will –

- Request a repeat blood group from the infant to confirm the result.
- Notify Welsh Blood Service.
- Initiate a local investigation by submitting a Datix.

NB – A false positive result is where screening has predicted the RhD group of the fetus to be positive, but the cord blood results show the infant to be RhD negative.

- **All discrepant results will be reported to the Serious Hazards of Transfusion (SHOT) haemovigilance scheme either by the local blood bank or Welsh Blood Service.**

Explanation regarding discrepancies:

Women who have accepted cffDNA screening will be aware that this is a screening test and although very accurate, is not 100% accurate.

Women who have a false negative result will not have had anti-D at 28 weeks or for any sensitising events during the pregnancy. We estimate that this will be approximately 1 in 1000 women. These women will have been at risk of becoming sensitised during their pregnancy and birth. It's estimated that 1% of women become sensitised if they receive post-natal anti-D but do not receive antenatal anti-D at 28 weeks.

Women who have a false positive result will have had anti-D unnecessarily at 28 weeks and for any sensitising events during pregnancy. The small false positive rate when using the cffDNA, means that approximately only 1.26% of D negative women will receive antenatal prophylaxis unnecessarily, rather than 40% without using cffDNA screening.

References:

https://onlinelibrary.wiley.com/doi/full/10.1111/tme.12091

https://www.nice.org.uk/guidance/ta156/documents/pregnancy-rhesus-negative-women-routine-antid-review-overview2#:~:test=The%20base%2Dcase%20sensitisation%20rate.and%20therefore%20be%20at%20risk

High-throughput, non-invasive prenatal testing for fetal rhesus D status in RhD negative women: a systematic review and meta-analysis – PubMed (nih.gov)

Links to Antenatal Screening Wales Information for Professionals

https://nhswales365.sharepoint.com/sites/PHW_ScrInfProComms/SitePages/Blood-group-andantibodies(1).aspx

Cell Free Fetal DNA (cffDNA) screening test: A guide for Health Professionals - Link

Links to Antenatal Screening Wales Patient Information and Leaflet

https://phw.nhs.wales/services-and-teams/screening/antenatal-screeningwales/informationresources/leaflets

https://phw.nhs.wales/services-and-teams/screening/antenatal-screening-wales/informationresources/leaflets/information-if-you-are-pregnant-and-d-negative-blood-group