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Fetal D Group DNA Screening and Routine Anti-D Prophylaxis (RAADP) for Non-Sensitised RhD Negative Pregnant Women/People Guideline

Summary statement: How does the document support patient	By providing evidence based guidance for staff with regard to Fetal testing for D group, and Anti D administration; both prophylactically and following a sensitising event.
Staff/stakeholders involved in development:	Leads for Maternity (Obstetric and Midwifery), Blood Transfusion Leads, Joint Obstetric Guidelines Group
Division:	Women and Children's
Department:	Maternity
Responsible Person:	Chief of Service
Author:	Transfusion Practitioner and Antenatal Clinical Manager
For use by:	Midwifery and Medical Obstetric staff Blood Transfusion laboratory staff
Purpose:	To provide guidance for staff on the safe and timely fetal DNA testing and appropriate administration of Anti D for non-sensitised RhD negative pregnant women/people
This document supports:	British Committee for Standards in Haematology, 2014 RCOG, 2011 NICE technology appraisal guidance 156, 2008
Key related documents:	UH Sussex (SRH&WH): Intra-operative Cell Salvage Policy Patient Identification Policy Midwives Exemptions Document
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Version	Date	Author	Status	Comment
1.0	August 2011	R. O'Donnell & J. Hargreaves	Archived	Updated Trust Guideline
2.0	May 2012	R. O' Donnell & H. Clarke	Archived	Guideline amended to include site differences in Anti D dose
3.0	February 2014	R. O' Donnell & H. Clarke	Archived	Guideline amended to update change to using the same dose of Anti D for all cases
3.1	March 2015	R. O' Donnell & H. Clarke	Archived	Minor updates
4.0	March 2017	R. O'Donnell	Archived	Admission of missed Anti D doses
5.0	July 2017	R O'Donnell	Archived	New process for cffDNA added
6.0	August 2019	R. O'Donnell	Archived	Minor re-wording of section 7.3
7.0	July 2022	R. O'Donnell, Transfusion Practitioner	Archived	3 year review. PSE section updated to incorporate current NICE guidance.
8.0	October 2022	J. Birrell, Gynaecology Matron	LIVE	Table of sensitising events amended to include surgical management of miscarriage and MVA.

The interpretation and application of clinical guidelines will remain the responsibility of the individual clinician.
If in doubt contact a senior colleague or expert.

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1.0 Abbreviations Used Within This Guideline

cffDNA - Cell-free Fetal DNA (RhD testing)	**DAT -** Direct Antiglobulin Test
EDD - Expected Delivery Date	**FC -** Flow Cytometry
HDFN - Haemolytic Disease of Fetus and Newborn	**G&S -** Group & Save (Antibody screen)
FMH - Feto Maternal Haemorrhage	**HCP** – Health Care Professional
IM - Intramuscular	**ICS -** Intraoperative Cell Salvage
IV - Intravenous	**IU -** International Units
RAADP - Routine Antenatal anti-D prophylaxis	**NHSBT -** NHS Blood and Transplant
D - RhD	**Sp-ICE** - NHSBT electronic reporting system
MIS - Maternity Information System	**SHOT** - Serious Hazards of Transfusion
MVA - Manual Vacuum Aspiration	

2.0 Aim

The aim of this guideline is to provide healthcare professionals with guidance to:

- Promote Fetal DNA D group testing,
- Aid safe and appropriate administration of anti-D,
- Avoid sensitisation to the D antigen during pregnancy and at birth for the prevention of HDFN.

Note: Throughout this document RhD is referred to simply as D

3.0 Scope

- Registered HCPs involved in the care of **non-sensitised** D negative pregnant women/people.
- Women/people with weak expression of the D antigen, between weak D Types 1 to 3, do not normally form allo anti-D, so do not require anti-D immunoglobulin prophylaxis. Please refer to their NHSBT report.
- Women/people with indeterminate D typing results should be treated as D negative until confirmatory testing is completed.
- If the D status of the fetus is unknown, it should be assumed that the fetus is D positive and the mother/birthing parent treated accordingly.

4.0 Responsibilities

Management are expected to ensure that the guideline is:

- Reviewed as required in line with Trust and National Recommendations.
- Accessible to all relevant staff.

The obstetrician or midwife responsible for antenatal care is expected to:

- Access, understand and follow this guidance.
- Use their professional judgement in the application of this guideline.
- Undergo regular training in the process.
- Discuss Cell Free DNA (cfDNA) testing and RAADP with the woman/person so that that they can make an informed choice about treatment. This discussion should include the risks, benefits and the options open to the woman/person.
- Ensure the woman/person is directed to online maternity information on anti-D as soon as D group, Expected Delivery Date (EDD) & viability confirmed. If the woman/person has been previously sensitised and is exempted from RAADP this should be recorded in the maternal and medical notes.
- Explain to women/people who may have a D positive fetus that if they have a sensitising event during pregnancy they need additional anti-D; and that giving Anti D does not protect against the development of other antibodies that cause HDFN.
- Provide written requests to the transfusion laboratory for anti-D in a timely manner with identification of the recipient and their clinical details.
- Ensure that gestation and reason for testing is made clear on request forms.
- Provide written requests and suitable samples for Kleihauer testing.

5.0 Introduction

5.1 Haemolytic Disease of the Fetus and Newborn (HDFN)

D negative mothers/birthing parents are is at risk of developing antibodies to D positive fetal red cells after a feto-maternal haemorrhage (FMH) which can occur during pregnancy or at birth. HDFN is caused by a blood group incompatibility and can affect D positive babies of D negative mothers/birthing parents. HDFN ranges in severity from only being detectable in laboratory tests, through to stillbirth, birth of infants with severe disabilities or death of newborns from anaemia and jaundice.

Anti-D administration prevents the mother/birthing parent producing anti-D antibodies by coating any D positive red cells that reach the maternal/birthing parent circulation before the immune system is sensitised.

5.2 Cell-free DNA Fetal D testing

Around 15% of a Caucasian population are D negative, of whom around 40% will have D negative babies (these women/people do not benefit from the blanket administration of anti D). Maternal/birthing parent plasma DNA includes free-floating fragments of DNA from the mother/birthing parent and fetus, known as cfDNA. CfDNA testing for the presence of fetal D group enables us to give anti D only to those women/people who have a D positive fetus.

It is the responsibility of the test requester to ensure that patient consent has been obtained.

Results Availability – samples are sent to NHSBT for testing and results available within 14 days. If the result is not returned within this time the midwife must contact the transfusion laboratory. The midwife must check result availability on Sp-ICE: After 14 days if not available on Sp-ICE then contact the Transfusion lab to ensure sample received and sent to the NHSBT: The Transfusion Lab will follow up.

Multiple Births – a positive result means at least one of the fetuses is D positive and the woman/person should receive RAADP. A negative result means that all of the fetuses are D negative.

Late bookers and transfers – may enter into the cfDNA testing pathway if their samples are sent for testing no later than 26 weeks. The lab however will test beyond this date on a case by case basis (please contact the lab). If anti-D has been given elsewhere state how much, when and where given on request form.

Test reliability

- Test reliable from 11+2 weeks gestation (crown rump length > 45mm).
- Up to 8% of samples may give result an “inconclusive” result. In these cases treat the fetus as D positive.
- Rate of false negatives is approximately 0.1% causes include insufficient fetal DNA and wrong patient’s blood in sample tube (misidentification errors).
- Women/people who have already received Anti-D can be tested (Anti-D does not interfere with the test.)
- Ensure the test result you have refers to the current pregnancy.
- Not suitable for people who have made anti G.
- Due 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.

Sample requirements

- NHSBT Fetal Genotype Screening request form.
- 6mL EDTA tube (minimum 4mL).
- Samples must be correctly and fully labelled or they will be rejected.
- Samples over 7 days old (by the time received by NHSBT) will not be tested.

- The form must also state the EDD.
- Haemolysed samples will not be tested as they may contain a high background of maternal DNA which could interfere with detection of fetal DNA.

5.3 Prescribing Anti-D immunoglobulin

Rhophylac ® (CSL Behring): available as 1500 IU prefilled syringe, for IM or IV use, stored at 2-8°C. Note: The manufacturer's guidance says that for women/people with a body mass index (BMI) 30 or more consider IV administration. If there is any doubt that the injection will not be intramuscular (IM) this must be discussed with the woman/person and the option of IV administration offered.

Anti-D can be supplied and administered under Midwives Exemptions as a routine (i.e. at 28/40 and postnatally). This should be documented as a STAT dose on the electronic prescription chart. It must be prescribed by a doctor following a potential sensitising event. Anti D administration should also be documented on MIS.

Within this guideline, all anti-D doses are 1500IU unless otherwise stated

6.0 Anti D administration

- The woman/person will be counselled and given their consent for anti D administration.
- The anti-D is documented on the electronic prescription chart and Maternity Information System (MIS).
- Take a G&S before administering anti-D.
- Occasionally anti-D causes an allergic response; therefore administration should take place in hospital where there is access to treatment (ensure adrenaline is available in case of anaphylactic reaction). Women/people should be monitored for early signs of hypersensitivity reactions including hives, generalised urticaria, chest tightness, wheeze, hypotension and anaphylaxis. All women/people should wait in the clinic for at least 20 minutes after administration.
- IM anti-D should be given into the deltoid muscle.
- A verbal identification check of the woman/person against the dispensed product and prescription must be completed prior to administration in accordance with the Trust Patient Identification Policy.
- Check the expiry of the Anti D.
- Anti D should be given as soon as possible after a sensitising event but always within 72 hrs. If it is not given before 72 hrs, every effort should still be made to administer Anti D, as a dose given within 10 days may provide some protection.
- Women/people with severe thrombocytopenia (platelets ≤30×10^9/L) or a history of a bleeding disorder e.g. Von Willebrand disease (vWD), should be administered anti-D IV or subcutaneously depending on whether a preparation suitable for IV

use is available. Please discuss these women/people with the consultant haematologist. Women/people with significant bleeding disorders such as vWD should be managed jointly with a Haemophilia Centre.

- Universal precautions should be exercised when handling blood products.
- Complete the transfusion traceability paperwork and return it to the transfusion laboratory within 24hrs of administration
- Document the details of administration in the patient record and Maternity Information System (MIS).
- Complete the anti-D prophylaxis section in the woman/person's notes and complete the laboratory traceability.

7.0 Process

(Also see Appendix 1)

- At Booking all pregnant women/people will have a G&S sample taken. It is important that the hospital site is on the form as this is used to send results to the ANC team.
- D negative individuals will be identified by the transfusion laboratory.
- At 14-16 weeks Expected Delivery Date (EDD) and viability is confirmed, ANC to send:
  - A letter to all non-sensitised women/people containing their D group and an ANC appointment (to discuss management and offer cfDNA test).
  - Signposting to online maternity information on cfDNA D testing and Anti D.
- At Worthing - CMW will discuss CFF DNA test, pathway and treatment plans. Blood for CFF DNA will be taken at this point if consented to. (They do not have a hospital appointment for this) CMW emails ANC who check results, make Anti D appointments, and update MIS.
- At St Richard's - The booking blood group result is checked by ANC and will contact the pregnant woman/person regarding fetal DNA blood test. This is performed with the combined screening blood test. ANC will book routine anti-D if indicated.
- Enter the woman/person into Pathway 1 or Pathway 2.

NB It is the responsibility of the community midwife at the 16 week appointment to check the D result and check that the woman/person is aware of their group and the proposed pathway of testing and treatment.

7.1 Pathway 1 - cfDNA D Group Testing

This pathway should be followed if the woman/person consents to fetal testing.

- Document discussion & consent in notes.
- Take cfDNA sample, use specific NHSBT form, send to the transfusion lab.
- After 14 days check results on Sp-ICE.

If fetus is D negative:

- At 28 Weeks take a G&S, no anti D is required, at birth take a cord blood and Kleihauer (to check cfDNA results).
- Place "Fetal D neg sticker" Appendix 2 in Green notes and, record on MIS.

If the fetus is D positive:

- Record on MIS, follow Pathway 2
- Inform the woman/person that they will require additional Anti D for any PSE.

7.2 Pathway 2 - Routine Antenatal Anti D Prophylaxis (RAADP)

This pathway should be followed if the:

- Woman/person declines cfDNA testing.
- cfDNA testing has failed.
- cfDNA testing shows the fetus is D positive.
- Woman/person is a late booker (after 26 weeks) and there are no cfDNA results.

28 Week Anti D appointment

- Take a G&S sample **before** administering anti D.
- Ideally check result of G&S however it is not necessary to wait for results, prior to administering anti-D. Obtain informed consent before administration.
- If the woman/person fails to attend her appointment the midwife should ensure that all attempts are made to contact them and reschedule another appointment as soon as possible. Anti-D should be given between 28-30 weeks; if this has not been possible seek advice from the obstetrician and report through Datix (and SHOT if appropriate).

NB If anti-D is detected, further history and investigation is required to establish whether it is immune or passive. The outcome will inform clinical decisions regarding anti-D prophylaxis and antenatal follow-up. If no clear conclusion can be reached as to the origin of the anti-D, then prophylaxis should continue to be administered in accordance with guidelines for women/people who have not formed immune anti-D.

7.3 Birth

All births regardless of previous pathways.

Required samples:

Baby: Cord blood for Group. If this is not collected for any reason, a heel prick sample can be obtained. In situations where this is not possible and there is no cffDNA result available (or cffDNA result predicts the baby to be D pos), it should be assumed the baby is D positive

for the purposes of FMH determination and the administration of anti-D Ig. In cases where the cffDNA result predicts the baby to be D negative and no cord blood sample is taken it is a clinical decision whether to accept the cffDNA result solely and not administer anti D to the woman/person. DAT is not required unless HDFN is suspected (a DAT will is however routinely tested as part of the baby group).

Mother/birthing parent: Kleihauer (if a G&S is indicated send a separate sample) (Take Kleihauer more than 30-45 mins after birth, but ideally within 2 hours of birth).

If infant is D Positive: ensure Anti D1500IU is prescribed initially and given to the mother/birthing parent within 72 hours of birth. Women/people who give birth at home must come into hospital to receive anti-D.

If FMH more than 4ml, additional anti-D may be required (see section on follow up of sensitising event). The mother/birthing parent must not leave the hospital until the anti-D has been given; or without a robust documented plan for them to receive this.

Cell Salvage (ICS): if a woman/person has undergone ICS, where cord group is confirmed as D positive (or unknown), anti-D should be administered following the re-infusion of salvaged red cells. To check if more anti-D is needed take a maternal/birthing parent sample for estimation of FMH no sooner than 30 mins after the reinfusion of blood.

8.0 Potentially Sensitising Events (PSE) if fetus is D positive or unknown

Amniocentesis, Chorionic Villus Biopsy & Cordocentesis	**Antepartum Haemorrhage/Uterine (PV) Bleeding in pregnancy**
External Cephalic Version	**Abdominal Trauma** (sharp/blunt, open/closed)
Therapeutic Termination of Pregnancy	**Intrauterine Death (IUD) & Stillbirth** – for IUD, anti-D should be given immediately following the **diagnosis** of IUD **and** a further dose of anti-D administered after birth.
Ectopic pregnancy if managed by surgical procedure	**In-utero Therapeutic Interventions** (transfusion, surgery, insertion of shunts, laser)
Miscarriage, Threatened Miscarriage	**Birth** – normal, instrumental, or Caesarean Section
Surgical Management of miscarriage including manual vacuum aspiration (MVA) and evacuation of molar pregnancy	**Intra-operative Cell Salvage**

D negative pregnant women/people must be aware of the PSEs and have been signposted to online maternity information.

Anti-D should be given as soon as possible within 72 hours of the event. However, in exceptional circumstances, some protection may be offered up to 10 days after the event.

8.1 PSEs before 12 weeks gestation (confirmed by ultrasound)

Anti D is indicated for the following procedures:

- Medical termination of pregnancy from 10 weeks gestation.
- Surgical termination of pregnancy at any gestation.
- Surgical management of miscarriage, including MVA and molar pregnancies.
- Surgical management of ectopic pregnancy.

A Keilhauer is **not** required.

8.2 PSEs at 12-20 Weeks Gestation (if fetus is D positive or unknown)

Take a G&S to check D group and the presence of anti-D. If anti-D is identified, further history should be obtained, and investigation undertaken to determine whether this is immune or passive. If no clear conclusion can be reached as to the origin of the anti-D detected, then the woman/person should continue to be offered anti-D prophylaxis on the assumption that it may be passive. Kleihauer not required.

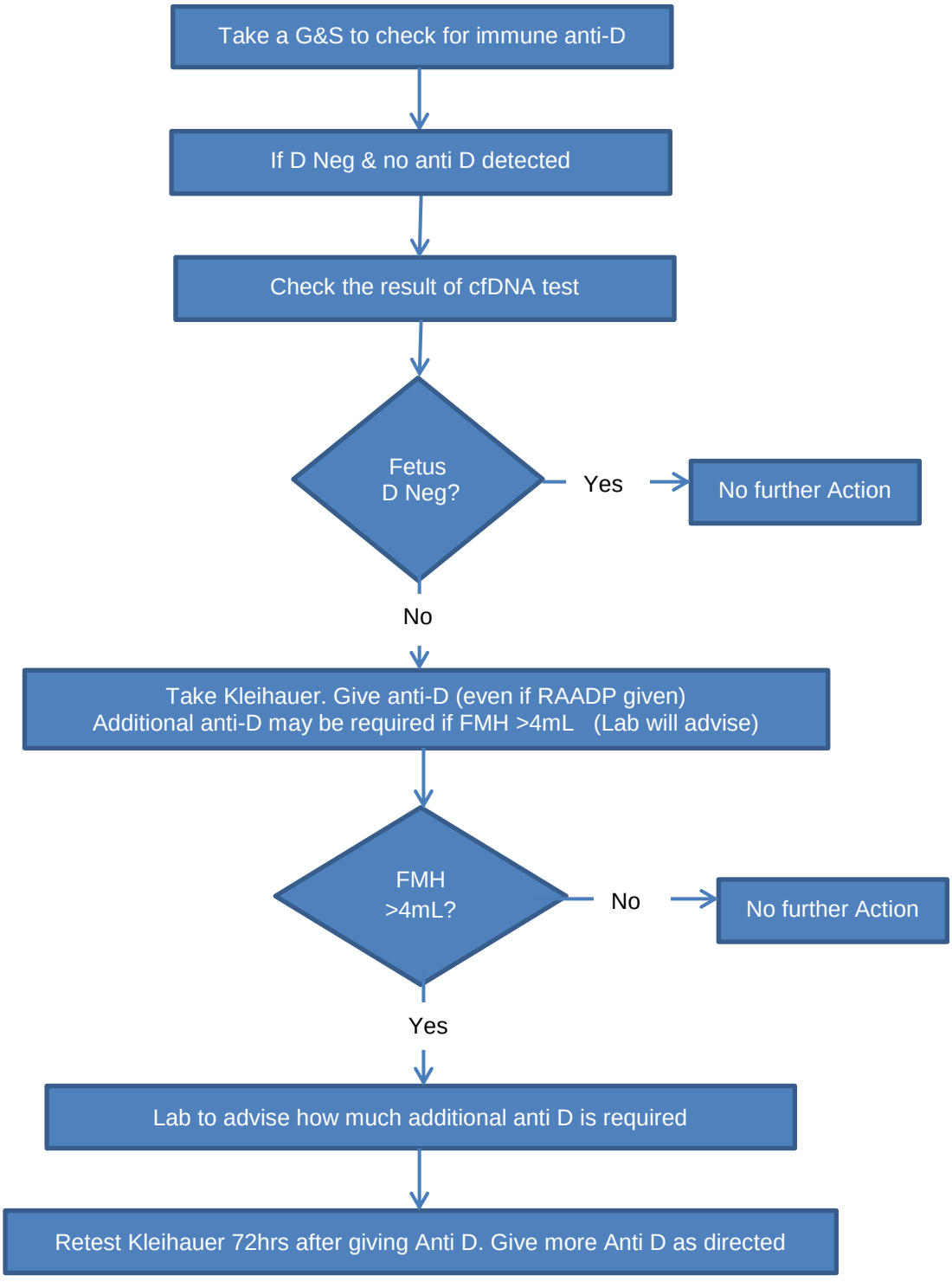
If the woman/person is D negative with no anti D detected check the result of the cfDNA test, **if fetus is D negative no further action is required.** Women/people with indeterminate D typing results should be treated as D negative until confirmatory testing is completed. Otherwise give anti D for all PSEs including medical and surgical miscarriages, termination of pregnancy and ectopic/molar pregnancies from 12 weeks gestation confirmed on ultrasound scan.

Continual Bleeding

In the event of continual uterine bleeding which is **clinically judged to represent the same sensitising event, with no features suggestive of a new presentation or a significant change in the pattern or severity of bleeding, such as the presence of abdominal pain or another clinical presentation,** anti-D should be given at six weekly intervals.

A Kleihauer should be carried out at every 2 weeks from 20 weeks onwards and additional anti D given as appropriate. Advice can also be sought from Consultant. If NHSBT Consultant discussion required, call the Transfusion lab for contact details.

8.3 PSEs after 20 weeks gestation



Continual Bleeding– See section above Kleihauer should be taken every 2 weeks and if positive give additional doses of anti-D regardless of the presence or absence of passive anti-D then retest Kleihauer after 72 hours.

9.0 Feto-Maternal Haemorrhage (FMH) confirmation

A Kleihauer is required if a D negative woman/person has a D positive fetus (or the D group of the fetus is unknown) and she experiences a potentially sensitising event after 20 weeks gestation or after the birth of a D positive baby.

If the Kleihauer shows a bleed of >2mL the lab will contact the clinical area and send the sample to a reference lab for flow cytometry to confirm these potentially significant bleeds.

9.1 Follow-up of confirmed FMH

FMH <4mL and routine anti-D has been given, no further testing is required.

FMH 4-11mL (confirmed by Flow Cytometry) is covered by the 1500IU IM anti-D dose already given; additional anti-D is not required. However, there should be a follow-up maternal sample to check for clearance of fetal cells.

FMH ≥12mL or more give extra doses (for IM administration calculate the additional anti-D according to the formula 125IU for every 1mL fetal cells) rounded up to the nearest vial size, taking into account the postnatal anti-D dose already given (but not RAADP dose). Use the same batch of anti-D if possible. Take follow-up maternal/birthing parent samples (after 72hrs post dose if given IM or 48hrs if given IV) to check for clearance of fetal cells. For large FMH the use of IV anti-D should be considered and if used, seek specialist advice from a haematologist or reference centre before administration.

Note the dose calculation is different for IV anti-D (67.5 IU / mL).

Check the Haemoglobin of the baby in these cases.

If fetal cells have not cleared on follow-up sample:

- Confirm that the anti-D has been given.
- Confirm that the baby is D positive.
- Send the follow-up maternal EDTA sample for testing by flow cytometry.
- Give further anti-D as dictated by the volume of fetal red cells remaining and repeat the Kleihauer 72 hrs after the repeat anti-D injection (48 hrs if anti-D was given IV).
- Repeat this sequence of immunisation and testing until no fetal cells are seen in the FMH test.

9.2 Longer term follow-up after birth

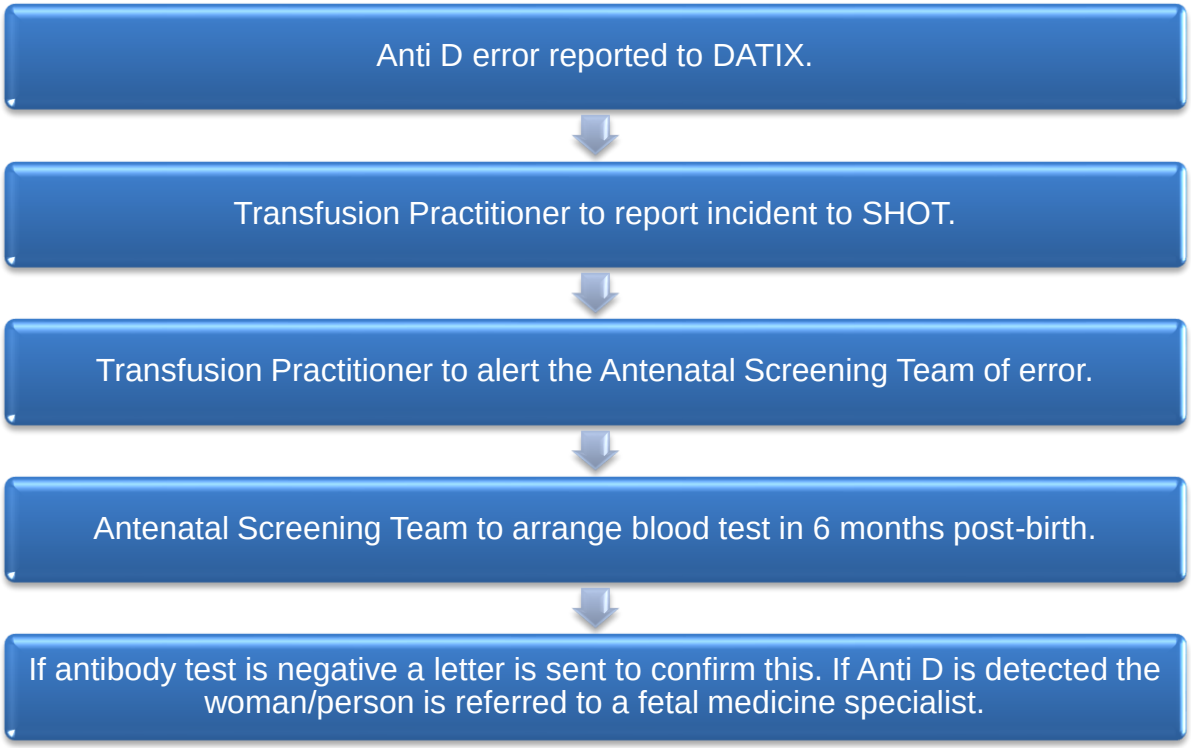
A maternal G&S should be taken 6 months post-birth (this will be decided on an individual patient basis dependent on the size of FMH and NHSBT advice). This follow up provides the

opportunity to counsel the woman/person about the possibility of sensitisation to the D antigen and the nature of HDFN. It is important to note that the absence of immune anti-D 6 months post-birth does not mean that the woman/person has not been sensitised. Women/people who have had FMH greater than 4mL detected should have this highlighted at booking in subsequent pregnancies.

10.0 Missed Anti D doses

If for any reason a dose of Anti D is missed or administered late, the woman/person should be a counselled about the possibility of sensitisation to the D antigen and the nature of HDFN. A follow-up maternal G&S sample should be taken 6 months post-birth. It is important to note that the absence of immune anti-D at 6 months post-birth does not mean that the woman/person has not been sensitised. All women/people who have missed anti D should have this highlighted at booking in subsequent pregnancies.

10.1 Process for missed Anti D follow-up:



11.0 Monitoring / Audit

Deviations from this guideline will be reported through DATIX and to SHOT as appropriate. Errors will be tracked and trended by the Transfusion Practitioner through the Trust Patient Blood Management Committee.

References

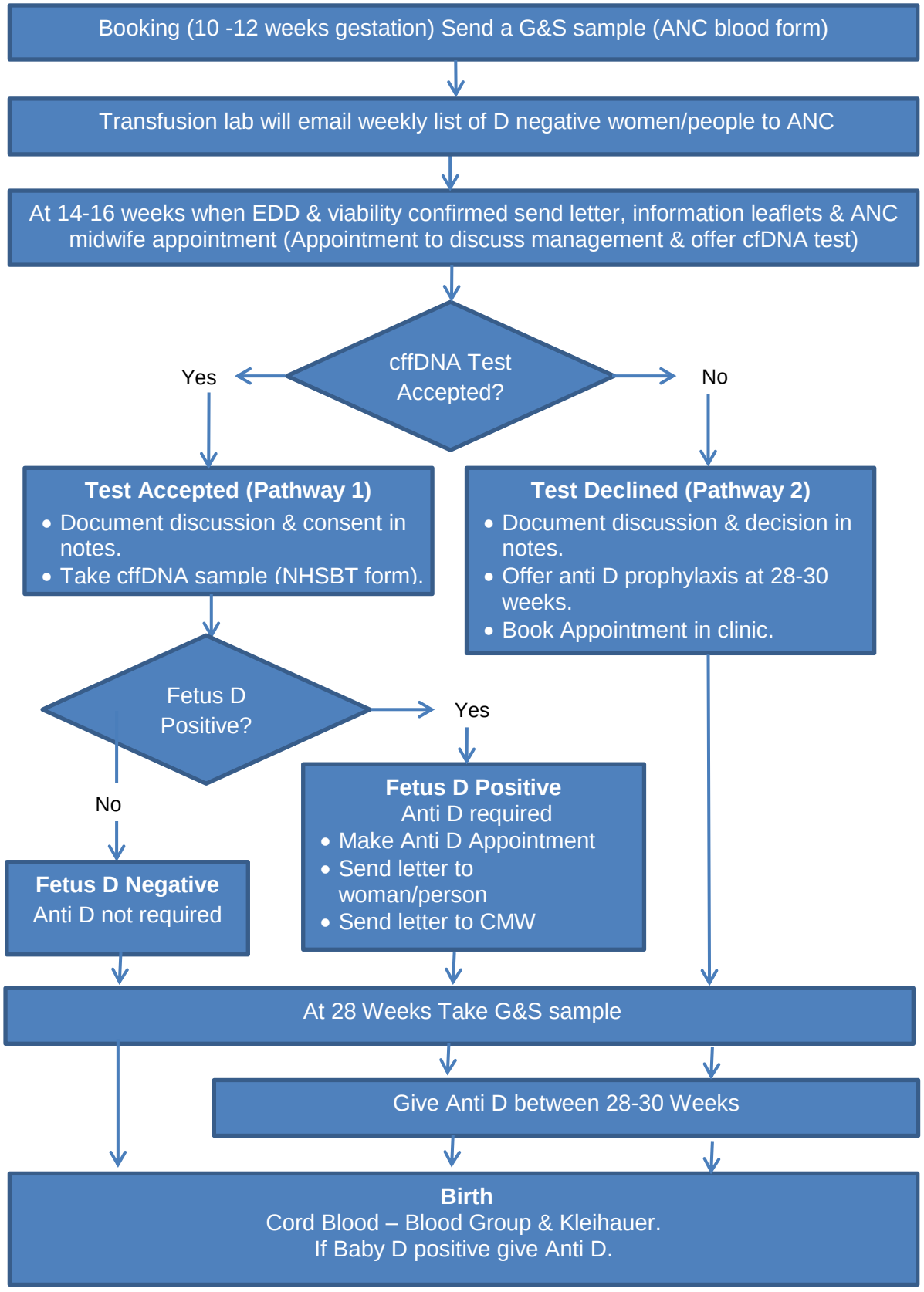
Guidelines for the Estimation of Fetomaternal Haemorrhage. BCSH 2009 [accessed 25/06/19]

BCSH guideline for the use of anti-D immunoglobulin for the prevention of haemolytic disease of the fetus and newborn 2014 [accessed 27/06/22]

NHSBT Fetal RHD Screening User Guide 2017] [accessed 22/06/2022]

NICE guidance documents can be found at: NG 126 and NG 140 [accessed 27/06/22]

Appendix 1: Process flowchart



Appendix 2: Example of D Negative sticker for patient notes