Pregnancy management of Rh Negative Women Application of ffDNA Testing & Anti-D Prophylaxis

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Approved by:	Maternity Governance Meeting	
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Key Amendments

Date	Amendments	Approved by

Introduction:

Patients who are RhD negative have for many years received antenatal prophylactic anti-D to help prevent sensitisation to the D antigen from small bleeds between the fetus and maternal circulations during the final trimester. However, it is also recognised that up to 40% of RhD negative patients will be carrying a fetus who is also RhD negative. This makes the administration of prophylactic anti-D unnecessary so making the risk associated with the administration of a blood product greater than the advantages experienced by receiving the product.

In the 1980's, a technique was developed whereby the fetal DNA which circulates within the maternal circulation could be extracted from a maternal peripheral blood sample. This Free Fetal DNA can then be sequenced to determine if the fetus is RhD positive or RhD negative. This allows the clinical teams to omit the administration for prophylactic anti-D to those patients carrying a RhD negative fetus.

NHSBT has offered a service determining the RhD type of a fetus for a number of years although this is been undertaken manually and as such was expensive and only used to determine the RhD status of a fetus when maternal anti-D was present. In 2015, NHSBT began to routinely offer a more automated service which allows all RhD negative patients to be tested to determine the RhD status of the fetus and permit more individualised treatment.

From 2018, WAHT changed the policy to offer free fetal DNA (ffDNA) testing to all Rh negative pregnant women.

Scope of this Guideline:

This guideline outlines the care pathway for management of Rh negative pregnant women. This covers the principles behind the ffDNA testing and Anti –D Prophylaxis. This provides guidance to the following group of staff members

- Medical and midwifery staff in countywide antenatal clinics and ward areas
- Community teams - midwives and primary care teams
- Other specialties including A&E and EGAU staff

ffDNA testing:

The benefits of this testing would be twofold. There is substantial reduction in the use of anti-RhD immunoglobulin, an expensive blood product in short supply. Women with an RhD negative fetus would be spared unnecessary exposure to this pooled human blood product with its associated discomfort and perceived risk from viral or prion contamination. This risk is exemplified by the infection of hundreds of women with hepatitis C virus transmitted by anti-RhD immunoglobulin in Ireland in 1977-8.

Pathway for ffDNA testing in Rh Negative Women:

See the flow chart in the appendix 1 for care pathway for Rh negative women. Rh status of all pregnant mothers is usually confirmed between 8-12 weeks' gestation via National Blood Service. The Rh negative women are then given information about ffDNA test and get referred to the Anti-D clinics. The Anti D clinics in Worcester, Kidderminster, Evesham and Redditch ANC will undertake ffDNA testing of these women after referral from Community.

Timing of ffDNA test:

Women who consent to bloods for ffDNA are offered a blood test at 15-17 weeks gestation. Sample from gestation <11 weeks may give inaccurate results and repeat sample will be required. Late booking women who present after 22 weeks' gestation will be offered routine anti –D prophylaxis.

The result of the FFDNA test is only valid for the current pregnancy- ffDNA needs to be repeated for each pregnancy. If the woman agrees to the FFDNA testing bloods 6ml sample is taken in a pink top bottle and sent to the laboratory with correct request form. This is documented in the maternity records and a red sticker is placed in case notes. The details of each test are recorded in a database (Paper copy). See appendix 2

Transport of sample and results reporting:

Samples should be sent at room temperature and must be received at NHSBT in Bristol within 7 days of venepuncture. NHSBT transport is used to transfer samples from any pathology laboratory in the country within the specified time via our established routine rounds. The turnaround time for the fetal RHD screening test is currently 14 days but this will be reduced once an increased volume of samples are being analysed.

All results are available via Sp-ICE, the electronic NHSBT reporting system, which enables hospitals to have direct access to reports on-line. Hard copies may also be sent to the requesting pathology laboratory if required. The midwives in ANC inform the result to woman by letter. See appendix 3 and 4. Electronic copies are sent to GP and CMW. The results are then transferred to database

Accuracy of testing:

The overall accuracy and particularly the sensitivity of the fetal RHD screening test are very high. Women with a rhesus‑D (D) positive fetus: only 2 to 4 in 1,000 such women will have a negative test result and so be at risk of sensitisation because they would not be offered antenatal anti‑D immunoglobulin. The causes of false negatives include insufficient fetal DNA and wrong blood in tube.

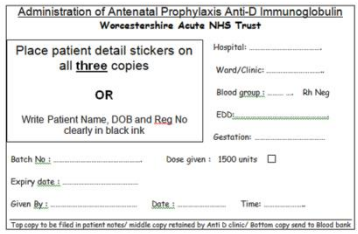
NIPT for fetal RhD genotype is slightly less accurate among women with a D‑negative fetus; between 13 and 57 in 1,000 such women will have a positive test result and so be offered antenatal anti‑D unnecessarily. (1-6% false positive with ffDNA testing compared to about 40% without RHD screening).

Inconclusive result or no result: The reported rate for inconclusive rate is 8%. The recommendation is to treat these results as D positive**.** Follow up has shown that 80% of these cases do result in an RhD positive fetus and therefore recommendation to receive anti-D is correct.

Anti-D Prophylaxis for Rhesus Negative Women:

- **Indications:**
    - Rh negative women who decline ffDNA testing;
    - Fetus tested Rh positive on ffDNA testing
    - Inconclusive result on ffDNA testing
    - Rh negative women who book late at 22 weeks or more
- The midwife will obtain verbal consent from the woman and an appointment for 28 weeks will be made directly with each Anti-D clinic site**.**
- Women who **decline** Anti-D should still be offered antibody screening at **both 28 and 34 weeks**, and should be carefully counselled. Detailed documentation is necessary.
- Anti-D will be administered as Midwife Exemption (DA/ME/11) in the dedicated Anti-D clinic. This maybe within a hospital or community setting provided adequate medical cover and resuscitation equipment is available.

Timing of Anti-D injections:

When	Blood test for Antibody Screen	Dosage and Route
28 weeks (up to **30** weeks) Doses of Anti-D given after 30 weeks can not be guaranteed to provide full cover.	**YES**, **prior** to Anti-D injection Anti-D Data Sheet  Administration of Antenatal Prophylaxis Anti-D Immunoglobulin Worcestershire Acute NHS Trust Place patient detail stickers on all three copies OR Write Patient Name, DOB and Reg No clearly in black ink Hospital: Ward/Clinic: Blood group : Rh Neg EDD: Gestation: Batch No : Dose given : 1500 units Expiry date : Given By : Date : Time: Top copy to be filed in patient notes/ middle copy retained by Anti D clinic/ Bottom copy send to Blood bank	1500 units IM **Deltoid Muscle** Women should remain within the clinic for a period of 15-20 mins following injection for observation in the unlikely event of any reactions occurring.

- The midwife should complete the **Anti-D data sheet** (See table above) and return copies as instructed on form.
- Documentation of the Anti-D batch number and date of administration should be clearly documented in the hand held notes.
- Any woman not attending for this appointment will be notified to the designated community midwife and followed up accordingly.
- Late Bookers should be offered Prophylactic Anti-D and the single dose can be administered according to guideline.

Potentially sensitising events:

Sensitisation can happen at any time during pregnancy, but is most common in the third trimester and during childbirth. Sensitisation can follow events in pregnancy known to be associated with Feto-maternal Haemorrhage, such as H/O fall, abdominal trauma, Obstetric events (Amniocentesis, cordocentesis, Antepartum haemorrhage/ vaginal bleeding in pregnancy, External cephalic version, Intrauterine death and still birth), In-utero therapeutic interventions (transfusion, surgery), Miscarriage, Therapeutic termination of pregnancy.

All women should be reviewed by medical staff following a sensitising event. If a sensitising event occurs and if the ffDNA result shows Fetus is Rh negative, Anti D is not necessary (unless requested by the woman). Kleihauer blood test does not need taken routinely unless requested by medical staff. If fetus is Rh positive, Anti D will be necessary and a Kleihauer blood test advised

If the result is not known or the woman declined ffDNA testing in pregnancy, following a sensitising event she should be reviewed by medical staff and advised to have blood taken for Kleihauer. She will need Anti-D 500iu injection.

Role of Kleihauer test:

Kleihauer test	
Why:	To quantify FMH – (Feto Maternal Haemorrhage)- detection of fetal cells in maternal circulation and to decide the correct dose of Anti D needed. This is indicated if fetal Rh status is not known or ffDNA confirms fetus is Rh positive.
When:	After any Potentially Sensitising Events in Pregnancy in Rh Negative women after 20 weeks of gestation
Timing:	During the admission and assessment: EDTA sample Within 30-45 minutes after the sensitising event. (It takes that time for the fetal cells to get distributed in maternal circulation)
Timing of Anti D injection:	Anti-D (standard dose 500 iu IM) should be given as soon as possible after the potentially sensitising event but always within 72 hours. If FMH >4 mls, patient needs additional Anti D. A 'standard' anti-D immunoglobulin dose of 500iu covers an FMH of up to 4mL fetal red cells, 1250iu up to 10mL and 1500iu up to 12mL when administered intramuscularly. (Should be managed by the Obstetric team)
Risk management issues	It is the responsibility of the Obstetric team who reviews the patient in A&E or in Maternity Triage to follow up the result of Kleihauer test. There should be a thorough handover between the teams to ensure results are followed up.

Frequently Asked Questions:

Can ffDNA test be used in multiple pregnancies?

Yes, this test can be used in Rh negative women 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 of the babies are D negative.

Can samples be tested when pregnant women have alloantibodies other than Anti-D?

Samples can be tested for cell free fetal DNA in the screening test which establishes the predicted RhD status of the fetus.

Can samples be tested when pregnant women have already received Anti-D Ig?

Samples can be tested for cell free fetal DNA. The Anti-D Ig does not interfere with this test.

Which blood tube do I send, pink or purple top?

For the fetal RHD screening test use a 6mL tube; with a pink top.

What is IBGRL's (International Blood Group Reference Laboratory) minimum sample size?

4Ml; This guideline recommends 6 ml of sample collected in a pink top bottle.

What will happen if Rh Negative women present to Triage before test results available?

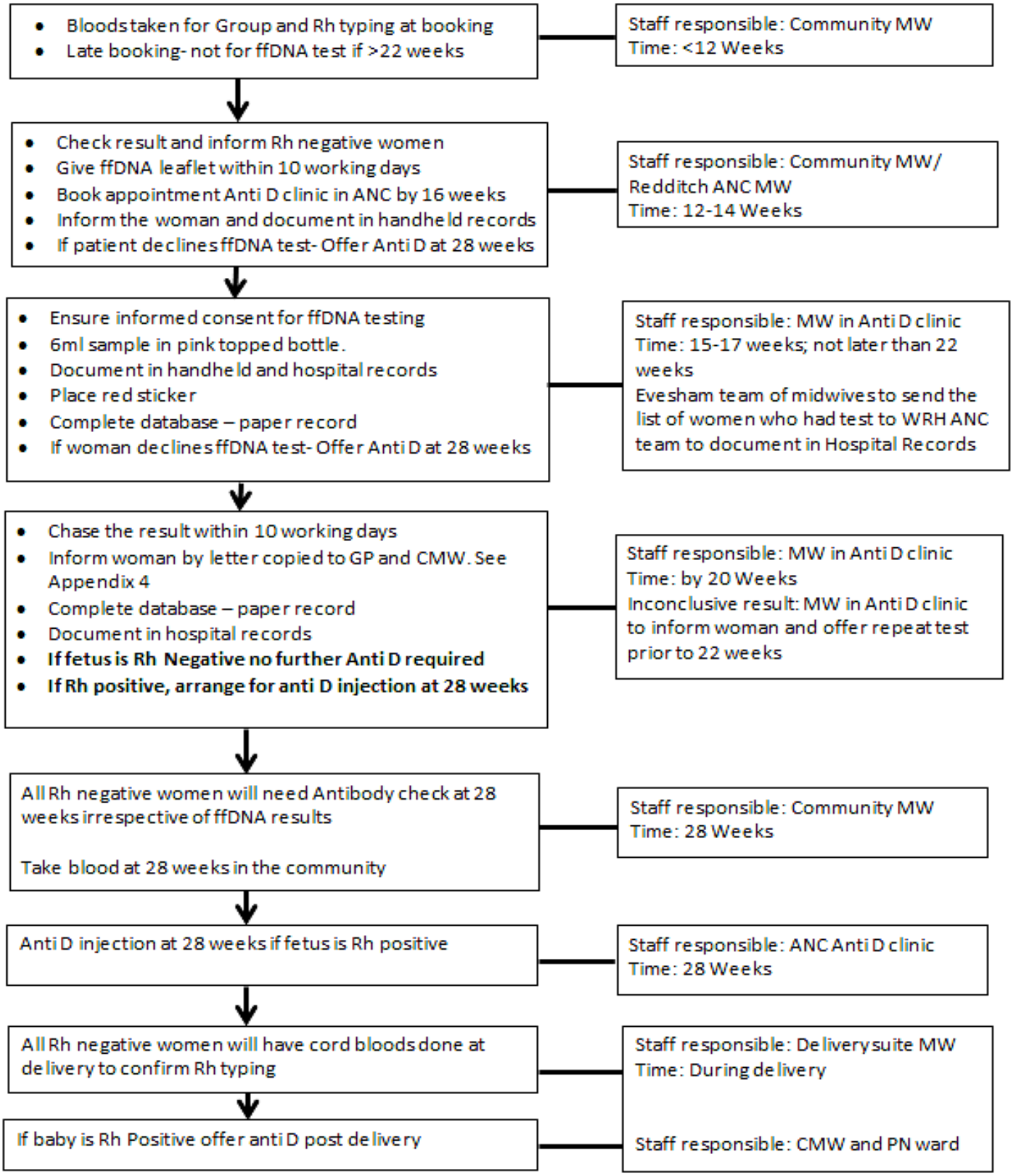
The advice is to give Anti D if indicated clinically and wait for the results.

Would IBGRL ever ask for repeat samples, if yes in which circumstances would they do this?

If the sample could not be tested due to one of the following:

- Insufficient patient identifiers on tube and referral form
- Insufficient sample
- Sample more than 7 days old
- Sample damage e.g. broken tube
- EDD not confirmed by scan
- Sample taken before 11 weeks gestation.

Appendix 1- Pathway for ffDNA testing in Rh Negative Women



Appendix 2

Database - ff DNA testing

Patient label	Date of sample	Gest. Age	EDD	Parity Singleton/ Multiple	Blood taken by:	Results	Checked by: Date:	Anti- D appointment needed or not	Date Letter sent

PRIVATE & CONFIDENTIAL

Worcestershire Royal Hospital
Charles Hastings Way
Worcester
WR5 1DD

Date

Name
Hospital Number
Address

Dear .....

Many thanks for attending ANC Anti-D Clinic for ff-DNA testing on .....We now have the results available.

The test is reported that your baby is **Rh Negative** this means you will not need any Anti-D injections during pregnancy or after delivery.

The midwife who is looking after you during delivery will send cord blood samples to confirm that the baby is Rh Negative.

Keep this letter in your green hand held records for further reference. Kindly let the medical team and the midwife know the test result when it is needed during any hospital admission.

Yours sincerely

Antenatal Clinic Midwife

Name

Signature:

PRIVATE & CONFIDENTIAL

Worcestershire Royal Hospital
Charles Hastings Way
Worcester
WR5 1DD

Date

Name

Hospital Number

Address

Dear .....

Many thanks for attending ANC Anti-D Clinic for ff-DNA testing on .....We now have the results available.

The test is reported that your baby is Rh Positive. We will offer you Anti-D injection at 28 weeks. The appointment will come to you by post.

The midwife who is looking after you during delivery will send cord blood samples to confirm that the baby is Rh Positive

You will also need Anti-D injections in the following circumstances

- Episode of vaginal bleeding
- Obstetric interventions such as amniocentesis, external cephalic version
- Abdominal injury after a fall/blow to the abdomen/traffic accident

Keep this letter in your green hand held records for further reference. Kindly let the medical team and the midwife know the test result when it is needed during any hospital admission.

Yours sincerely

Antenatal Clinic Midwife

Name Signature: