Standard Operational Procedure for Cell Free Fetal DNA (cff-DNA) Testing in Pregnancy, the use of Anti-D Prophylaxis and Sensitising Events

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Version:	V1
Status:	Ratified
Effective from:	26th November 2020
Review:	26th November 2023

cffDNA Pathway 1-Initial Management

Booking appointment with MW (under 24/40)
Group & Antibody screen as per routine Booking Bloods.
Clinic to obtain results as per guideline

Mother Rhesus Positive

No further action needed

Routine Group & Antibody screen at 28 wks

Mother Rhesus NEGA TIVE
Antenatal Clinic to discuss @ first trimester screening appointment.

ANC provides P.I.L and offers cffDNA testing at 16 wk MW appointment.

ANC inform CMW of the need to offer testing at 16 wk appointment. Provide CMW with path request form and pink top bottle.

CMW undertakes 16/40 appointment
Discusses cffDNA

Woman accepts- 7ml pink top bottle sent to lab with form requesting cffDNA testing with EDD clearly stated on form.

Inform clinic that blood has been taken, clinic to check result in 14-16 days and continue on-going management as per pathway 2 for **Fetus Predicted RhD pos** or **RhD neg**

Woman declines
Inform clinic of decision. On-going management as per pathway 2 for **Fetal Blood Group Unknown**

Late Booking Appointment between 24-26 wks
Group & Antibody screen as booking bloods.
Plus offer additional blood sample for cffDNA (7ml EDTA pink top) If accepted by woman, inform clinic of need to check result in 14-16 days and continue on-going management as per pathway 2 either-
Fetus Predicted RhD pos
Fetus Predicted RhD neg
If woman declines cffDNA follow on-going Fetal Group Unknown

Booking appointment with MW (over 26 wks)
Group & Antibody screen as part of routine booking bloods.
Clinic to check results as per guideline

Mother Rhesus Postive
No further action needed

Mother Rhesus Negative
On-going management as per pathway 2 for **Fetal Group Unknown**

★ See cffDNA pathway 2 for On-going Management

cffDNA Pathway 2- Ongoing Management

Following on from cffDNA Pathway 1

Fetus predicted to be RhD Neg

(RhD neg Mother)

- Repeat Group and Antibodies at 28 weeks
- No routine Anti-D prophylaxis necessary
- No need for Anti-D after sensitising events
- Cord blood after birth to confirm baby's blood group
- Maternal Kleihaur **NOT** necessary

Fetus predicted to be RhD pos

(RhD neg Mother)

- Repeat Group and Antibodies at 28 weeks (prior to giving prophylactic Anti-D)
- Anti-D prophylaxis (1500iu) to be given at 28 weeks
- Anti-D required for all potential sensitising events
- At delivery- take maternal blood for kleihaur **AND** Cord blood as it is required as a control for maternal Kleihaur
- Postnatal Anti-D (500iu) to be offered within 72 hours

Fetal blood group unknown

(RhD neg Mother)

- Repeat Group and Antibodies at 28 weeks (prior to giving prophylactic Anti-D)
- Anti-D prophylaxis (1500iu) to be given at 28 weeks
- Anti-D required for all potential sensitising events
- At delivery- take maternal blood for kleihaur **AND** cord blood to determine baby's blood group
- If baby found to be RhD pos, Postnatal Anti-D (500iu) to be offered within 72 hours

1. Purpose/Background:

The purpose of this document is to provide guidance on the offer of cell free fetal DNA (cff-DNA) testing for women who have been identified as having a rhesus negative blood group. This will determine the unborn baby's blood group and therefore prevent unnecessary Anti-D medication being administered to mothers carrying a RhD negative baby. It explains the steps required to support the maternal choice.

It will also cover the administration of routine antenatal Anti-D prophylaxis (RAADP) as a treatment option to women with a confirmed RhD positive baby, or women who decline, or who present too late to have the cff-DNA test.

2. Scope:

This Standard Operating Procedure (SOP) aims to guide all staff in the appropriate use of cff-DNA testing and Anti-D prophylaxis

This SOP applies to all healthcare professionals providing care at St Mary's Maternity Services to RhD-negative women.

3. Responsibilities

It is the responsibility of all Midwifery Nursing and medical staff to:

- Access read understand and apply this SOP
- Attend any mandatory training pertaining to the SOP

It is the responsibility of the department to:

- Ensure the SOP is reviewed as required in line with trust and national recommendations

Ensure the SOP is accessible to all relevant staff

4. Procedure:

4.1 Identification of RhD negative women suitable for cff-DNA testing

- All pregnant women should have their ABO blood group, RhD type and antibody titres checked at booking, and repeated at 28 weeks.

- Staff in the screening office will access results of all booked women and refer to a consultant or monitor appropriately any woman with an antibody positive screen.
- Antenatal Clinic midwives are responsible for checking the booking blood results and identifying if a woman is RhD negative at the first scan appointment.
- Women who are identified to be RhD negative and have **not** been sensitised, are informed following their first scan by the midwife who will explain the implications.
- The women will be offered cff-DNA blood testing and be given a PIL regarding this.

4.2 If a woman declines cff-DNA

- Document her decision and inform her the test can be re-offered until 26+0.
- Antenatal clinic to book woman an appointment to attend Anti-D Clinic for prophylactic administration of 1500iu Anti-D at 28 weeks of pregnancy.
- On-going management of maternal care as for unknown fetal RhD factor (see cff-DNA pathway on Page 2).

4.3 Cff-DNA Testing Accepted

The Antenatal Clinic Midwife will inform the Community Midwife via the Community Co-ordinator.

At the 16 week antenatal appointment the community midwife will:

- Discuss cff-DNA and take consent
- Take a 7ml blood sample in a pink top bottle
- Send blood to the laboratory with cff-DNA request form (Appendix 1).
- Inform Antenatal Clinic that blood test has been performed

Antenatal clinic will check the result in 14-16 days as per local reporting policy. When the result has been obtained, the clinic midwife will inform the woman and the community midwife via Community Co-ordinator.

- If the baby is predicted to be **RhD Negative**:
  - No need for Anti-D after sensitising events.
  - No routine Anti-D prophylaxis necessary.
  - Repeat 28 week Group and Antibodies with Full Blood Count as with all antenatal women.
  - At delivery, cord blood will be required to confirm baby's blood group
  - No maternal blood, as Kleihaur not necessary
- If Baby predicted to be **RhD positive**:
  - Anti-D will be required for all potential sensitising events
  - Anti-D (1500 IU) prophylaxis to be given at 28 weeks
  - At delivery, maternal blood will be required for Kleihaur
  - Cord blood needed as control for maternal Kleihaur
  - Offer postnatal Anti-D within 72 hours

4.4 Administration of RAADP at 28 Weeks Gestation for women with a predicted RhD positive baby, or those that decline cff-DNA testing

- Patient issued Anti-D will be ordered by the Antenatal Clinic Midwife from blood bank prior to each Anti-D clinic. It should be stored in the drug fridge in Antenatal clinic until required and the temperature must be maintained between 2℃ and 8℃.
- 28 week blood samples for FBC (purple top bottle) and group and antibody screen (pink top bottle) must be taken **PRIOR** to administration of RAADP. Samples taken after the administration could result in passive anti-D being detected which may be mistaken for immune anti-D.
- Obtain consent from the woman for RAADP.
- Administer 1500iu of anti-D, this is in addition to any anti-D already received following potentially sensitising events during pregnancy.
- Clearly document the date of administration, batch number of anti-D and injection site in the woman's hand held notes. A prescription sheet should also

be completed. Anti-D can be prescribed by the midwife and is covered under the midwives exemptions.

- The issue form must be signed by the midwife administering the anti-D. The peel off part of the compatibility label should be applied to the woman's hand held notes and the other part of the red label returned to Blood Bank for traceability of the vial. A pink Anti-D administration sticker should be completed and applied to the woman's hard copy as a failsafe.
- The woman must be advised to remain in the clinic for 20 minutes after administration, in case of adverse reactions.
- Allergic responses to anti-D immunoglobulin are rare (0.01 – 0.1%). Women should be informed of the early signs of hypersensitivity reactions.
- **I**f the 28 week appointment is missed, the woman must be contacted and a new appointment made for administration of anti-D at the next available opportunity. It is the responsibility of the staff in the Anti-D clinic to follow up missed appointments.

4.5 RAADP Late in Pregnancy

Women presenting after 28 weeks gestation

- The single dose of 1500iu of anti-D may be administered in accordance with the RAADP programme at any gestation during the pregnancy, following the same procedure as stated above.
- If the woman presents during labour, and is found to be RhD negative, a kleihaur and cord blood will need to be taken following delivery. Anti-D will be given post-partum if the baby is RhD positive.

4.6 If the woman declines RAADP

- The reasons for her decline should be explored, documented in the maternity notes by the midwife caring for her, and the Antenatal Screening Co-ordinator informed.
- Blood should be then taken for antibody screen at 28 weeks and following any sensitising event (see 5.4).

4.7 Potentially Sensitising Events in Women:

After the following potentially sensitising events during pregnancy, Anti-D immunoglobulin should be given to all non-sensitised RhD negative women with cff-DNA testing Predicting a RhD positive baby (or women who have declined cff-DNA). This is in addition to any anti-D already received.

- Pregnancy loss involving uterine evacuation or termination of pregnancy
- Threatened or complete miscarriage after 12 weeks of gestation
- Ectopic pregnancy
- Invasive prenatal procedure i.e. amniocentesis or chorion villus sampling
- External cephalic version
- Antepartum haemorrhage
- Abdominal trauma/fall/accident
- Intrauterine death
- Stillbirth
- Delivery of RhD positive infant

4.7.1 Sensitising events less than 12 weeks of gestation

Anti-D prophylaxis is not required for bleeding or miscarriage at less than 12 weeks of gestation, unless surgical intervention is required. The risk of Fetal Maternal Haemmorrhage is minimal at these gestations. However, if bleeding is persistent, heavy or gestation is doubtful, women should be referred for further assessment to EPAU and the gestation should be confirmed by ultrasound.

4.7.2 Sensitising events after 12 weeks gestation

- Following sensitising events the following must be sent to the laboratory:
  - Over 20 weeks gestation, a 3ml (purple top) bottle for Kleihauer test, (this is not required and **should not** be requested below 20 weeks).
  - 6 ml (pink top) bottle for Blood Group/Antibody screen.

- Haematology form – clearly write: gestation and 'potential sensitising event' in clinical details section (please avoid using abbreviations) and whether the woman has had Anti-D administered in this pregnancy

- A dose of 500iu of anti-D immunoglobulin is recommended **for each and every** sensitising event.
- Once it has been administered (see 5.5) an 'Anti-D Administration Form' (Appendix 2) should also be completed and sent to the laboratory.

4.8 Postnatal Prophylaxis

4.8.1 Samples required from Mother:

- Maternal blood is **not required** when baby predicted to have a RhD Neg blood group
- If the baby's blood group is predicted to be RhD positive or it is unknown, the following **must be taken 1-2 hours after birth** and sent the laboratory (prior to administration of Anti-D):
- 3 ml (purple top) bottle for Kleihauer test/Full Blood Count
- 6ml (pink top) bottle for Blood Group /Antibody screen
- Pre-stamped Haematology form

4.8.2 Samples from the cord

- Cord blood following delivery is required for **ALL** babies of RhD negative women.
- In cases where cff-DNA testing has predicted a RhD negative baby, it is to confirm the baby's blood group.
- In cases when cff-DNA has predicted baby to be RhD positive, it is used as a control for the maternal Kleihaur.
- Cord blood should be taken using a syringe and needle to take the blood from an umbilical cord vessel. The following is required by the Path lab:
    - 3 ml (purple top) bottle for Blood Group with a yellow 'cord blood' sticker around the top of the sample bottle.

- Pre-stamped Haematology form.
- The baby's IW number must be on the haematology form

- At least 500iu of anti-D should be given to every non sensitised RhD negative women **within 72 hours** of delivery of a RhD positive baby with maternal consent. Once it has been administered an 'Anti-D Administration Form' should also be completed and sent to the laboratory.

If the pregnancy is non-viable and no sample can be obtained from the baby, 500iu anti-D should be administered to non-sensitised RhD negative woman.

If the IW number is not obtainable (e.g. due to computer failure), Labour ward staff should contact A&E who can issue a Unique Identifier (UI) number to be used until an IW number can be generated.

4.9 Administration of Anti-D

4.9.1 Storage and suitability

- 500iu of Anti-D is held as a stock item in the Omnicell fridge on Maternity ward which must be maintained between 2 and 8°C, and the temperature checked and documented daily.
- Anti-D should always be given as soon as possible after the sensitising event and **always within 72 hours**. If it is not given before 72 hours, every effort should still be made to administer the anti-D, as a dose given within 9-10 days may provide some protection.
- Women who are already sensitised should not be given anti-D.
- Women who have haemoglobinopathy or clotting disorders should be referred to an obstetrician and receive anti-D immunoglobulin via the subcutaneous or intravenous route.
- If a large Fetal Maternal Haemorrhage has occurred, blood bank will, where indicated, prescribe the amount of additional Anti-D required.

4.9.2 Process for Anti-D administration from maternity

- When Anti-D is required, remove a dose from the omnicell, and check the dose, expiry date and Lot number with a second registered colleague.
- The Mother's identity, hospital number, blood group and reason for administration also need to be confirmed with the 2nd checker.
- An 'Anti D administration form' needs to be fully completed including documenting any previous doses of Anti-D that have been given in the pregnancy and sent to blood bank.
- The Anti-D should be given in the deltoid muscle and the Mother encouraged to remain on the ward for 20 minutes after the dose is given.
- Following administration of the Anti-D, complete either an Ante-natal or Postnatal Administration sticker (Appendix 3), and place in the handheld notes.
- The dose also needs to be documented in e-care-logic to allow blood bank be able to see previous doses that have been administered (please see appendix 4 for correct procedure).

4.10 Consent

All women that are offered anti-D should be made aware that it is a blood product and verbal consent should be obtained before administering. This particularly concerns women who would prefer not to receive blood products e.g. Jehovah Witnesses.

5. Implementation/training/awareness

- This is a review of a current document and it formalises current practice.
- Once ratified it will be available in all clinical areas within the Maternity Unit and on the intranet.
- All new, reviewed and ratified documents are notified to staff via the monthly maternity newsletter

6. Auditable Standards

Quarterly audit of 5 sets of postnatal notes by ANNB screening coordinator to monitor:

Antenatal

- Blood group and antibody status documented in hand held notes
- PIL given
- Anti D given within 72 hours of sensitising event
- Kleihauer taken if APH after 20wks
- Blood taken for antibodies prior to RAADP administration.
- RAADP given between 28- 30 wks.- documentation of date, time, batch no, and consent
- Given into deltoid muscle.

Postnatal

- Cord and maternal blood (Kleihauer) taken after birth.
- Anti D administration within 72 hours

Notable practice and lessons learned will be disseminated via the screening newsletter.

Any high risk assessments will be escalated to the risk management.

7. Related Documents:

Guidelines /SOP's

- Standard Operating Procedure for the provision of Antenatal Care
- Patient Information Leaflet 'D Negative mother's Blood Test to check her unborn baby's blood group' NHS Blood & Transplant Feb 2019

8. References:

- National Institute for Clinical Excellence (2008) *Routine antenatal anti-D prophylaxis for women who are rhesus D negative. Review of NICE technology appraisal guidance 41* London: NICE

- Royal College of Obstetricians and Gynaecologists (2011) *Green top guideline No 2. 'The Use of anti-D Immunoglobulin for Rhesus D Prophylaxis.* London: RCOG

9. DISCLAIMER

It is the responsibility of staff to check the Trust intranet to ensure that the most recent version/issue of this document is being referenced.

DOCUMENT HISTORY					
Date of Issue	**Version No.**	**Next Review Date**	**Date Approved**	**Director Responsible for Change**	**Nature of Change**
Nov 2005	1.0	November 2007	November 2005	D Reece	Ratified
Dec 2011	2.0	20th December 2014	20th December 2011	A Pearson	Ratified
Nov 2016	3.0	May 2019	November 2016	Anya Wright	Ratified
July 2020	4.0	August 2021	31st July	Amanda Rendell/MCSG	Ratified
Nov 2020	SOP v1.0	Nov 2023	26th Nov 2020	MCSG	Converted to SOP, reviewed and ratified

Appendix 1 cffDNA Blood Form

Place labelled speciment in bag, remove protective strip, fold flap onto bag and seal firmly.

Request for cell free fetal DNA (cffDNA) Screen
RhD Fetal Genotyping Service

NHS Blood and Transplant

This form is only to be used for RhD negative pregnant women.

Please DO NOT USE this form for samples from women who have anti-D antibodies. For those cases, please speak to the Fetal Maternal Unit first (a different form and sample volume are required).

At least three points of matching identification must be used on form and sample tubes

Mother's Details:

NHS No. ..... **or* Hospital No.** .....

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

Surname .....

First name .....

Address .....

.....

.....

.....

.....

DOB ..... **EDD from scan*** .....

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

Please provide 6ml EDTA blood sample from the mother (store at room temperature)

Date of sample taken ..... **Name of person taking sample** .....

Hospital and Requester Details:

Full Hospital Trust Name ..... **Hospital NHS Code*** .....

*ODS code (Formerly NACS code)

Midwife code ..... **Practice code** .....

Sender's name and address Telephone: Email:	For Hospital Laboratory use Date received:
SEND SAMPLE WITH THIS FORM TO THE PATHOLOGY LABORATORY **Instructions for Laboratory Reception** Follow Hospital Trust SOP. See sample labelling and transport instructions on the reverse of this form.	For NHSBT use Date received:

FRM5197/1.1 Effective: 16/11/15 1516474

Appendix 2: Anti-D Administration Form:

Anti-D Administration Form-500iu

(RhD Neg Patients Only)

Patient Surname : .....

Forename : .....

DOB : .....

Hosp. No : .....

Ward : .....

Consultant : .....

Blood Group : .....

Reason for administration : Sensitising Event: ☐ Post Delivery: ☐

Baby's predicted blood group (via cff-DNA) : RhD Pos: ☐ RhD Neg: ☐ Unknown: ☐

Antenatal - Gestational age : .....

Postnatal –

Date and time of delivery : __ __/__ __/__ __ __ __ __ __:__ __ hrs

Cord blood taken (all baby's): ☐ Kleihaur taken (if baby RhD Pos or blood group unknown): ☐

Previous Anti-D given : Yes / No

If Yes, date given : __/__/__ __ __ __ Dose __ __ __ IU

If baby RhD. Pos - Anti-D given : Dose __ __ __ IU Lot No _____

Expiry Date: __ __/__ __/__ __ __ __

Date and time given : __ __/__ __/__ __ __ __ ____:__ __ hrs

Given by : Dr/Midwife .....

Signature : .....

Lab use only

Lab Number:

KL: Foetal cells seen? : Yes / No / N/A (Baby RhD Neg)

Controls as expected : Yes / No

Date & time KL tested : __ __/__ __/__ __ __ __ __ __:__ __ Sig: .....

Further Anti-D required : Yes / No

If Yes, Date and Time Maternity contacted : __ __/__ __/__ __ __ __ __ __:__ __ hrs

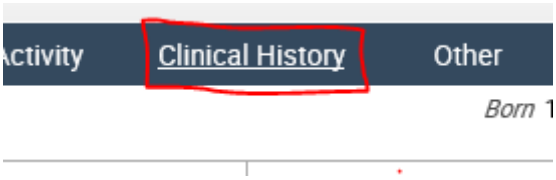
Appendix 3: Anti-D Administration stickers:

ANTE-NATAL ANTI-D ADMINISTRATION	
DATE OF ADMINISTRATION:	
TIME OF ADMINISTRATION:	
DOSE:	
LOT NO:	
EXPIRY DATE:	
KLEIHAUR AND ANTIBODY SCREEN SENT:	**DATE:** **TIME:**
ANTI-D ADMINISTRATION FORM COMPLETED AND SENT:	☐
DOCUMENTED ON E-CARE LOGIC:	☐
SIGNATURE 1:	**SIGNATURE 2**

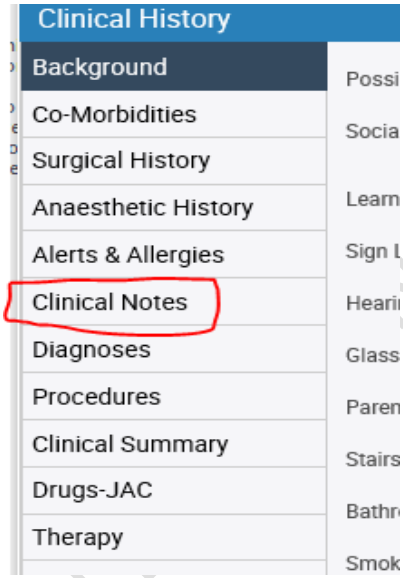
POSTNATAL ANTI-D ADMINISTRATION	
DATE OF ADMINISTRATION:	
TIME OF ADMINISTRATION:	
DOSE:	
LOT NO:	
EXPIRY DATE:	
KLEIHAUR AND ANTIBODY SCREEN SENT:	**DATE:** **TIME**
CORD BLOOD SENT:	**DATE:** **TIME:**
ANTI-D ADMINISTRATION FORM COMPLETED AND SENT:	☐
DOCUMENTED ON E-CARE LOGIC:	☐
SIGNATURE 1:	**SIGNATURE 2:**

Appendix 4- Process for inputting Anti-D dose on E-care Logic

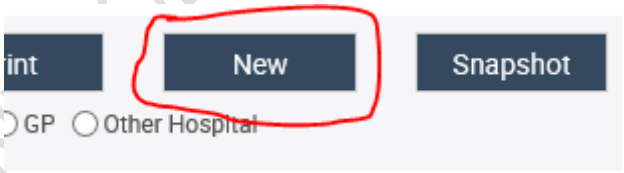
Open E-Care Logic and search for patient
Once correct patient record selected Click on '**CLINICAL HISTORY**'



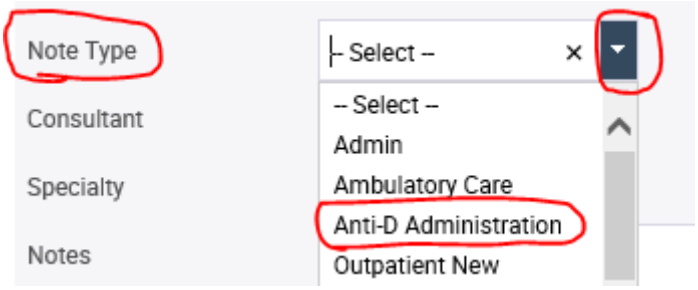
Click on '**CLINICAL NOTES'**



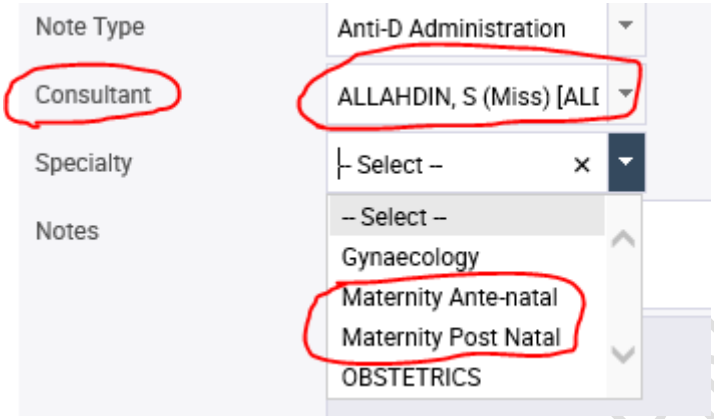
Click on '**NEW**'



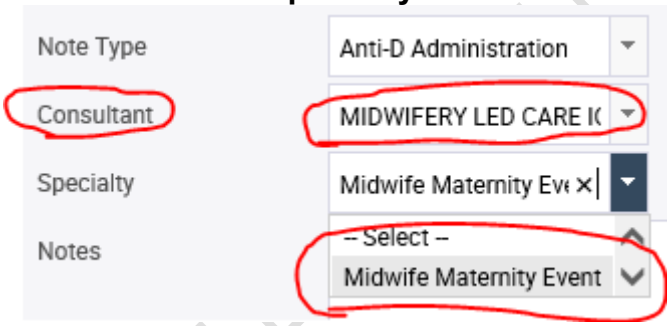
On the '**NOTE TYPE**' dropdown menu, select '**ANTI-D ADMINISTRATION'**



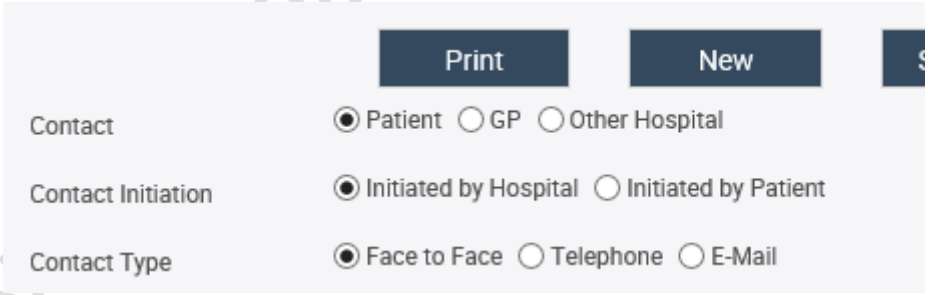
Ensure the patients correct Consultant is selected and under '**SPECIALITY**' select either '**MATERNITY ANTE-NATAL/POSTNATAL'** depending on timing of Anti-D administration



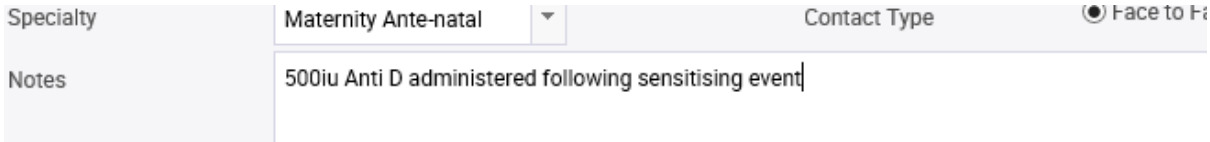
If the patient is Midwife Led Care the '**speciality'** is '**Midwife Maternity Event'**



Click on '**Patient**', '**Initiated by Hospital**' and '**Face to Face**' as below



Write dose and reason for administration in '**Notes**' box



Click '**SAVE'**