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.

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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.

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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.

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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.

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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)

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