

Accuracy of non-invasive fetal blood group genotyping and sexing at the International Blood Group Reference Laboratory, NHS Blood and Transplant – information sheet for service users

Definition of “false negative result”

For fetal blood group genotyping tests a false negative result means that NHSBT has predicted an antigen negative fetus when the baby is found to be antigen positive at birth. For fetal sexing, a false negative result means that NHSBT has predicted a female fetus when the baby is male (Y chromosome positive).

Definition of “false positive result”

For fetal blood group genotyping tests a false positive result means that NHSBT has predicted an antigen positive fetus when the baby is found to be antigen negative at birth. For fetal sexing, a false positive result means that NHSBT has predicted a male fetus when the baby is female (Y chromosome negative).

How is accuracy calculated?

Assays were initially validated using maternal plasma samples from pregnancies in which invasively derived fetal material was available to verify the results. The accuracy of our test results are monitored and all incorrect results notified to us are investigated as quality incidents (with the exception of false positive results for our fetal *RHD* screening test). Verification of correct results are also recorded. These records are periodically reviewed to revise our accuracy statistics. However, we do not receive feedback on the vast majority of tests performed, particularly for blood groups other than RhD. Therefore, it is not possible to provide accurate sensitivity and specificity of all tests.

The importance of feedback on discrepancy between genotype and phenotype – what should I do if I identify a discrepancy?

Your feedback on our test results is vital to maintain accurate data on how our tests perform. If you identify an incorrect result, please contact molecular.diagnostics@nhsbt.nhs.uk as soon as possible and retain cord blood and maternal blood.

What will NHSBT do if they are notified of an incorrect result?

NHSBT will ask you to send a sample of cord and maternal blood to IBGRL for investigation. Please note that we will not investigate false positive results for the fetal *RHD* screening test but would like to ask you to report this to us. IBGRL will check for wrong blood in tube and will re-test the cord sample to confirm genotype and phenotype. If possible, the original plasma sample will be re-tested. The case will be investigated as a quality incident (or hospital complaint) in the NHSBT quality management system and if error in procedure is identified, corrective and preventative actions will be put into place. You will be notified of the findings and any recommended further action.

Reasons for incorrect results

Fetal DNA in maternal plasma represents a very small fraction of the total DNA in plasma. The amount of fetal DNA increases during pregnancy. However, it can vary between individuals and in some cases the amount of fetal DNA may be too low to detect, especially in early pregnancy. This can cause a false negative result.

Other causes of false negative results may be:

- Error (human or mechanical) in testing
- Wrong blood in tube

False positive results may, on rare occasions, be caused by presence of genes which are not expressed on red cell surface (i.e. the phenotype does not reflect genotype). Some blood group genes are inactivated by mutations distinct from the blood group gene itself. Other causes of false positive results may be:

- Extraneous contamination of the blood sample
- Extraneous contamination of testing reagents (all normal precautions are taken to ensure this does not occur)
- Error (human or mechanical) in testing
- Wrong blood in tube
- Vanishing twin

ACCURACY RATES FOR NON-INVASIVE FETAL BLOOD GROUP GENOTYPING AND SEXING

Fetal *RHD* screening

Based on a prospective, population-based cohort study (Chitty *et al*, *BMJ* 2014: 349) in which cord blood group data was collected at birth, sensitivity for detection of fetal RHD positivity was 99.83%, 99.67%, 99.82% and 100% at completed weeks gestation 11-13, 14-17, 18-23 and >23 respectively. After 11 weeks gestation the incidence of false negative results was 0.1%.

The false negative rate is constantly monitored based on customer feedback of cord blood group discrepancy with predicted phenotype and is updated each time a false negative result is confirmed.

Current false negative rate is at or is below 0.1%.

Diagnostic fetal blood group genotyping and fetal sexing

Non-invasive fetal RHD genotyping

Between 2001 and 2014 a total of 2514 fetal *RHD* genotyping tests were performed of which 2 tests were reported to have been false negatives and 5 as false positives. This equates to a sensitivity of 99.8% and specificity of 99.2%.

Non-invasive fetal Rh C genotyping

Fetal C genotyping was performed in 184 pregnancies between 03/08/06 and 30/09/18. We received confirmation that 4 tests predicting a C negative baby were correct; we have not been informed of any incorrect results.

Non-invasive fetal Rh c genotyping

Fetal c genotyping was performed in 816 pregnancies between 2003 to 31/12/17. 48 results were confirmed to be correct following cord blood testing (40 correctly confirmed c positive, 8 correctly confirmed c negative). We have not been notified of any incorrect results.

Non-invasive fetal Rh E genotyping

Fetal E genotyping was performed in 782 pregnancies between 2003 to 31/12/17. 66 results were confirmed to be correct following cord blood testing (26 correctly confirmed E positive, 40 correctly confirmed E negative). We have not been notified of any incorrect results.

Non-invasive fetal Kell (K1) genotyping

An audit was performed of all samples referred between 1st Jan 2004 and 30th Sept 2017. Only 4% of women had their cord blood Kell group confirmed at birth. 6 women were found to have false negative results during pregnancy which equates to a false negative rate of 0.5% for fetal K1 genotyping (95% CI 0.24%-1.2%).

Non-invasive fetal sexing

A review of fetal sexing was performed: Hill *et al*, *Clinical Genetics*, 2011:80; 68-75. In phase 2 of this audit the fetal sexing statistics for IBGRL were 99.2% accuracy, 98.5% sensitivity, 100% specificity.

A NHSBT clinical audit was performed in 2011 following change in standard operating procedure: accuracy rate in this audit was 100%. We have not been notified of any incorrect results since this time.