SHOT Bite No. 28
Cell-free fetal DNA (cffDNA) screening errors

SHOT Serious Hazards of Transfusion
November 2023

During pregnancy, fetal DNA is shed into the maternal blood system. This is referred to as cell-free fetal deoxyribonucleic acid (cffDNA). The cffDNA is cleared from the maternal circulation soon after delivery. Fetal DNA can be extracted from a maternal blood sample allowing for non-invasive prenatal testing (NIPT) for a variety of screening and diagnostic assays, including predicting the fetal D-type. In 2016, the National Institute for Health and Care Excellence (NICE) recommended high-throughput NIPT for fetal *RHD* genotype. In non-immunised women, the cffDNA screening testing predicts the fetal D-type for the current pregnancy so that D-negative pregnant mothers can avoid receiving antenatal anti-D Immunoglobulin (Ig) if carrying a D-negative baby. Since 2018, SHOT has been collecting data on anti-D Ig errors relating to cffDNA screening to provide recommendations for improvements in practice.

Useful facts relating to the cffDNA screening test

Fetal *RHD* screening service is available from 11^{+2} weeks gestation in D-negative pregnancies

This test is not indicated for pregnant women with immune anti-D
In these cases, samples should be tested for non-invasive fetal genotyping (diagnostic testing)

The assay has limitations, with sensitivity of 99.3% (95% confidence interval [CI] 0.982-0.997) and specificity of 98.4% (95% CI 0.964-0.993) (Mackie et al. 2017), leading to a small risk of false-positive or false-negative cffDNA screening results

False-positive and false-negative cffDNA screening results should be reported to SHOT and to the test provider to assess accuracy of the test

False positives can be due to vanishing twin, extraneous assay or sample contamination, wrong blood in tube (WBIT), error (human or mechanical) in testing and presence of genes but antigen not expressed on red cell. False negatives can result from insufficient fetal DNA, WBIT or error (human or mechanical) in testing

Errors related to interpretation, reporting by hospitals or availability of cffDNA screening results should also be submitted to SHOT

Summary of cases reported to SHOT 2019-2022: SHOT analysed 127 cases relating to cffDNA screening during this period

Failure to check cffDNA screening results prior to order, release or administration of anti-D Ig leading to inappropriate administration of anti-D Ig to mother with D-negative fetus	47
Cord blood D-type discrepant with predicted D-type – false positive leading to inappropriate administration of anti-D Ig to mother with D-negative fetus	34
Cord blood D-type discrepant with predicted D-type – false negative leading to omission of anti-D Ig	24
Misinterpretation or misunderstanding cffDNA screening results causing unnecessary administration or omitted administration of anti-D Ig	14
Results not available to the clinical team due to a laboratory delay in entering cffDNA screening results into the laboratory information management system (LIMS) leading to inappropriate administration of anti-D Ig to mother with D-negative fetus	2
The cffDNA result checked prior to administration of anti-D Ig was from a previous pregnancy causing unnecessary administration or omitted/late administration of anti-D Ig	2
Miscellaneous – incorrect advice from laboratory, WBIT (cord sample), transcription error and patient insistence on received anti-D Ig despite cffDNA screening predicting D-negative fetus	4

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SHOT key messages and recommendations (Annual SHOT Reports 2020-2022)

Non-invasive prenatal testing for fetal D-type should be made available to all D-negative women in the UK during pregnancy

Processes should be in place to ensure that cffDNA screening D-type results are reviewed prior to order or administration of anti-D Ig for routine antenatal anti-D Ig prophylaxis (RAADP) or for potential sensitising events (PSE)

Cases where the cord D-type is discrepant with the D-type predicted by cffDNA screening test should be investigated in a timely manner to ensure appropriate administration of anti-D Ig, if required

Ensure that cffDNA screening results are for the current pregnancy, and available results should be entered into the LIMS in a timely manner to avoid unnecessary issuing of anti-D Ig

Focus on information technology (IT) to improve management of D-negative pregnancies

IT supports safe and appropriate management of anti-D Ig based on the cffDNA screening result

IT can access:
- Pregnancy status
- Maternal D-type
- cffDNA result

And support →

- Clinical decision-making
- Checklists for appropriate administration
- Failsafes to prevent omissions and delays
- Flags and alerts for anti-D Ig administration
- Reminder for appointments

Case study – misinterpretation of cffDNA results: *Inconclusive* is different to *predicted D-positive*

Call received from maternity requesting 1500IU anti-D Ig → BMS checked cffDNA results for current pregnancy → BMS assumed that an inconclusive cffDNA result would trigger the same actions as predicted D-positive → BMS issued 1500IU anti-D Ig before testing cord sample → Baby's group was D-negative and unnecessary anti-D Ig injection administered

Benefits of cffDNA screening test
- Preventing unnecessary administration of anti-D Ig and associated risk for D-negative mothers when the fetal D-type is predicted as D-negative
- Reducing the number of antenatal anti-D Ig prophylactic clinic appointments needed, and the amount of anti-D Ig used
- Increasing the availability of anti-D Ig for use after PSE in pregnancy when the screening result for fetal D-type is positive or unknown
- Providing information to allow D-negative mothers to make an informed decision about whether to have treatment with anti-D Ig

Useful Resources

SHOT Bite No 2: Anti-D Ig Administration: https://www.shotuk.org/resources/current-resources/shot-bites/
SHOT video Anti-D Ig and Immune anti-D (part 1 and part 2): https://www.shotuk.org/resources/current-resources/videos
IT supports anti-D Ig management in pregnancy: https://www.shotuk.org/resources/current-resources/
Template for investigation of discrepant cffDNA results in hospitals: https://www.shotuk.org/resources/current-resources/
Rh-D Haemolytic Disease of the fetus and newborn – The role of SHOT in improving care: https://www.rcpath.org/profession/publications/college-bulletin/july-2021/rh-d-haemolytic-disease-of-the-fetus-and-newborn-the-role-of-shot-in-improving-care.html
Fetal RhD screening of maternal blood to support targeted anti-D prophylaxis – the story so far: https://www.rcpath.org/profession/publications/college-bulletin/october-2023/fetal-rhd-screening-of-maternal-blood-to-support-targeted-anti-d-prophylaxis-the-story-so-far.html
Mackie et al. (2017) The accuracy of cell-free fetal DNA-based non-invasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis: https://pubmed.ncbi.nlm.nih.gov/27245374/