

Feto-maternal haemorrhage estimation

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Significance

One of the known complications of pregnancy is the transfer of fetal red cells into the maternal circulation, therefore, feto-maternal haemorrhage (FMH). While there are certain situations (sensitising events) where there is a known increased risk of a FMH occurring, studies suggest that bleeds of less than 1ml of fetal blood into maternal circulation occur in 96 per cent of normal pregnancies and losses of 30ml in 0.3 per cent of pregnancies.¹

'Any fetal red cells that cross the placenta into the maternal circulation and are incompatible with the maternal blood group have the potential to stimulate maternal red cell antibody production.'

Any fetal red cells that cross the placenta into the maternal circulation and are incompatible with the maternal blood group have the potential to stimulate maternal red cell antibody production. These antibodies can then cross the placenta and cause haemolytic disease of the fetus or newborn (HDFN). Most commonly, this has been associated with an RhD negative mother and RhD positive fetus, with the D antigen on fetal red cells stimulating production of maternal anti-D. This leads to destruction of fetal red cells and in severe cases fetal death. It is known that as little as 0.1ml of fetal red cells is sufficient to immunise an RhD negative mother.²

In 2003, the Australian National Health and Medical Research Council (NHMRC) released a guideline recommending the use of prophylactic RhD immunoglobulin (RhD Ig) for all RhD negative women without pre-formed anti-D at 28 and 34 weeks gestation and within 72 hours of delivering an RhD positive baby.³ RhD Ig should also be given for any antenatal sensitising event (see Table 1). This has decreased the incidence of RhD immunisation during pregnancy to 0.2 to one per cent.⁴ One cause for the continued low immunisation rate could be an FMH larger than the protection offered by a single dose of RhD Ig. Administration of 20ug (100IU) of RhD Ig is known to provide protection against 1ml of RhD positive red cells.⁵ Therefore, a dose of 125ug/625IU would provide protection against 6ml of fetal red cells. For an FMH larger than 6ml, it is necessary to administer a larger dose of RhD Ig to prevent immunisation.

Indication for FMH estimation

Any RhD negative woman presenting with a sensitising event at greater than 12 weeks gestation should have a FMH estimation (FMHE) performed. The test should also be performed immediately postpartum. In the event of a large FMH, the FMHE is useful to determine how many doses of RhD Ig should be given to protect the woman from RhD immunisation. RhD Ig is ideally given within 72 hours of the event but may provide protection if given later. A large FMH may be associated with unexplained fetal death in utero (FDIU) near term and unexplained fetal anaemia. In these cases, FMHE may be useful for RhD positive women to aid in determining the cause of a fetal death in utero or fetal anaemia. At the Royal Women's Hospital (RWH) in Melbourne, FMHE is performed as requested for pregnancies greater than 12 weeks gestation presenting with a sensitising event. It is not usually considered an urgent test and all requests are batched and performed once per day. RWH performs an average of 136 FMHE per month. In the last year, there has been eight FMHs of greater than 6ml, including two greater than 100ml, requiring additional doses of RhD Ig to be administered. Remembering that as little as 0.1ml of red cells can sensitise an RhD negative woman, it is vital to give the extra vials of RhD Ig for any FMH greater than 6ml.

Table 1. Antenatal sensitising events as an indication for feto-maternal haemorrhage.

Sensitising event	Sensitising event
Miscarriage	Retained products of conception
Fetal blood sampling	Dilation and curettage
Fetal blood sampling	Query concealed antepartum haemorrhage
Threatened miscarriage	Termination of pregnancy
Antepartum haemorrhage	Ectopic pregnancy
Incomplete abortion	Delivery of an RhD positive or Du positive baby (alive or stillborn)
Bleeding placenta praevia	Extra cephalic version
Missed abortion	Fetal death in utero
Fall/abdominal trauma	Chorionic villous sampling
Complete abortion	Cordocentesis amniocentesis
Motor vehicle accident	

Feto-maternal haemorrhage estimation

There are currently two techniques available at RWH to estimate the volume of a feto-maternal haemorrhage: the acid elution test (commonly referred to as the Kleihauer-Betke test) and estimation by flow cytometry. At RWH, the Kleihauer-Betke test is used to screen for FMH. Any FMH of greater than 5ml detected by the Kleihauer-Betke test is then tested by flow cytometry to quantitate the exact volume.

Acid elution (Kleihauer-Betke) test

The acid elution test first described by Kleihauer *et al*⁶ differentiates red cells containing adult haemoglobin from those containing fetal haemoglobin (HbF). When a thin blood smear is exposed to acid,

haemoglobin is eluted from adult red cells, whereas fetal cells retain HbF. When counterstained, the fetal red cells appear bright pink and the adult red cells appear as grey 'ghost' cells (see Figures 1, 2 and 3).

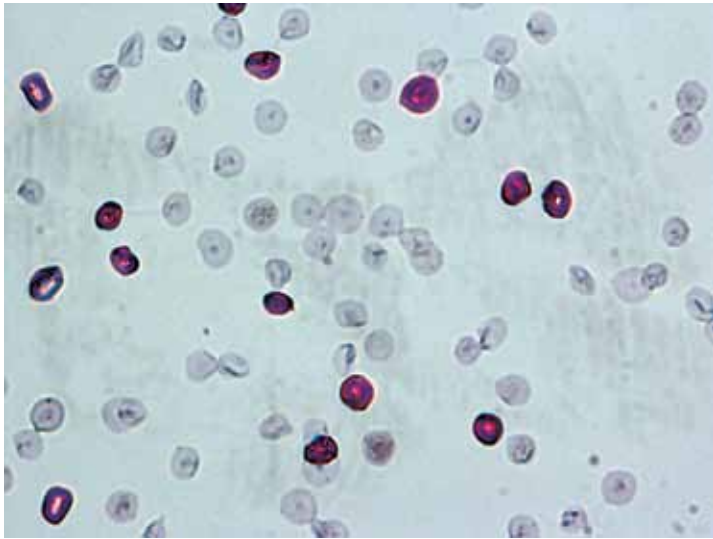


Figure 1. Positive control slide Kleihauer-Betke test – fetal cells appear bright pink and adult cells appear grey.

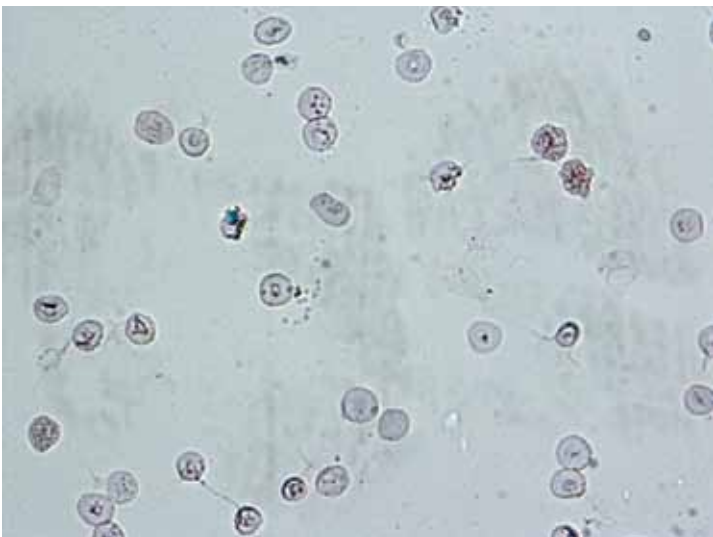


Figure 2. Negative control slide Kleihauer-Betke test – all adult cells.

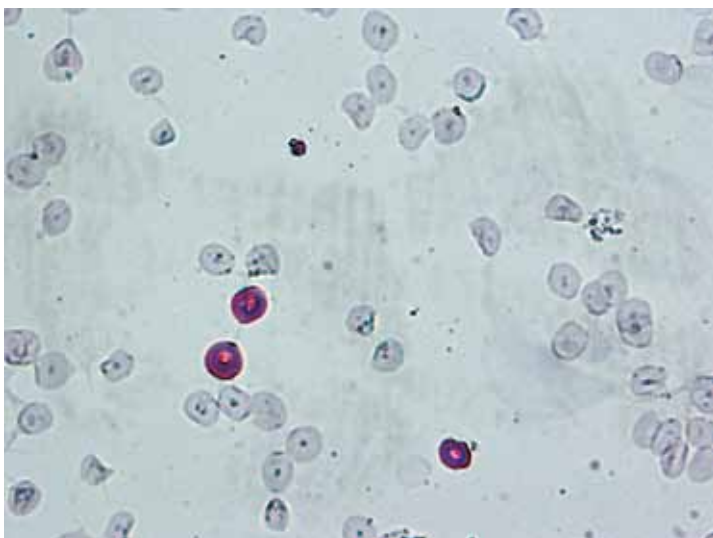


Figure 3. Kleihauer-Betke test slide showing an 11 ml fetomaternal haemorrhage – fetal cells appear bright pink and adult cells appear grey.

The Kleihauer-Betke test is not dependent of the presence of RhD positive fetal red cells, requires only basic laboratory equipment and is relatively inexpensive to perform.

However, there are significant disadvantages to the test. The Royal College of Pathologists of Australasia's external fetomaternal quality assurance program has highlighted wide variation in results between participating laboratories. The test is dependent on time, temperature and pH. The examination of the stained blood smear is subjective and dependent on the quality of slide preparation, experience of the operator and the method used to count the fetal cells. A study in the United Kingdom showed a variation of up to 500 per cent between operators.⁷ Kleihauer-Betke tests are known to result in falsely high results in a large FMH (greater than 6ml). Another complication of the Kleihauer-Betke test is that HbF levels in pregnant women are known to often be increased.⁸ Some patients with haemoglobinopathies also have increased levels of HbF. Adult HbF is difficult to distinguish from fetal HbF in the Kleihauer-Betke test so the test is not suitable in these cases. For all of these reasons, any large FMH (greater than 6ml) is referred for flow cytometric analysis.

Flow cytometry

Flow cytometers have the ability to quantitate small numbers of cells present in large cell populations. Flow cytometers can count a large number of cells and therefore should be more sensitive and accurate than other techniques. Australian quality assurance program results show that in comparison to the Kleihauer-Betke test, flow cytometry testing produces results within a narrower range, smaller standard deviation and a reduced co-efficient of variation (CV). However, flow cytometry testing is relatively expensive to perform and requires staff with specific expertise to perform the assay.

Assessment of FMH can be performed by flow cytometry using fluorescently conjugated antibodies to the RhD antigen or HbF. However, the anti-D based assay can only be used where the mother is RhD negative and the baby is RhD positive. The anti-D assay is similarly of no value when the mother has been given prophylactic RhD Ig that is still detectable in the plasma. The method of choice then is the anti-HbF assay. However, it can also be difficult to distinguish between adult and fetal HbF cells using the anti-HbF flow cytometry method. In the flow cytometry method, fetal HbF positive cells are detected by fluorescently conjugated anti-HbF. The positive fetal HbF cells are represented on single parameter histograms by their increased mean channel fluorescent number compared to adult HbF and HbF negative cells (see Figures 4, 5 and 6).

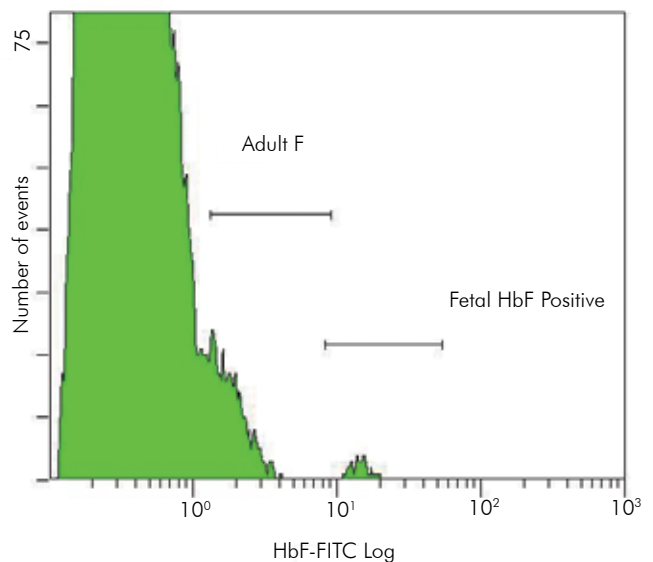


Figure 4. Positive fetal control. Fetal HbF positive cells show increased fluorescent intensity staining with anti-HbF-FITC conjugated antibodies compared to adult HbF and HbF negative cells.

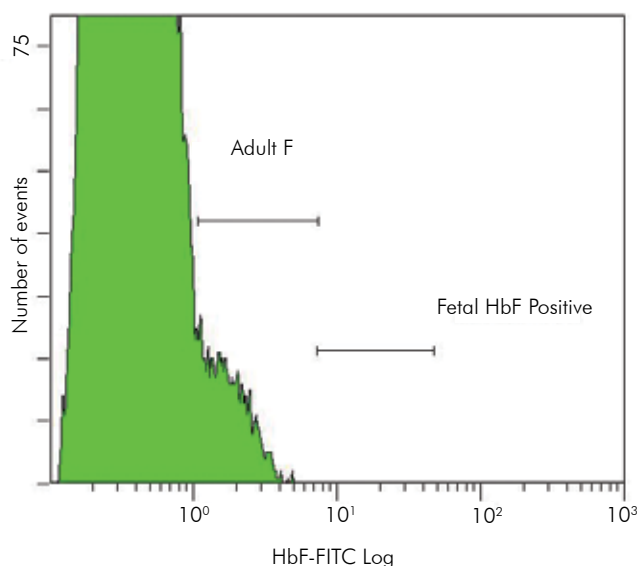


Figure 5. Sample negative for fetal HbF.

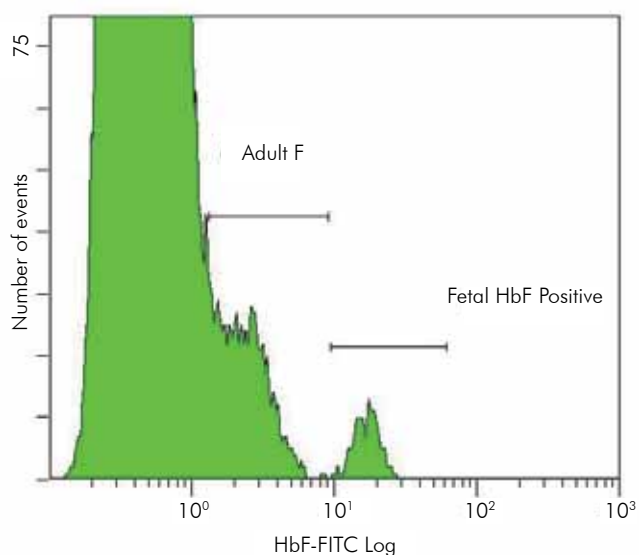


Figure 6. Patient sample with 0.53 per cent fetal HbF positive cells, equating to an 11.0 ml FMH.

Conclusion

The determination of a FMH plays a vital role in the reduction of RhD immunisation in RhD negative pregnant women. An adequate dose of RhD Ig is effective in preventing RhD immunisation in most cases. As the consequences of RhD immunisation for a fetus can be catastrophic, it is necessary to accurately quantify the FMH to ensure that an adequate dose of RhD Ig is administered. The Kleihauer-Betke test has been shown to be a suitable, convenient method for screening for FMH, but flow cytometry based tests are the preferred methods for quantitating large fetomaternal haemorrhage (greater than 6ml).

References

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