Proposed Changes to BCSH Guidelines and NICE review of anti-D use

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NHSBT, Imperial College Healthcare NHS Trust and UK NEQAS!!

Guideline Review
Why does it all take so long?

- All guidelines have a 'sell-by' date
- Formal BCSH guideline process
  - Transfusion Taskforce reviews need for new guidelines or revisions
  - Writing group, sounding board, website
- Individual guideline review may result in previous guidance being 'out-of-date'

Classification of evidence levels

<table>
<thead>
<tr>
<th>Evidence obtained from:</th>
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<tbody>
<tr>
<td>Ia Meta-analysis of RCTs</td>
<td></td>
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<tr>
<td>Ib At least one RCT</td>
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<tr>
<td>Iia At least one well-designed controlled study</td>
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<tr>
<td>Iib At least one other type of well-designed quasi-experimental study.</td>
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<tr>
<td>III Well-designed non-experimental descriptive studies, such as comparative studies, correlation studies and case studies.</td>
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<tr>
<td>IV Expert committee reports or opinions and/or clinical experience of respected authorities</td>
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Grades of Recommendations

A Requires at least one RCT addressing the specific recommendation. (Evidence levels Ia, Ib)
B Requires the availability of well controlled clinical studies but no RCTs on the topic of recommendations. (Evidence levels IIa, IIb, III)
C Requires evidence obtained from expert committee reports or opinions and/or clinical experiences of respected authorities. (Evidence level IV)

Good practice point. Recommended best practice based on the clinical experience of the guideline development group.

Guidelines Relevant to the Prevention of HDFN

- British Committee for Standards in Haematology
  - Use of Anti-D Immunoglobulin 2006
  - Blood Transfusion in Obstetrics 2007
  - Compatibility Guidelines 2004
  - Antenatal Serology 2006
  - Fetomaternal Haemorrhage 1999/2008
- Royal College O&G Green Top Guidelines
  - Transfusion in obstetrics 2007
- National Institute Clinical Excellence
  - Routine Antenatal Anti-D Prophylaxis 2002/2008

Prevention of HDFN

- Identify D negative women, check for anti-D
- Give right blood components to D- women
- Counsel about sensitising events in pregnancy
- Give right dose of anti-D immunoglobulin at the right time
  - Sensitising events, RAADP and post delivery
- Do a test for FMH after 20 weeks gestation
Guideline Implementation

Who needs to know?
- Midwives
- Obstetricians
- Transfusion scientists
- Haematologists
- General practitioners
- Women of childbearing age

• SHOT category for anti-D errors
• BBT3 recommendations re anti-D

Guideline Update

• BCSH anti-D and Antenatal Serology Guidance reviewed 2006
  - FMH guidelines missed being launched at same time although FMH testing still required

• NICE guidance on routine antenatal anti-D prophylaxis up for review
  - Continued to support RAADP and FMH testing

<table>
<thead>
<tr>
<th>Sensitising event</th>
<th>Minimum dose anti-D Ig</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERPC and miscarriage</td>
<td>250 units up to 20 weeks</td>
</tr>
<tr>
<td>CVS</td>
<td>500 units after 20 weeks</td>
</tr>
<tr>
<td>Amniocentesis, cordocentesis</td>
<td>500 units initially FMH test</td>
</tr>
<tr>
<td>APH</td>
<td>500 units initially FMH test</td>
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<tr>
<td>Abdo trauma, fall</td>
<td>500 units initially FMH test</td>
</tr>
<tr>
<td>ECV</td>
<td>500 units initially FMH test</td>
</tr>
<tr>
<td>Post delivery</td>
<td>500 units initially FMH test</td>
</tr>
<tr>
<td>RAADP</td>
<td>500 units at 28 and 34 weeks</td>
</tr>
</tbody>
</table>

125 units anti-D Ig im covers 1mL fetal red cells

1% will have FMH more than 4mL and 0.3% more than 10mL
Routine Antenatal Anti-D Prophylaxis
NICE Technology Appraisal 156 August 2008 (updated from 2002)

- RAADP continues to be recommended
  - evidence of both clinical and cost effectiveness
- Preparation with lowest associated costs
  because no evidence that one dose is different to two
  - D-gam 500 iu } x2 at 28 and 34
  - Partobulin SDF 1250 iu } weeks
  - Rhophylac 1500 iu x1 at 28-30 wks
- Review 2011

Sensitisation Still Occurs

- D negative women not identified and counselled*
- D positive blood components transfused*
- RAADP not given either because it is missed, refused or not offered*
- Anti-D Ig not given for sensitising events in pregnancy, or post delivery if baby D group incorrect*
- FMH occurs in the absence of observable sensitising events so anti-D not given
- Insufficient anti-D given because FMH test not done accurately (or at all)*

* Reportable to SHOT/SABRE

Sensitising Events in Pregnancy

- 1st and 2nd trimester
  - Miscarriage
  - Ectopic pregnancy
  - Therapeutic TOP
  - CVS and amniocentesis
- After 20 weeks
  - Antepartum haemorrhage
  - Placental abruption
  - Abdominal trauma
  - Amniocentesis
  - External cephalic version

Small volume and infrequent:
- 0.05 mL in 5%
- 0.5 mL in 2%

More frequent:
- <2 mL in 98%
- >30 mL in 0.03%

Updated: 6th December 2009
**FMH at Delivery**

- Fetal cells in the circulation of 50% of women at normal delivery
  - 3 mL or more in 1%
  - 10 mL or more in 0.3%

- Frequency and volume increased by caesarean section and manual removal of placenta

**Massive FMH (more than 25mL)**

- FMH as a proportion of newborn blood volume
  - Blood volume at term ~270 mL \( \times 1.4 \) = 388 mL
  - Placental blood volume ~120 mL

**THE CAUSE OF MOST MASSIVE FMH IS UNKNOWN**

- 50% with FMH >25 mL have no history of 'known' risk factors
- Silent/spontaneous FMH in D positive women may go unnoticed

**Delivery of targeted anti-D Ig is improving**

- Early studies showed 1.2-1.8% sensitisation to D antigen without RAADP but later studies show that this is reducing in control and RAADP groups

**But RAADP is variable:**

- A UK survey in 2005, only 75% of obstetric units were using RAADP
- Retrospective audit (Chaffe et al 2007) 86.5% of 207 D negative women in two centres had both doses of a RAADP regime
**BCSH FMH Guidelines**

- Standardised methods for detecting and estimating the volume of fetal cells in the maternal circulation
  - Acid elution based on HbF (the Kleihauer Test)
  - Flow cytometry based on monoclonal anti-D
- Calculation based on Mollison’s formula
- Recommendations for follow-up
  - Confirmation of original sample with 2nd method if positive
  - Repeat maternal samples after additional anti-D

**UK NEQAS FMH Exercises**

- Pilot scheme showed poor analytical accuracy particularly in AE methods
- Improvement after guidelines published 1999
- Ongoing issues:
  - High CVs for acid elution compared to FC
  - Lower method median values for flow cytometry compared to AE
  - Questionnaires show unvalidated screening methods in use

**BCSH FMH Guideline 2008**

- **Writing Group**
  - Eric Austin
  - Stephan Bates
  - Mahes de Silva
  - Diane Howarth
  - Anatole Lubenko
  - Megan Rowley
  - Eric Thomas
  - Jenny White
  - Mark Williams
- **BCSH Transfusion Taskforce**
  - Derek Norfolk (chair)
  - Keith Wilson (secretary)
  - Jenny White (NEQAS)
  - And others
Significant Changes

- Accuracy and quality assurance in FMH testing remains important but there is increased focus in these guidelines on the further testing of FMH greater than 2mL and the timely and effective communication of results to clinicians so that appropriate action is taken.
- Other situations where AE and FC tests are undertaken is included and it is stated where FMH testing is not required.

“An anti-D immunoglobulin injection is given in specific clinical situations in a standard dose according to local protocols. The FMH test is to determine whether an additional dose is required.”

This is a common misunderstanding! FMH test is should not be used to see whether any anti-D is required.

Post Delivery
D- Woman, D+ Baby

- Babies should be typed with anti-D reagents used for routine patient testing (saline reacting, IgM reagents which do not detect D\textsuperscript{v}) with no additional tests required.
- However, if found to be weak D or D variant using these reagents, a baby should be treated as D positive for the purposes of anti-D administration to the mother and FMH testing.
**During Pregnancy:**

**Sensitising Events**

- **Less than 20 weeks**, no FMH test
- **After 20 weeks**, a maternal EDTA sample is required for FMH estimation
  - Up to 28 weeks confirm maternal blood group and perform antibody screen
  - After 28 weeks of gestation, anti-D immunoglobulin is still required even if RAADP has been given but antibody screening is not necessary

**Recurrent PV Bleeding**

**After 20 weeks**

- Anti-D immunoglobulin will be required at a minimum of 6 weekly intervals
- An FMH test should be performed every 2 weeks and if FMH is detected:
  - additional anti-D will be required regardless of the presence or absence of passive anti-D
  - follow-up samples should be taken after 48 - 72 hours to check that the fetal cells have cleared

**Persistent Positive FMH Test in Pregnancy**

- Blood group of the fetus is unknown during pregnancy and anti-D immunoglobulin will only clear D positive fetal cells
- Flow cytometry using anti-D can be performed to determine whether the fetal cells remaining in the maternal circulation are D positive or not
  - It is envisaged that this situation will occur relatively infrequently

A test for FMH is not routinely recommended for D positive women with PV bleeding or ante-partum haemorrhage (APH)
**FMH Tests Not Required**

- When the sensitising event is before 20 weeks
  - The fetal blood volume is insufficient to exceed that covered by the minimum standard anti-D Ig
- When the woman has immune anti-D.
  - Distinguishing between passive and immune anti-D may be difficult. *If unsure, do an FMH test*
- When the fetus/baby is known to be D

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**Kleihauer Tests in D Positive Women?**

In D positive women with unexplained abdominal pain or PV bleeding in late pregnancy, Kleihauer (AE) tests are of limited diagnostic use

- More sensitive and specific tests exist to investigate suspected placental abruption
- Do a test for FMH by AE in D positive women when occult FMH is suspected and event is otherwise unexplained
  - Intrauterine death or stillbirth
  - Severe anaemia at birth

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**Uses of Flow Cytometry**

- Following a D positive RBC transfusion to a D negative woman of childbearing potential.
  - To estimate or confirm the dose of anti-D Ig required to prevent sensitisation
- In solid organ transplantation when the donor is D positive and recipient D negative with childbearing potential
  - Anti-D Ig can be administered prophylactically with a flow cytometry test at 24 hours post transplant
**Labelling and Risk of Transposition**

- Ensure cord and maternal samples are correctly labelled and not transposed
- Maternal and cord samples are often taken at the same time by the same member of staff
- If the maternal and cord D group is the same:
  - ABO group
  - MCV on FBC
  - Alkali denaturation test ('APT' test) to distinguish between fetal and maternal haemoglobin

**Separate Maternal Samples for FMH Test and ABO & D?**

Centrifugation of red cells for blood group may cause discrepancies
- larger fetal RBCs closer to the plasma:RBC interface
- theoretical risk that an FMH test performed on the same sample could underestimate the true FMH
- makes thorough mixing for subsequent FMH testing more difficult

Inconsistencies in maternal blood grouping could occur if there is a large FMH

**Timing of Samples**

“The maternal sample for FMH estimation should be taken when sufficient time has elapsed to allow fetal cells to be distributed within the maternal circulation following delivery, manual removal of placenta or sensitising event”

A period of 30-45 minutes is considered adequate
Laboratory Testing of Samples

“The sample should be processed and results reported in sufficient time to ensure that a supplementary dose of anti-D immunoglobulin could be given within 72 hours of delivery* or sensitising event if the FMH estimation exceeds the ‘standard’ anti-D immunoglobulin dose”

Methods of Estimation of FMH

- The AE method is most suited to screening for FMH and initial quantification of the volume of fetal cells, if FMH is detected
- The FC method is the recommended reference method to confirm the volume of FMH after an initial positive AE screen

Kits for AE

- 87% of laboratories use a commercial kit for AE (Questionnaire sent with UK NEQAS exercise 0503F 2005)
- MHRA evaluation of kits (Parker-Williams and Carpenen 2005)
- Some recently described modifications of the AE technique are reported to make the counting of the maternal RBCs easier by eluting half the slide (Howarth 2002)

Slide preparation, staining and controls - no change
Screening

The 1999 guidelines stated that: “if any fetal cells were seen whilst screening 25 low power fields (x10 eyepiece and x10 objective) then quantification should be performed”

This recommendation should continue to be followed, unless the criteria for the below are semi-quantitative screen are met.

Validation of the Screening Method

- The minimum number of adult cells counted in a high power field is 100
- The number of cells in a low power field (using x10 eyepiece and x10 objective) is 16 times that in a high power field (x10 eyepiece and x40 objective)
- The minimum number of adult cells in a low power field, from the same area of the film, can be extrapolated to be 1600
- This allows the number of cells per low power field to be validated each time the test is performed.
The Following Criteria Must Be Met

- Use a x10 eyepiece and x10 objective (low power field)
- Initial validation should be performed to ensure that the area viewed in a low power field is at least 16 times greater that in a high power field
- Each time this method is performed, adult cells in one high power field (x10 eyepiece and x40 objective) should be counted in the same area of the film as is to be screened, and there must be at least 100 adult cells present in this high power field
- At least 25 low power fields should always be screened

Semi-Quantitative Screening – Validation Step

- Scan control slides for fetal cells under low power field
- Scan centre and edges of test slides for fetal cells under low power
- Is the film evenly spread? Are the fetal cells evenly distributed? Are fetal cells clearly stained?
- Count adult cells in one high power field

Semi-Quantitative Screening – Screening Step

- Count fetal cells in 25 low power fields
- More than 10 fetal cells per 25 low power fields
- Proceed to quantification
- No further testing required
- Report
Quantification

- For accurate quantification it is recommended that a minimum of 10,000 maternal red cell ghosts are examined using an x40 objective.
- The use of a Miller square or an indexed grid is necessary to obtain an accurate ratio of fetal to maternal cells in each blood film.

A historical assumption of the number of maternal ghost cells per field must not be relied upon for FMH estimation.

Mollison’s Formula

The fetal bleed should be calculated as follows:

Number of fetal cells per high power field × 1800 × 122 × 100
Number of maternal cells per high power field 100 92

OR CAN BE SIMPLIFIED TO:

Number of fetal cells per high power field × 2400
Number of maternal cells per high power field

Assumptions:
- The maternal red cell volume is 1800mL
- Fetal cells are 22% larger than maternal cells
- Only 92% of fetal cells stain darkly

Confirmation

WHY?
Inaccuracy of FMH estimation at critical decision-making point.

Systematic evaluation of difference between AE and FC methods at low (as well as high) bleed volumes.
Flow Cytometry

- For the purpose of quantifying a minor population of D positive cells, a fluorochrome conjugated IgG monoclonal anti-D reagent is used to label the D positive fetal cells.
- Flow cytometry is used to detect and quantify the minor population in the D negative maternal sample.
- Other tests using antibodies to alternative markers may be available but are not currently in routine use.

The original maternal EDTA sample should be used for confirmatory FMH testing by Flow Cytometry.

Staining – Choice of Reagents

Direct staining using a fluorochrome conjugated IgG monoclonal anti-D with high avidity for the D antigen.

Specificity of the anti-D:
- React with D phenotypes capable causing anti-D**
- When discrepancies arise between the results of AE and FC on a single sample consider the possibility that the anti-D reagent may not have detected D variant cord cells.

**reagents currently available may not meet this criterion but familiarity with reagent characteristics and anti-D specificity is important.

Follow-Up of Positive AE Test

Why 48-72 hours? Should it be 2-3 days?
Too early, fetal cells will not have cleared
Too late, missed opportunity to give additional anti-D
Presence of Fetal Cells at Follow-Up

- May indicate that insufficient anti-D immunoglobulin has been administered
- Confirm that the anti-D immunoglobulin has been administered
- Confirm that the blood group of the baby is D positive
- Send the follow-up maternal EDTA sample for testing by FC

**At this final stage of follow-up test for anti-D in the maternal circulation, using a routine IAT antibody screening technique, to confirm that free anti-D is present testing until no fetal cells are seen in the FMH test**

Longer Term Follow-Up

- Repeat maternal RBC antibody screen at six months? Desirable but not always practicable.
- Opportunity to counsel the woman and to perform an Rh phenotype on the partner
- Absence of immune anti-D six months does not mean that the woman has not been sensitised
- All women who have had FMH greater than 4mL detected should have this highlighted at booking in subsequent pregnancies
Reporting Results to Clinicians

It is important that FMH results are timely and effectively communicated. This will allow clinicians to manage the woman appropriately.

Where an estimated FMH exceeding 2mL is detected, a preliminary result should be communicated to the ward, prior to the results of the confirmatory test, to alert clinicians to the possible need for additional anti-D immunoglobulin prior to discharge.

The Report Format

Needs to Communicate the Following:

- The reason for the sample
- The result of the FMH test in 'mL fetal red cells' rounded to the nearest mL
- Whether any supplementary anti-D immunoglobulin is required
  - Advice about the anti-D immunoglobulin dose required to cover the reported bleed

Suggested report format either if no fetal cells or less than 2mL fetal cells present on semi-quantitative screen

FMH test by acid elution
Give standard post-natal anti-D immunoglobulin dose
Less than 2mL fetal cells seen; this FMH will be covered by the standard dose of (insert dose) iu anti-D immunoglobulin
No further testing is required

The use of the term ‘negative’ is not used in this context because of the potential of communicating the wrong message to clinicians who may assume that a standard dose of anti-D immunoglobulin is not required.
Suggested report format if significant numbers of fetal cells detected and a quantification has been performed

FMH test by acid elution

- Fetal cells seen
- Estimated \( \text{insert volume rounded to nearest whole mL} \) mL fetal red cells by acid elution
- This has been sent for confirmation \( \text{results of confirmatory test including methodology} \)
- Either: This will be covered by the standard dose of \( \text{insert dose} \) iu anti-D immunoglobulin
- Or: Give a further dose of \( \text{insert dose} \) iu anti-D immunoglobulin immediately
- Send a further maternal EDTA sample in 48 - 72 hours to check for clearance of fetal red cells

If Follow-Up Is Required

The report should contain:

- Advice regarding follow-up samples to check for clearance of fetal cells
- Results of confirmatory tests from the reference lab
- Or from repeat in-house testing by AE where FC results are not available within 72 hours and the AE results are acted on in the interim

Audit

Clinical audit

- All D negative women have had a FMH test at the appropriate time and, if the test is positive, that the appropriate action has been taken

Laboratory audit

- Of the results of confirmatory tests for FMH compared to the initial quantification

Combined laboratory and clinical audit

- Correct confirmatory and follow-up procedures in women requiring additional anti-D Ig
Summary

- In the UK FMH testing is still required whatever the dose of anti-D Ig in use and the preferred screening method is acid elution
- A semi-quantitative AE screening method has been introduced which is quick and validated
  - This should be used instead of discounting a ‘few’ fetal cells as non-significant
- Historical adult cells counts should not be used in quantification by AE

Summary

- Any FMH >2mL should be quantified and confirmed by flow cytometry
  - with ongoing audit
- Reporting of FMH test results should be clear and directive to support clinical decision making
- Follow-up algorithms have been changed
  - 48-72 hours after anti-D Ig reflects the current knowledge about clearance of D positive cells whilst remaining practical and clinically applicable

Transfusion Update Meeting

anti-D Day

Royal Society of Medicine
Saturday, 6th December 2008