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Feto-Maternal Haemorrhage Practice Report
Questionnaire issued May 2016

Introduction

The purpose of this questionnaire was to gather detailed information on routine feto-maternal haemorrhage (FMH) procedures, including testing techniques using acid elution and flow cytometry, and policy on anti-D Ig prophylaxis.

Return rate and data analysis

The questionnaire was sent to 258 laboratories, 222 in the UK and Republic of Ireland (ROI) and 36 overseas. Of these laboratories, 52 have two registrations, one for acid elution and another for flow cytometry; some combined their responses, whilst others submitted separate responses for each registration; the latter have been combined for the purposes of analysis. Duplicate entries have been removed (with the most recent entry kept for inclusion in the analysis), as have entries from 17 hospital laboratories that did not answer any questions regarding details of testing. Data from 146 (56.6%) participating laboratories has been included in the following analysis, 139 from the UK and ROI and 7 overseas. Some questions were not answered by all respondents.

Results

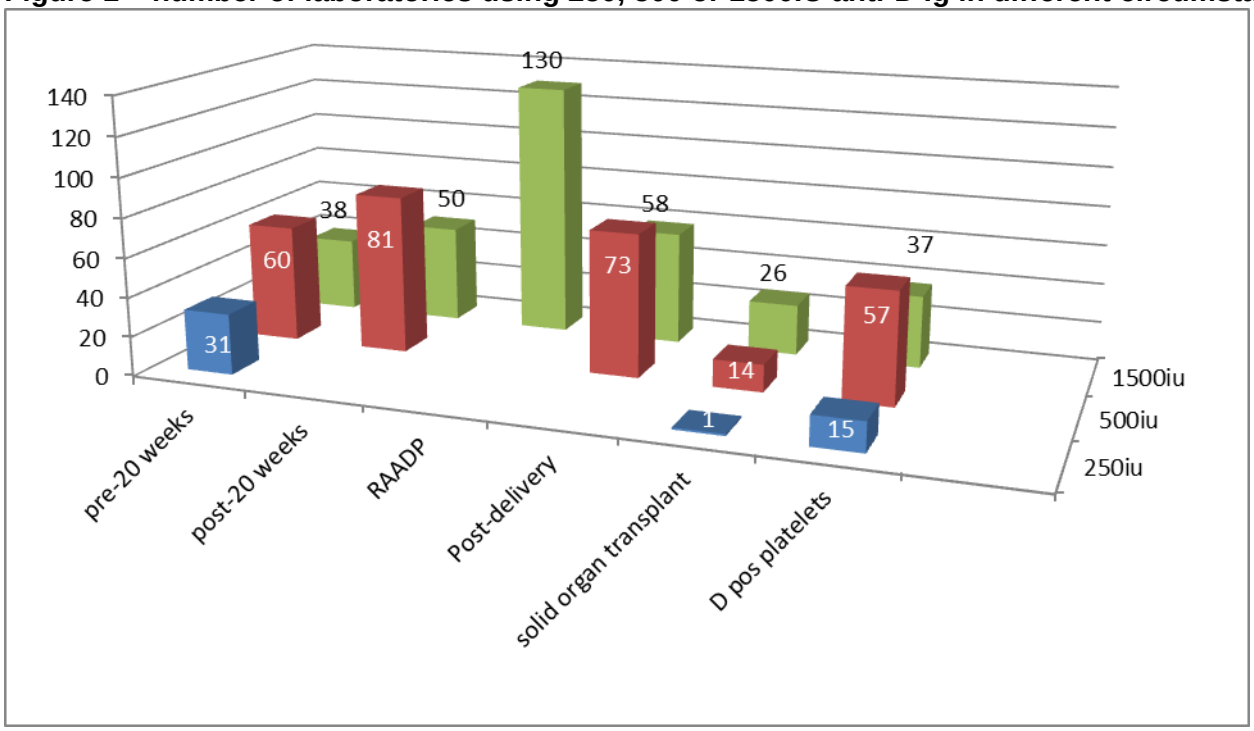
Reasons for testing

FMH testing to guide anti-D prophylaxis

142/146 (97.3%) respondents undertake FMH testing to guide anti-D Ig prophylaxis in various circumstances. Of the remaining four, one reference laboratory undertakes some confirmatory testing and research, and three non-UK laboratories cited testing for abruptions and intra-uterine deaths.

Figure 1 shows the standard dose of anti-D suggested in six different circumstances. In addition (not shown in the figure, one non-UK laboratory uses a standard dose of 1000IU, and one UK laboratory said they give 1250iu for RAADP, although this may have been a data entry error. It should be noted that some of this data is likely to be out of date, as availability of some products has changed since the questionnaire was distributed. Eight respondents did not state a dose for any of the categories, with five of these stating that the hospital blood transfusion laboratory makes the decision re dosing.

Figure 1 – number of laboratories using 250, 500 or 1500IU anti-D Ig in different circumstances



FMH testing in other circumstances

Table 1 shows the circumstances, other than for anti-D Ig prophylaxis, for which FMH testing is undertaken. Four did not answer the question.

Table 1: other circumstances in which FMH testing is undertaken

Reason for FMH testing (n=142)	Number (%)
Suspected placental abruption during pregnancy	74 (52.1%)
Unexplained intrauterine death	117 (82.4%)
Unexplained anaemia in a neonate	3 (2.1%)
Other [1]	6 (94.2%)
None – anti-D prophylaxis only[2]	14 (9.9%)

[1] - includes suspected fetal bleed at delivery; trauma during pregnancy; neonate post IUT; 16-20 week miscarriage; if authorised by consultant haematologist.
[2] - includes four who occasionally but not routinely undertake FMH for other reasons.

Number of FMH tests undertaken per year

Table 2 shows the number of tests undertaken per year in-house (requested from within the institution) and number of referrals to another laboratory, and Table 3 shows the number of tests undertaken which have been referred from elsewhere.

Table 2: Number of tests/year undertaken in-house and number of referrals to another institution

No. tests per year requested from *within* the Institution	None / no answer	1-10	11-20	21-50	51-100	>100
Acid elution (AE) screens	24	1	0	4	6	111
AE quantification	45	31	36	15	9	10
Flow cytometry quantifications	117	7	8	5	2	7
Referrals to elsewhere	38	55	40	12	0	1

Table 3: Number of tests/year referred from elsewhere

Number of tests per year requested from *outside* the Institution	None / no answer	1-10	11-20	21-50	51-100	>100
Acid elution (AE) screens	137	2	4	1	0	2
AE quantification	142	3	0	1	0	0
Flow cytometry quantifications	131	1	1	6	2	5

Testing by acid elution

Who undertakes the testing?

131/146 (89.7%) undertake testing by acid elution. In the majority of hospitals the testing is usually undertaken by staff from the blood transfusion department:

- 103 (78.6%) blood transfusion staff
- 17 (13.0%) haematology staff
- 11 (8.4%) staff from both of the above or combined blood sciences

Screening by acid elution

Counting

- 87/128 (68.0%) use the BSH 'semi-quantitative' screen
- 18 (14.1%) said that they do not, and 23 (18.0%) were not sure
    - 23/41 (56.1%) use a method based on counting the number of both fetal and adult cells
    - 14/41 (34.1%) use a method based on counting the number of fetal cells per field, but using an historical estimate of the number of adult cells
    - 4/41 (9.8%) refer for flow cytometry if they see any fetal cells

Decision making

- 66/127 (52.0%) have the slides examined by more than one individual
- 57/127 (44.9%) have the slides examined by one individual only
- 4/127 (3.1%) do something else
    - 2 use two individuals whenever possible
    - 2 have a second individual examine the film, if the first person sees any fetal cells.

Quantification by acid elution

Counting and calculating the FMH

- 107/131 (81.7%) undertake quantification by acid elution
- 100/101 (99.0%) use a graticule for counting
- 87/101 (86.1%) examine an area containing at least 10,000 adult cells as recommended by BSH guidelines[1]. Of the remaining 14, seven count 6000 adult cells, and the other seven use a variety of either number of adult cells or number of fields
- 98/100 (98.0%) use the BSH formula[1] to calculate the FMH. The other two probably also use this formula but may have included the calculation for using the graticule in the formula

Decision making

- 70/101 (69.3%) have the slides counted by more than one individual and report an average
- 21/101 (20.8%) have the slides counted and reported by one individual
- 10/101 (9.9%) do something else:
    - 5 have two people count the slides but report one or other result (e.g. the highest; the first)
    - 3 don't report FMH quantification results, but continue to count to maintain competency
    - 2 did not specify

Is additional anti-D Ig issued without a quantification result?

Table 4 details the circumstances in which laboratories would issue anti-D Ig on the basis of an AE result only, and Table 5 details any additional testing that would be undertaken.

Table 4: issue of additional anti-D Ig on the basis of an AE quantification only

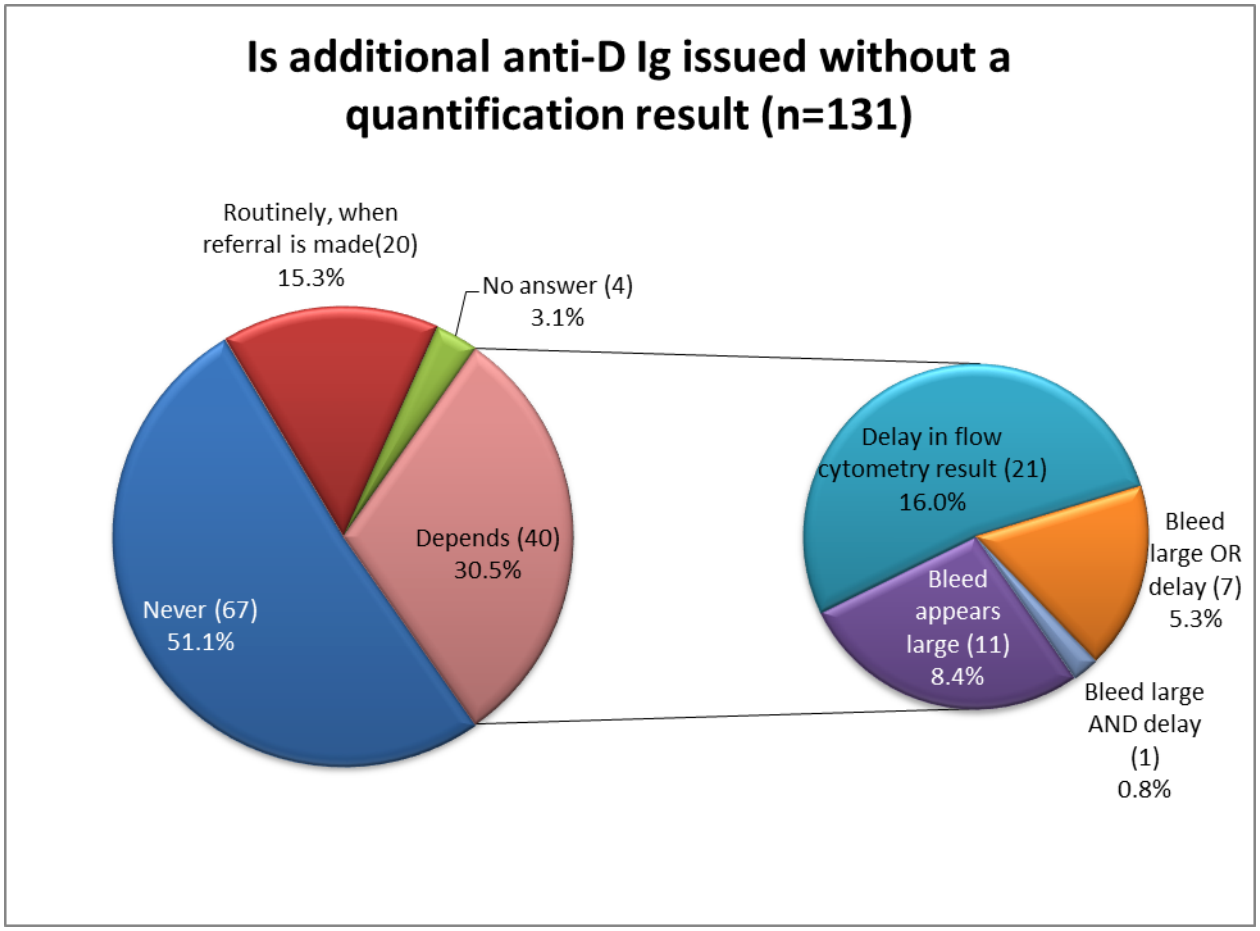
Is additional anti-D Ig (> standard dose) ever issued on the basis of an AE quantification result only?	**(n=102)**
Never	21 (20.6%)
As routine practice where a referral is made for flow cytometry	21 (20.6%)
If there is a delay in receipt of the flow cytometry result	36 (35.3%)
Where there appears to be an FMH greater than that covered by the standard anti-D Ig dose	32 (31.4%)

Table 5: Testing undertaken where additional anti-D Ig is issued based on the AE quantification

If additional anti-D Ig is issued based on the AE quantification result, is a second AE test performed before issue?	**(n=81)**
No repeat testing	38 (46.9%)
Slides recounted	20 (24.7%)
Test repeated from the start by possibly the same person	12 (14.8%)
Test repeated from the start by second person	10 (12.4%)
Counted by second person unless out of hours	1 (1.2%)

Is additional anti-D Ig issued without a quantification result?

Figure 2 – additional anti-D Ig issued without a quantification result (acid elution or flow cytometry)



Testing by flow cytometry (FC)

25/143 (17.5%) undertake testing by flow cytometry in-house; 22 are in the UK (including two in ROI) and three outside of the UK.

Who undertakes the testing?

The testing is undertaken by the following departments:

- 10 (40.0%) blood transfusion
- 7 (28.0%) haematology
- 2 (8.0%) immunology
- 2 (8.0%) blood transfusion and immunology
- 4 (16.0%) other
    - 2 red cell immunohaematology (reference)
    - 1 clinical chemistry
    - 1 flow cytometry

Do staff testing for FMH by FC have other expertise in FC?

- 2 (9.5%) no (one UK and 1 ROI)
- 10 (47.6%) yes, all staff
- 9 (42.9%) yes, some staff

The testing process - screening and quantification

In 23 centres, a full quantification is performed on all samples received, whilst in two (one UK and one non-UK) a screen is undertaken by flow cytometry to identify samples requiring quantification.

The UK centre which performs flow cytometry screening tests samples exclusively to guide anti-D prophylaxis. The centre uses an anti-D marker without the isotype control, counting 100,000 - 500,000 events, and performs a quantification if the screen result is >2mL. The reasons cited for choosing to screen by flow cytometry were objectivity, speed, specificity and ease of use.

The non-UK centre which undertakes flow cytometry screening tests samples to guide anti-D prophylaxis and for investigation of cases of unexplained intrauterine death. The centre uses an anti-HbF marker and counts <100,000 events. No details of the criteria for proceeding to a full quantification were given.

Number of events collected for quantification

- 12 (66.7%) collect 500,000 events
- 2 (11.1%) collect >500,000 events
- 4 (22.2%) collect 100,000 – 500,000 events
    - All UK and ROI laboratories are testing to guide anti-D prophylaxis

Details of sample preparation

The questions regarding replicate testing (the whole process including sample preparation) and replicate counts on each of these tests was answered by 21 centres.

- 3 (14.3%) prepare more than one test for each sample and count each of these more than once
- 7 (33.3%) prepare more than one test for each sample, and perform a single count on each one
- 2 (9.5%) prepare a single test for each sample and perform more than one count on it
- 9 (42.9%) prepare a single test per sample and perform a single count on it

Markers in use

- 20 (80.0%) use anti-D (+/- white cell marker) only
    - 17 IBGRL BRAD3 FITC / AEVZ FITC
    - 1 IBGRL BRAD3 FITC / AEVZ FITC + CD45 PERCP (BD)
    - 1 IBGRL BRAD3 FITC / AEVZ FITC / BIRMA 17C PE 'Classic Plus' kit
    - 1 not stated

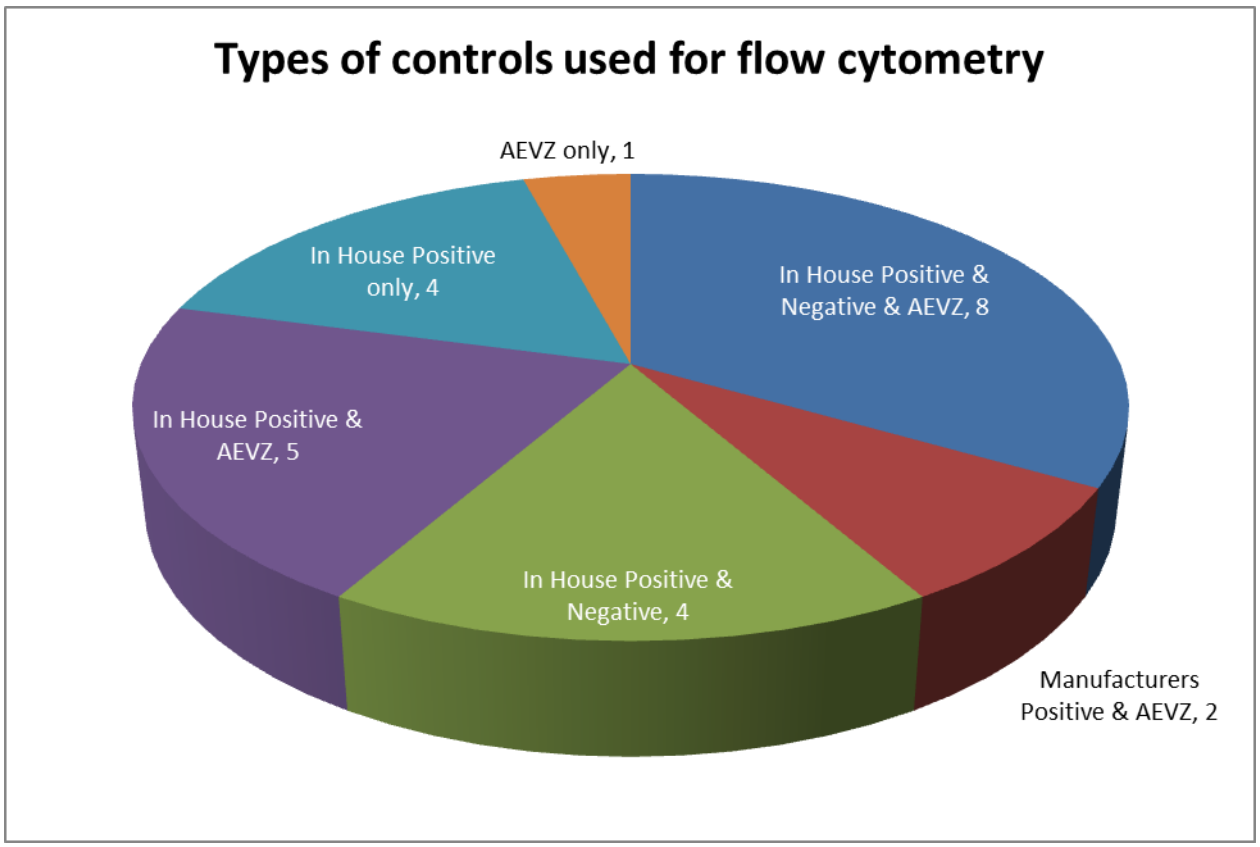
- 3 (12.0%) use anti-HbF (+/- carbonic anhydrase) only (all non-UK)
    - 2 IQ products 'Fetal cell count' kit anti-HbF / carbonic anhydrase
    - 1 anti-HbF, FITC-conjugated (BD) (in-house method)

- 2 (8.0%) use both anti-D and anti-HbF methods
    - 1 IBGRL BRAD3 FITC / AEVZ FITC / BIRMA 17C PE 'Classic Plus' kit and IQ products 'Fetal cell count' kit anti-HbF / carbonic anhydrase
    - 1 IBGRL BRAD3 FITC / AEVZ FITC / BIRMA 17C PE 'Classic Plus' kit and in-house anti-HbF method
    - Both UK reference centres use the anti-D marker as a first line test if the patient is D Negative. The HbF marker is used for D Positive patients and in the event that there is a significant discrepancy between the estimated acid elution result and the flow cytometry result (i.e. to investigate Hereditary Persistence of Fetal Haemoglobin (HPFH)).

Use of controls for methods

Figure 3 shows the different combinations of controls used when testing by flow cytometry

Figure 3 – Controls used for flow cytometry



% fetal cells used for control(s)

19 laboratories answered this question and there was wide variation in the % fetal cells used:

- 6 (31.6%) used 1% and 0.2%
- 8 42.1%) used more than one positive control, ranging from 0.1% to 100%, with 7 including 1%
    - 3 also included a 0mL bleed
- 5 (26.3%) used a single positive control (0.2%, 0.5%, 5%, simulated 12mL bleed, >50mL)

Is the value obtained for the negative control subtracted from the test value before reporting?

- 6 (25.0%) No
    - 2 anti-HbF and 4 anti-D
- 16 (66.7%) Yes, isotype matched control
    - 15 anti-D
    - 1 anti-HbF
- 2 (8.3%) Yes, negative control using a sample known not to contain fetal cells
    - Both anti-D and both UK

Acceptable value for controls

- 6 (40.0%) stated 2SD (including 1 2SD based on previous 3 months, and one 2SD or 2%)
- 8 (53.3%) specified a range of values (variable depending on % control)
- 1 (6.7%) stated that a range was applied to controls but did not specify the range

Gating and Uncertainty of Measurement (UoM)

Is the gating fixed, or reviewed and adjusted if necessary for each sample tested?

- 7 (29.2%) fixed
- 1 (4.2%) fixed for anti-D analysis and adjusted in HbF if necessary
- 6 (25.0%) reviewed for each sample and adjusted, if necessary, by any operator
- 10 (41.7%) reviewed for each sample and adjusted in consultation with staff with expertise in FMH by FC

Only one laboratory (using an anti-D marker) noted problems with differentiating cell populations, and this was probably due to the presence of WBC.

Have you established uncertainty of measurement?

- 7 (33.3%) No
- 1 (4.8%) for AE only
- 7 (33.3%) for FC only
- 6 (28.6%) for AE and FC

Reporting testing by flow cytometry

Formula for calculating FMH

- 20 use the BSH formula[1]
- 1 uses the formula '% positive erythrocytes X 1800 X 1.3*/100' (non-UK)

*The 1.3 multiplication factor is similar to the 1.22 figure used in the UK to correct for the potential difference in size of the maternal and fetal cells.

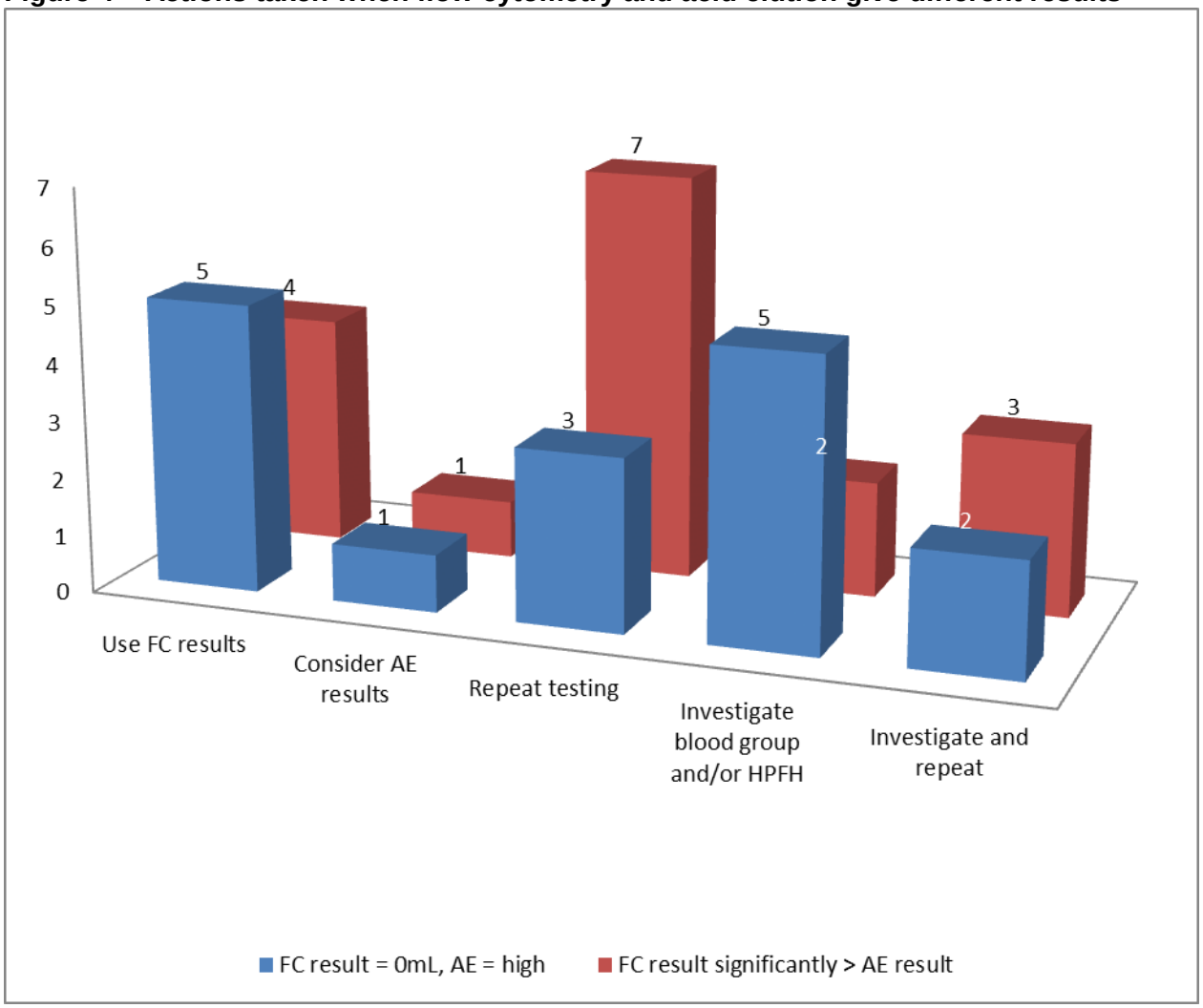
Do you routinely recommend an anti-D Ig dose?

- 14 (66.7%) Yes
- 7 (33.3%) No (6 UK including 1 non-clinical laboratory, and 1 non-UK)

  5 stated who is responsible for anti-D Ig dosing:
  - 1 NHSBT/Haematology consultants
  - 1 Clinical Immunology
  - 2 Transfusion BMS staff
  - 1 Not applicable for the work conducted

Policy where there are significant discrepancies between AE and FC

Figure 4 – Actions taken when flow cytometry and acid elution give different results



Reporting of FMH results >4mL to clinicians

- 11 (52.4%) always round up to a whole number
- 3 (14.3%) round up or down as appropriate to the nearest whole number
- 6 (28.6%) report to one decimal place
- 1 (4.8%) reports mL of fetal cells and anti-D Ig dose to administer

13/14 that have established measurement of uncertainty do not report it with clinical results; one (non-UK laboratory) does report it with the FC result if the FMH is <6mL.

Would you be interested in a UK NEQAS flow cytometry workshop?

- 20 yes
- 1 no (UK)

DISCUSSION

Acid elution testing

The majority of acid elution testing is performed by staff in blood transfusion laboratories, and all use acid elution to guide anti-D prophylaxis. Approximately 50% of UK laboratories also use acid elution testing to investigate potential placental abruption. However, in D positive women with unexplained abdominal pain in late pregnancy, FMH tests (by AE) are of limited diagnostic use and more sensitive and specific tests are now available to investigate suspected placental abruption[1].

The majority of laboratories use an acid elution screening method that involves counting both adult and fetal cells. However, 10% participants reported using a screening method based on a historical estimate of the number of maternal cells, and this can introduce inaccuracy in the maternal cell count and therefore the FMH measurement[1].

All but one laboratory (in the UK) use a graticule for quantification of FMH. In 75% of laboratories more than one individual counts the FMH slides, with the majority of these reporting the average result. Twelve laboratories reported count less than 10,000 cells, with seven counting 6,000. In 2009, the BSH guideline for estimation of FMH[1] the recommended number of cells to be counted during quantification was updated to 10,000 (from 6,000 in the 1999 version), to improve accuracy given that the presence of a fetal cell is a 'rare event'. Only 34% of laboratories undertaking quantification by acid elution quantify more than 20 samples per year. A low volume of work may present difficulties in maintaining staff competency for this test.

Issue of additional anti-D Ig prior to confirmation of FMH

Approximately half of those screening (but not quantifying) by acid elution, issue anti-D Ig in addition to their 'standard' dose, prior to receipt of a quantification result. Two thirds of these only do so in specific circumstances, i.e. where there is delay in results from a referral and/or a 'large' FMH by acid elution. The majority of these laboratories use an antenatal and / or postnatal standard anti-D Ig dose of 500IU.

Additional anti-D Ig is issued before receipt of the flow cytometry result by 87% of those quantifying by acid elution, with 47% of these undertaking no repeat re-testing or re-counting by acid elution in the interim.

Flow Cytometry testing

The blood transfusion laboratory undertakes FMH testing by flow cytometry in approximately half of the centres, whilst a variety of departments are responsible for testing in the remainder.

In 48% of laboratories, two sample aliquots are prepared and tested; this is in line with the 2009 BSH guideline[1]. Testing the same aliquot twice would not necessarily detect operator error, such as decanting fetal cells with the supernatant during washing, which can be a significant cause of FMH underestimation.

A wide variety of % fetal cells used for positive controls was reported, and there was also variation in the acceptance criteria for controls; within 2 SD was the most common answer.

Four laboratories count fewer than 500,000 cells; and this can adversely affect the accuracy of the result, with FMH being a 'rare event' analysis. There is variation in practice regarding the subtracting of the result of a negative control from the test result before reporting, with incomplete correlation with the type of marker used.

All UK laboratories are using the formula recommended in the 2009 BSH FMH guidelines[1] (adapted from the Mollison formula, assuming a maternal blood volume of 1800mL) for calculating the bleed volume, whilst one non-UK laboratory uses a different formula. Further analysis of data submitted for recent FMH exercises, shows that the calculation used to obtain the bleed volume based on the fetal cell % varies for non-UK laboratories. This has prompted a change to the instruction paperwork (as of 1704F) stating that for EQA samples, the estimated maternal blood volume should be assumed to be 1800mL, and it is hoped that this will improve comparability of results of EQA samples.

Discrepancy between acid elution and flow cytometry results

Where the acid elution result was high but the flow cytometry result found to be 0mL, five laboratories would proceed based on the flow cytometry result alone. Discrepancies may occur for a number of reasons, e.g. D variant fetal cells, or testing an incorrect sample, and there are risks involved in reporting either the acid elution or flow cytometry result without further investigation and / or obtaining a repeat sample.

Uncertainty of Measurement (UoM)

Whilst 67% of laboratories have established UoM for FMH testing, none routinely report this with clinical results.

FMH workshops

There was clear interest from flow cytometry laboratories for UK NEQAS to deliver a flow cytometry workshop. In view of this, a flow cytometry workshop will be considered following on from a trial acid elution workshop that is planned for late 2017.

REFERENCE

[1] British Committee for Standards in Haematology (BSH) (2009): E Austin, S Bates, M de Silva, D Howarth, A Lubenko, M Rowley, M Scott, E Thomas, J White, M Williams. Guidelines for the Estimation of Fetomaternal Haemorrhage. http://www.b-s-h.org.uk/guidelines/