



*Biennial Report*  
**2013 - 2014**

*UK NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME  
for Feto-Maternal Haemorrhage*

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## **1. INTRODUCTION TO THE REPORT**

This is a biennial report covering the calendar years of 2013 and 2014.

## **2. THE SIGNIFICANCE OF FMH QUANTIFICATION**

Quantification of fetal D positive cells in the circulation of D negative women after delivery is essential to ensure that an adequate dose of prophylactic anti-D immunoglobulin is prescribed. The protective effect of anti-D immunoglobulin is dose-dependent and 125 iu/mL of packed fetal red cells is recommended when given by the intramuscular route<sup>1</sup>. Studies suggest that 0.65% women have a fetomaternal haemorrhage (FMH) >4 mL, whilst 0.3% women have an FMH of >10 mL. The majority of hospitals in the UK give a standard dose of 500iu post-delivery, with a growing number giving 1500iu. Irrespective of the anti-D regime employed for post-partum prophylaxis, quantification of FMH is required to ensure that sufficient anti-D immunoglobulin is given to reduce the probability of alloimmunisation.

Traditionally, most clinical laboratories have used a variation of the Kleihauer-Betke test to quantify FMH, based upon the differential staining of adult and fetal cells, following the preferential acid elution of adult rather than fetal haemoglobin. The technique has been known to have significant inter-laboratory and inter-observer variations, as the counting process is subject to human error and interpretation. Flow cytometry has been reported to improve the accuracy of quantification of FMH, and is generally accepted to be the reference method. BCSH guidelines for estimation of FMH were updated in 2009<sup>2</sup> and provide a semi-quantitative screening method, with a recommendation to refer bleeds of >2mL for quantification by flow cytometry.

## **3. BACKGROUND TO UK NEQAS (FMH) SCHEME**

FMH became a substantive scheme from April 1998, as a joint venture between the Haematology and Blood Transfusion Schemes. It is advised by the Steering Committee for Blood Transfusion Laboratory Practice, which is supported by the Specialist Advisory Group for Feto-Maternal Haemorrhage. Current membership is shown in Appendix 1.

#### 4. STAFF

*Scheme Co-Directors* – Dr Megan Rowley and Professor Keith Hyde  
*Scheme Co-Managers* – Mrs Clare Milkins and Mrs Barbara De la Salle  
*Scheme Deputy Managers* - Ms Jenny White, Mr Paul McTaggart

*Executive Assistant* – Ms Isabella De-Rosa

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*Chair of the BTLP Steering Committee* – Dr Peter Baker, Royal Liverpool Hospital

#### 5. ANALYTES

- Estimation of feto-maternal haemorrhage:
  - i. Quantification; mL packed cells
  - ii. Screening test only.
  
- Additional data collected which contributes to performance monitoring:
  - i. Suggested dose of anti-D
  - ii. Referral for flow cytometry
  - iii. Request for repeat sample.

#### 6. NUMBER OF PARTICIPANTS

At the end of 2014 there were a total of 286 participating laboratories; details are shown in table 1.

**Table 1 – Laboratory participation by method**

Method	UK	Non-UK & Misc
Quantification by acid elution (AE) only	141	19
Quantification by flow cytometry (FC) only	14 <sup>1</sup>	18 <sup>2</sup>
AE and FC	19	4
Screening only	43 <sup>1</sup>	5 <sup>2</sup>

<sup>1</sup>4 labs screen by acid elution and quantify by flow cytometry

<sup>2</sup>2 labs screen by acid elution and quantify by flow cytometry

## 7. MATERIAL

Adult blood is obtained from group AB D negative blood donors, whilst cord blood (in CPD) is obtained from the NHSBT Cord Bank. Both are tested by the supplier and found negative for all mandatory viral markers.

Each survey comprises two specimens, simulating post-delivery D negative maternal specimens with varying levels of FMH. This is achieved by adding an appropriate volume of D positive cord whole blood to D negative adult whole blood, in accordance with the following assumption and calculation, to create a 'target' value, expressed in mL packed cells. This 'target' value is intended for internal purposes only and does not represent the expected result, since no correction factors (as used in Mollison's formula) are used in its calculation and it is not validated.

**Assumption made:** 1800 mL = red cell volume (RCV) of a pregnant woman,  
e.g. 6 mL FMH = 0.33% adult RCV

**Calculation to prepare a 6 mL 'target' bleed:**  $X = 0.33 \times \frac{\text{adult haematocrit}}{\text{cord haematocrit}}$

where X is the volume of whole cord blood to be added to each 100 mL of adult whole blood.

## 8. DATA MANIPULATION AND PERFORMANCE MONITORING

### 8.1 Calculation of analytical performance score

The median for each method and the SD, derived from the method inter-quartile range, is used to produce a deviation index (DI). The DI is used to calculate the analytical performance score.

There are three steps involved in the calculation of the score:

1. The deviation index is calculated using the formula

$$DI = \frac{(R-M)}{SD}$$

Where:

*R*=Laboratory Result

*M*=Method Median

*SD*=Standard Deviation

2. The absolute value of the DI is taken (ignoring the sign). Any DI values greater than 3.5 are rounded down to 3.5, to avoid very high values having an excessive effect on the calculation.
3. The resulting DI values for the six most recent scored specimens for which results have been returned are added together and then multiplied by a multiplication constant, set at 7 for flow cytometry and 8 for acid elution during this reporting period, to give the analytical performance score.

## 8.2 Clinical Significance Errors

### ***a) In laboratories registered for screening only:***

Participants are requested to state whether the initial 'screen' would trigger quantification. If the answer is 'No' and insufficient anti-D has been prescribed to cover the flow cytometry method median, this is defined as an episode of unsatisfactory performance.

### ***b) In laboratories registered for quantification by acid elution:***

The same algorithm applies as detailed in a) above, but in addition, there is a second algorithm following quantification: if insufficient anti-D is prescribed and no referral is made for flow cytometry or a repeat sample requested, this is defined as an episode of unsatisfactory performance.

## 8.3 Outlying results – acid elution only

A grossly outlying result, defined as a DI of  $<-2$  or  $>3.5$  constitutes an episode of unsatisfactory performance.

## 9. RESULTS

### 9.1 Summary of Survey Data by Method

Table 2 summarises the median and interquartile range (IQ Range) for results by acid elution and flow cytometry, and the reported sample quality.

**Table 2 – Summary of overall results by method**

Survey	Date distributed	Reported satisfactory sample quality	Acid Elution			Flow Cytometry		
			Returns <sup>2</sup> No.	Median (mL)	IQ Range (mL)	Returns <sup>2</sup> No.	Median (mL)	IQ Range (mL)
1301F - 1 <sup>1</sup> - 2 <sup>1</sup>	8 Jan 13	97.6%	191	9.9	8.3 – 12.1	51	6.6	6.3 – 7.0
		98.0%	191	9.8	8.2 – 12.5	51	6.6	6.4 – 6.8
1302F - 1 - 2	5 March 13	99.0%	189	5.4	4.7 – 6.8	51	4.5	4.3 – 4.8
		99.0%	189	23.9	21.0 – 26.6	51	20.9	19.9– 21.7
1303F - 1 - 2	30 April 13	98.0%	179	15.7	13.3 – 17.8	53	12.7	12.0 – 13.2
		95.2%	60 <sup>3</sup>	1.2	0.7 – 2.1	45	0.0 <sup>3</sup>	0.0 – 0.2
1304F - 1 - 2	2 July 13	97.3%	179 <sup>3</sup>	4.2	3.3 – 5.3	52	2.8	2.6 – 3.1
		97.3%	185	26.4	23.3 – 30.0	52	24.7	22.7 – 25.8
1305F - 1 - 2	3 Sept 13	98.3%	18	0.5	0.0 – 0.9	54	0.0 <sup>3</sup>	0.0 – 0.1
		98.0%	181	24.4	21.9 – 27.3	54	24.3	23.5 – 25.2
1306F - 1 - 2	12 Nov 13	97.3%	183	12.2	10.8 – 14.2	53	9.9	9.6 – 10.3
		96.2%	182 <sup>3</sup>	3.5	2.8 – 4.5	53	2.7	2.4 – 3.1
1401F - 1 - 2	14 Jan 14	97.6%	187	10.8	9.1 – 12.4	52	10.0	9.1 – 10.4
		97.9%	186	14.8	13.4 – 17.0	52	12.0	11.4 – 12.7
1402F - 1 <sup>1</sup> - 2 <sup>1</sup>	4 March 14	97.6%	181	24.1	21.4 – 28.1	55	20.9	19.7 – 21.9
		98.3%	181	24.5	21.5 – 27.5	55	20.9	19.5 – 21.5
1403F - 1 - 2	13 May 14	98.6%	183	8.1	6.9– 9.6	56	6.8	6.6 – 7.2
		97.6%	37 <sup>3</sup>	0.7	0.4 – 2.6	56	0.0 <sup>3</sup>	0.0 – 0.2
1404F - 1 - 2	1 July 14	97.3%	176	6.8	5.8 – 8.0	51	5.3	5.0 – 5.7
		98.3%	179	25.1	22.0 – 27.6	51	23.5	21.6 – 24.9
1405F - 1 - 2	2 Sept 14	99.3%	180	13.2	11.8 – 15.2	52	10.9	10.3 – 11.5
		97.6%	173 <sup>3</sup>	4.6	3.7 – 5.7	52	3.1	2.9 – 3.2
1406F - 1 <sup>1</sup> - 2 <sup>1</sup>	4 Nov 14	96.2%	177	13.7	12.0 – 15.7	53	11.2	10.8 – 11.5
		94.5%	176	14.0	12.4 – 15.9	53	11.2	10.9 – 11.6

<sup>1</sup> - Specimens within the survey were prepared from the same pool

<sup>2</sup> - Excludes returns where no numerical value was given, e.g. <4mL

<sup>3</sup> - Specimens not scored where flow cytometry median <4mL or where bleed is 0mL.

## 9.2 'At Risk' Results

Table 3 shows the number of acid elution results which would have put a woman at risk of sensitisation to the D antigen had the same set of results have been reported for a similar clinical sample, i.e. insufficient anti-D to cover the flow cytometry median, combined with no follow up.

**Table 3 – No. (%) of episodes of 'women being put at risk of sensitisation'**

Survey	No. Returns Quantification/screen only	Flow cytometry median (mL)	No. 'at risk' Quantification	No. 'at risk' Screen only
1301F - 1	191/43	6.6	0	0
1301F - 2	191/43	6.6	0	1
1302F - 1	189/41	4.5	4	0
1302F - 2	189/41	20.9	0	0
1303F - 1	179/45	12.7	1	1
1304F - 2	185/45	24.7	1	0
1305F - 2	181/45	24.3	1 <sup>1</sup>	0
1306F - 1	183/45	9.9	0	0
1401F - 1	187/44	10.0	1	1
1401F - 2	186/44	12.0	0	0
1402F - 1	181/48	20.9	0	0
1402F - 2	181/48	20.9	0	0
1403F - 1	183/48	6.8	0	0
1404F - 1	176/47	5.3	3	1
1404F - 2	179/47	23.5	2	0
1405F - 1	180/47	10.9	1	0
1406F - 1	177/48	11.2	0	0
1406F - 2	176/48	11.2	1	0
<b>Total</b>	<b>3294/817</b>	<b>N/A<sup>2</sup></b>	<b>15<sup>3</sup></b>	<b>4</b>

<sup>1</sup> – Transposition error

<sup>2</sup> – Not applicable

<sup>3</sup> – Nine are non-UK

### **Participants registered for quantification**

Over this two-year period, there were 15 episodes where participants registered for quantification using acid elution, potentially placed a 'patient' at risk of sensitisation, as a consequence of an inadequate recommended dose of anti-D Ig coupled with no follow-up. This translates to an 'error' rate for UK NEQAS surveys of 0.46%. Nine of these related to laboratories outside of the UK, where different criteria may be used for determination of the appropriate dose of anti-D (e.g. 100IU/mL rather than 125IU/mL as recommended in the UK).

### **Participants registered for screening only**

During this two-year period, a maximum of 48 participants were registered for screening only; these laboratories perform an initial 'screen' using an acid elution technique, and based on the result, decide whether quantification would be undertaken (presumably by referring for flow cytometry). During this period, there were four episodes where a 'patient' was placed at risk of immunisation to the D antigen, through quantification not being



triggered and insufficient anti-D being prescribed. This translates into an 'error' rate of 0.49%.

### 9.3 Outlying Results

Table 4 shows the number of outlying acid elution results reported excluding samples not subject to performance monitoring. There were a total of 87 outlying results due to underestimation, and 42 due to overestimation, giving rates of 2.6% and 1.3% respectively.

**Table 4 – No. (%) of outlying acid elution (AE) results**

Survey	No. Participants	AE median (mL)	No. (%) outliers DI <-2	No. (%) outliers DI >3.5
1301F - 1	191	9.9	4	3
1301F - 2	191	9.8	2	4
1302F - 1	189	5.4	2	3
1302F - 2	189	23.9	6	3
1303F - 1	179	15.7	4	0
1304F - 2	185	26.4	5	2
1305F - 2	181	24.4	6	3
1306F - 1	183	12.2	2	4
1401F - 1	187	10.8	4	2
1401F - 2	186	14.8	3	6
1402F - 1	181	24.1	6	2
1402F - 2	181	24.5	7	1
1403F - 1	183	8.1	1	2
1404F - 1	176	6.8	6	3
1404F - 2	179	25.1	11	0
1405F - 1	180	13.2	7	3
1406F - 1	177	13.7	3	0
1406F - 2	176	14.0	8	1
<b>Total</b>	<b>3294</b>	<b>N/A</b>	<b>87</b>	<b>42</b>

## 9.4 Reporting of 0 mL bleeds

Two exercises, 1305F and 1403F, included one sample comprising adult red cells only, to simulate a 0mL bleed. The donation used in each case did not indicate the presence of HbF and in-house acid elution testing did not demonstrate any staining of adult cells. However, several participants reported seeing fetal cells:

### **1305F**

Fifty laboratories reported seeing fetal cells by acid elution. Twelve were registered for screen only, and six of these reported that they would have referred for flow cytometry. Of those registered for quantification, 13/38 proceeded to quantify the bleed, reporting values of between 0.1 to 1.8mL.

Significantly more laboratories using an Inverclyde or Guest kit, reported seeing fetal cells, than those using a Clintech kit ( $P=0.001$ ). Numbers using other kits were too small to be statistically valid.

### ***Results***

Fetal cells seen by:

27/82 (33%) using an Inverclyde kit

10/31 (32%) using a Guest kit

9/76 (12%) using a Clintech kit

### **1403F**

Eighty-one laboratories reported seeing fetal cells by acid elution. Seventeen were registered for screen only, and eight of these reported that they would have referred for flow cytometry. Of those registered for quantification, 30/64 proceeded to quantify the bleed, reporting values of between 0.1 to 10.7mL, with a median of 1.1mL.

Significantly more laboratories using an Inverclyde or Guest kit, reported seeing fetal cells, than those using a Clintech kit ( $P<0.001$ ). Numbers using other kits were too small to be statistically valid.

### ***Results***

Fetal cells seen by:

40/85 (47%) using an Inverclyde kit

18/31 (58%) using a Guest kit

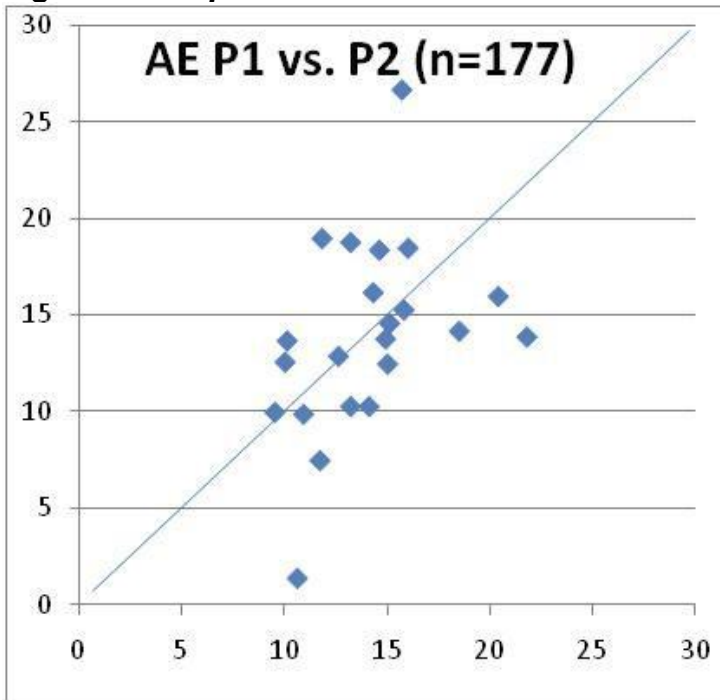
12/71 (17%) using a Clintech kit

In house testing confirmed differences in staining properties between the kits. The Inverclyde and Guest kits provide a clearer differential between fetal and adult red cells, but less so between fetal red cells and maternal white cells. Adult white cells are easy to identify with the Clintech kit even under low power, but the fetal red cells are less well defined than with the other two kits. When undertaking FMH estimation, the test slide should be compared with the control slide, which must be made at the same time; any stained cells that are not easily identifiable under low power, should also be examined under high power.

## 9.5 Intra-laboratory precision in replicate testing

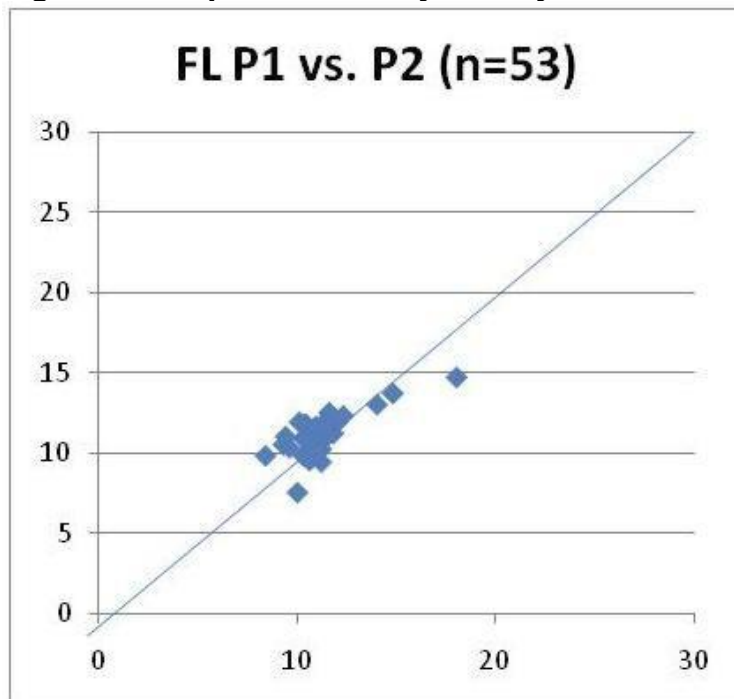
In exercise 1406F, both samples were prepared from a single pool. Figures 1 and 2 show the acid elution and flow cytometry results as dot plots for 'Patients' 1 and 2 within individual laboratories. As would be expected, the flow cytometry results show a better correlation than the acid elution results.

**Figure 1: Dot plot for acid elution results for 1406F**



Acid elution method median:  
13.7mL for P1  
14.0mL for P2

**Figure 2: Dot plot for flow cytometry results for 1406F**



Flow cytometry method median:  
11.2mL for both P1 and P2

## **10. SCHEME PROGRESS AND DEVELOPMENT**

### **10.1 Accreditation**

The Scheme last underwent a CPA inspection in July 2012 and this included a pre-assessment visit by UKAS for the new ISO 17043 proficiency testing standards. The Scheme, as part of the Unit at Watford, submitted an application for inspection against the ISO standards in April 14. The inspection date is planned in 2015. In the meantime the Scheme retains its accreditation status.

### **10.2 IT systems**

The level of registration for web-entry remained at 98% overall. 100% of non-UK participants were registered and all but five UK participants. The scheme will be 100% web entry starting in April 2015.

### **10.3 In-house testing protocols**

Following 1305F, where 50 participants reported seeing fetal cells in a 0mL bleed, the decision was taken to introduce in-house acid elution testing using the kits most commonly used by participants. This allows us to better advise participants who are having problems with their technique and also to make more specific educational comments in the reports. Testing with these kits started in 2014.

### **10.4 Quality Improvement Plan**

Suggestions by participants for improvement are added to the Quality Improvement Plan. Two were logged during 2013:

- A screen-only laboratory suggested that it would be useful to see the full acid elution report. Similarly a laboratory registered for quantification by acid elution requested to see the flow cytometry reports. This was implemented with 1402F.
- Some participants have said that they would like the offer of a pre-paid place at the BTLP Annual Participants' Meeting for FMH participants as well as BTLP participants. This is being implemented as a trial in 2015 at the National Motorcycle Museum in Birmingham, as this venue has the capacity for a bigger audience

### **10.5 Separate specimens for flow cytometry and acid elution**

A small, but increasing number of laboratories are registering for screening by acid elution and quantification by flow cytometry. The participants receive a single set of samples, but are requested to undertake quantification by flow cytometry even if the screen is negative. This allows complete assessment for flow cytometry, but does not reflect clinical practice. It also provides for a potential reporting conflict, where the flow cytometry indicates a substantial bleed (above the trigger for quantification) but the screen result is negative. In order to resolve this issue, the scheme is considering sending separate samples for assessment by the two different methods. This change would also allow the scheme to better target the bleed sizes to assess proficiency at more critical levels for each technique. This option will be taken to the SAG and Steering Committee for discussion during 2015.

## 10.6 Performance monitoring

### a) **Redefinition of UP and PUP for scores >100**

Following approval by the Steering Committee, SAG and NQAAP, the definition of Unsatisfactory Performance (UP) and Persistent Unsatisfactory Performance (PUP) relating to the scoring element of the scheme were amended in 2013. The new definitions are shown in Table 5.

**Table 5 – New definition of UP and PUP**

<b>Score and trend</b>	<b>Performance status</b>
80-99	Borderline
100+	UP
100+ and falling	UP
100+ and rising or not falling (inc non-return)	PUP
100+ on two occasions in one 12 month period	PUP

### b) **Change of constant for calculation of DI and score**

Previously, a constant of 6 has been used as the DI multiplication factor for determining the score, for both acid elution and flow cytometry. It was notable that there were no laboratories in the UK with a score of 100+ during 2011, and only one during 2012.

The scores for 2012 were remodelled using a constant of 7, 8, and 9 and compared with those obtained with the constant of 6.

Following a thorough review of the impact on participants, we concluded that a constant of 7 should be used for scoring flow cytometry results and 8 for acid elution results (the lower constant required to elevate the scores for flow cytometry reflects the tighter CVs for flow cytometry).

Overall, 11% of flow cytometry and 1% of acid elution laboratories would have reached a score of >100 during 2012, using constants of 7 and 8, respectively. Given that flow cytometry is the reference method and there is no other means of detecting unsatisfactory performance, it was deemed as reasonable to identify more laboratories as UP by the analytical performance score than for acid elution. The change was implemented in 2013.

### c) **Scoring of bleeds <4mL by Flow Cytometry**

It was agreed by the Steering Committee that bleeds of <4mL should be scored for flow cytometry, even though they are withdrawn for scoring for acid elution. This was implemented in April 2013.

### d) **0mL bleeds**

Following 1305F, where 12 screen-only laboratories reported the presence of fetal cells in a 0mL bleed, and six of these reported that they would have made an unnecessary referral for flow cytometry, we implemented a new system to request these laboratories to review their procedures and complete a CAPA form. We also offer to review their slides, if they wish to submit them.

## 11. KEY PERFORMANCE INDICATORS

The Scheme met all of its KPIs in 2013 and 2014. Table 6 details the targets and achievements.

**Table 6 – Key Performance Indicators**

Category	No. of Events	Target	Target Achievement Rate	Actual Achievement Rate
Exercise Distributions	12	On schedule	100%	<b>100%</b>
Report Distributions	12	Within 4 days of C/D	75%	<b>100%</b>
Complaints	12	Dealt with in 4 weeks	70%	<b>100%</b>
Reported Sample Quality	24	≤5% unsatisfactory	75% of samples	<b>96% (mean USQ 2.4%)</b>
Integrity of Samples	7030	≤0.5% unsuitable for testing per exercise	75% (i.e. 3/4 exercises)	<b>100% (mean 0.1%)</b>

## 12. EDUCATION AND PUBLICATIONS

UK NEQAS (FMH) has been represented on or associated with the following committees/organisations etc. during the reporting period:

- BCSH Transfusion Task Force
- BCSH guideline writing groups for:
  - Anti-D immunoglobulin prophylaxis
  - Antenatal testing guidelines

## 13. REFERENCES

1. WHO (1971) Prevention of Rh Sensitisation. Technical Report Series 468:
2. BCSH (2009) Guidelines for estimation of fetomaternal haemorrhage  
[www.bcshguidelines.org](http://www.bcshguidelines.org)

## **Appendix 1**

### **Membership of the BTLP Steering Committee at end 2014**

Dr Peter Baker (Chair), Royal Liverpool University Hospital  
Mr Martin Maley, RCI, NHSBT, Newcastle  
Mrs Anna Capps-Jenner, Ealing Hospital and TDL  
Mr Ray Melanaphy, Northern Ireland BTS  
Ms Catherine Almond, Kent & Canterbury Hospital  
Dr Rekha Anand, NHSBT, Birmingham  
Dr Mallika Sekhar, Royal Free NHS Foundation Trust  
Mr Malcolm James (co-opted), NHSBT Reagents, Birmingham  
Mrs Debbie Asher (Observer - NQAAP representative),  
Mrs Clare Milkins (Secretary), Scheme Manager, UK NEQAS  
Dr Megan Rowley, Scheme Director, UK NEQAS  
Ms Jenny White, Deputy Scheme Manager, UK NEQAS

### **Membership of the FMH SAG at end 2014**

Dr Mark Williams (Chair), NHSBT, Leeds  
Mr Stephan Bates, Retired (ex Cheltenham General Hospital )  
Professor Marion Scott, IBGRL, Bristol  
Mrs Diane Howarth, Leeds General Infirmary  
Mrs Lynne Porter, WBS  
Dr Sylvia Armstrong-Fisher, SNBTS  
Mr Colin Barber, Royal London Hospital  
Dr Megan Rowley, Scheme Co-Director, UK NEQAS  
Ms Jenny White (Secretary), Deputy Scheme Manager, UK NEQAS  
Mrs Barbara De la Salle, Scheme Co-Manager, UK NEQAS  
Mrs Clare Milkins, Scheme Co-Manager, UK NEQAS