UK NEQAS
Haematology and Transfusion

Annual Report
2015

UK NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME
for Feto-Maternal Haemorrhage

UK NEQAS (FMH)
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1. INTRODUCTION TO THE REPORT

This is an annual report covering the calendar year of 2015. This will be the last separate annual report for FMH, as in future years the FMH report will be incorporated into the BTLP report.

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[1,2]. 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[3] 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. Membership for the reporting period is shown in Appendix 1.

4. STAFF

Scheme Director – Dr Megan Rowley
Scheme Co-Managers – Mrs Clare Milkins and Mrs Barbara De la Salle
Scheme Deputy Managers - Ms Jenny White, Mr Paul McTaggart
Office Manager – Mrs Pinky Bambhra
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 2015 there were a total of 255 participating laboratories, with 286 registrations; details are shown in table 1.

Table 1 – Laboratory participation by method

Method	UK	Non-UK & Misc
Quantification by acid elution only	136	17
Quantification by flow cytometry only	16[1]	23[2]
AE and FC	18	4
Screening only	38	3

[1]6 laboratories also screen by acid elution
[2]3 laboratories also screen by acid elution

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 method DI is calculated using the formula

$$DI = \frac{x_i - x_{pt}}{SD_{pt}}$$

Where x_i is the laboratory result
x_{pt} is the consensus trimmed mean value or median value
SD_{pt} is the estimated SD*

*The Estimated SD (Est SD) is calculated using the equation:

$$Estimated\ SD = \frac{IQR}{1.349}$$

Where: IQR=Interquartile range
1.349 = the spread for the standard Gaussian distribution at the IQR

2. The absolute value of the Method DI is taken (ignoring the sign) and 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 specimens for which results have been returned are added together and then multiplied by a constant to give the Analytical Performance Score. The constant is currently set at 8 for acid elution and 7 for flow cytometry.

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 to cover the flow cytometry method median 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

Tables 2 and 3 summarise the results by acid elution and flow cytometry, respectively.

Table 2 – Summary of distributions and results for acid elution

Survey	Acid Elution					
	No. Returns Analysed	Median (mL)	Interquartile Range (IQR) (mL)	Estimated SD (IQR\1.349)	Full Range (mL)	Uncertainty of Median [5]
1501F - 1	182	6.0	5.1 – 7.0	1.4	2.4 – 10.8	0.13
- 2	183	24.0	21.5 – 26.6	3.7	8.9 – 39.9	0.35
1502F - 1 [2]	183	4.2	3.4 – 5.0	1.2	2.2 – 12.0	0.11
- 2	182	13.2	11.5 – 15.3	2.8	4.2 – 26.9	0.26
1503F - 1 [1]	177	13.2	11.6– 14.8	2.4	2.8 – 43.6	0.22
- 2 [1]	177	13.0	11.8 – 15.0	2.4	4.1 – 43.0	0.22
1504F - 1	179	14.0	12.2 – 15.7	2.6	2.7 – 30.0	0.25
- 2 [2]	178	4.3	3.7 – 5.5	1.3	1.6 – 22.6	0.13
1505F - 1 [1]	173	26.0	23.0 – 30.0	5.2	7.0 – 48.4	0.49
- 2 [1]	173	27.0	24.0– 30.5	4.8	10.0 – 44.1	0.46
1506F - 1	173	6.9	5.8 – 8.4	1.9	2.0 – 24.0 [4]	0.18
- 2 [3]	71	1.8	1.1 – 2.3	0.9	0.0 – 17.6 [4]	0.13

[1] Prepared from the same pool
[2] Specimens not intended for scoring as FC median <4mL
[3] Specimen not intended for scoring as 0mL bleed (adult cells only)
[4] One lab appears to have transposed the samples or results
[5] According to ISO 13528:2015, the uncertainty of the assigned value may be considered to be negligible if it is less than 0.3 times the SD

Table 3 – Summary of distributions and results for flow cytometry

Survey	Flow Cytometry					
	No. Returns Analysed	Median (mL)	Interquartile Range (IQR) (mL)	Estimated SD (IQR\1.349)	Full Range (mL)	Uncertainty of Median [6]
1501F - 1	54	4.7	4.4 – 4.9	0.4	1.8 – 7.2	0.06
- 2	54	22.5	21.4 – 23.7	1.71	6.8 – 25.5	0.28
1502F - 1	57	2.8	2.5 – 3.1	0.4	0.7 – 9.5	0.07
- 2	57	11.7	11.2 – 12.3	0.8	3.5 – 25.5	0.14
1503F - 1 [1,5]	61	11.6	11.0 – 12.0	0.7	1.0 – 23.7	0.12
- 2 [1,5]	61	11.4	10.8 – 12.0	0.9	1.0 – 23.7	0.14
1504F - 1	61	13.5	12.7 – 14.0	1.0	8.6 – 20.7	0.15
- 2	61	3.1	2.9 – 3.5	0.4	0.0 [4] – 13.4	0.07
1505F - 1 [1]	60	24.4	23.5 – 25.3	1.3	14.5 – 28.0	0.22
- 2 [1]	60	24.4	23.2 – 25.2	1.5	15.4 – 28.3	0.25
1506F - 1	58	5.3	5.1 – 5.7	0.4	0.0 – 10.2 [3]	0.07
- 2 [2]	58	0.0	0.0 – 0.2	0.1	0.0 – 4.6 [3]	0.02

[1] Prepared from the same pool
[2] Specimen not intended for scoring as 0mL bleed (adult cells only)
[3] Three laboratories appear to have transposed the samples or results
[4] One non-UK laboratory
[5] Withdrawn from scoring, due to the presence of an unacceptable high background count detected during in-house testing
[6] According to ISO 13528:2015, the uncertainty of the assigned value may be considered to be negligible if it is less than 0.3 times the SD

9.2 'At Risk' Results

Table 4 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. The table only shows results for samples which were scored.

Table 4 – 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
1501F - 1	182/46	4.7	2 [1]	1
1501F - 2	183/46	22.5	1 [1]	0
1502F - 2	182/45	11.7	0	0
1503F - 1	177/49	11.6	0	0
1503F - 2	177/49	11.4	0	0
1504F - 1	179/47	13.5	0	0
1505F - 1	173/48	24.4	0	0
1505F - 2	173/48	24.4	0	0
1506F - 1	173/50	5.3	2 [1,2]	0
Total	**1599/428**	**N/A**	**5**	**1**

[1] *– One non-UK*

[2] *– One transposition error*

Participants registered for quantification

During this period, there were five 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 0.3%. One of these related to a transposition error, and another three related to laboratories outside of the UK, where different criteria may be used for determination of the appropriate dose of anti-D (e.g. 100 IU/mL rather than 125 IU/mL as recommended in the UK).

Participants registered for screening only

During this period, a maximum of 50 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 was one episode 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 0.2%.

9.3 Outlying Results

Table 5 shows the number of outlying acid elution results reported, excluding samples not subject to performance monitoring. There were a total of 52 outlying results due to underestimation, and 25 due to overestimation, giving rates of 3.3% and 1.6% respectively.

Table 5 – No. (%) of outlying acid elution (AE) results

Survey	No. Participants	AE median (mL)	No. (%) outliers DI <-2	No. (%) outliers DI >3.5
1501F - 1	182	6.0	3	1
1501F - 2	183	24.0	8	2
1502F - 2	182	13.2	6	2
1503F - 1	177	13.2	9	3
1503F - 2	177	13.0	6	8
1504F - 1	179	14.0	6	2
1505F - 1	173	26.0	3	2
1505F - 2	173	27.0	7	1
1506F - 1	173	6.9	4	3
Total	**1599**	**N/A**	**52**	**24**

9.4 Reporting of 0mL bleeds

1506F included one sample comprising adult red cells only, to simulate a 0mL bleed. The adult red cell donation used did not have elevated levels of HbF and was leucodepleted. In-house acid elution testing did not demonstrate any staining of adult cells,

Excluding a probable transposition error, 108/222 (49%) laboratories reported seeing fetal cells by acid elution. This included:

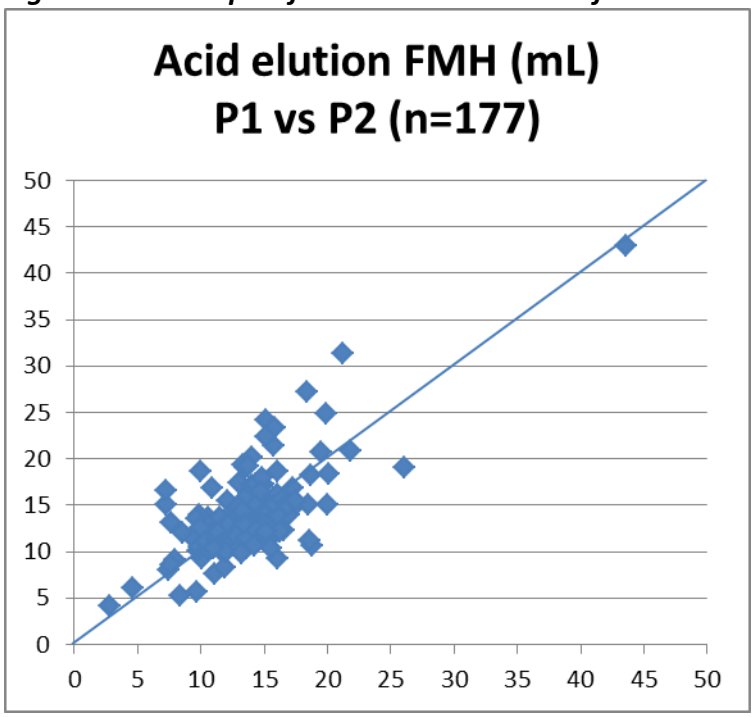
- 17/50 (34%) laboratories registered for screen only
    - 9/17 (53%) reported that they would have referred for flow cytometry.
- 91/172 (53%) laboratories registered for quantification
    - 64/91 (70%) proceeded to quantify the bleed
        - 32/64 would have referred for flow cytometry.

Reported bleed volumes ranged from 0.4mL to 17.6mL, with a median of 1.8mL. There was no obvious association with the kit used.

9.5 Intra-laboratory precision in replicate testing

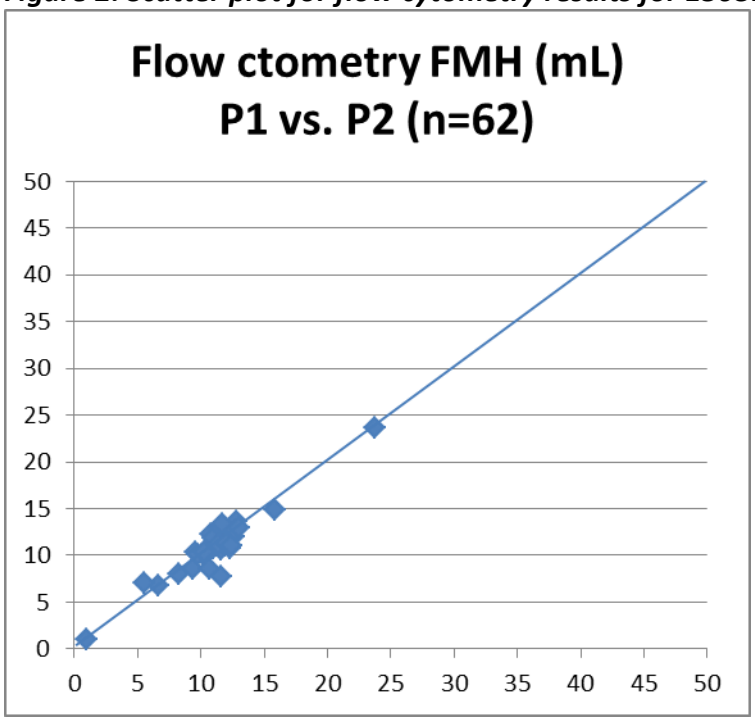
In exercise 1503F and 1505F, both samples were prepared from a single pool. Figures 1 and 2 show the acid elution and flow cytometry results as dot plots for 'Patients' 1 and 2 within individual laboratories for 1503F, and Figures 3 and 4 the same data for 1505F. As would be expected, the flow cytometry results show a better correlation than the acid elution results.

Figure 1: Scatter plot for acid elution results for 1503F



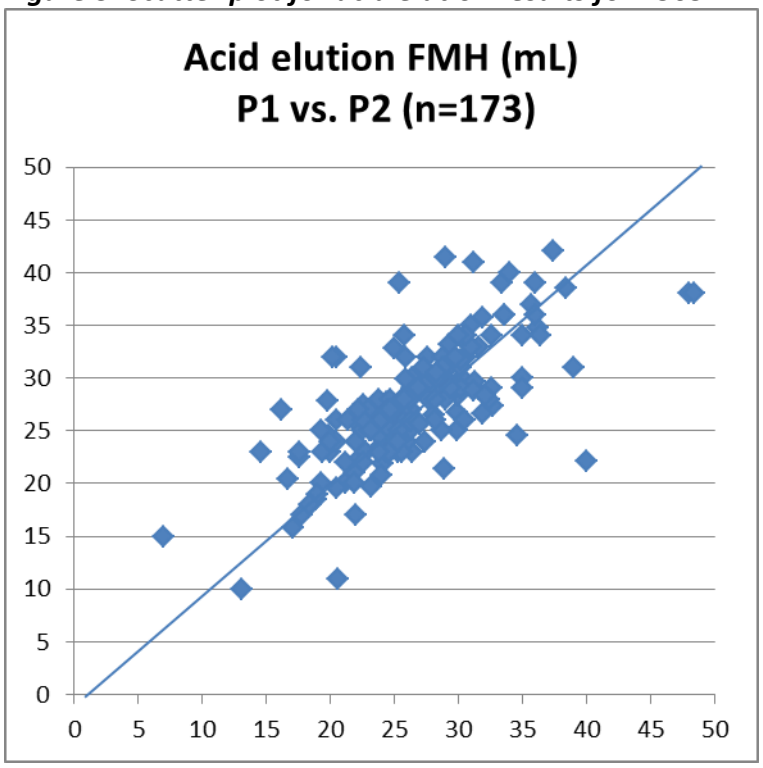
Acid elution method median:
13.2mL for P1
13.0mL for P2

Figure 2: Scatter plot for flow cytometry results for 1503F



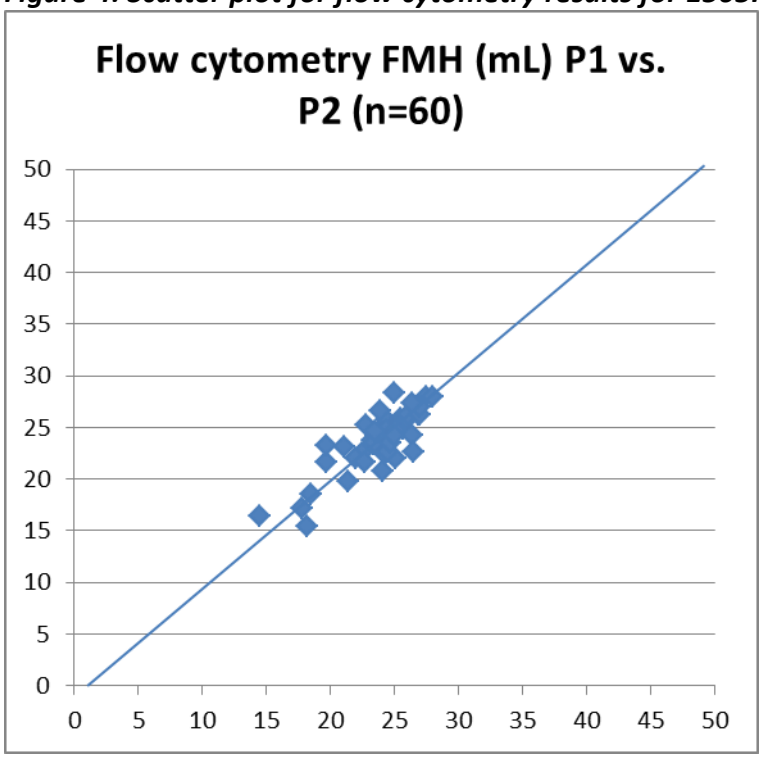
Flow cytometry method median:
11.6mL for P1
11.4mL for P2

Figure 3: Scatter plot for acid elution results for 1505F



Acid elution method median:
26.0mL for P1
27.0mL for P2

Figure 4: Scatter plot for flow cytometry results for 1505F



Flow cytometry method median:
24.4mL for both P1 and P2

10. SCHEME PROGRESS AND DEVELOPMENT

10.1 Accreditation

The Centre underwent a successful assessment visit by UKAS in June 2015 against ISO 17043 standards. As a result of making a single application from the Schemes based at Watford General Hospital, a single 'trading name' and logo was required to be used in conjunction with the UKAS accreditation symbol. The Centre is now accredited under the name: West Herts Hospitals NHS Trust, operating UK NEQAS Haematology and Transfusion.

10.2 IT and communications

- A new information website was launched in 2015. It provides easy navigation and access to content, and will evolve into both an important new communication avenue for the schemes, and a useful tool for the participants. During 2016, we are planning to develop it further, to include:
    - A dedicated feedback page for general feedback, criticisms, suggestions or features that participants would like to see implemented.
    - A secure area for Steering Committee and SAG communications.
    - Facility for participants to be able to make amendments to their own registration details.
    - Facility for participants to print certificates of registration and annual summaries of performance.

10.3 Quality Improvement Plan

Suggestions by participants for improvement are added to the Quality Improvement Plan. One was logged during 2015:

- One participant said they would like to see their % fetal cells on FC reports. This has been agreed and will be implemented in 2016.

10.4 Separate specimens for flow cytometry and acid elution

As reported last year, a small 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. During 2015, the SAG and Steering Committee discussed an option for distributing different specimens for testing by each technique. After long discussions and a risk assessment, it was decided that it might be preferable to split screening from quantification, rather than acid elution from flow cytometry, and this option will be thoroughly assessed during 2016.

<u>BCSH FMH guidelines</u>

A review of the current 2009 BCSH FMH guidelines was started at the end of 2015 and will progress during 2016. As previously, this is being led by the UK NEQAS FMH SAG, with additional writing group members being co-opted as appropriate.

11. KEY PERFORMANCE INDICATORS

The Scheme met all of its KPIs in 2015. 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	6	On schedule	100%	**100%**
Report Distributions	6	Within 4 days of C/D	75%	**100%**
Unsatisfactory performance letters	6 surveys	Posted before the subsequent exercise closes	100%	**100%**
Complaints	1	Acknowledged within one week and full response in 4 weeks	70%	**100%**
Reported Sample Quality	12	≤5% unsatisfactory	75% of samples	**100% (mean USQ 1.9%)**
Integrity of Samples	3496	≤0.5% unsuitable for testing per exercise	75% (i.e 9/12 exercises)	**83% Mean (0.2%)**

12. EDUCATION AND PUBLICATIONS

- UK NEQAS (FMH) was represented on the BCSH Transfusion Task Force during the reporting period in respect of the following guidelines:
  - Blood group and antibody testing during pregnancy
  - Estimation of fetomaternal haemorrhage
  - Anti-D immunoglobulin prophylaxis (addendum to the 2014 published guidelines)

13. REFERENCES

1. WHO (1971) Prevention of Rh Sensitisation. Technical Report Series 468: 6 http://apps.who.int/iris/bitstream/10665/40894/1/WHO_TRS_468.pdf (last accessed 26/09/16)
2. BCSH guideline for the use of anti-D immunoglobulin for the prevention of haemolytic disease of the fetus and newborn. H. Qureshi, E. Massey, D. Kirwan, T. Davies, S. Robson, J. White, J. Jones & S. Allard. Transfusion Medicine 2014, **24**, 1, 8 - 19
3. BCSH (2009) Guidelines for estimation of fetomaternal haemorrhage www.bcshguidelines.org (last accessed 10 August 2016)

Appendix 1

Membership of the BTLP Steering Committee at end 2015

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

Meeting dates (all face-to-face):
23 March 2015
5 July 2015
30 November 2015

Scientific Advisory Group at end 2015

Mr Matthew Hazell (Chair), IBGRL, Bristol (replaced Professor Marion Scott December 2015)
Mrs Diane Howarth, St James' Hospital, Leeds
Ms Lynne Porter, Welsh Blood Service
Dr Sylvia Armstrong-Fisher, SNBTS
Mr Dan Pelling, St Mary's Hospital, London
Mr John Eggington, NHSBT, Liverpool
Ms Jenny White, (Secretary), UK NEQAS
Mrs Clare Milkins UK NEQAS
Dr Megan Rowley, UK NEQAS
Mrs Barbara De La Salle, UK NEQAS
Mr Jon Sims, UK NEQAS

Meeting Dates
26 March 2015 – teleconference
15 December 2015 – face-to-face meeting