UK NEQAS

# *Biennial Report*
# ***2011 - 2012***

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

**UK NEQAS (FMH)**
**PO Box 1000**
**Watford**
**WD18 0WP**

**INDEX**


| | | Page Number |
|---|---|---|
| **1** | **INTRODUCTION** | **1** |
| **2** | **THE SIGNIFICANCE OF FMH QUANTIFICATION** | **1** |
| **3** | **BACKGROUND TO THE SCHEME** | **1** |
| **4** | **STAFF** | **2** |
| **5** | **ANALYTES** | **2** |
| **6** | **NUMBER OF PARTICIPANTS** | **2** |
| **7** | **MATERIAL** | **3** |
| **8** | **DATA MANIPULATION AND PERFORMANCE MONITORING** | **3-4** |
| **9** | **RESULTS** | |
| | Summary of Survey Data by Method | **5** |
| | 'At risk' results | **6** |
| | Outlying results | **7** |
| **10** | **SCHEME PROGRESS AND DEVELOPMENT** | **7-9** |
| **11** | **KEY PERFORMANCE INDICATORS** | **9** |
| **12** | **EDUCATION AND PUBLICATIONS** | **9** |
| **13** | **REFERENCES** | **9** |

**APPENDICES**

| | | |
|---|---|---|
| 1 | Membership of Steering Committee and SAG | **10** |
| 2 | Example of a CAPA form | **11** |

## 1. INTRODUCTION TO THE REPORT

This is a biennial report covering the calendar years of 2011 and 2012.

## 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]. Studies suggest that 0.65% women have a fetomaternal haemorrhage (FMH) >4 mL and will be insufficiently protected by a prophylactic dose of 500 iu, whilst 0.3% women have an FMH of >10 mL and will require more than a standard dose of 1250 iu. Consequently, 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; 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 [2] 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

Following a series of pilot exercises in 1996/97, FMH became a substantive scheme from April 1998 and 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 Dr 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


Telephone: 01923 217933
Fax: 01923 217879
Email fmh@ukneqas.org.uk
Web (for result entry): www.ukneqasfmh.org


*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 2012 there were a total of 259 participating laboratories; details are shown in table 1.

**Table 1 Participation by method**

| Method | UK | Non-UK & Misc |
|---|---|---|
| **Acid elution only** | 144 | 17 |
| **Flow cytometry only** | 10 | 10 |
| **AE and FC** | 26 | 6 |
| **Screening only** | 40 | 6 |

## 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 6 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

In April 2012 there was agreement from the FMH SAG on a change in the FMH scheme design, moving from four surveys per year each comprising three samples, to a six surveys per year each comprising two samples. In the calendar year 2012, five surveys were distributed, one in March, with three samples, and four more with two samples in May, July, September and November.

## 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 | | |
|---|---|---|---|---|---|---|---|---|
| | | | No. Returns [2] | Median (mL) | IQ Range (mL) | No. Returns | Median (mL) | IQ Range (mL) |
| 1101F - 1 [1] | **8 Mar 11** | 96.9% | 205 | 8.5 | 7.3 – 9.9 | 43 | 6.8 | 6.6 – 7.4 |
| - 2 [1] | | 96.6% | 205 | 8.5 | 7.2 – 9.7 | 43 | 6.9 | 6.6 – 7.3 |
| - 3 | | 97.6% | 206 | 11.0 | 9.3 – 13.1 | 43 | 8.3 | 7.8 – 8.9 |
| 1102F - 1 | **7 June 11** | 98.0% | 198 | 7.6 | 6.5 – 9.1 | 48 | 5.6 | 5.3 – 6.0 |
| - 2 | | 99.0% | 199 | 13.8 | 11.5 – 16.0 | 48 | 11.2 | 10.7 – 11.8 |
| - 3 | | 98.6% | 198 | 24.6 | 20.2 – 27.9 | 48 | 22.0 | 20.8 – 22.8 |
| 1103F - 1 | **6 Sept 11** | 92.2% | 184 | 29.7 | 26.0 – 33.7 | 41 | 28.3 | 27.1 – 28.8 |
| - 2 | | 92.6% | 183 | 29.1 | 24.9 – 32.9 | 41 | 28.2 | 27.1 – 29.0 |
| - 3 [3] | | 92.2% | 176 | 4.2 | 3.5 – 5.3 | 41 | 3.3 | 3.0 – 3.4 |
| 1104F - 1 [3] | **6 Dec 11** | 98.3% | 48 | 0.0 | 0.0 – 0.8 | 46 | 0.0 | 0.0 – 0.2 |
| - 2 | | 98.3% | 196 | 7.8 | 6.7 – 9.4 | 47 | 5.9 | 5.5 – 6.5 |
| - 3 | | 98.0% | 196 | 10.2 | 8.9 – 11.7 | 47 | 7.7 | 7.4 – 8.1 |
| 1201F - 1 | **6 Mar 12** | 97.6% | 197 | 25.8 | 22.9 - 29.9 | 48 | 24.6 | 23.4 – 26.8 |
| - 2 [1] | | 96.3% | 195 | 10.5 | 8.7 – 12.1 | 48 | 9.7 | 9.0 – 10.0 |
| - 3 [1] | | 97.0% | 195 | 10.5 | 8.6 – 12.3 | 48 | 9.6 | 9.0 – 10.0 |
| 1202F - 1 [3] | **1 May 12** | 97.0% | 177 | 3.7 | 2.9 – 4.2 | 49 | 2.9 | 2.6 – 3.1 |
| - 2 | | 92.3% | 184 | 18.6 | 14.8 – 22.1 | 49 | 15.2 | 14.5 – 15.7 |
| 1203F - 1 | **3 July 12** | 94.6% | 181 | 10.8 | 8.9 – 13.0 | 51 | 8.4 | 7.8 – 8.8 |
| - 2 | | 95.6% | 183 | 14.0 | 12.0 – 16.5 | 51 | 12.4 | 12.0 – 13.1 |
| 1204F - 1 [3] | **4 Sept 12** | 98.0% | 181 | 4.5 | 3.5 – 5.3 | 51 | 3.4 | 3.0 – 3.7 |
| - 2 | | 97.6% | 183 | 24.1 | 21.0 – 26.7 | 51 | 21.2 | 20.3 – 22.1 |
| 1205F - 1 [3] | **6 Nov 12** | 95.2% | 163 | 2.2 | 1.7 – 3.0 | 52 | 1.0 | 0.8 – 1.2 |
| - 2 | | 97.3% | 188 | 30.5 | 26.7 – 34.2 | 52 | 27.9 | 26.9 – 29.0 |

[1] *- Specimens within the survey were prepared from the same pool*
[2] *- Excludes returns where no numerical value was given, e.g. <4mL*
[3] *- Specimens not scored as flow cytometry median <4mL.*

## 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[1] Quantification/screen only | FC median (mL) | No. 'at risk' Quantification | No. 'at risk' Screen only |
|---|---|---|---|---|
| **1101F - 1** | 205/33 | 6.8 | 1 | 0 |
| **- 2** | 205/33 | 6.9 | 1 | 0 |
| **- 3** | 205/33 | 8.3 | 0 | 1 |
| **1102F - 1** | 198/34 | 5.6 | 0 | 0 |
| **- 2** | 198/34 | 11.2 | 1 | 0 |
| **- 3** | 198/34 | 22.0 | 2 | 0 |
| **1103F - 1** | 183/37 | 28.3 | 0 | 0 |
| **- 2** | 182/37 | 28.2 | 2 | 0 |
| **1104F - 2** | 195/38 | 5.9 | 0 | 1 |
| **- 3** | 195/38 | 7.7 | 0 | 0 |
| **1201F - 1** | 197/39 | 24.6 | 0 | 0 |
| **- 2** | 195/39 | 9.7 | 0 | 0 |
| **- 3** | 195/39 | 9.6 | 2 | 0 |
| **1202F - 2** | 184/40 | 15.2 | 2 | 0 |
| **1203F - 1** | 181/40 | 8.4 | 1 | 1 |
| **- 2** | 183/40 | 12.4 | 4 | 1 |
| **1204F -2** | 183/44 | 21.2 | 1 | 1 |
| **1205F -2** | 188/44 | 27.9 | 2 | 0 |

[1] *- Excludes participants who did not state a dose of anti-D*

### *Participants registered for quantification*

Over this two-year period, there were 19 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 0.55%.

### *Participants registered for screening only*

During this two-year period, a maximum of 45 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 five 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 0.75%.

### 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 74 outlying results due to underestimation, and 40 due to overestimation, giving rates of 2.3% and 1.2% respectively.

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

| Survey | No. Participants | AE median (mL) | No. (%) outliers <-2 | No. (%) outliers >3.5 |
|---|---|---|---|---|
| **1101F - 1** | 205 | 8.5 | 6 | 2 |
| **- 2** | 205 | 8.5 | 4 | 4 |
| **- 3** | 206 | 11.0 | 1 | 3 |
| **1102F - 1** | 198 | 7.6 | 3 | 3 |
| **- 2** | 199 | 13.8 | 1 | 1 |
| **- 3** | 198 | 24.6 | 7 | 1 |
| **1103F - 1** | 183 | 29.7 | 4 | 1 |
| **- 2** | 182 | 29.1 | 6 | 5 |
| **1104F - 2** | 196 | 7.8 | 2 | 2 |
| **- 3** | 196 | 10.2 | 7 | 4 |
| **1201F - 2** | 195 | 10.5 | 2 | 0 |
| **- 3** | 195 | 10.5 | 4 | 0 |
| **1202F - 2** | 184 | 18.6 | 1 | 1 |
| **1203F - 1** | 181 | 10.8 | 4 | 4 |
| **- 2** | 183 | 14.0 | 5 | 1 |
| **1204F - 2** | 183 | 24.1 | 7 | 3 |
| **1205F - 2** | 188 | 30.5 | 10 | 5 |

## 10. SCHEME PROGRESS AND DEVELOPMENT

### 10.1 Accreditation

The Scheme underwent a CPA inspection in July 2012, and maintains full accreditation with CPA (UK) Ltd. The inspection included a pre-assessment visit by UKAS for the new ISO 17043 proficiency testing standards.

### 10.2 IT systems

- The level of registration for web-entry of results has increased over the past 24 months from 82% to 94% overall, and from 84% to 94% in the UK.
- On-line re-registration for participation in the Scheme was continued in 2012.

### 10.3 Source of material

In the past, donors from a selected pool of AB D negative males were invited to donate whole blood at a specified session at the West End Donor Centre. However, AB male plasma is in great demand as a clinical component and is no longer readily available for EQA exercises. We have collaborated with the donor consultant at NHSBT Colindale, to identify a suitable panel of AB D negative female donors, whose donations might otherwise not be required for therapeutic use.

## 10.4 Stability of cord cells

Following the problems with deterioration of cord cells leading to two surveys being withdrawn from performance monitoring in 2010 /11, two trials were undertaken at the International Blood Group Reference Laboratory (IBGRL) to look at the stability of cord cells over time. Several different cord samples were used to prepare FMH samples in the same way as they are prepared at UK NEQAS and the samples were tested sequentially by flow cytometry. The aim was to try to establish a time limit up to which all examples of cord cells tested are stable. The first trial using five cord samples at 1-5% monitored over 27 days showed a steady slow linear decrease in % fetal cells up to 20-25 days, at which point the background signal increased as a result of the adult cells beginning to deteriorate. The second trial using four cords tested at more frequent intervals did not show the same rate of deterioration for three of the four cords.

There is no clear conclusion, except that there is some accelerated deterioration of cord cells compared to adult cells over time, and variation between individual cords. The current strategy of using cord cells as fresh as possible seems reasonable and will continue. Exercise material, which has been subjected to the postal system, is tested in-house by flow cytometry and acid elution on the closing date. If deterioration is noted, within pre-defined limits, the EQA sample is not subject to penalty scoring.

## 10.5 Teaching Slides

In March 2011, a trial was started as a collaborative project between UK NEQAS and a BSc student at St Mary's Hospital, Imperial College Healthcare NHS Trust, London. The aim was to test stability of stained, fixed and unfixed slides over a three- month period, with a view to validating use of slides prepared following each UK NEQAS exercise, to be used by laboratories for training against local SOPs. Such slides would be useful for teaching and training, because the method median would have been established by participants in the corresponding UK NEQAS exercise. Selected clinical laboratories registered for quantification by acid elution were provided with one stained and three unstained slides (prepared from one of the samples from 1101F, but this was not disclosed at the time). Each was asked to undertake sequential FMH estimation over a three-month period to assess the stability and/or deterioration of the slides. Additionally, opinions were sought as to the general benefit to laboratories of this resource for training in FMH estimation. Initial results showed that unstained, fixed slides were unsuitable for examination, even one month after preparation, in all laboratories. Slides fixed and stained by the participating laboratories on day one were still suitable for examination by all throughout the trial, with good sample quality at two months and moderate quality at three months. In a parallel project at Imperial, frozen slides (fixed or unfixed and wrapped in tin foil) showed less deterioration than fully prepared slides. The FMH SAG will use this information to develop advice for participants on how to prepare and store teaching slides for the two months between UK NEQAS FMH exercises.

## 10.6 Performance Monitoring

A review of the performance monitoring for acid elution (AE) was undertaken in 2011 followed, in 2012, by a major review of flow cytometry (FC) results and performance monitoring. These reviews highlighted that very few laboratories were reaching a score of 100, despite submitting outlying results.

Following discussions at the SAG meeting, it was agreed that proposals would be made to the National Quality Assurance Advisory Panel for the following changes:

- To increase the constant used in the calculation to identify 1-5% of participants as unsatisfactory.
- To redefine the terms Unsatisfactory Performance (UP) and Persistent Unsatisfactory Performance (PUP) in relation to numerical scoring.
- To score flow cytometry laboratories for bleeds of < 4mL, but > 0mL.

Extensive work was undertaken on remodelling the 2011 and 2012 data using a variety of different constants, and following a review of the data by the senior staff team, new constants have been defined to present to the NQAAP for approval at the annual meeting in March 13.

A Corrective and Preventive Action (CAPA) form designed for BTLP participants to download when they have made an error has been adapted and piloted for FMH and is proving to be successful. See Appendix 2 for an example.

## 11. KEY PERFORMANCE INDICATORS

The Scheme met all of its KPIs in 2011 and 2012. Table 5 details the targets and achievements.

***Table 5 – Key Performance Indicators***

| Category | No. of Events | Target | Target Achievement Rate | Actual Achievement Rate |
|---|---|---|---|---|
| Exercise Distributions | 9 | On schedule | 100% | **100%** |
| Report Distributions | 9 | Within 4 days of C/D | 75% | **100%** |
| Complaints | 11 | Dealt with in 4 weeks | 70% | **100%** |
| Reported Sample Quality | 23 | ≤5% unsatisfactory | 75% of samples | **96%** |
| Integrity of Samples | 6392 | ≤0.5% unsuitable for testing per exercise | 75% (i.e. 3/4 exercises) | **90%** |

## 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

# Appendix 1

## Membership of the BTLP Steering Committee at end 2012

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
Mrs Samantha Harle-Stephens, Derriford Hospital, Plymouth
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
Mr Allan Morrison (Observer - NQAAP representative), Addenbrookes Hospital, Cambridge
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 2012

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
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

## Appendix 2 – Example of CAPA form

# EQA CAPA Summary (PRN xxxxx)

| Details of unsatisfactory performance | | | |
|---|---|---|---|
| Exercise Code: | 1203F and 1205F | | |
| Sample(s): | Both | | |
| Area of assessment (tick appropriate box) | Potential for sensitisation | Outlying result √ | Score >100 |
| Result Reported: | **1203F:**<br>Overestimation for P1: 22.5mL *cf* median of 10.8mL<br>**1205F:**<br>Overestimation for P1: 52.8mL *cf* median 2.2mL (non scored)<br>Underestimation for P2: 2.4mL *cf* median of 30.5mL | | |

| Details of laboratory investigation |
|---|
| |
| **Participant's assessment of the cause of unsatisfactory performance** |
| |
| **Potential for impact in clinical situation** |
| |
| **Details of CAPA** |
| |

| Signature (as appropriate) / Date | |
|---|---|
| Laboratory Manager | |
| Consultant Haematologist | |
| Quality Manager | |