

FMF Certification of Biochemical Laboratories

The requirements for certification are:

1. Compliance with the FMF Good Laboratory Guidelines (see below) and a signed Declaration of Conformity to the GLP guidance.
2. Satisfactory laboratory Standard Operating Procedure (SOP) for prenatal screening.
3. Your UKNEQAS Laboratory number. This is to confirm satisfactory participation and monitoring of your ongoing performance; it will be kept solely by the FMF Director of Biochemical Screening (Prof Kevin Spencer) and will not be given to any other person inside or outside of the FMF.

Full certification is conditional on the demonstration of acceptable audit results after six months. Continued certification depends on acceptable external quality assurance performance and participation in an annual FMF audit. Please note that with the new release of the FMF algorithms the software will allow calculation of risks only if the operator holds the FMF Certificate of competence in measurement of nuchal translucency thickness and has carried out regular annual audits.

Recommendations for good laboratory practice

Laboratory registration

1. All laboratories must participate in the United Kingdom National External Quality Assessment Service (UKNEQAS) scheme for First Trimester Downs Syndrome Screening. The performance in this scheme must be such that the analytical bias from the Method Mean for free β -hCG and PAPP-A does not deviate by more than 10% on an ongoing basis.

Information on this Program can be obtained from:

Andy Ellis
UK NEQAS for Peptide Hormones
Clinical Chemistry Department
Royal Infirmary
Edinburgh EH16 4SA
UK

Phone: 44 131 242 6848

Fax: 44 131 242 6882

Email: UKNEQAS@ed.ac.uk

Web Page: www.ukneqas.org.uk/Directory/CC/dsfirst.htm

2. When appropriate laboratories should be accredited by the Clinical Pathology Accreditation (UK) Ltd in the UK or by an equivalent body in other countries. Clinics who are not part of an organised laboratory service providing combined screening using FMF approved automated systems for the measurement of free β -hCG and PAPP-A may be exempt from this accreditation, but should employ an experienced laboratory person as an advisor.

Sample collection

1. Laboratories must ensure that whole blood is received within 48 hours of collection and serum samples within 72 hours of collection. Measurement of free β -hCG in samples collected beyond these limits are unreliable.

- The samples must be collected between 11 weeks 0 days and 13 weeks 6 days and accompanied by the following information:

| | |
|-----------------|-----------------------------|
| Patient | Full name |
| | Referring clinician |
| | Date of birth |
| | History of previous trisomy |
| | Ethnicity |
| | Weight |
| | Smoking |
| Blood Sample | Date of collection |
| | Reference number |
| Ultrasound scan | Date |
| | Name of sonographer |
| | Fetal crown – rump – length |
| | Fetal nuchal translucency |
| | Number of live fetuses |

Analysis of samples and internal quality control

- Laboratories must use analytical systems and assays for free β -hCG and PAPP-A that are supported by the manufacturer for the purpose of first trimester screening for Down's syndrome and have proven clinical performance for this use. Please see below for Assay Systems Specification. At present the FMF algorithm only supports both Kryptor analytical platforms, Kryptor and Kryptor compact (www.kryptor.net) from Brahms Aktiengesellschaft, Berlin (www.brahms.de), the PerkinElmer Manual Delfia, Auto Delfia and Delfia Express analytical platforms (www.perkinelmer.com) and Roche Elecsys and Cobas E analytical platforms (www.roche.com).
- Laboratories should perform Internal Quality Control (QC) procedures with each batch of samples analysed – or on a daily basis. Three level QC should be performed for free β -hCG and PAPP-A and the between day coefficients of variation (CV) should be as follows:

| | free β -hCG conc | CV | PAPP-A conc | CV |
|---------|------------------------|-----|-------------|-----|
| Level 1 | 85 | 3.0 | 0.30 | 3.5 |
| Level 2 | 20 | 3.0 | 1.50 | 3.5 |
| Level 3 | 8 | 3.5 | 4.0 | 3.0 |

- Prenatal screening laboratories should monitor the overall median MoM for Free β -hCG and PAPP-A on a monthly basis. This should be within the limits of 1MoM + 10%.
- Prenatal screening laboratories should monitor the medians for individual completed weeks on a 3 monthly basis to ensure they do not deviate from the expected values by more than 10%.

Calculation of risk

- All risk calculations should only be performed using software approved by the Fetal Medicine Foundation (see 'Registered Software' below) and all laboratories must ensure that they only take nuchal translucency measurements from sonographers who hold the

FMF Certificate of Competence in the 11-13+6 weeks scan (see 'Certification in the 11-13+6 weeks scan').

2. The percentage of total screened cases identified with a risk of 1 in 300 or greater should be monitored on a monthly basis. The screen positive rate should be between 3% and 6%, depending upon the age of the population being screened.
3. Laboratories should monitor the variability of the risk derived from a fixed maternal age, fixed gestational age and fixed NT using results from the Level 1 and Level 2 controls. For a target risk of 1 in 250 a 10% CV of the risk should be achievable.
4. Laboratories should follow up the outcome of all pregnancies screened or at least those identified with a risk of 1 in 300 or greater.

Continuing audit and certification

1. Laboratories should supply the Fetal Medicine Foundation with regular audit data. Initially this is done at six monthly intervals, but once satisfactory results have been demonstrated will be carried annually.
2. Continued certification depends on acceptable external quality assurance performance and participation in an annual Fetal Medicine Foundation audit.
3. Laboratories should analyse over 1000 screening samples per year for rigorous quality assurance.

Specifications for assay systems in order to meet FMF standards

1. Assays must be supported by the company for use in First Trimester Prenatal Screening.
2. Assays must be standardised against the relevant International Reference Preparation (IRP) for Free Beta hCG and PAPP-A and be expressed in IU/L and in mass or molar units if appropriate.
3. Within day and between day %CV's at the following concentrations must be demonstrable:

| Free Beta hCG | Within Day CV % | Between Day CV % |
|---------------|-----------------|------------------|
| 85iu/l | 3.0 | 5.0 |
| 20iu/l | 3.0 | 5.0 |
| 8iu/l | 4.0 | 6.0 |
| PAPP-A | Within Day CV % | Between Day CV % |
| 0.30iu/l | 4.0 | 6.0 |
| 1.50iu/l | 4.0 | 6.0 |
| 4.00iu/l | 3.0 | 5.0 |

4. Performance must be assessed against both individual methods and the ALTM with the UKNEQAS scheme for First Trimester Down's Syndrome Screening.
5. A Bias of less than +/- 10% from the ALTM must be demonstrable.
6. Assays should have a minimum working range without need for dilution of 150iu/l for Free Beta hCG and 6.0iu/l for PAPP-A.
7. Recovery of IRP added to non pregnancy serum must be 100% +/-10% across the whole assay range.
8. Dilution recovery of high samples must be within +/-10% of the neat material.
9. Assays should have clearly defined performance in the presence of jaundice, haemolysis and lipaemia.
10. Performance in the presence of excess antigen (hook effect) must be clearly detailed.
11. Manufacturers should provide guidelines for the median levels at 11,12, 13, 14 weeks gestation. A minimum of 150 samples at each gestational week must be analysed.
12. The manufacturer must provide the distribution parameters for the normal data set as overall median MoM, log₁₀ MoM and its standard deviation. In addition correlation of log PAPP-A MoM with log Free Beta hCG MoM must be provided. Item 11 and 12 should be provided from one single testing center.

13. The manufacturer should provide evidence of acceptable clinical performance of the assays with a panel of sera from known cases of trisomy 21. A study in one center must comprise of at least 50 cases of trisomy 21. The T21 cases must come from a routine first trimester screened population. The manufacturer must state for each case the reasons for diagnosis and if identified pre or post delivery.
14. From the above population the manufacturer must provide the median MoM, the mean Log MoM and its standard deviation and the correlation coefficient between log PAPP-A MoM and log Free Beta hCG MoM.
15. Using the population parameters for the unaffected population and from the Trisomy 21 population the manufacturer must simulate the detection rate and false positive rate using a standardised age population such as that in England & Wales (2000). A detection rate better than 60% at a 5% false positive rate must be demonstrated. If the manufacturer cannot provide such a simulation from an authoritative source then the raw data must be made available to the FMF in order for this to be established.
16. The assay minimum detection limit and functional sensitivity should be described.

Further information on the Laboratory Certification process can be obtained from:

Prof Kevin Spencer
Director of Biochemical Screening
Fetal Medicine Foundation
Email: KevinSpencer1@aol.com