

Portfolio Submission for ACS Certificate of Attainment

- **Report**
- **Appendix of supporting material**
- **Competency document**

Xxxxx Xxxxx

**Modality: Clinical Genetics
Sub-modality: Molecular Genetics**

COMPETENCES REQUIRED FOR CLINICAL SCIENTISTS TO ATTAIN STATE REGISTRATION			
MODALITY:	Clinical Genetics	SUBMODALITY: (if applicable)	Molecular Genetics
			APPLICANT'S NAME:
			Xxxxx Xxxxx

APPENDIX 1

This set of documents must be completed and returned in your portfolio.
Please complete the three header sections above on each page.
Refer to the Specific Competences document for guidance in completing this document.
Use typescript or black ink and block capitals for all sections.

EXPERIENCE: The candidate should be able to demonstrate that he/she has worked in an environment that has enabled the individual to receive training and gain experience relevant to the competences set out below.	
SCIENTIFIC	
AREA OF COMPETENCE	INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED
<ul style="list-style-type: none"> understanding the science that underpins the speciality (modality) and the broader aspects of medicine and clinical practice demonstrating a strong base of knowledge appropriate to the speciality and to the investigations and therapeutic options available experience of searching for knowledge, critical appraisal of information and integration into the knowledge base ability to apply knowledge to problems associated with the routine provision, and development, of the service ability to identify the clinical decision which the test/intervention will inform ability to make judgements on the effectiveness of procedures application of the knowledge base to the speciality (modality) and to the range of procedures/investigations available 	<p>Report: 17-21, 27, 44, 60, 63, 64 Appendix: 1, 2, 4, 12</p> <p>Report: 12, 38, 47, 57, 60 Appendix: 1, 2</p> <p>Report: 6, 11, 15, 26, 42, 60 Appendix: 7, 9</p> <p>Report: 9, 10, 13, 57 Appendix: 5, 6, 14</p> <p>Report: 27, 35, 48, 58, 63, 64 Appendix: 9</p> <p>Report: 5, 12, 14, 26, 38, 43, 52 Appendix:</p> <p>Report: 39, 43, 47 Appendix: 14</p>

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CLINICAL	
AREA OF COMPETENCE	INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED
<ul style="list-style-type: none"> ability to provide interpretation of data and a diagnostic (therapeutic) opinion, including any further action to be taken by the individual directly responsible for the care of the patient 	Report: 36, 47 Appendix: 8, 10, 13
<ul style="list-style-type: none"> understanding of the wider clinical situation relevant to the patients presenting to his/her specialty 	Report: 27, 63, 64 Appendix:
<ul style="list-style-type: none"> ability to develop/devise an investigation strategy taking into account the complete clinical picture 	Report: 13, 47, 57 Appendix: 14
<ul style="list-style-type: none"> understanding of the clinical applications of his/her specialty and the consequences of decisions made upon his/her actions/advice 	Report: 13, 27, 63 Appendix:
<ul style="list-style-type: none"> awareness of the evidence base that underpins the use of the procedures employed by the service 	Report: 6, 11, 15, 23, 33, 40 Appendix: 7

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TECHNICAL		INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED
AREA OF COMPETENCE		
<ul style="list-style-type: none"> understanding of the principles associated with a range of techniques employed in the modality 		Report: 4, 7, 9, 12, 16, 22-26, 28, 34, 38, 41, 43 Appendix:
<ul style="list-style-type: none"> knowledge of the standards of practice expected from these techniques 		Report: 47, 54, 57, 59 Appendix: 15, 16
<ul style="list-style-type: none"> experience of performing these techniques 		Report: 4, 5, 7, 9, 12, 13, 16, 24, 25, 29, 34, 39, 52, 55, 57 Appendix: 5, 6, 8, 15
<ul style="list-style-type: none"> the ability to solve problems that might arise during the routine application of these techniques (troubleshooting) 		Report: 9, 10, 34, 57 Appendix: 15
<ul style="list-style-type: none"> understanding of the principles of quality control and quality assurance 		Report: 47, 54, 59, 60 Appendix: 10, 13
<ul style="list-style-type: none"> experience of the use of quality control and quality assurance techniques including restorative action when performance deteriorates 		Report: 47, 49, 52, 54, 66 Appendix: 10, 11, 14

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RESEARCH AND DEVELOPMENT	
AREA OF COMPETENCE	INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED
<ul style="list-style-type: none"> ability to read and critically appraise the literature 	Report: 15, 42 Appendix: 7, 9
<ul style="list-style-type: none"> ability to develop the aims and objectives associated with a project 	Report: 43 Appendix: 9
<ul style="list-style-type: none"> ability to develop an experimental protocol to meet the aims and objectives in a way that provides reliable and robust data (i.e. free of bias) 	Report: 43, 48, 52, 58 Appendix: 9
<ul style="list-style-type: none"> ability to perform the required experimental work ability to produce and present the results (including statistical analysis) 	Report: 10, 43 Appendix: 9
<ul style="list-style-type: none"> ability to critically appraise results in the light of existing knowledge and the hypothesis developed and to formulate further research questions 	Report: 10, 43 Appendix: 5, 6, 9
<ul style="list-style-type: none"> ability to present data and provide a critical appraisal to an audience of peers – both spoken and written 	Report: 10, 44, 64 Appendix: 5, 6, 12

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COMMUNICATION	
AREA OF COMPETENCE	INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED
<ul style="list-style-type: none"> ability to assess a situation and act accordingly when representing the specialty 	Report: 37, 47, 50, 67 Appendix:
<ul style="list-style-type: none"> ability to respond to enquiries regarding the service provided when dealing with clinical colleagues 	Report: 37, 47, 53, 65 Appendix:
<ul style="list-style-type: none"> ability to communicate with patients, carers and relatives, the public and other healthcare professionals as appropriate 	Report: 44, 50, 64 Appendix: 12
<ul style="list-style-type: none"> ability to communicate the outcome of problem solving and research and development activities 	Report: 10, 44, 64 Appendix: 5, 6
<ul style="list-style-type: none"> evidence of presentation of scientific material at meetings and in the literature 	Report: 10, 50 Appendix: 3, 5, 6, 12

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PROBLEM SOLVING	
AREA OF COMPETENCE	INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED
<ul style="list-style-type: none"> to assess a situation 	Report: 52, 57 Appendix:
<ul style="list-style-type: none"> determine the nature and severity of the problem 	Report: 49, 52 Appendix:
<ul style="list-style-type: none"> call upon the required knowledge and experience to deal with the problem 	Report: 49, 52 Appendix: 11
<ul style="list-style-type: none"> initiate resolution of the problem 	Report: 49, 52, 57 Appendix:
<ul style="list-style-type: none"> demonstrate personal initiative 	Report: 52, 57 Appendix:

MANAGEMENT	
AREA OF COMPETENCE	INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED
<ul style="list-style-type: none"> to understand the principles of management 	Report: 51, 58, 60, 62, 68 Appendix:
<ul style="list-style-type: none"> to understand the principles of quality assurance, audit, safety and accreditation relevant to a specific discipline 	Report: 49, 59, 60 Appendix: 11

Note:

The above are the generic competences that must be met by all Clinical Scientists. These competences have also been mapped onto specific subjects. Copies of these can be obtained from the ACS Administrative Office.

ACS Portfolio Report

Grade A training

- 1 I began my training in October 1998 as a supernumerary Grade A Trainee Clinical Scientist in Molecular Genetics for the xxxxx Region. The first 12 months were spent in the Molecular Genetics Department at xxxxx Hospital, under the supervision of the Head of Department, Dr xxxxx.

Introductory Module

- 2 In the Introductory module, I was introduced to the basic workings and organisation of the laboratory. Before undertaking any practical work, I was trained in and read about the health and safety procedures in the laboratory. This comprised general conduct in the laboratory, first aid procedures, hazards associated with handling human blood samples, confirmation of appropriate vaccinations, COSHH assessments of all potentially hazardous substances in the laboratory. Training in the correct handling of radioactivity consisted of reading the local rules, training by the laboratory's Radiation Protection Supervisor, and attendance of the Medical Physics radioactivity introductory course.
- 3 For a period of approximately 4 weeks, I was given the responsibility of all booking on, under the supervision of the Medical Technical Officer with routine responsibility, as well as the Clinical Scientists.
- 4 I learnt the various extraction procedures that were routinely carried out in the laboratory: a Nucleon II protocol for extraction of blood samples of 2.5-15ml blood, a Nucleon I protocol for the extraction of small volumes of blood (0.5-2ml), and a crude sodium hydroxide/boiling extraction for 200µl blood samples used for high-throughput diseases (cystic fibrosis, fragile X syndrome, haemochromatosis). Again, for a period of 2-4 weeks I undertook these extractions myself, after appropriate training and supervision.
Competencies: Tech.
- 5 As a mini-project, I evaluated three different procedures for extracting DNA from blood. The aim was to evaluate the current extraction procedure (Nucleon II) against two alternatives: the 'Crumlin' protocol, using home-made reagents (based on the Puregene DNA isolation kit) and Camgen genomic kit. The protocols were compared on a number of criteria: DNA yield from different volumes of blood (measured by UV spectrophotometry), DNA quality for use in diagnostic testing (evaluated crudely by use in the CAG repeat assay for Huntington disease, considered a sensitive assay), cost of reagents, time taken to perform extraction, ease of use. None of the three protocols were reliable in extracting from small quantities of blood (1-1.5ml), confirming the necessity for a separate protocol (Nucleon I). The Nucleon II protocol appeared to be the best of the three options, despite its relatively high costs. It produced good yields of high quality DNA from a range of blood volumes, and the protocol was relatively straight-forward, attributes that are essential in the setting of a diagnostic laboratory, where a large number of samples are received which vary widely, and where failures must be kept to an absolute minimum.
Competencies: Scient, Tech.

Huntington Disease Module

- 6 I studied and wrote about the clinical features of the disease (motor disturbance, dementia and psychiatric changes); its genetic basis (expanded polyglutamine-encoding CAG repeat in exon 1 of the IT15 gene, different phenotype/penetrance depending on size of expansion; repeat expansion only via paternal transmission); the testing carried out by the laboratory (PCR and, if necessary, Southern Blotting); and the current theories of molecular pathogenesis (intracellular aggregates/ inclusions, the possible role of proteolytic cleavage and/or ubiquitination of the abnormal huntingtin protein).
Competencies: Scient, Clin.
- 7 Practical work was carried out on archive samples initially, but later in the module I gained experience of testing live diagnostic samples. PCR was used as the initial screen, using primers HD1 and HD3; in most cases a normal result could be determined from this test alone (i.e. 2 alleles in normal size range). In cases where only one allele was observed, another PCR could be carried out using primers HD1 and HD2, which includes a second polymorphic sequence CCG, and this could separate two alleles giving a homozygous result on the HD1-HD3 assay, or with a possible primer-site mutation under HD3. Individuals with a suspected large expansion that would not be detected by PCR and those showing only one allele on HD1-HD3 and HD1-HD2 PCR assays were analysed further by Southern Blot analysis (DNA digested with PstI and 4G6P1.7 probe used). Theoretical consideration was given to prenatal exclusion testing, where one partner of a pregnant couple has a parent with molecularly confirmed Huntington disease, does not wish to know their own status via presymptomatic testing, but does not wish to have a child who will develop the disease later in life. DNA markers from the family members are taken and tested using polymorphic markers closely flanking the IT15 gene in order to identify the haplotypes present in the affected family member. The fetus may then be tested for the presence or absence of one of the affected grandparental haplotypes.
Competencies: Tech.
- 8 The clinical and ethical considerations of Huntington disease testing were covered, with regard to its late onset and implications of a positive result for any children, and the importance of informed consent for any testing, and careful counselling before any presymptomatic/predictive testing.
- 9 Several case studies were undertaken. In one instance, a patient with an uncertain diagnosis of Huntington disease was found by PCR to have a CAG expansion of approximately 39-43 repeats, on the border between the region of reduced penetrance (36-39 repeats) and the definite disease range (≥ 42 repeats). It was necessary to size the CAG repeat precisely, which could not be done by the standard method on non-denaturing acrylamide gel. Therefore, the PCR was repeated using a fluorescently-tagged primer and the products run out on a fluorescent gel system (ALF Express). A control sample of known and validated repeat size was run alongside, and also the two samples were mixed and run out in order to aid an exact sizing of the patient's expanded allele. It was found that the repeat was forty-two CAGs in size, giving a firm confirmation of the diagnosis.
Competencies: Scient, Tech.
- 10 Two other patients were investigated whose sample gave unusual results on the standard HD1-HD3 PCR assay. These investigations formed the basis of a

project whose initial findings I presented at the CMGS Spring meeting in xxxxx in xxxxx (**SEE APPENDIX A.5**). Further analysis lead to conclusions which were presented as a poster at the BSHG York meeting in xxxxx (**SEE APPENDIX A.6**). Both cases involved the use of alternative primers to identify the cause of the unusual results, and then sequencing to confirm the presence of nucleotide changes affecting HD1 and /HD3 primer-binding sites.

Competencies: Scient, Tech, R+D, Comm.

Fragile X Syndrome Module

- 11 I covered the theoretical aspects of the module: the clinical features of the disease; the basis of the disease being a CGG expansion in the promoter region of the FMR-1 gene which becomes methylated when abnormally expanded (>~200 repeats) and silences the gene copy; the normal, intermediate, premutation and affected ranges for CGG repeat sizes; repeat size expansion via maternal transmission only and normal transmitting males; the occurrence of premutation/full mutation and normal/full mutation mosaic individuals; the existence of the FraXE expansion as a cause of mild mental retardation.
Competencies: Scient, Clin.
- 12 I gained practical experience of all the techniques used in diagnostic testing: PCR across the CGG repeat to identify normal and small premutation alleles, Southern Blot analysis to identify large premutations and full expansions, with the importance of including appropriate controls. The xxxxx laboratory carries out three different Southern Blot analyses depending upon the referral and the PCR results: the methylation-sensitive EcoRI+EagI blot with OX1.9 probe distinguishes large premutations from full expansions by assessing the methylation status of the allele(s), while the smaller fragment PstI blot with OX0.55 probe can help to size premutations and hence assess their risk of expanding upon transmission via a female. The BglII blot with OX1.9 probe is used occasionally to detect very large expansions that appear as a very faint smear on the EcoRI+EagI blot. I tested, interpreted and reported approximately twenty-five individuals as 'live' cases, including unaffected and affected males and females, intermediate allele males, premutation males and females and prenatal samples.
Competencies: Scient, Tech.
- 13 One prenatal case I studied in detail involved a mother who was a premutation carrier and had one son affected with a full expansion and affected with Fragile X syndrome. The mother was pregnant and a chorionic villus biopsy (CVB) sample was sent to the laboratory for testing. Prior to the CVB sample arriving, samples from the mother and affected son were tested using the flanking markers AC1 and AC2 (and fluorescent gel analysis on the ALF Express) to identify the 'high risk' allele. These were uninformative so a further marker, DXS548, was typed and found to be informative. Upon receipt of the CVB sample, I observed the procedure for extracting DNA and then carried out several analyses: the amelogenin PCR allowed sexing of the fetus, since the cytogenetic analysis had failed (fetus was male); direct PCR analysis of the CGG repeat region identified one allele in the normal size range of the same size as the mother's normal allele; the DXS548 marker indicated that the fetus had inherited the alternative maternal allele to the affected son (this also excluded maternal contamination of the CVB sample); EcoRI+EagI Southern Blot analysis showed no evidence of a premutation or full mutation. From this extensive analysis, it was concluded that

the fetus had not inherited the high-risk allele from its mother and hence was at low risk of developing Fragile X syndrome. I wrote a report to this effect.

Competencies: Scient, Clin, Tech.

- 14 I wrote a piece regarding the problems in writing definitive reports in cases of prenatal diagnosis of Fragile X syndrome. The factors I considered important were that methylation patterns are not fully established at the time of chorionic villus sampling (10-11 weeks gestation) and so negative Southern Blot results may not be reliable and absolute sizing of CGG repeats must be used instead; maternal contamination of the CVB sample may well make any assessment of the fetus' mutation status impossible; the variable penetrance and expressivity of females with a full mutation prevents a conclusive report being written in this situation; mosaicism can cause several problems in that the detection of mosaicism in the CVB sample may not reflect the situation in the fetus (confined placental mosaicism) or true mosaicism prevents an accurate assessment of the predicted phenotype of the fetus, or mosaicism in the fetus may not be present in the CVB sample.

Competencies: Scient.

Myotonic Dystrophy Module

- 15 I wrote a detailed essay on Myotonic Dystrophy (**SEE APPENDIX A.7**), including the clinical features, the cloning and identification of the causative mutation, the structure of the DMPK gene, genotype-phenotype correlations, the origin of the myotonic dystrophy expansion mutation, theories on the molecular pathogenesis of myotonic dystrophy and the finding of a second myotonic dystrophy locus DM2. This involved a review of the current literature.

Competencies: Scient, Clin, R+D.

- 16 The majority of the module was taken up with 'live' diagnostic work, including diagnostic referrals, carrier testing and presymptomatic/predictive testing. The analysis comprised direct PCR across the CTG repeat to identify normal alleles, then two Southern Blot analyses: EcoRI digest with the M10M6 probe, which shows large expansions but includes a 1kb insertion/deletion polymorphism that can confuse interpretation, and PstI digest with the M10M6 probe, which identifies smaller expansions and does not include the 1kb insertion/deletion polymorphism. An example of this analysis is included in the **APPENDIX A.8**.

Competencies: Tech.

Cytogenetics Module

- 17 I spent four weeks in the neighbouring Cytogenetics department. Initially, I learnt the organisation of the department and relevant safety aspects, then reception and booking on of samples and use of the database.

Competencies: Scient.

- 18 In the postnatal section, I set up blood cultures, harvested them, stained the chromosomes for G-banding then photographed and karyotyped them.

Competencies: Scient.

- 19 In the prenatal section, I observed and took part in the preparation of slides from amniotic fluid and CVB samples.

Competencies: Scient.

- 20 Two weeks were spent studying the theoretical and practical aspects of fluorescent in-situ hybridisation (FISH), and how it is used in the detection of conditions such as the Prader-Willi and Angelman syndromes, DiGeorge syndrome and other microdeletion syndromes. I also learnt of the role of FISH in

malignancy, in detecting the more common rearrangements that aid the sub-classification of leukaemias and allow some prediction of prognosis. I became aware of other FISH-based techniques like chromosome painting and reverse chromosome painting (for identifying the origins of a rearranged or marker chromosome), primer in-situ labelling (PRINS – a rapid technique for detecting repeated sequences), comparative genomic hybridisation (CGH – detects changes in copy number of chromosomal regions), and multi-FISH/spectral karyotyping (SKY – each chromosome is simultaneously hybridised with a unique colour).

Competencies: Scient.

- 21 I made extensive notes on practical methods, the theory of chromosomal rearrangements and translocations, the diseases and syndromes caused by cytogenetic abnormalities, and analysed, with help, a number of karyotypes with abnormalities.

Competencies: Scient.

ABI377 DNA Sequencer Module

- 22 This module was undertaken in the Molecular Genetics Department at xxxxx. It gave experience of different fluorescent technology in the diagnostic setting, but also some insight into how different diagnostic laboratories can be organised.

Competencies: Tech.

- 23 The practical work was based around a rare disease called Albright's Hereditary Osteodystrophy (AHO) which is a 'speciality' offered by the xxxxx laboratory. It is characterised by short stature, obesity, developmental delay, mental retardation and subcutaneous ossification, hypocalcaemia and hyperphosphataemia. The causative gene is the α subunit of the G_s G-protein (GNAS1); unusual features are that, while inherited loss-of-function mutations cause AHO (though in addition a parent-of-origin effect leads to different sub-types of AHO), somatic gain-of-function mutations lead to a different disorder, McCune-Albright syndrome.

Competencies: Clin, Tech.

- 24 Sequencing analysis was carried out on a panel of AHO patients by di-deoxy terminator sequencing. Practical techniques included PCR, preparation of sequencing reactions and the setting-up and running of ABI377 gels. Appropriate use of software packages (Sequence Analysis, Sequence Navigator and Factura) allowed the identification of GNAS1 mutations in the affected patient samples and an assessment of their likely effect.

Competencies: Tech.

- 25 Fragment analysis was used to study a family with multiple cases of AHO in order to establish linkage with the GNAS1 locus. Again, specific techniques for sample preparation were required, and alternative software packages (Genescan, Genotyper) were needed to analyse the results and to prove that the condition segregating in the family was linked to GNAS1.

Competencies: Tech.

- 26 I wrote an essay discussing the benefits of automated/fluorescent sequencers over manual methods, and compared three different fluorescent sequencers used in diagnostic laboratories – the ABI377, the ALF Express and the ABI310 capillary sequencer.

Competencies: Scient, Tech.

Clinical Genetics Visit

- 27 At my own request, I spent three days in the neighbouring Clinical Genetics department at xxxxx Hospital. I spoke to clinicians and nurses regarding their roles areas of expertise, sat in on departmental meetings and was allowed to observe in a number of interviews with patients. This was a hugely valuable experience and gave me a brief insight into the work of Clinical Genetics and the impact of the laboratory's work peoples lives.

Competencies: Scient, Clin.

Duchenne/Becker Muscular Dystrophy (DMD/BMD) Module

- 28 The key feature of this module was the use of fluorescent dosage PCR to analyse the dystrophin gene, as a result of the high proportion of exon deletions and duplications found in affected individuals. The assay comprised two multiplex dosage PCRs (covering the 5' and 3' deletion hot-spots) which were run on the ALF Express fluorescent gel system and analysed by the dosage quotient method in an Excel spreadsheet. The method was far more sensitive and prone to failure than standard PCR and so more stringent control of each experiment was required: samples were amplified and run in triplicate and several control samples (normal, deletion male, deletion female, duplication female) were run in each experiment.

Competencies: Tech.

- 29 I tested a range of 'live' diagnostic samples, both negative and positive for mutations, including one case with a single exon deletion which had to be confirmed by designing a second set of primers for that exon and repeating the dosage experiments in order to exclude the possibility of a primer-site mutation.

Competencies: Tech.

- 30 Two known families were analysed using flanking and intragenic microsatellite markers (detected by silver-staining or radioactive labelling), and brought up issues calculating risks for individuals with unidentified dystrophin mutations and intragenic recombinations.

- 31 A review of DMD/BMD referrals from the Clinical Genetics department raised interesting features and complications, such as the calculation of residual carrier risks, the inclusion of creatine kinase test results in such calculations, and germline mosaicism. Further sample questions and QA problems from previous years illustrated the complexities of analysing, interpreting and reporting DMD/BMD cases.

Cystic Fibrosis Module

- 32 This module, and the rest of my training, was carried out at the xxxxx Molecular Genetics Service in xxxxx NHS Trust.

- 33 Study of the clinical aspects of the disease included the wide spectrum of disease caused by mutations in the CFTR gene: classical pancreatic-insufficient CF, milder pancreatic-sufficient CF, bronchiectasis, congenital bilateral absence of the vas deferens (CBAVD), echogenic bowel, meconium ileus. The genotype-phenotype correlations were noted, and the effects of different mutations on the consequence for production of CFTR protein from that gene copy.

Competencies: Clin.

- 34 There was a wide range of assays that were required in providing the CF diagnostic service, and I gained practical experience in all of them: the $\Delta F508$ PCR assay, the Elucigene CF12 and CF20 kits (multiplex ARMS PCR technology), poly-thymidine tract PCR and digest assay, SSCP analysis of CFTR

exons, intragenic microsatellite marker analysis. This not only gave experience in a variety of techniques but also required troubleshooting in the introduction of new assays and the re-optimisation of old ones.

Competencies: Tech.

- 35 The range of referrals for the disease, and so the appropriate combination of assays, was extremely varied, including diagnostic queries, carrier testing in known CF families, infertile males, carrier screening for females undergoing fertility treatment, screening of pregnant couples with echogenic bowel detected in the fetus, prenatal diagnosis for at-risk fetuses. The laboratory also forms part of the xxxxx CF Neonatal Screening programme, where blood spots taken at day 6 are tested biochemically for immunoreactive trypsinogen (IRT) levels, an indicator of possible CF, and 'high-risk' samples come to the laboratory for mutation analysis. This had to be carried out and reported each week, requiring rapid turnaround times and close liaison with the Neonatal Screening laboratory.

Competencies: Scient.

- 36 The wide range of referrals and family situations allowed me to greatly develop interpretive and reporting skills, and required the regular calculation of carrier risk prior to and after molecular testing, using Bayesian calculations.

Competencies: Clin.

- 37 I was given responsibility, under supervision, for all routine CF testing towards the end of the module, and kept this responsibility for the remainder of my training, giving me valuable preparation for handling a full diagnostic workload as a clinical scientist.

Competencies: Comm.

Breast Cancer (BRCA)/Familial Adenomatous Polyposis (FAP) Module

- 38 This module introduced the inherited cancer predisposition syndromes, and I researched the clinical features, genetic characterisation, genotype-phenotype correlations and clinical management. However, a major aspect of the module was the use of the Protein Truncation Test (PTT) technology as a screen for mutations. These diseases are suited to PTT analysis firstly because the vast majority of mutations result in the premature truncation of the protein, and a large proportion of the mutations are located in very large exons (so genomic DNA template can be used) which encode the majority of the proteins. The technique involved use of long-range PCR, handling of ³⁵S radioactivity, coupled transcription-translation kit, SDS-PAGE and autoradiography.

Competencies: Scient, Tech.

- 39 As part of the 'live' diagnostic work undertaken for the breast cancer service, I carried out predictive tests in two families with known BRCA2 mutations by designing restriction enzyme-based assays, thereby obviating the need for expensive and time- and labour-intensive sequencing, which ideally was only necessary for the characterisation of the familial mutation in the proband.

Competencies: Scient, Tech.

Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS) Module

- 40 This final disease-specific module demonstrated the role of genomic imprinting in human genetic disease: both disorders map to the same chromosomal region (15q11-13) where several genes are imprinted (i.e. methylated and transcriptionally silenced) on one of the parental chromosomes. PWS appears to be a contiguous gene syndrome where loss of expression of paternally

expressed genes causes disease; AS is caused by loss-of-function of the maternally-expressed gene UBE3A. The mutational mechanisms observed in patients are large deletions, uniparental disomy (UPD) and imprinting centre mutations.

Competencies: Clin.

- 41 In the laboratory, methylation-sensitive Southern Blot analysis using a XbaI and NotI digest with the KB17 probe identified ~99% of PWS cases and ~75% cases of AS by revealing lack of paternal or maternal contribution respectively. In positive cases, analysis of the affected child and their parents by microsatellite markers flanking and within the chromosomal region allow distinctions to be made between deletion, UPD and imprinting centre mutation. I studied previously analysed families that demonstrated all three situations.

Competencies: Tech.

Project – Setting up a Diagnostic Assay for Osteogenesis Imperfecta Type I

- 42 My project was based around the disease Osteogenesis Imperfecta ('brittle bone disease'), as the laboratory was about to develop a full diagnostic service, the first comprehensive one in the UK. A comprehensive review of the literature was required to explore the clinical features and classifications as well as the techniques previously used in its study.

Competencies: Scient, R+D.

- 43 The project compared two methods for detecting loss of COL1A1 expression from one allele. The first technique was relatively simple and published, while the second was a novel assay, using real-time PCR technology, which I designed, tested and improved. At the time, lack of positive control samples prevented the full validation of the novel technique, but subsequent development of the service by other colleagues in the laboratory has confirmed the validity and usefulness of the assay. The summary of my project write-up is included in the **APPENDIX (A.9)**.

Competencies: Scient, Tech, R+D.

- 44 Throughout my training, I attended weekly lab meetings and regular journal clubs, where I contributed and tried to make the most of the opportunities to develop my presentation and liaison skills. I also attended relevant lectures and seminars held at various hospitals and universities in xxxxx and xxxxx.

Competencies: Scient, R+D, Comm.

- 45 I completed my training in May 2001 and was awarded the Postgraduate Certificate of Competence from the Clinical Molecular Genetics Society.

Grade B8-10 Clinical Scientist

- 46 I was fortunate enough to obtain first a temporary, then a permanent post within the xxxxx Laboratory in xxxxx. Initially, I continued my responsibility for providing the Cystic Fibrosis service, as I had throughout the latter part of my grade A training. In addition I took on the testing and reporting for Fragile X syndrome (PCR only) and haemochromatosis, two services with relatively high numbers of referrals but simple genetic testing protocol.

- 47 In providing the cystic fibrosis service, I was given extensive experience in dealing with a wide range of referrals, working to fairly short reporting times, writing and adapting reports to address the individual issues of each referral, and using Bayesian calculations. For urgent referrals and prenatal diagnosis cases, it was essential to carry out the laboratory work quickly and effectively, and to

liaise closely with the referring clinicians. I also dealt with External Quality Assessment (EQA) scheme samples; one example of the reports written is included in the **APPENDIX (A.10)**.

Competencies: Scient, Clin, Tech, Comm.

- 48 One development of the neonatal CF screening programme which I took part in was the expansion of the molecular testing carried out. The established protocol was to test samples with a raised IRT level for the $\Delta F508$ mutation only; the modification was to test any samples found to be heterozygous for the $\Delta F508$ mutation for a further 19 CFTR mutations. The rationale for this was that compound heterozygous CF individuals could be identified and treated more rapidly, possibly improving their long-term outcome. The technical difficulty was to produce from dried blood spot samples DNA of sufficient quality to be used successfully in the Elucigene CF20 assay (based on multiplex ARMS PCR). Several methods were compared, and a commercial kit was found to give the most consistent results, and hence was included in the standard protocol.

Competencies: Scient, R+D.

- 49 After the first six months of this modified testing protocol (Jan – Jun 2001), I carried out an audit of neonatal screening samples. The aims were firstly to ascertain the pick-up rate for compound heterozygotes, and also to address concerns over some delays in testing and reporting of samples. The analysis and summary are included in the **APPENDIX (A.11)**.

Competencies: Tech, ProbSolv, Manag.

- 50 In July 2002, I was asked to give a presentation regarding the xxxxx CF neonatal screening programme at a national Newborn Metabolic Screening meeting. The aim of the meeting was to compare the seven CF neonatal screening programmes currently in operation within the UK with the aim of reaching some consensus on a protocol that could be applied nationally. A copy of the presentation given is included (**APPENDIX A.12**).

Competencies: Comm.

- 51 In the later stages of my period with the cystic fibrosis service, I played a major role in the training of two grade A trainee clinical scientists in this disease. This involved training and supervision of practical work, regular discussions regarding progress, any problems and theoretical background and evaluation of written work.

Competencies: Manag.

- 52 I undertook one development of the Fragile X syndrome service: the primers used for the established PCR assay gave relatively large PCR products which could be difficult to score accurately, were somewhat prone to failures and limited the size of premutation that could be detected. I assessed an alternative pair of primers, giving shorter PCR products, that another laboratory had presented in poster form at the York BSHG conference – both non-fluorescent (visualised by silver-staining) and fluorescent (run on the LICOR system) versions were attempted. The assay required some optimisation to abolish non-specific products and the inclusion of the fluorescent label made optimisation more difficult to achieve. A non-fluorescent version was found to be reliable and able to amplify reasonably large premutations (~70-85 CGG repeats); it is now used as the standard diagnostic assay.

Competencies: Scient, Tech, R+D, ProbSolv.

- 53 Later in my grade B8-10 position, disease responsibilities were rotated between the laboratory's clinical scientists. I retained responsibility for Cystic Fibrosis but took on the services for Spinal Muscular Atrophy (SMA), Familial Adenomatous

Polyposis (FAP) and supervision of paediatric leukaemia post-transplant chimerism service.

Competencies: Comm.

- 54 The SMA service involved relatively small numbers of referrals but unusual genetics in terms of the presence of a pseudogene (SMN2) in addition to the causative gene SMN1, the mutations involved (large deletions, gene conversions) and genotype–phenotype correlations. The laboratory’s testing protocol does not detect SMA carriers and so I was involved in some liaison with the DNA laboratory in Liverpool for the testing of such samples. I also carried out the testing of and wrote reports for SMA samples from the External Quality Assessment (EQA) scheme (see **APPENDIX A.13**). I attended a best practice meeting for SMA, where colleagues from laboratories across the country shared experiences and opinions regarding diagnostic testing for SMA.

Competencies: Tech.

- 55 The first major task for the FAP service was to characterise a significant number of mutations in individuals with truncations identified in exon 15 of the APC gene. I developed a framework of PCR primers to cover the PTT fragment with overlapping PCR products that could be sequenced in both directions. All patient mutations were identified, with the exception of one where a false positive PTT result was revealed. This further emphasised the importance of confirming and fully characterising mutations before issuing any report.

Competencies: Tech.

- 56 An audit of all FAP families was undertaken in collaboration with the Genetic Counselling service. The aim was to identify those families with no identified APC mutation but with a strong clinical diagnosis that would warrant full mutation screening of the APC gene.

- 57 One FAP case illustrated the care and attention to detail needed in analysing and interpreting results. In this case, both the PTT analysis and the initial sequencing gave only subtle indications of the presence of a mutation. (See **APPENDIX A.14**)

Competencies: Scient, Clin, Tech, ProbSolv.

- 58 The paediatric leukaemia post-transplant chimerism service uses microsatellite markers to assess relative levels of donor and recipient cells in recipients of bone marrow transplants as a method of monitoring the success of the procedure. I further developed the panel of microsatellite markers available and optimised their quantitative analysis on the LICOR fluorescent gel system. The practical aspects of the service were then handed on to a Medical Technical Officer (MTO); I was therefore responsible for supervising their training and work, and for writing the reports for each analysis. This gave me a very valuable learning experience in communication, teamwork, organisation and management.

Competencies: Scient, R+D, Manag.

- 59 I had an active role in the preparation for the laboratory’s first inspection by the Clinical Pathology Accreditation (CPA) Board in November 2001; the laboratory now has conditional CPA accreditation. The process of preparing for the inspection illustrated all the workings and procedures within the laboratory, and the challenges of bringing them together to form a rational and efficient service. An example of a Standard Operating Procedure updated for the inspection is enclosed (**APPENDIX A.15**).

Competencies: Tech, Manag.

- 60 As part of my career development, I attended the MRCPath Part I Self-Help

course for one day every month between Sep 2000 and Mar 2001. The tutorial-style sessions required significant reading and preparation of notes, handouts and presentations. Diverse areas were covered, ranging from the identification of disease-causing genes and mutation detection techniques to EQA and audit and Bayes calculations. As well as providing a hugely useful learning experience, it was an opportunity to meet and talk with colleagues and to exchange ideas and experiences.

Competencies: Scient, Tech, Manag.

61 The laboratory received funding from the Cancer Services Implementation Group to purchase an automated fluorescent capillary sequencer. Three possible systems were considered and each was brought into the laboratory to allow comparative trials to be performed. I was involved with this, along with many other members of the laboratory, and both prepared samples for analysis on the instruments and set up and performed some of the runs. Sequencing and microsatellite marker analysis were carried out on a large standard set of samples in order to give an objective comparison of the quality of results generated. A decision is to be made soon.

62 Throughout my grade B8-10 position, I was in charge of one area of the laboratory's ordering: those companies where we had set up standing orders. This gave insights into budgeting issues and laboratory management. In addition, responsibility for primer ordering included organising the storage system for the primers, allowing a standardised method of ordering, storage and retrieval.

Competencies: Manag.

63 Every week joint meetings between the DNA laboratory and the Genetic Counselling service are held, where discussion can take place regarding individuals or families currently being tested, urgent samples due to come into the laboratory in the near future and various issues regarding the ethics, availability and financial issues pertaining to molecular testing. This liaison is vital to ensuring the smooth running of the laboratory, and also puts the testing performed in the laboratory in the context of the real situation of the individual/family.

Competencies: Scient, Clin.

64 Joint journal clubs and case presentations with the Cytogenetics department and Genetic Counselling service gave me an opportunity to present various articles and cases, and also to learn more about the work carried out by these other related disciplines.

Competencies: Scient, Clin, R+D, Comm.

Grade B11-13 Clinical Scientist

65 In October 2002, I gained promotion within the xxxxx Molecular Genetics Service to a grade B11-13 position. My responsibilities changed and I have become responsible for all the cancer testing services offered (excluding the paediatric leukaemia service). This includes breast/ovarian cancer, FAP, Multiple Endocrine Neoplasia type 1 (MEN1), *RET* gene testing and *WT1* gene testing. This marks a change in the type of service I am directly involved in: previous responsibilities mainly involved diseases with high referral numbers but straightforward testing. The cancer services have a lower referral rate but the molecular testing is more complex and requires a higher level of organisation of time and resources.

Competencies: Comm.

- 66 My initial aim has been to consolidate and standardise all the core cancer testing offered, including a consistent, reasonably rapid reporting time of 3 months. Testing strategies and Standard Operating Procedures have been updated and formalised (see example, introduction only, in **APPENDIX A.16**).
Competencies: Tech.
- 67 The recent appointment of a Cancer Co-ordinator to the xxxxx Genetics Service emphasise the importance of the cancer services and the great need to develop and operate in the most effective ways. I am liaising with the Cancer Co-ordinator regarding the evaluation of breast/ovarian cancer families for extended *BRCA1/BRCA2* screening. This liaison will continue to develop in various areas of the cancer services.
Competencies: Comm.
- 68 I am supervising the Cystic Fibrosis service, which is being provided by a non-supernumerary grade A trainee clinical scientist. I also have some supervision of an MTO in the provision of the *WT1* genetic testing for individuals with suspected Denys-Drash syndrome. I therefore have ample scope for developing further my management skills.
Competencies: Manag.
- 69 One future challenge is to develop a full mutation screening service for FAP to be offered to the whole of the xxxxx region, as part of an integrated, consortium-based approach to cancer molecular testing.

Appendix

Reference	Document Description
A.1	CV
A.2	Academic certificates: - Postgraduate Certificate of Competence in Clinical Molecular Genetics - MSc Medical Genetics - MA Natural Sciences
A.3	Abstract of Research Paper: <i>Xxxxx et al. (1999) Mitochondrial genetic analyses suggest selection against maternal lineages in bipolar affective disorder. A Journal 65(2):508-18.</i>
A.4	List of Meetings/Courses
A.5	Huntington Disease – Spoken presentation of project, CMGS Meeting, Xxxxx
A.6	Huntington Disease - Poster presentation of project update, BSHG Conference, Xxxxx
A.7	Myotonic Dystrophy – Review of disease and literature
A.8	Myotonic Dystrophy – Example of Southern Blot analysis
A.9	Osteogenesis Imperfecta project - Summary
A.10	EQA reports – Cystic Fibrosis, 2001 scheme
A.11	Audit of CF Neonatal Screening CF20 testing
A.12	Oral presentation on Xxxxx Neonatal Screening programme at Newborn Metabolic Screening meeting
A.13	EQA reports – Spinal Muscular Atrophy, 2002 scheme
A.14	Case study of FAP mutation detection
A.15	Standard Operating Procedure – Spinal Muscular Atrophy
A.16	Standard Operating Procedure – BRCA1/BRCA2 testing
A.17	Supporting letter from Head of Department