Post Doctoral Fellowship in Cytogenetics

Duration of the Course: One year

Syllabus:

Cytogenetics is an evolving field. Obtaining chromosomes for study requires cell culture and is labour-intensive. Training in morphology and pattern recognition is necessary to be able to identify chromosomes correctly. Abnormalities have to be described using the international system for human cytogenetic nomenclature (ISCN).

The course will focus on the following skills that are necessary to enable the trainee to be a competent diagnostician and to function in a supervisory capacity in a clinical cytogenetics laboratory.

- Cell culture techniques to obtain chromosomes for study.
- Methods to identify and characterize chromosomal abnormalities.
- The morphology of normal and abnormal chromosomes.
- Performance and interpretation of fluorescence in situ hybridization tests.
- Use of cytogenetic nomenclature (ISCN) to write reports for the above tests.
- Performance and interpretation of related molecular diagnostic techniques.

Teaching Methods:

- Seminars/ Lectures
- Case Discussions
- Laboratory Posting for hands on experience with cytogenetic techniques

Theory

- Structure and organisation of the human genome
- DNA structure, variation, replication and repair
- Transcription, translation and post translational processing
- Structure of genes and functional DNA elements
- Regulation of gene expression
- Various types of repeats in human genome
- Types of polymorphisms in the human genome
- Mutation – types and effects
- Epigenetics, imprinting and DNA methylation
- Cell cycle, mitosis, and meiosis, recombination
- Gametogenesis and fertilization
- Uniparental disomy
- Chromosome structure, morphology and variation
- Lyon hypothesis and X inactivation
- Autosomal and sex chromosome aneuploidy syndromes

- Structural abnormalities of chromosomes
- Microdeletion syndromes
- Chromosome breakage syndromes
- Cytogenetics of spontaneous miscarriage and infertility, polysomies
- Sexual differentiation and intersex
- Prenatal diagnosis – amniocentesis, chorionic villus sampling, cordocentesis
- Pseudomosaicism, mosaicism and chimerism
- Role of cytogenetics in cancer
- Chromosomal abnormalities in haematological malignancies
- Chromosomal abnormalities in solid tumours
- The genetic and molecular basis of cancer
- Inherited cancer predisposition
- Indications for chromosome analysis
- Cytogenetic nomenclature (ISCN)
- Mendelian inheritance
- Atypical patterns of inheritance
- Single gene disorders
- Common disorders with multifactorial inheritance
- Molecular basis of genetic disease
- Role of genes and cellular and molecular mechanisms in development
- Genotypes and phenotypes in populations
- Hardy Weinberg equilibrium
- Genetic drift and founder effect
- Calculation of conditional probability and genetic risk
- Bayesian probabilities
- Methods of mutation analysis
- Mapping and disease gene identification
- Molecular cytogenetics – fluorescence in situ hybridisation, multiplex ligation dependent probe amplification, DNA microarrays,

Practical skills

Cytogenetic techniques and microscopy to analyse chromosomes from

- blood
- bone marrow
- amniotic fluid
- chorionic villus samples
- solid tissues including products of conception, tumours and skin

Specifically, the candidate must be able to

- Initiate, maintain and harvest in-vitro suspension and adherent cultures of diagnostic human specimens from a variety of tissues as listed above
- Prepare metaphase spreads from human chromosomes.
- Perform appropriate staining techniques (G-,R-, Q-,C-, NOR, distamycin-DAPI) know their uses and limitations and be able to interpret the findings .
- Perform other cytogenetic tests such as Mitomycin C testing for chromosome breakage and sister chromatid exchange.
- Perform fluorescence in-situ hybridization **(FISH) analysis** for
  - aneuploidy
  - microdeletion
  - translocation
  - amplification
  - in constitutional and acquired abnormalities and interpret the result.
- Prepare reagents required for these tests
- Use light, phase contrast, fluorescence and inverted microscopes and be able to maintain them.
- Use automated karyotyping systems for karyotyping and FISH.
- Detect and correct errors in the above techniques.
- Maintain quality control systems in the laboratory.

Interpreting and communicating the findings of cytogenetic analysis .

- to develop a professional opinion as to the causation, severity, and likely outcome of the abnormality.
- to write a written report which clearly conveys diagnostic information and recommendations to the requesting physician.
- to communicate with clinicians to seek or provide appropriate information and inferences about a patient by oral (face-to-face or telephone) communication and follow up patient outcomes by consultation with clinicians.
- To be aware of when to seek further expert opinion.

Other skills:

To advise the referring physicians regarding the

- most appropriate test for a particular clinical question
- relative diagnostic strengths and the limitations of any proposed investigation.
- relevant samples and anticoagulants or transport media used for specific samples, eg., use of sodium heparin versus EDTA for blood, bone marrow aspirates, buccal smears, chorionic villus and amniotic fluid samples, products of conception, skin biopsy, muscle biopsy, lymph node biopsy, and cerebrospinal or any other body fluid.

- requirement for duplicate samples, and positive and negative control samples
- specimen transport conditions (time after collection, temperature and container) for the dispatch of samples.

- **Maintain patient** confidentiality and privacy.

- **Contribute** appropriately to teaching, clinico-pathological conferences, quality control and other relevant meetings.

Laboratory management

To be able to run a laboratory with regard to current standards and to

- **handle specimens appropriately with respect to**
  - transport time and conditions to ensure specimen integrity and reduce the likelihood of a suboptimal /failed culture.
  - receipt, identification and allotting a laboratory accession number.
  - data entry including the recording of all relevant information on both patient and sample .
  - safe handling, storage, retention and disposal of samples.

- **organise and monitor**
  - workflow to ensure that routine samples are processed and analysed as per the stated turn-around times and priorities urgent or out-of-hours work.
  - staff for best use of time and maintain appropriate instrumentation & automation systems.

  - **maintain** a standard operating procedure manual.
  - **Ensure** that specimens are selected, indexed and stored appropriately.
  - **Keep** accurate records.
  - **Retrieve** specimens or records promptly when required for examination and review.
  - **Be** familiar with, and maintain the requirements for quality control, calibration of equipment and standardisation of tests.
  - Be aware of costs of reagents, their preparation and usage ,sources of patient and laboratory variation.
  - **Maintain** a safe environment including methods of waste disposal as per prescribed standards.

  - **trouble-shoot** and validate in-house laboratory tests.
  - **train** personnel as required.

Related molecular cytogenetics / genetics skills

Prepare nucleic acids as required for testing programs, including cDNA and modified / substituted DNA, to an appropriate purity standard.

Perform standard polymerase chain reactions.

Be able to advise on the appropriate use of PCR / hybridisation based testing (Southern analysis)

- DNA sequencing
- linkage studies
- microsatellite based studies
- M-FISH/SKY/ microarrays
- analytical methods for known mutations /common mutation screen

Books Suggested for reading :

- Principles of Clinical Cytogenetics by Steven L Gersen and Martha B Keagle
- Thompson and Thompson Genetics in Medicine by Robert L Nussbaum, Roderick R McInnes, Huntington F Willard
- Chromosome abnormalities and genetic counseling by R J McKinlay Gardner and Grant R Sutherland
- The AGT cytogenetics laboratory manual by Margaret J Barch, Turid Knutsen, and Jack L Spurbeck
- International System for Human Cytogenetic Nomenclature (ISCN) 2013, L G Shaffer, ML Slovak and N Tommerup

Journals Suggested :

- American Journal Of Human Genetics
- BMC genetics
- BMC medical genetics
- Human Molecular Genetics
- Clinical Dysmorphology
- Journal of Genetics
- Molecular Cytogenetics
- Cancer Genetics and Genome Research
- Genes, Chromosomes and Cancer
- Nature genetics

Departments involved in the training programme:

- **Cytogenetics** – Ten months to gain adequate exposure to constitutional as well as cancer cytogenetics* with an option of spending one month in a laboratory in a recognised institution.

- **Haematology** – six weeks in the molecular genetics and the flow cytometry laboratories for familiarity with the related laboratory tests.

- **Pathology and Clinical Pathology** – two weeks for familiarity with bone marrow morphology and with molecular pathology.

In addition, the candidate must do either of the following:

1. Submit an article (cases series, not case report) for publication in an indexed journal.

2. Submit a case-book of 10 cases as follows: at least one prenatal, one postnatal, one haematology /oncology and one FISH study from cases sent for diagnosis during the training period. One patient may appear only once. Each case should be given a unique identity number so that details may be checked. Word limit: 25,000, excluding references.
Patient names/ identification must not be present, but the cases should be numbered so that they can be identified.

Practical Skills

1. Cytogenetics techniques, microscopy and related skills

- Set up, maintain and harvest in vitro culture for diagnostic human specimens.
- Prepare metaphase spreads according to laboratory guidelines.
- Select, perform and interpret routine and special stains, and detect and correct errors in these processes.
- Maintain laboratory microscopes and use light, phase contrast, fluorescence and inverted microscopy appropriately.
- Be able to use automated karyotyping systems.
- Specifically should to be able to perform accurate identification of normal and abnormal chromosomes and common aberrations.

2. Perform cytogenetic analysis of

- blood
- amniotic fluid cells
- chorionic villus samples
- products of conception
- normal tissues (skin or other) for fibroblast cytogenetics
- bone marrow and other malignant tissues

3. Perform FISH analysis for

- aneuploidy
- microdeletion
- translocation
- chromosome painting

4. Provide a professional opinion on each case, (based on the available information**)**

- As to the nature, causation, severity, and likely outcome of the condition.
- Should be able to access appropriate information to assist in the interpretation of data.

5. Communicate an opinion

- By preparing a written report which contains all appropriate diagnostic information recommendations to the requesting clinician in a timely fashion,
- Where appropriate, be able to discuss a case to the referring clinician
- Be aware of when to seek further expert opinion.

Other related skills

1. Be able to advise clinicians regarding the

- appropriate choice and selection of tests, given the clinical question to be answered,
- Relevant samples and preservatives required for specific tests: eg blood, bone marrow aspirates, chorionic villus, and amniotic fluid, products of conception, skin, lymph nodes, body fluids and buccal swabs.
- Relative diagnostic strengths and the limitations of any proposed investigation.
- Requirement for duplicate samples, and positive and negative control samples.

- Specimen transport conditions for the dispatch of samples, including timeliness and temperature.

2. Request provision of pedigree information for familial conditions if

required. 3. Maintain patient confidentiality and privacy.

4. Contribute appropriately to teaching, clinico-pathological conferences, morbidity and mortality reviews, quality and audit committees and other similar meetings.

Handling of Specimens

- Ensure specimen transport time and conditions have been appropriate to guarantee specimen integrity and timeliness with reference to the relevant laboratory procedure
- Apply the principles of appropriate specimen receipt, identification and laboratory accession.
- nsure procedures for data entry include the recording of adequate information on both patient and sample for repeat future family testing.
- Apply principles of safe sample handling, storage, retention and subsequent disposal.
- Apply laboratory-specified workflow procedures to prioritize routine, urgent and out of hours work.

Production, Validation, Analysis, Reporting and Storage of Laboratory Data

i) Be familiar with the requirements for
- specimens
- quality control
- calibration of equipment
- standardisation of tests
- sources of patient and lab variation
- reagent preparation and usage
- waste disposal as per existing standards
- costs
- record keeping
- training of personnel as required

ii) Design, trouble-shoot and validate in-house laboratory tests.

iii) Analyse, interpret, record, report, compile and check morphological, qualitative and quantitative test results in the context of the clinical question.
iv) Maintain a standard operating procedure manual.
v) Be able to use and maintain appropriate instrumentation & automation systems**.**
vi) Ensure that specimens are selected, indexed and stored appropriately.
vii) Be able to retrieve specimens, records and showing examples of specific diseases or processes for examination and review.

Laboratory environment

- Be able to run a laboratory with regard to current standards
- Maintain a safe environment
- Organize staff
- Participate in continuing education

Related molecular genetics skills

- Prepare nucleic acids as required for testing programs, including cDNA and modified / substituted DNA, to an appropriate purity standard.
- Design oligonucleotide primers to maximize PCR sensitivity and specificity.
- Establish PCR reagent mixes for different PCR applications.
- Perform standard PCRs.
- Be familiar with, and be able to advise on the appropriate use of
  - standard and allele restricted PCR
  - modified PCR for methylation studies
  - modified PCR for mutation screening
  - quantitative PCR methodologies
  - hybridisation based testing (Southern analysis)
  - DNA sequencing
  - linkage studies
  - microsatellite based studies
  - M-FISH/SKY
  - analytical methods for known mutations and common mutation screens

Detailed syllabus

Course Content – Post Doctoral Fellowship in Cytogenetics

Theory

Genome and Genetic structures

- DNA structure and variation
- Epigenetics and DNA methylation
- Chromosome structure, morphology and variation
- Structure and organisation of the human genomes, & comparisons with other phyla
- Structure, variation, function and identification of genes and functional DNA elements
- Mapping, linkage disequilibrium and haplotypes in the human genome
- Various types of repeats in human genome

Molecular and cellular physiology

- DNA replication and repair
- RNA transcription and processing
- Translation and post translational processing
- Mitosis and the cell division cycle
- Meiosis, recombination and chromosomal segregation
- Fertilisation, early embryogenesis and malformation
- Mosaicism and chimerism
- Twins and twinning

Mutation / polymorphisms

- Mutation and polymorphisms
- Microdeletions & microduplications
- Biological basis of non-disjunction & aneuploidy
- Segregation of chromosomal structural anomalies
- Types of polymorphisms in the human genome
- Mutation scanning techniques for known and unknown variants

Inheritance

- Standard patterns and modifiers of inheritance
- Complex, multifactorial and quantitative traits
- Population genetics & Hardy Weinberg equilibrium Methods of mutation analysis

Cancer genetics

- The biological basis of cancer
- Inherited cancer predisposition
- Molecular aberration in cancer

Principles of genetic testing

- Direct and indirect laboratory testing
- Clinical: molecular correlates. Phenotype/genotype relationships
- Calculation of conditional probability and genetic risk
- Bayesian probabilities
- Relevant aspects of epidemiology and statistics

- Population screening

Cytogenetics

- Uses of karyotyping
- Chromosomal preparations and banding techniques
- Cytogenetic nomenclature
- FISH techniques
- Molecular techniques with direct relevance to cytogenetic analysis
- Assessment of chromosome breakage
- Karyotyping systems in clinical cytogenetics

Clinical relevance of constitutional and acquired chromosome abnormalities

- Constitutional chromosomal disorders
- Autosomal and sex chromosome aneuploidy
- Polysomies
- Structural abnormalities of chromosomes
- Cryptic chromosomal translocations
- Microdeletion syndromes
- Uniparental disomy
- Abnormal segregation of structural anomalies

Pregnancy

- Fertility and infertility
- First trimester screening
- Prenatal testing by amniocentesis
- Prenatal testing by CVS
- Mosaicism and pseudomosaicisim
- Cytogenetics of spontaneous miscarriage

Development

- Embryogenesis and malformations
- Sex determination & differentiation
- Chromosome causes of intersex
- Chromosome breakage syndromes

Cancer Cytogenetics

- Molecular basis of cancer
- Common karyotypic abnormalities in leukemia
- Significance of karyotype in leukemia
- Significance of karyotype in solid tumours

Nature genetics