RCPA

The Royal College of Pathologists of Australasia

Extension of Scope of Practice in Molecular Genetics (NPAAC Supervision Certification Modules)

CHEMICAL PATHOLOGY

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GLOSSARY

CPDP	RCPA Continuing Professional Development Program
(F)RCPA	(Fellow of the) Royal College of Pathologists of Australasia
IANZ	International Accreditation New Zealand
MDT	Multi-disciplinary team
NATA	National Association of Testing Authorities
NPAAC	National Pathology Accreditation Advisory Council
PPD	Personal Professional Development
RCPAQAP	RCPA Quality Assurance Programs Pty Ltd
SOP	Standard Operating Procedure
WHS	Workplace Health and Safety

SECTION 1

INTRODUCTION

The Royal College of Pathologists of Australasia (the College) offers a number of certification modules for Fellows of the Royal College of Pathologists of Australasia who have completed Fellowship in the discipline of Chemical Pathology.

The development of these certification modules has been required to satisfy the recent NPAAC requirements (http://health.gov.au/internet/main/publishing.nsf/Content/health-npaac-docs-supervision.htm), particularly as applicable to supervision of testing involving molecular diagnostics and genomics.

To adequately medically supervise a molecular/genomic service, chemical pathologists need to have adequate and detailed knowledge of the wet and dry laboratory aspects of the technology and bioinformatics analysis. It is recognised that this knowledge may not be the same as the hands-on experience of the scientist in the genomics laboratory; however, supervising pathologists should be familiar with the limitations and strengths of the methodology, the ethical considerations of data use and reporting and the clinical relevance for assessing appropriate requesting and reporting.

It is anticipated that in the next few years, genomic analysis will increasingly become integral to the work of pathology laboratories. These modules are designed to provide all chemical pathologists with the opportunity to participate in accordance with NPAAC requirements.

GENERAL AIMS OF THE TRAINING PROGAM

The genetics/ genomics certification modules build on discipline-specific Fellowship training. The current discipline-specific trainee curriculum is regarded to be the current standard and determines the Scope of Practice for each respective discipline, which with Chemical Pathology includes genotyping for *AAT*, *APOB*, *APOE*, *BCHE*, *HFE*, *TPMT*, *UGT1A1,* etc. As well as gaining additional competencies in genetics/ genomics relevant to Chemical Pathology, candidates are expected to extend further their skills in management, research, scholarship, as well as the professional qualities they have been developing during their pre- and post-Fellowship years and will continue to develop during their professional life.

This Handbook outlines requirements for the Chemical Pathology Certification Modules. It is based on a common approach for Fellows in all pathology disciplines to develop/ demonstrate the minimum professional competencies required for safe clinical service provision of genetic/ genomic testing. As such, the certification modules and associated competency standards outlined within this handbook have shared features with the modules outlined in the equivalent handbooks for other disciplines.

The purpose of the modules outlined in this Handbook is to offer Fellows the opportunity to gain certification of expertise for a graduated range of genetic/ genomic testing categories specifically for clinical applications within their specific pathology discipline.

Completion of one or more modules would result in the extension of scope of practice within Chemical Pathology to the limits defined for each module. The scope of practice would not extend into other discipline areas – for example, investigation of intellectual disability or prenatal diagnostic testing. Please note: non-invasive prenatal screening and testing is within the scope of Chemical Pathologists who have completed the relevant module, while all other prenatal testing is not.

An essential part of sub-specialty genetics/ genomics training is for practitioners to gain sufficient understanding of the breadth of the field; are aware of the limits of their own knowledge and skills and appreciate when it is in the best interests of patients to refer onto, or formally consult with genetic pathologists or other appropriately credentialed colleagues.

GENETIC AND GENOMIC TESTING

Genetic and genomic testing includes targeted analysis for presence/ absence of predefined mosaic or clonal genomic variation; screening for undefined variants in a single gene; screening for undefined variants in a limited number of specified genes, and screening for undefined variants in a large number of specified genes.

Testing can also include untargeted screening of all chromosomes (karyotyping), higher resolution screening of all chromosomes (chromosomal microarray), whole exome screening, and whole genome screening.

Specialised genetic, genomic, epigenetic and gene expression tests are also emerging. They include targeted testing for uniparental disomy, chimerism, methylation anomalies and other epimutations, and gene expression profiling.

The goal of all genetic/ genomic investigations is to identify variants that are of direct relevance to the clinical phenotype in question. A growing number of tests may also simultaneously detect additional disease-causing variants that are not directly relevant to the clinical indication for the specified test. Instead, these unanticipated findings may indicate a heightened risk of either having, or developing in the future, another unrelated illness. Additionally, tests that involve screening specified genomic sequences for undefined variants will inevitably identify variants that are of uncertain clinical significance.

To address adequately the wide range of professional competencies required to cover these activities, it is proposed that post-graduate training and assessment is delivered in three modules. The modules are a practical response to the gradient of technical and clinical complexity within genetic and genomic testing, as well the differing practical skill sets required across the spectrum of methods now available.

It must be acknowledged that there is a close relationship between the Biochemical Genetic Pathology and Chemical Pathology, with possible boundary overlap. The aim of these Certification Modules is not to cause angst, but to ensure that the respective scopes of practices are upheld. Chemical Pathologists can work towards upskilling in that area and then present their credentials to be assessed via a training determination and the necessary training requirement to be granted an additional scope of practice. Further information surrounding this process, can be obtained from the College.

SECTION 2

LEARNING OUTCOMES AND RECCOMMENDED TRAINING ACTIVITIES

Module 1 – Targeted testing for presence/ absence of predefined genomic variation by molecular methods.

Rescinded in December 2018

The Curriculum Development Working Group met in December 2018, and it was agreed upon that Module 1 content and professional competency was now adequately covered within the standard Trainee Curriculum for the discipline of Chemical Pathology.

Applicants for the Recognition of Prior Learning pathway are not required to address the contents of this Module.

Module 2 – Targeted screening for undefined variants in genes associated with specified clinical phenotypes.

Examples of typical clinical applications for this module would include:

- Targeted gene panels for diagnosis/predictive testing for pre-defined inherited conditions (e.g. cystic fibrosis, familial hypercholesterolaemia, hereditary haemochromatosis, alpha-1 antitrypsin deficiency, butyrylcholinesterase deficiency, etc)

The unifying concept of this module is the detection of unknown genomic variation within a pre-defined list of genes for analysis (focused on a specific biochemical phenotype/clinical context) which allows for the practice and development of discipline-specific expertise.

Typically, these assays are 20-30 gene amplicon panels, which are run for disease diagnosis or stratification. They may also include single gene- or single exon-sequencing for specific diseases.

Types of genomic variation covered

Targeted panels and associated bioinformatic pipelines may be designed to detect sequence variants and copy number changes involving pre-defined genes/regions on the targeted panel, depending on the nature of the genomic variations contributing to the defined clinical phenotype.

Methodologies covered

- Sanger sequencing
- Multiplex-ligation primer amplification (MLPA)
- Massive parallel sequencing with a range of library preparation/ bioinformatic filtering
    - Targeted amplicon enrichment
    - Hybridisation-based enrichment.

Knowledge and practical skills

General considerations

- This is complex and specialised training, which builds upon the sound genomic basics within the Chemical Pathology Trainee Curriculum.
- Knowledge of specific quality issues, validation and requirements of supervision of MPS (i.e. *Requirements for human medical genome testing utilising massively parallel sequencing technologies, National Pathology Accreditation Advisory Council, 2017*)
- Knowledge of potential sources of error arising from MPS assays

Wet lab considerations

- General practical skills understanding of nucleic acid preparation method(s), quantification/purity/intactness, storage/archiving (as per core module 1)
- Knowledge of technical performance, limitations and quality issues associated with different library preparation methodologies (amplicon, hybridisation based, use of unique molecular identifiers (UMI), etc)
- Knowledge of technical performance, limitations and quality issues associated with different sequencing technologies.

Dry lab considerations

- Knowledge of primary, secondary and tertiary analysis, variables and limitations
- Knowledge of relevant bioinformatics issues including performance and limitations of demultiplexing/alignment tools/variant callers, variant annotation strategies, bioinformatic methods of structural variant detection, reference generation strategies for copy number assessment, performance and validation of copy number calling algorithms
- Knowledge of data architecture, computing/processing/capacity issues and data security/privacy
- Knowledge of cloud based secondary and tertiary analysis systems.

Analysis considerations

- Detailed working knowledge of HGVS/ISCN nomenclature and its practical application
- Knowledge and application of pathogenicity classification systems for germline variants
- Management of incidental genomic findings more generally, and variant associated with significant clinical outcomes unrelated to the purpose of testing)
- Integration of the genomic variations detected with accompanying biochemical analyte data and the clinical context of the patient in order to provide a clinically appropriate genomic report
- Knowledge of ethical, clinical and regulatory structures/framework around germline testing for inherited disease/predictive testing.

Post-Analytic Considerations

- Ability to provide clinically appropriate advice regarding contents of genomic reports including diagnostic implications, prognostic implications and therapeutic implications of detected genomic variations
- Advice on appropriate follow-up genomic testing/other modalities as required (including testing of family members)
- Involvement and ability to communicate in multidisciplinary meetings with pathologists, physicians, scientists and other referring specialists.

Professional Competency

- **Log book** - summarising all lab-based experiences, which includes supervising the analysis, data interpretation and reporting of at least 200 cases and 100 disease-associated variants. *Log book to include test name; assay type; number of assays/ runs; test failures requiring review*
- **Portfolio Requirements** - should also include:
    - *Fifteen (15) technically challenging or unusual cases/consultations involving laboratory data. * See below for a list of examples of technical considerations/ challenges. NOTE: evaluation of variants of uncertain significance and assessment of variant pathogenicity MUST be included.*
    - *Five (5) primary requestor one-on-ones (telephone),*
    - *Eight (8) quality assurance activities (including two (2) compulsory), and*
    - *One (1) significant/critical laboratory incident report relevant to the module*
- **Case-based Discussions** - d*etailed discussion (at least one (1) page description of case in addition to CbD cover sheet) on five (5) "challenging" cases that required use of multiple skills, including consideration of laboratory data. At least one (1) CbD should be on further workup/management of unusual such as an "additional" or "off target" finding.*
- **Supervisor sign-off**
    - With sign-off indicating:
        - the principles of the method are understood
        - working knowledge of instrument processes and maintenance requirements
        - successful generation of results from each method, at a quality level sufficient for reporting
        - strong understanding of QC procedures for the method, including internal and external QA
        - working knowledge of method anomalies and associated trouble-shooting requirements
- **Dry Practical** (practical cases)
- **Structured Oral**

*** Examples of technical considerations:**

- Investigations/actions when DNA is of inadequate quantity for testing
- Sanger sequencing primer design
- Sequence with poor quality Phred score
- Trouble-shooting poor-quality sequence
- Challenges associated with homopolymer runs
- Challenges with assay controls – positive and negative
- Trouble-shooting an MPS run with poor quality control metrics – wet lab and/or dry lab MLPA probe design
- Issues with sample depurination
- Trouble-shooting MLPA results – quantitation, standard deviation, abnormal marker patterns, low probe signals, apparent single exon deletion, etc.
- Investigations/actions when control samples perform adequately but one or more samples does not pass QC metrics

- Investigations/actions when testing performed at another laboratory does not match with the result in your laboratory
- Validation process for Sanger sequencing/ MLPA
- Validation process for MPS, including validation of bioinformatic pipeline
- Maintenance of bioinformatic pipeline, including version control, verification of new versions
- Assessment of variants of uncertain clinical significance (must be included)
- Consultation with colleagues regarding an incidental/secondary finding
- Investigations/actions when an external QA program does not award full marks for genotyping
- Investigations/actions when an external QA program does not award full marks for result interpretation/reporting
- Consultation with expert colleagues regarding an incidental/secondary finding, or other complex finding.

Module 3 – Sequence-based screening for known and unknown variants in multiple genes, including genes potentially linked to clinical phenotypes that have not been previously diagnosed in the patient.

The unifying concept of this module is the application of massively parallel methodologies to screen for disease-causing and disease-associated genomic variants in multiple genes, including genes which can be linked to biochemical phenotypes that have not been previously diagnosed in the patient.

The purpose of genome-wide testing would be to bioinformatically extract panels of genes relevant to biochemical phenotypes, or for genome-wide analysis of biochemical disorders. Noting the limitation of the Chemical Pathologists from unfocussed (genome wide) DNA and RNA analysis of samples from patients with these conditions – i.e. the purpose of this module is the diagnosis of phenotypes related to chemical pathology.

Examples of typical clinical applications include: –

- Diagnosis of biochemical disorders with diverse genetic aetiologies
- Whole genome/ exome sequencing for inherited biochemical disorders

The approach used to detect disease-linked variants will also yield the following outcomes:

- False positive results
- False negative results
- Detection of variants of uncertain clinical significance
- Detection of variants with partial penetrance or expressivity
- Findings that may allow reproductive choices

Wide scope sequencing may also yield other additional or “off-target” findings:

- Medically treatable disorders
- Serious, incurable conditions
- Variants conferring susceptibility to disease
- Variants with pharmacogenetic implications
- Mis-attributed paternity
- Close consanguinity

Types of genomic variation covered

Bioinformatic “pipelines” can be targeted to screen MPS output for nucleotide variants, copy number changes and structural variants/fusions across a large number of specified genes up to the level of all exons of all known genes, or even the whole genome.

Methodologies covered

MPS using amplicon-based and hybridisation capture-based assays.

Knowledge and practical skills

General considerations

- This is complex and specialised training and builds upon modules 1 and 2.
- Sound working fluency in dealing with the range of expected challenging outcomes and additional, “off-target” findings.
- Knowledge of specific quality issues, validation and requirements of supervision of MPS (i.e. *Requirements for human medical genome testing utilising massively parallel sequencing technologies, National Pathology Accreditation Advisory Council, 2017*)

Wet lab considerations

- Refer to module 2.

Dry lab considerations

- Refer to module 2.

Analysis considerations

- Refer to module 2.

Post-Analytic Considerations

- Refer to module 2.

Additionally:

- Close link with a clinical genomics service, particularly for whole genome/ exome services.

Professional Competency

- **Log book** – summarising all lab-based experiences, which includes supervising the analysis, data interpretation and reporting of at least 200 cases with 100 unique variants. *Log book to include test name; assay type; number of assays/ runs; test failures requiring review*
- **Portfolio Requirements** - should also include:
    - *Fifteen (15) technically challenging or unusual cases/consultations involving lab data. * See below for a list of examples of technical considerations/ challenges.*
    - *Five (5) MDT attendances,*
    - *Eight (8) quality assurance activities (including two (2) compulsory), and*
    - *One (1) significant/critical laboratory incident report relevant to the module*
- **Case-based Discussions** *(**note:** discipline-specific discussion topics to be defined) - detailed discussion (at least one (1) page description of case in addition to CbD cover sheet) on five (5) "challenging" cases that required use of multiple skills, including consideration of lab. data. At least one (1) CbD should be on further workup/management of unusual such as an "additional" or "off target" finding.*
- **Supervisor sign-off**
    - With sign-off indicating:
        - the principles of the method are understood
        - working knowledge of instrument processes and maintenance requirements
        - successful generation of results from each method, at a quality level sufficient for reporting
        - strong understanding of QC procedures for the method, including internal and external QA
        - working knowledge of method anomalies and associated trouble-shooting requirements
- **Dry Practical** (variant curation, practical cases)
- **Structured Oral**

*** Examples of technical considerations:**

- Evaluating primary specimen for suitability for molecular testing
- Investigations/actions when signal is detected in a no-template control
- Investigations/actions when DNA is of inadequate quantity for testing
- Investigations/actions when DNA is of inadequate quality/amplifiability for testing
- Investigations/ actions when sex discrepancy, discordant result, sample mix up identified
- Investigations/actions when results may be confounded by repetitive DNA sequences/ pseudogenes
- Investigations/actions when testing performed at another laboratory does not match with the result in your laboratory
- Trouble-shooting a whole exome/genome sample/run with poor quality control metrics – wet lab and/or dry lab metrics

- Validation process for a targeted assay based on bioinformatic filtering of whole genome/exome sequencing data
- Panel selection from WES/WGS data, including technical and clinical considerations
- Approach to targeted genes which are not adequately covered for clinical reporting
- Investigations/actions when an external QA program does not award full marks for genotyping
- Investigations/actions when an external QA program does not award full marks for result interpretation/reporting
- Consultation with expert colleagues regarding an incidental/secondary finding, or other complex finding.

Module 4 – Cell Free DNA (cfDNA) and Single Nucleotide Polymorphisms (SNPs) for the purpose of Non-Invasive Prenatal Screening (NIPS)

Under development

The Curriculum Development Working Group is currently developing this module for the Recognition of Prior Learning Pathway. The module content and professional competency will be released shortly to allow Fellows to apply for PRL prior to the 01 August 2019 implementation date of the revised *Requirements*.

