HANDBOOK

RCPA
The Royal College of Pathologists of Australasia

Extension of Scope of Practice in Molecular Genetics

(NPAAC Supervision Certification Modules)

Anatomical Pathology

Contents

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

1.1 Introduction

The Royal College of Pathologists of Australasia (the College) offers three certification modules in genetic/genomic anatomical pathology for those who have attained FRCPA.

These modules have been designed to satisfy the recent NPAAC Requirements, particularly as applicable to supervision of testing involving molecular diagnostics and genomics.

To competently supervise a molecular/genomic service, Anatomical 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 be integral to the work of anatomical pathology laboratories and thus the general Fellowship training program. These modules are designed to provide currently practising anatomical pathologists with the opportunity to participate in accordance with NPAAC requirements.

1.2 General aims

The genetics/genomics certification modules applicable to Anatomical Pathology build on earlier Fellowship training. They are designed to build base knowledge, analytical and interpretive skills, quality assurance and communication capabilities relevant to genetics/genomic testing in Anatomical Pathology.

This Handbook 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, although different disciplines have varied the number of modules considered appropriate to that discipline.

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 Anatomical Pathology.

It is recognised that some genetic tests (for example assessing for pre-defined genetic variants in specific cancer biomarker genes by PCR-based methodologies, or the localization of genetic targets in tissues by in situ hybridization methodologies) are now so widespread in Anatomical Pathology laboratories, that they are considered part of the core knowledge expected of a Fellow; the Anatomical Pathology Fellowship Curriculum is in the process of being updated to include details of expected competencies related to such testing. Tests of this nature are not included in this Handbook and are also considered exempt from requiring “recognition of prior learning” at the initiation of this Certification project (see 1.4 below). Pathologists involved in reporting and supervising such tests must fulfil all appropriate NPAAC requirements related to appropriate training of staff, verification of test performance in their laboratory, maintenance of documentation, satisfactory performance in relevant Quality Assurance modules and ongoing appropriate continuing education. The Modules outlined in this Handbook relate to more complex testing, where the potential for unexpected findings and the need for more detailed curation is required.

Completion of one or more modules would result in extension of scope of practice within Anatomical Pathology to the limits defined for each module. It should be noted that the scope of practice does not extend into other discipline areas.

It is necessary that they 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. Close collaboration between Anatomical Pathologists and appropriately

1.3 Genetic and genomic testing

The scope of genomic testing relevant to neoplastic and non-neoplastic tissue samples includes targeted analysis for the presence or absence of predefined genomic variations, sequencing- and copy-number-based screening of genomic sequences for undefined variants in one or several specified genes, untargeted screening of all chromosomes by chromosomal microarray, as well as massively parallel sequencing-based whole exome and whole genome screening.

It also includes a growing range of specialised genetic, genomic, epigenetic and gene expression tests, including targeted testing for microsatellite instability, circulating tumour DNA (ctDNA) analysis including minimum residual disease and tumour mutational burden analysis, oncogenic HPV nucleic acid testing, chimerism, methylation anomalies, other epimutations, uniparental disomy and gene expression profiling.

A crucial aspect of tumour-directed genetic and genomic testing is that supervising pathologists have attained the practical standards required to ensure that appropriate histology and cytology material is submitted for genetic testing and that account is taken of important parameters such as the effects of formalin fixation, neoplastic cell content and requirements for macro and/or micro dissection as well as their interpretation in the correct tumour and clinical context.

The goal of all genetic/genomic investigations is to identify variants that are of direct relevance to the disease in question in the context of the morphological and immunophenotypic profile of the disease. They generally provide information to establish or refine the diagnosis, to further subclassify tumours and to predict prognosis or likelihood of response to targeted therapies. 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.

1.4 Types of genetic testing considered outside the certification process for Anatomical Pathology

It is expected that Anatomical Pathology Fellows have a working knowledge of “simple” genetic testing involving the detection of known genetic variants within a pre-defined list of genes (which are typically linked to a specific cancer or disease). It may include the detection of defined somatic variants in one or several genes (specified variants at the small nucleotide level), identification of copy number variations or structural rearrangements, targeted DNA methylation analysis, clonality assays and micro-satellite instability (MSI) testing.

Applications are mainly related but not limited to cancer diagnosis/classification, prognostication and targeted therapy selection. Examples of typical clinical applications include:

- Detection of somatic “gain of function” variants (e.g. activating mutations in KRAS, BRAF and EGFR)
- Assessment of minimal residual disease

- Assessment of genome mutability (instability)
- Detection of oncogenic fusion genes/gene rearrangements/gene amplifications

There are a broad range of nucleic acid amplification-dependent assays available for targeted testing of genetic alterations, each being useful in different diagnostic circumstances. These include end-point PCR and associated read-out methods, real-time PCR (e.g. Cobas 4800), droplet digital PCR (ddPCR), oligonucleotide ligation assays, mutagenically separated/allelic discrimination PCR (e.g. Taqman) and single nucleotide primer extension (mini-sequencing). Methylation specific-PCR provides a means of assessing aberrant DNA methylation of specific genes. Mass-spectrometry based arrays for defined variants in one or more genes provide an alternative methodology to PCR-based platforms. Capillary electrophoresis and fragment analysis may be utilized for MSI testing and clonality testing.

Microscopy-based in-situ hybridization methods involving analysis of tissue sections (SISH/FISH) are also widely-used to assess for targeted genomic/ chromosomal deletions, duplications, structural rearrangements and amplifications, and are also considered part of the core knowledge expected of an Anatomical Pathologist.

1.5 Registration

Applicants must complete a registration form, obtainable from the RCPA website and submit this with the application fee. The form must clearly state the modules(s) being applied for. Applications for partial exemption from assessments (recognition of prior learning) must be submitted with supporting documentation at the time of registration.

1.6 Training structure and requirements

The modules are outcomes-based, with no fixed time requirements for completion. Candidates must participate in a range of laboratory-based experiences to achieve the listed outcomes. All specified assessment tasks must be completed satisfactorily to achieve certification.

1.7 Supervision

All training must be supervised by an approved Fellow of the RCPA with the required expertise. Supervisors are expected to monitor and provide regular feedback on the candidate's developing competence. Formal meetings with the candidate are expected to occur at least every three months.

The supervisor will complete a Supervisor Report at the end of the training period, along with a completed and signed portfolio summary sheet. If the training period exceeds twelve months, an additional annual Supervisor report will be required.

The supervisor will carry out workplace-based assessments or may delegate this responsibility to another suitably qualified pathologist or senior scientist.

1.8 Assessment

Assessment consists of a range of activities and workplace-based assessments to be documented in a portfolio, and formal examinations as prescribed for each module.

Portfolios may be maintained in paper-based and/or electronic format with back-up. Portfolios do not need to be submitted to the College. The supervisor will verify that all entries and assessment tasks have been completed on the portfolio summary sheet to be submitted with the final Supervisor Report for the module.

1.9 Continuing professional development

Activities carried out and documentation associated with the modules may be included, along with general anatomical pathology activities, in the relevant categories of the RCPA Continuing Professional Development Program (Professional Performance Framework). Following certification, specialists are expected to undertake ongoing professional development relevant to the modules for which they are certified.

SECTION 2

Learning outcomes and recommended training activities

Module 1: Screening for known and unknown somatic genome variants in gene(s) associated with specified clinical phenotypes

This is complex and specialised training which builds upon a sound basic knowledge of cell and molecular biology as well as specialized molecular biological techniques and assays as outlined in the Basic Pathological Science and Anatomical Pathology trainee curricula.

This category involves targeted analysis of the genomic sequences of one or multiple genes, all of which are associated with specified clinical phenotypes.

Typical clinical applications include targeted “gene panel” testing for specified cancer presentations (e.g. targeted gene panels for specific cancer diagnosis, prognostication and targeted therapy selection). Applications also include targeted gene panels for diagnosis/predictive testing for pre-defined inherited conditions (i.e., familial cancer risk) and use of sequencing techniques for clonality testing.

The unifying concept of this module is sequence- and copy number-based screening for genomic variation within a pre-defined list of genes, all of which are known to contribute to the occurrence and clinical outcomes associated with specific phenotypes. Such panels will generally identify known genetic variants but can potentially identify unknown or less well characterised variants.

Typically, these assays (many of which are available commercially) include panels with less than 50 genes which are run for disease diagnosis or stratification. They may also include single gene or single exon sequencing for specific tumour types.

Note that if the testing process is divided between different sites e.g. wet laboratory aspect of the assay is performed separate to the processing, interpretation and reporting of data, each site needs to have appropriately credentialed supervising and governance arrangements.

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

1.1 Learning outcomes

1.1.1 Theoretical and Technical Knowledge

- Methods covered
  - Sanger sequencing of whole genes
  - Multiplex-ligation primer amplification (MLPA)
  - Focused massively parallel sequencing (MPS/NGS) with a range of library preparation/ bio-informatic filtering including:
    - Targeted amplicon enrichment
    - Hybridization-based enrichment text
- Knowledge of theory and processes relating to Wet Lab:

    - General practical skills and understanding of nucleic acid preparation method(s), quantification/purity/intactness, storage/archiving
    - 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
    - Knowledge of potential sources of error arising from massively parallel sequencing assays designed specifically for formalin-fixed, paraffin-embedded tissues
- Knowledge of theory and processes relating to Dry Lab:
    - 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, including awareness of data security and privacy issues
- Knowledge of somatic variant curation strategy including understanding of variant annotation, advantages and limitations of cancer (e.g. COSMIC) and healthy population databases (e.g. gnomAD), curation of literature with regard to diagnostic, prognostic and targeted therapies, advantages and limitations of *in silico* prediction tools and splice prediction tools
- Knowledge of current somatic and constitutional reporting guidelines

1.1.2 Analytical and interpretive skills

- Detailed working knowledge of HGVS/ISCN nomenclature and its practical application in reporting tumour-specific variants
- Knowledge and application of pathogenicity classification systems for somatic and germline variants
- Management of incidental genomic findings (e.g. germline variants of significance detected during somatic testing; more generally, any variants associated with significant clinical outcomes unrelated to the purpose of testing) in accordance with international guidelines (such as consensus ACMG and AMP)
- Knowledge and application of bioinformatics pipelines to identify and characterize known somatic variants as well as novel uncharacterized variants using databases such as COSMIC (https://cancer.sanger.ac.uk/cosmic), My Cancer Genome (https://www.mycancergenome.org) and IARC TP53 (http://p53.iarc.fr/).
- Ability to distinguish between tumour-specific somatic variants as opposed to known polymorphisms using common population databases such as dbSNP and ExAc (exome aggregation consortium).
- Integration of the genomic variations detected with accompanying morphological, immunophenotypic, cytogenetic and clinical context of the patient in order to provide a clinically appropriate genomic report
- Synthesis and clinical interpretation of laboratory data taking account of the clinical presentation, morphology, immunophenotype and other relevant non-genomic and genomic investigations

1.1.3 Quality assurance

- Knowledge of specific quality issues, validation and requirements of supervision of massively parallel sequencing (ie. Requirements for human medical genome testing utilising massively parallel sequencing technologies, NPAAC, 2017)
- Knowledge of potential sources of error arising from massively parallel sequencing assays designed for formalin-fixed, paraffin-embedded tissues
- Knowledge of NGS workflow, selection of appropriate sample, practical application of macro/microdissection techniques and their pros and cons, sample adequacy, appropriate triage and use of cytology and body fluid material for NGS and knowledge of cancer biology in relation to the patient's sample phenotype
- Knowledge of ethical, clinical and regulatory structures/framework around germline testing for inherited disease/predictive testing

1.1.4 Communication and Consultation

- Ability to communicate findings with scientists, pathologists and other relevant clinicians, including in multidisciplinary meetings
- Ability to provide clinically appropriate advice regarding contents of genomic reports including diagnostic, prognostic and therapeutic implications of detected genomic variations
- Ability to provide advice on appropriate follow-up genomic testing/other modalities as required (including testing of family members)
- Recognition and troubleshooting of challenges
- Competence in monitoring data quality and result verification

1.2 Specific considerations/challenges to be addressed in assessment

- 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 a massively parallel sequencing 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 massively parallel sequencing, 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

1.3. Assessment

1.3.1 Portfolio requirements

Item	Requirements (number/type)	Documentation
Case Log	Summaries of all lab-based experiences, including analysis and reporting of at least 100 cases with 50 unique variants.	Log book to include test name; assay type; number of assays/ runs; test failures requiring review. (See Appendix 2)
Clinical Consultations	Fifteen (15) technically challenging or unusual cases/consultations involving lab data. Should address challenges as listed in paragraph 1.2.	Clinical Consultations Sign-Off Form (Appendix 2)
Process-based Discussions (PdD)	• Detailed discussion (at least one (1) page description of case in addition to PbD cover sheet) on five (5) "challenging" cases that required use of multiple skills, including consideration of lab data. • At least one (1) PbD should be on further workup/management of unusual such as "additional" or "off target" finding.	PbD Assessment Form (Appendix 2)
MDT Participation	Five (5) MDT attendances	Multidisciplinary Meeting Sign-Off Form (Appendix 2)
Quality Assurance (QA) Activities Log	Five (5) quality assurance activities. (**note**: You may include a significant/critical laboratory incident relevant to the module as a QA Audit)	Quality Assurance Activities Log (Appendix 2)
QA Reflection	Reflection on each of above	QA Reflection Form (Appendix 2)
Supervisor Report	One final report, plus additional annual report if module not completed in <12 months.	With sign-off indicating: • successful fluorescent-labelling of slides, at a quality level sufficient for reporting; • strong understanding of QC procedures for the methods, including internal and external QA; • working knowledge of method anomalies and associated trouble-shooting requirements. (Supervisor Report, Appendix 2)
Portfolio Summary sheet	To accompany final Supervisor Report	Portfolio Summary Form

1.3.2 Formal examinations

See Appendix 1

1.3.3 Assessment matrix

See Appendix 3

Module 2 – Screening of all chromosomes for known and unknown genomic variants (microscopy-based karyotyping and chromosomal microarray)

This is specialised testing with an associated significant potential for complex and challenging results, which require high-level interpretive reporting. Knowledge of specific quality issues, validation and requirements for supervision of cytogenetics testing are addressed elsewhere (see Requirements for Cytogenetic Testing, NPAAC, 2013).

Examples of typical clinical applications for this module would include:

- G-banded karyotyping of dissociated malignant tissue samples
- Sequential G-banding to FISH analysis of malignant tissue samples
- Multicolour (M)-FISH and multicolour (m)-BAND analysis of malignant tissue samples
- CGH-array and SNP-array analysis for selected malignancies
- Array analysis of atypical placental tissues including suspected molar or trisomic gestations

As the module involves untargeted screening for disease-linked genomic variants the following outcomes may occur:

- 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

Additionally, other additional or “off-target” findings may also be detected:

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

Types of genomic variation covered
Autosomal and sex chromosome aneuploidy, polysomies, structural anomalies, translocations and other balanced rearrangements, copy number changes, absence/loss of heterozygosity and uniparental disomy, identify-by-descent (distant and close) and chimerism.

2.1 Learning outcomes

2.1.1 Theoretical and Technical Knowledge

- Knowledge of theory and processes relating to Wet Lab:
  - Cell culture, selection and processing for whole cell-based genetic analysis
    - Processing of samples referred for cytogenetic and molecular analysis; solid tumour biopsies, lymph node and bone marrow
    - Cell culture and selection
    - Culture, synchronization, mitogens, harvest and fixing of metaphase cells for cytogenetic analysis
    - Slide-making and banding
  - Microarray
    - Knowledge of appropriate sample selection, preparation and nucleic acid extraction from a range of biological sources including fresh tissue, cytological specimens and formalin-fixed paraffin embedded tissues
    - Evaluation of sample suitability for microarray testing including nucleic acid quantity and quality indicators

    - Understanding of requirement for paired non-tumoural samples in assessment of neoplastic samples
    - Awareness of the unique characteristics of the specific array technology being employed
- Knowledge of theory and processes relating to Dry Lab:
  - Bright-field and fluorescence microscopy
  - Karyotypic analysis
  - Metaphase and interphase FISH in samples other than paraffin sections
  - Image capture and analysis systems for G-banding and (M)-FISH
  - Array technologies and analysis
- Detailed working knowledge of ISCN/HGVS nomenclature and its application
- Knowledge of major genome browsers and databases required to interpret karyotyping and CGH and SNP array findings

2.1.2 Analytical and interpretive skills

- Competence in monitoring data quality and result verification
- Competence in the assessment of chimerism and somatic and germline mosaicism
- Clinical evaluation of somatic genomic anomalies detected by karyotyping and array
- Clinical evaluation of constitutional genomic anomalies detected by karyotyping and array
- Knowledge and application of pathogenicity classification systems for somatic and constitutional variants
- Synthesis and clinical interpretation of laboratory data taking account of the clinical presentation, morphology and other relevant non-genomic and genomic investigations
- Assessment of familial recurrence risks arising from chromosomal anomalies

2.1.3 Quality assurance

- Knowledge of ethical, clinical and regulatory structures/framework around germline testing for inherited disease/predictive testing
- Knowledge of specific quality issues, validation and requirements of supervision
- Knowledge of potential sources of error
- Knowledge of technical performance, limitations and quality issues
- Recognition and troubleshooting of challenges
- Competence in monitoring data quality and result verification

2.1.4 Communication and Consultation

- Guidance regarding follow-up testing including result validation and testing of other family members
- Ability to provide clinically appropriate advice regarding contents of reports including diagnostic, prognostic and therapeutic implications of the results.
- Ability to communicate results and provide interpretative discussion to referring specialists and in multidisciplinary meetings
- Ability to recognise when complex test results mean that patient safety is best served by consultation with an expert colleague e.g. a genetic pathologist

2.2 Specific considerations/challenges to be addressed in assessment

- Investigations/actions when microarray data is not fit for clinical purpose (e.g. microarray quality control metrics indicate increased risk of false negative or positive results)
- Further investigation of an abnormality detected using microarray by application of microscopy-based methods (FISH, karyotype) to further elucidate the clinical significance of a finding (and vice versa)
- Approach to a germline secondary finding, detected when testing for somatic variants (e.g. evidence of consanguinity; detection of a sex chromosome abnormality; detection of a pathogenic variant in a dominant disease gene unrelated to the purpose of testing)
- Approach to assessment and reporting of variants of uncertain significance in the somatic and germline context
- Approach to assessment and reporting of 'susceptibility variants' or risk alleles in the somatic and germline context
- Investigations/actions when testing performed at another laboratory does not match with the result in your laboratory
- Validation process for microarray testing
- Consultation with expert colleagues regarding an incidental/secondary finding, or other complex findings
- 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

2.3. Assessment

2.3.1 Portfolio requirements

Item	Requirements (number/type)	Documentation
Case Log	Summaries of all lab-based experiences, including analysis and reporting of at least 100 cases with 50 unique variants. (If array technology is being assessed, include at least 50 microarrays with 50% abnormal variants, including variants of uncertain significance)	Log book to include test name; assay type; number of assays/ runs; test failures requiring review. (See Appendix 2)
Clinical Consultations	Fifteen (15) technically challenging or unusual cases/consultations involving lab data. Should address challenges as listed in paragraph 2.2.	Clinical Consultations Sign-Off Form (Appendix 2)
Process-based Discussions (PdD)	• Detailed discussion (at least one (1)-page description of case in addition to PbD cover sheet) on five (5) "challenging" cases that required use of multiple skills, including consideration of lab data. • At least one (1) PbD should be on further workup/management of unusual such as "additional" or "off target" finding.	PbD Assessment Form (Appendix 2)
MDT Participation	Five (5) MDT attendances	Multidisciplinary Meeting Sign-Off Form (Appendix 2)

Quality Assurance (QA) Activities Log	Five (5) quality assurance activities. (**note**: You may include a significant/critical laboratory incident relevant to the module as a QA Audit)	Quality Assurance Activities Log (Appendix 2)
QA Reflection	Reflection on each of above	QA Reflection Form (Appendix 2)
Supervisor Report	One final report, plus additional annual report if module not completed in <12 months.	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. (Supervisor Report, Appendix 2)
Portfolio Summary sheet	To accompany final Supervisor Report	Portfolio Summary Form

2.3.2 Formal examinations

See Appendix 1

2.3.3 Assessment matrix

See Appendix 3

Module 3 - Sequence-based testing for known and unknown variants in multiple genes, including genes potentially linked to clinical phenotypes not previously in the patient

This is complex and specialised training and builds particularly upon Module 1 which is considered a pre-requisite.

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, whole exomes and whole genomes, including genes associated with clinical phenotypes that have not been previously diagnosed in the patient.

Genome-wide sequencing with associated bioinformatic analysis targeted to large panels of genes relevant to a range of solid tumour phenotypes is now emerging, along with the prospect of comprehensive genome-wide analysis for a range of malignancies. This will include testing for disease diagnosis, to determine a possible primary site, to stratify patients in the context of clinical trials, to identify cancer predisposition genes and to identify potential therapeutic targets. These assays and techniques have an increased potential of finding variants of unknown significance including germline variants. Variants with partial penetrance or expressivity may be identified, as well as findings which may affect reproductive decision-making.

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 massively parallel sequencing 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.

3.1 Learning outcomes

3.1.1 Theoretical and Technical Knowledge

- Methods covered
  - Massively parallel sequencing with a range of library preparation/ bio-informatic filtering including
    - Targeted amplicon enrichment
    - Hybridization-based enrichment
    - 3rd generation sequencing technology (non-amplification based)
- Knowledge of theory and processes relating to Wet Lab:
  - General practical skills and understanding of nucleic acid preparation method(s), quantification, assessment of purity/intactness and storage/archiving
  - Knowledge of technical performance, limitations and quality issues associated with different library preparation methodologies (amplicon, hybridization-based, use of unique molecular identifiers (UMI), etc.)
  - Knowledge of technical performance, limitations and quality issues associated with different sequencing technologies
  - Knowledge of potential sources of error arising from massively parallel sequencing assays designed specifically for formalin-fixed, paraffin-embedded tissues

- Knowledge of theory and processes relating to Dry Lab:
  - Knowledge of primary, secondary and tertiary analysis for NGS workflow, including variables and limitations of each analysis
  - Knowledge of relevant bioinformatics issues including performance and limitations of demultiplexing/alignment tools/variant callers, variant annotation strategies, bioinformatic methods of structural variant detection, 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, including awareness of data security and privacy issues
- Knowledge of read depth and coverage issues whilst using whole exome or genome techniques for somatic variant analysis in cancer and /or in rare phenotypes/inherited conditions
- Knowledge of primary, secondary and tertiary analysis, variables and limitations
- Knowledge of relevant software and 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 and experience of QC parameters for massively parallel sequencing; bioinformatic pipelines to detect sequence variants, copy number changes and structural variants/fusions involving pre-defined genes/regions on the targeted panel, depending on the nature of the genomic variations contributing to the defined clinical phenotype is necessary.
- Knowledge of data architecture, computing/processing/capacity issues and data security/privacy including cloud-based systems
- Knowledge of somatic variant curation strategy including understanding of variant annotation, advantages and limitations of cancer (e.g. COSMIC) and healthy population databases (e.g. gnomAD), curation of literature with regard to diagnostic, prognostic and targeted therapies, advantages and limitations of in silico prediction tools and splice prediction tools, current somatic reporting guidelines
- Knowledge of constitutional/germline variant curation strategy including understanding of variant annotation, advantages, use and limitations of cancer and clinical trial databases (e.g., Catalog of Somatic Mutations in Cancer, My Cancer Genome, TCGA and International Cancer Genome Consortium).
- Knowledge of current somatic and constitutional reporting guidelines

3.1.2 Analytical and interpretive skills

- Detailed working knowledge of HGVS/ISCN nomenclature and its practical application in reporting tumour-specific variants
- Knowledge and application of bioinformatics pipelines to identify and characterize known somatic variants as well as novel uncharacterized variants using databases such as COSMIC (https://cancer.sanger.ac.uk/cosmic), My Cancer Genome (https://www.mycancergenome.org) and IARC TP53 (http://p53.iarc.fr/).
- Ability to distinguish between tumour-specific somatic variants as opposed to known polymorphisms using common population databases such as dbSNP and ExAc (exome aggregation consortium)
- Integration of the genomic variations detected with accompanying morphological, immunophenotypic, cytogenetic and clinical context of the patient in order to provide a clinically appropriate genomic report
- Use of other databases such as population frequency databases (e.g. Clinvar, gnomAD, 1000

Genomes Project, dbSNP, dbVar, ExAC), locus specific databases, segregation analysis, modes of inheritance, curation of constitutional literature, mosaicism, appropriate germline databases (HGMD, ClinVar), advantages and limitations of in silico prediction tools and splice prediction tools for an accurate interpretation of somatic variants

- Knowledge and application of pathogenicity classification systems for somatic and germline variants; gene function; interpretation and pathways involved; well powered studies with consensus, preclinical studies, case reports etc.
- Management of incidental genomic findings (e.g. germline variants of significance detected during somatic testing).
- Recommendations relevant to diagnosis, prognosis and therapeutics to be based on clinical impact of the somatic variant and available evidence. Evidence used for variant categorization should be based on current guidelines
- Integration of the genomic data with review of pathological diagnosis (i.e. tumour type, subtype, grade, stage) and family history / clinical context of the patient in order to provide a clinically appropriate genomic report
- Synthesis and clinical interpretation of laboratory data taking account of the clinical presentation, morphology, immunophenotype and other relevant non-genomic and genomic investigations

3.1.3 Quality assurance

- Knowledge of specific quality issues, validation and requirements of supervision of massive parallel sequencing (ie. *Requirements for Human Medical Genome Testing Utilising Massively Parallel Sequencing Technologies*, NPAAC, 2017)
- Sound working fluency in dealing with the range of expected challenging outcomes and additional “off-target” findings
- Knowledge of ethical, clinical and regulatory structures/framework around germline testing for inherited disease/predictive testing including patient consent
- Knowledge of potential sources of error arising from massively parallel sequencing assays designed for formalin-fixed, paraffin-embedded tissues
- Knowledge of NGS workflow, selection of appropriate sample, practical application of macro/microdissection techniques and their pros and cons, sample adequacy, appropriate triage and use of cytology and body fluid material for NGS and knowledge of cancer biology in relation to the patient’s sample phenotype
- Knowledge of technical performance, limitations and quality issues
- Recognition and troubleshooting of challenges
- Competence in monitoring data quality and result verification

3.1.4 Communication and Consultation

- 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 and molecular tumour boards with oncologists, other pathologists, geneticists, scientists and other referring specialists
- Knowledge of when to seek advice from genetic pathologists/ other relevant clinical experts in cases where findings are outside the expertise of the supervising anatomical pathologist
- Close link with a clinical genomics service, particularly for whole genome /exome services

3.2 Specific considerations/challenges to be addressed in assessment

- Evaluating primary specimen for suitability for molecular testing
- Normal cell contamination
- 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
- Trouble-shooting poor-quality sequence
- Challenges associated with homopolymer runs
- Challenges with assay controls - positive and negative
- Issues with sample depurination
- Maintenance of bioinformatic pipeline, including version control, verification of new versions
- 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
- Approach to assessment and reporting of variants of uncertain significance in the somatic and germline context
- Approach to assessment and reporting of 'susceptibility variants' or risk alleles in the somatic and germline context
- Cross-discipline consultation with expert colleagues regarding potential additional or "off-target" findings

3.3. Assessment

3.3.1 Portfolio requirements

Item	Requirements (number/type)	Documentation
Case Log	Summaries of all lab-based experiences, including analysis and reporting of at least 50 cases with 50 unique variants.	Log book to include test name; assay type; number of assays/ runs; test failures requiring review. (See Appendix 2)
Clinical Consultations	Fifteen (15) technically challenging or unusual cases/consultations involving lab data. Should address challenges as listed in paragraph 3.2.	Clinical Consultations Sign-Off Form (Appendix 2)
Process-based Discussions (PdD)	• Detailed discussion (at least one (1) page description of case in addition to PbD cover sheet) on five (5) "challenging" cases that required use of multiple skills,	PbD Assessment Form (Appendix 2)

	including consideration of lab data. (note: discipline-specific discussion topics to be defined) • At least one (1) PbD should be on further workup/management of unusual such as “additional” or “off target” finding.	
MDT Participation	Five (5) MDT attendances	Multidisciplinary Meeting Sign-Off Form (Appendix 2)
Quality Assurance (QA) Activities Log	Five (5) quality assurance activities. (**note**: You may include a significant/critical laboratory incident relevant to the module as a QA Audit)	Quality Assurance Activities Log (Appendix 2)
QA Reflection	Reflection on each of above	QA Reflection Form (Appendix 2)
Supervisor Report	One final report, plus additional annual report if module not completed in <12 months.	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. (Supervisor Report, Appendix 2)
Portfolio Summary sheet	To accompany final Supervisor Report	Portfolio Summary Form

3.3.2 Formal examinations

See Appendix 1

3.3.3 Assessment Matrix

See Appendix 3

SECTION 3

Appendices

Assessment

Assessment is by

- Formal examinations (see Appendix 1)
- A portfolio of evidence of having participated in a sufficient number and type of work activities (see Appendix 2)
- Satisfactory progress (supervisor reports) (see Appendix 2)

An assessment matrix is provided in Appendix 3.

Portfolio requirements

Portfolio activities are carried out in the workplace provides evidence that fellows have engaged in the appropriate number and type of work-based activities to build base knowledge, analytical and interpretive skills, quality assurance and communication capabilities relevant to genetics/genomic testing in Anatomical Pathology. Requirements or each module are summarised in a table at the end of the module in Section 2.

Appendix 2 contains the forms and logbook pages for recording the portfolio activities. Please file the hard copy forms in a **portfolio folder** with separate sections as in the table provided.

A soft copy **portfolio summary** (Excel spreadsheet) should also be compiled so that the fellow can keep track of what has been completed. The spreadsheet can be downloaded from the RCPA website. It is the fellow's responsibility to keep both hard and soft copy records up-to-date.

The portfolio summary spreadsheet should be apprehended to the annual supervisor reports and will be reviewed by the Registrar, Board of Education and Assessment and the Chief Examiner. Signatories and fellows may be contacted to confirm evidence of satisfactory completion.

Appendix 1: Examinations

There will be an oral examination consisting of three stations, each of twenty minutes, with two examiners for each. This will include stations focussing on practical cases; for example:

- Array-detected variant curation, cytogenetic- and array- based cases for Module 2;
- Practical cases for variant curation for Module 3.

Candidates will be allowed 30 minutes pre-reading time, during which they may review data and reports, making notes where applicable, prior to discussion with the examiners.

The examination will focus on ability to:

- Analyse and interpret findings
- Explain principles of the test methods used, identifying any limitations
- Apply quality management principles and troubleshooting methods to explain and prevent possible sources of error
- Communicate clinically relevant conclusions and advice to referring doctors, including discussion of uncertainties
- Discuss broader health implications and/or ethical considerations relevant to the testing performed

Appendix 2: Forms and log pages for Portfolio

The Royal College of Pathologists of Australasia	**Medical Genomics** **Anatomical Pathology** **Routine Case Log**

How to use this form

From the beginning of training, Fellows should log all laboratory-based experiences, including analysis and reporting. Only runs that the Fellow has been directly involved with should be logged.

Module 1&2: A minimum of 100 cases with 50 unique variants should be recorded during these Modules.

Modules 3: A minimum of 50 cases with 50 unique variants should be recorded during the Module.

The log book should include:

- Test name
- Assay type
- Number of assays/ runs
- Test failures requiring review

At the end of each Module, the log should be sighted and signed off on the Supervisor Report.

Fellow Name			**Fellow ID**	**Module of Training** (please tick) ☐ Module 1 ☐ Module 2 ☐ Module 3	
	Date	***Test name***	***Assay type***	***Number of assays/ runs***	***Test failures requiring review***
1					
2					
3					
4					
5					
6					

7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
Final Outcome (please tick) ☐ Competent ☐ Not Competent					
Signature of Assessor			**Signature of Fellow**		
Name of Laboratory					

Medical Genomics
Anatomical Pathology
Clinical Consultations Sign-Off Form

How to use this form

From the beginning of training, Fellows should log consultations with clinical colleagues that involve significant, difficult or unusual cases.

A minimum of (fifteen) 15 consultations should be recorded during the Module.

Consultation type should be noted on the form as: ***ORAL:*** *Telephone Outpatient* ***(TOP) OR*** *Telephone Inpatient* ***(TIP)***
WRITTEN: *Outpatient* ***(OP) OR*** *Inpatient* ***(IP)***

At the end of each Module, this form and appended case lists should be sighted by the supervisor and signed off.

Fellow Name			**Fellow ID**	**Module of Training** (please tick) ☐ Module 1 ☐ Module 2 ☐ Module 3	
	Date	***Headline summary of case***	***Issue(s) raised by the case***	***Consult type***	***Fellow's role in the case***
	example	***eg. G-banded karyotyping of dissociated malignant tissue samples***	***Clinician seeking guidance on diagnostic possibilities and investigations***	***TOP / TIP OP / IP***	***Advice offered; review of results; follow-up discussion with referring clinician***
1					
2					
3					
4					
5					
6					
7					
8					

9					
10					
11					
12					
13					
14					
15					
Final Outcome (please tick) ☐ Competent ☐ Not Competent					
Signature of Assessor			**Signature of Fellow**		
Name of Laboratory					

Medical Genomics Anatomical Pathology PbD Assessment Form

Process based Discussion

Fellow Name	**Fellow ID**	**Module of Training** (please tick) ☐ Module 1 ☐ Module 2 ☐ Module 3
Assessor Name	**Assessor position** ☐ Pathologist ☐ Other (please specify)	

Techniques/methods (tick the box that applies).

1. ☐ Sanger sequencing of whole genes
2. ☐ Multiplex ligation primer amplification
3. ☐ Focused massively parallel sequencing (MPS/NGS) employing targeted amplicon enrichment
4. ☐ Focused massively parallel sequencing (MPS/NGS) employing hybridization based enrichment
5. ☐ Non-targeted massively parallel sequencing (MPS/NGS) employing exome capture techniques (WES)
6. ☐ Non-targeted massively parallel sequencing (MPS/NGS) involving whole genome sequencing (WGS)
7. ☐ Routine cytogenetic karyotyping involving G-banding of chromosomes
8. ☐ Multicolour FISH or multicolour BAND analysis of chromosomes
9. ☐ Comparative genomic hybridization (CGH) array
10. ☐ Single nucleotide polymorphism (SNP) array

Please comment on whether these aspects of the Fellow's performance	**Yes**	**No**	**N/A**
Understands the principles of the method			
Has a working knowledge of instrument processes and maintenance requirements			
Has observed all phases of an assay successfully and been involved in the production of a valid result that can be reported			
Able to explain the Quality Controls procedures for this method, including internal and external Quality Assurance.			
Able to discuss anomalies and resolve uncertainties for the method			
Able to explain maintenance and trouble-shooting requirements for the method			

Please comment on other relevant aspects, especially on aspects for improvement (use the reverse side if insufficient room)

Final Outcome (please tick)

☐ Competent ☐ Not Competent

Signature of Assessor	**Signature of Fellow**

Name of Laboratory

Medical Genomics
Anatomical Pathology
Clinical/Multidisciplinary Meeting Sign Off Form

How to use this form

This form is to be used to record that the Fellow has fulfilled the following requirements:

Present cases at a minimum of five (5) clinical or laboratory meetings throughout the module.

Fellows should retain a list of the cases/entities presented at each meeting in the portfolio. At the end of the module, this form and appended case lists should be sighted by the supervisor and signed off.

Fellow Name:	**Fellow ID:**	Module of Training (please tick) ☐ Module 1 ☐ Module 2 ☐ Module 3

	Meeting date	**Brief description of meeting; subject(s) of discussion**	**Did Fellow present cases? Y/N**
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Final Outcome (please tick) ☐ Competent ☐ Not Competent

Signature of Assessor	**Signature of Fellow**

Name of Laboratory

Medical Genomics
Anatomical Pathology
Quality Assurance Activities Log

How to use this form
Eight (8) activities should be selected from the list. (One of these could be an incident report) Items 9 and 10 are compulsory.

Use the Reflection Form in this handbook to write a brief reflection on what you learned from doing each activity (photocopy as many copies of the form as you need). Keep the forms in your portfolio along with other specified documents if required
At the end of each rotation, the log should be sighted and signed off by the supervisor.

Fellow Name	**Fellow ID**	**Module of Training** (please tick) ☐ Module 1 ☐ Module 2 ☐ Module 3

	Quality activity	***Summary of Fellow's role in the activity or comment (where applicable)***	***Date***
1	Analyse the design and operating characteristics of a particular instrument or platform		
2	Work through the development of a new in vitro diagnostic test and the associated IQA processes		
3	Review relevant AS ISO standards (list documents reviewed)		
4	Review relevant NPAAC standards and guidelines (list documents reviewed)		
5	Review the laboratory's quality policy, including policy guiding response to unsatisfactory QAP results		
6	External QAP (particularly involvement with the HGSA/QAP, EMQN, ASoC programs)		
7	Active involvement in preparations for laboratory accreditation		

Medical Genomics
Anatomical Pathology
Quality Assurance Activities Log

How to use this form
Eight (8) activities should be selected from the list. (One of these could be an incident report) Items 9 and 10 are compulsory.

Use the Reflection Form in this handbook to write a brief reflection on what you learned from doing each activity (photocopy as many copies of the form as you need). Keep the forms in your portfolio along with other specified documents if required
At the end of each rotation, the log should be sighted and signed off by the supervisor.

Fellow Name	**Fellow ID**	**Module of Training** (please tick) ☐ Module 1 ☐ Module 2 ☐ Module 3

	Quality activity	***Summary of Fellow's role in the activity or comment (where applicable)***	**Date**
8	Participate in a workflow check of effective/ efficient laboratory function		
9	**MANDATORY ACTIVITY** Include reports in portfolio.		
10	**MANDATORY ACTIVITY** Significant incident: Involvement in assessment, reporting and review, focussing particularly on the quality issues that were identified and addressed. **Minimum of one**. OR Quality audits: Conduct. Where possible, include comparison with relevant national/international guidelines. **Minimum of one**. Include documentation in portfolio. Use the reporting form in supplied in this Handbook		

Supervisor name……..... Signature…….....…… Date…….....

The Royal College of Pathologists of Australasia	**Medical Genomics** **Anatomical Pathology** **Quality Assurance Reflection Form**	
Fellow Name	**Fellow ID**	**Module of Training** (please tick) ☐ Module 1 ☐ Module 2 ☐ Module 3
Nature of activity		
Your role in the activity		
Your reflection on what you learned from your involvement in this activity *Points to consider: -* - *Type of Activity* - *Actions undertaken* - *Findings* - *Resource considerations* - *Ethical considerations*		
Fellow signature		**Date**
Supervisor name (please print) and signature		**Date**
Name of Laboratory		

Medical Genomics Supervisor Report Anatomical Pathology

Please review the Fellow's portfolio and logbook before completing this report

Fellow Name (please print)	**Fellow ID**	**Module of Training** (please tick) ☐ Module 2 ☐ Module 2 ☐ Module 3
Name of Organisation		
Training period: _____/_____/_____ to ___/_____/_____		
Name of supervisor (please print)		Supervisor RCPA ID no.

Please inspect the forms in the Fellow's portfolio and use the Table below to record evidence of completion

Portfolio items for which there is a minimum requirement				
	Previous total (if applicable)	Number in current year/ module	Cumulative Total	Minimum for completion of module
Case Log				Module 1&2: 100 Module 3: 50
Clinical Consultations				15
Process-based discussion (PbD)				5
MDT Participation				5
QA Activities				8
QA Reflection				1 per each QA activity
Previous Supervisor's reports				1 report/ 12 months
Portfolio Summary				

Does the print-out of the portfolio summary spreadsheet accurately record the contents of the portfolio?
☐ Yes ☐ No

Please score the Fellow's performance using this scale

1 = Performance currently falls far short of expected standards for level of training.
There is a serious problem that may have implications for accreditation of the current training period. The problem must be stated clearly on the final page.

2 = Performance currently falls short of expected standards for level of training.
There is an area of lower than expected performance. The problem must be stated clearly on the final page.

3 = Performance is consistent with the expected level of training.
About 80% of fellows will merit this grade.

4 = Performance is better than expected for level of training.
About 10% of fellows will merit this grade.

5 = Performance is exceptional.
Very few fellows will merit this grade.

N/A = Not Applicable to this training period

	Score
The principles of all methods are understood	
Working knowledge of method anomalies and associated trouble-shooting requirements	
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 methods, including internal and external QA	
Working knowledge of method anomalies and associated trouble-shooting requirements	
Ability to communicate results and provide interpretative discussion to referring specialists and in multidisciplinary meetings	
Ability to provide clinically appropriate advice regarding contents of genomic reports	
Ability to provide advice on appropriate follow-up genomic testing/other modalities as required	

Overall evaluation

Have the outcomes of this module been satisfactorily achieved?
☐ Yes ☐ No

Is specific further professional development required? If yes, please outline process
☐ Yes ☐ No

Signatures

Fellow (please PRINT name and sign)	Date
Supervisor name (please PRINT name and sign)	Date
Other senior staff member/second supervisor (if applicable) (please PRINT name and sign)	Date
Head of Department (please PRINT name and sign)	Date

Comments by Fellow:

_____

_____

_____

_____

_____

_____

Please return this Supervisor's Report to:

The Royal College of Pathologists of Australasia
207 Albion Street
Surry Hills NSW 2010 AUSTRALIA

Faxed reports will not be accepted.

THE ROYAL COLLEGE OF PATHOLOGISTS OF AUSTRALASIA USE ONLY

Signature

Registrar/Deputy Registrar, Board of Education and Assessment (please PRINT name and sign)	Date

Appendix 3 – Assessment matrix

	Portfolio of workplace activities					Supervisor report	Oral exam
	Case Log	Consult-ations	PbD	MDT	QA Activity		
Theoretical & technical knowledge	Y	Y	Y	Y		Y	Y
Analytical and interpretive skills		Y	Y	Y	Y	Y	Y
Quality assurance			Y	Y	Y	Y	Y
Communication & consultation		Y	Y	Y	Y	Y	Y