RCPA

The Royal College of Pathologists of Australasia

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

# ANATOMICAL 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 |

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# 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 Anatomical Pathology.

The development of these particular certification modules facilitates extension of the scope of practice of Anatomical Pathologists in the area of molecular genetic testing in order to satisfy NPAAC requirements for appropriate supervision of such testing (http://health.gov.au/internet/main/publishing.nsf/Content/health-npaac-docs-supervision.htm).

To adequately supervise a molecular/genomic service, Anatomical Pathologists need to have sufficiently 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 of a particular result in order to ensure 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 Anatomical Pathologists with the opportunity to participate in accordance with NPAAC requirements.

# GENERAL AIMS OF THE TRAINING PROGAM

The genetics/genomics certification modules applicable to Anatomical Pathology build on earlier Fellowship training. As well as developing competencies in more complex genetics/ genomics relevant to Anatomical Pathology, candidates are expected to extend 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 the requirements for each of the Anatomical 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, 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 their specific pathology discipline.

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

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"recognition of prior learning" at the initiation of this Certification project (see Appendix A). Obviously, 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 – for example, investigation of haemoglobinopathies, intellectual disability, immunodeficiencies or prenatal diagnostic testing.

The pathologist has the ultimate responsibility for the test results, the quality and safety standards of the laboratory, advising clinicians on the interpretation of test results and on the further investigation of the patient.

An essential part of sub-specialty genetics/genomics training is for practitioners to gain sufficient understanding of the breadth of the field. 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. The necessity for close collaboration between Anatomical Pathologists and appropriately trained and accredited scientists involved in performing the testing is also essential.

# 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

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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 knowledge and 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 wide range of methods now available.

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# SECTION 2

# LEARNING OUTCOMES AND RECOMMENDED TRAINING ACTIVITIES



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

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.

**Methodologies 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

**Knowledge and practical skills**

*General considerations*

- 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.
- Requires knowledge of specific quality issues, validation and requirements of supervision of massive parallel sequencing (i.e. *Requirements for human medical*

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*genome testing utilising massively parallel sequencing technologies*, National Pathology Accreditation Advisory Council, 2017)
- Requires knowledge of potential sources of error arising from massively parallel sequencing assays designed for formalin-fixed, paraffin-embedded tissues
- Requires 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.

*Wet lab considerations*

- General practical 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

*Dry lab considerations*

- 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

*Analysis considerations*

- 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).
- 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 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

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significant clinical outcomes unrelated to the purpose of testing) in accordance with international guidelines (such as consensus ACMG and AMP)
- Knowledge of current somatic and constitutional reporting guidelines
- 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
- Knowledge of ethical, clinical and regulatory structures/framework around germline testing for inherited disease/predictive testing

*Post-Analytic Considerations*
- 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)

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**Professional Competency**

- **Log book** - summarising all lab-based experiences. *Log book to include test name; assay type; number of assays/runs; test failures requiring review.* It would be expected that in order to achieve competence in this module, a candidate would have reported approximately 100 cases with 50 unique variants.
- **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 MUST be included.*
    - *Five (5) MDT attendances,*
    - *Five (5) Quality Assurance activities 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 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

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- 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.

Comment: Pathologists seeking certification on the basis of "recognition of prior learning" are not expected to submit the above Log-book/Portfolio materials or to undergo formal assessment, however they are expected to demonstrate in written application that their experience and knowledge is comparable to that described above, including successful participation in Quality Assurance activities.

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

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 that will be identified**

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.

**Knowledge and practical skills**

*General considerations*

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, National Pathology Accreditation Advisory Council, 2013).

*Wet lab considerations*

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

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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

*Dry lab considerations*

- 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

*Analytic considerations*

- Competence in monitoring data quality and result verification
- Competence in the assessment of chimerism and somatic and germline mosaicism
- Detailed working knowledge of ISCN/HGVS nomenclature and its application
- Clinical evaluation of somatic genomic anomalies detected by karyotyping and array
- Clinical evaluation of constitutional genomic anomalies detected by karyotyping and array
- Knowledge of major genome browsers and databases required to interpret karyotyping and CGH and SNP array findings
- Knowledge and application of pathogenicity classification systems for somatic and constitutional variants

*Post-analytic considerations*

- 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
- Guidance regarding follow-up testing including result validation and testing of other family members
- Knowledge of the relevant regulatory framework(s)
- Knowledge of ethical, clinical and regulatory structures/framework around germline testing for inherited disease/predictive testing
- 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

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**Professional Competency**

- **Log book** - summarising all lab-based experiences. *Log book to include test name; assay type; number of assays/runs; test failures requiring review*. It would be expected that in order to achieve competence in this module, a candidate would have reported approximately 100 cases with 50 unique variants. If array technology is being assessed, the total would be expected to include around 50 microarrays with 50% abnormal variants (including variants of uncertain significance).
- **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 MUST be included.*
    - *Five (5) MDT attendances,*
    - *Five (5) Quality Assurance activities 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** (array-detected variant curation, array-based practical cases)
- **Structured Oral**

*** Examples of technical considerations:**

- 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

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- 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

Comment: Pathologists seeking certification on the basis of "recognition of prior learning" are not expected to submit the above Log-book/Portfolio materials or to undergo formal assessment, however they are expected to demonstrate in written application that their experience and knowledge is comparable to that described above, including successful participation in Quality Assurance activities.

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# Module 3 – Sequence-based testing for known and unknown variants in multiple genes, including genes potentially linked to clinical phenotypes not 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, 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.

**Methodologies 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 and practical skills**

*General considerations*

- This is complex and specialised training and builds particularly upon Module 1 which is considered a pre-requisite
- Knowledge of specific quality issues, validation and requirements of supervision of massive parallel sequencing (i.e. *Requirements for Human Medical Genome Testing*

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*Utilising Massively Parallel Sequencing Technologies, National Pathology Accreditation Advisory Council, 2017*)

- Sound working fluency in dealing with the range of expected challenging outcomes and additional "off-target" findings

*Wet lab considerations*

- Refer to Module 1.

*Dry lab considerations*

- Refer to Module 1.

Additionally:

- 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

*Analysis considerations*

- Refer to Module 1.

Particularly:

- 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 massive 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).
- 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 of ethical, clinical and regulatory structures/framework around germline testing for inherited disease/predictive testing including patient consent.
- 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.

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- 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

*Post-Analytic Considerations*

- Refer to Module 1.

Additionally:

- 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.

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**Professional Competency**

- **Log book** – summarising all lab-based experiences. *Log book to include test name; assay type; number of assays/runs; test failures requiring review*. It would be expected that in order to achieve competence in this module, a candidate would have reported approximately 50 cases with 50 unique variants.
- **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.*
    - *Five (5) MDT attendances,*
    - *Five (5) Quality Assurance activities and*
    - *One (1) significant/critical laboratory incident report relevant to the module*
- **Case-based Discussions** ***(note:** discipline-specific discussion topics to be defined)* - 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** (variant curation, practical cases)
- **Structured Oral**

*** Examples of technical considerations:**

- 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

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- 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.

Comment: Pathologists seeking certifications on the basis of "recognition of prior learning" are not expected to submit the above Log-book/Portfolio materials or to undergo formal assessment, however they are expected to demonstrate in written application that their experience and knowledge is comparable to that described above, including successful participation in Quality Assurance activities.

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# APPENDIX A

## 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.

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