KK Women's and Children's Hospital
SingHealth

| Please affix patient identification label or complete the following fields. |
|---|
| NAME:<br>NRIC/ID NO.:<br>ACCOUNT NO.:<br>DATE OF BIRTH:<br>GENDER: Male / female |

# MOLECULAR HISTOPATHOLOGY REQUEST FORM
## Department of Pathology and Laboratory Medicine

| **CLINICAL HISTORY:** | **SPECIMEN TYPE: (please tick)** |
|---|---|
| Tumour location:<br><br>Working diagnosis:<br><br>Relevant findings (e.g. FISH, IHC results): | ☐Snap frozen tumour<br>☐Paraffin block of tumour<br>☐Unstained sections (x10) with corresponding H&E-stained section<br><br>Specimen identification number: |
| **REQUESTING PHYSICIAN:** | **PURPOSE OF TESTING: (please tick all applicable statements)** |
| Signature:<br>Name:<br>MCR:<br>Telephone number:<br>Email address: | ☐This is a current clinical case and the results will be utilized for patient care<br>☐This test is performed for research / publication purposes<br>☐This is performed for verification of an existing molecular result |

| |
|---|
| ☐ Test 1: Medulloblastoma subgroup determination by NanoString nCounter gene expression profiling |
| ☐ Test 2: Gene fusion detection in paediatric low-grade gliomas by NanoString nCounter |
| ☐ Test 3: Gene fusion detection in paediatric tumours and sarcomas by NanoString nCounter |
| ☐ Test 4: Gene fusion detection in solid tumours by anchored multiplex PCR (Archer FusionPlex pan-solid assay) |
| ☐ Test 5: Microsatellite instability (MSI) testing |
| ☐ Test 6: *MLH1* promoter methylation analysis |
| ☐ Test 7: Molecular genotyping for identification of molar pregnancies |
| ☐ Test 8: *MYOD1* p. L122R mutational analysis for spindle cell/sclerosing rhabdomyosarcomas |
| ☐ Test 9: *POLE* full exons 9,13 and14 mutational analysis for endometrial carcinomas |
| ☐ Test 10: OncoScan SNP microarray FFPE tumour analysis |

Note: Please tick the requested test. All fields **MUST** be completed. For enquiries, please email molhisto@kkh.com.sg.

## Test 1
## Medulloblastoma subgroup determination by NanoString nCounter gene expression profiling.

### Background
The 2016 World Health Organization Classification of Tumours of the Central Nervous System recognizes genetically-defined medulloblastoma subgroups – WNT-activated, SHH-activated and non-WNT/non-SHH (groups 3 and 4). Combined morphological and molecular information provides optimal prognostic and predictive information to guide clinical management.

### Purpose of test
The test assigns a medulloblastoma molecular subgroup on the basis of the expression level of 22 medulloblastoma signature genes using NanoString nCounter technology.

### Specimen requirements
10 unstained sections of tumour and a corresponding H&E-stained histological section, OR a paraffin block of tumour.

### Turnaround time
The test is batched and performed every fortnight. The test itself takes 3 working days to complete.

### Caveats
RNA integrity and concentration must meet assay requirements. Low tumour content may result in an inaccurate result. A small proportion of cases cannot be classified into any of the four subgroups.

### Proficiency testing
Exchange with an overseas centre performing the same test.

### Reference
Northcott PA et al. Rapid, reliable, and reproducible molecular sub-grouping of clinical medulloblastoma samples. Acta Neuropathologica 2012; 123: 615-626.

## Test 2

## Gene fusion detection in paediatric low-grade gliomas by NanoString nCounter technology.

### Background

A proportion of paediatric low-grade gliomas have gene fusions and duplication events. Identification of such gene fusions are important for diagnosis and may provide prognostic and predictive information.

### Purpose of test

This test identifies any of 31 specific gene fusions and one duplication event known to be present in paediatric low-grade gliomas using NanoString nCounter technology.

| Low grade glioma fusion targets | | | | | |
|---|---|---|---|---|---|
| 1 | BRAF (exon 7)-MACF1 (exon 19) | 12 | KIAA1549 (exon 15)-BRAF (exon 10) | 23 | NACC2 (exon 4)-NTRK2 (exon 13) |
| 2 | CLCN6 (exon 2)-BRAF (exon11) | 13 | KIAA1549 (exon 15)-BRAF (exon 11) | 24 | NTRK3 (exon 10)-ETV6 (exon 5) |
| 3 | ETV6 (exon 1)-NTRK3 (exon 18) | 14 | KIAA1549 (exon 15)-BRAF (exon 9) | 25 | QKI (exon 1)-RAF1 (exon 14) |
| 4 | FAM131B (exon 1)-BRAF (exon 10) | 15 | KIAA1549 (exon 16)-BRAF (exon10del74) | 26 | QKI (exon 2)-MYB (exon 16) |
| 5 | FAM131B (exon 2)-BRAF (exon 9) | 16 | KIAA1549 (exon 16)-BRAF (exon 11) | 27 | QKI (exon 6)-NTRK2 (exon 16) |
| 6 | FAM131B (exon 3)-BRAF (exon 9) | 17 | KIAA1549 (exon 16)-BRAF (exon 9) | 28 | RNF130 (exon 3)-BRAF (exon 9) |
| 7 | FGFR1 (exon 17)-TACC1 (exon 7) | 18 | KIAA1549 (exon 18)-BRAF (exon 10) | 29 | SRGAP3 (exon 11)-RAF1 (exon 8) |
| 8 | FGFR3 (exon 17)-TACC3 (exon 4) | 19 | KIAA1549 (exon 19)-BRAF (exon 9) | 30 | SRGAP3 (exon 12)-RAF1 (exon 10) |
| 9 | FXR1 (exon 13)-BRAF (exon 10) | 20 | MKRN1 (exon 4)-BRAF (exon 11) | 31 | ST6GAL1 (exon 2)-WHSC1 (exon 4) |
| 10 | GNAI1 (exon 1)-BRAF (exon 10) | 21 | MYB (exon 6)-MAML2 (exon 4) | 32 | MYBL1 duplication |
| 11 | KIAA1549 (exon 13)-BRAF (exon 9) | 22 | MYB (exon 9)-PCDHGA1 (exon 2) | | |

### Specimen requirements

10 unstained sections of tumour and a corresponding H&E-stained histological section, OR a paraffin block of tumour.

### Turnaround time

The test is batched and performed every fortnight. The test itself takes 3 working days to complete.

### Caveats

RNA integrity and concentration must meet assay requirements. Low tumour content may result in an inaccurate result. The specific gene fusion present needs to be expressed at high levels for detection.

### Proficiency testing

Exchange with an overseas centre performing the same test.

### Reference

Ryall S. Multiplex Detection of Pediatric Low-Grade Glioma Signature Fusion Transcripts and Duplications Using the NanoString nCounter System. J Neuropathol Exp Neurol 2017; 76: 562–570.

## Test 3

# Gene fusion detection in paediatric tumours and sarcomas by NanoString nCounter technology.

### Background

A proportion of paediatric solid tumours and sarcomas have gene fusions. Identification of specific gene fusions are important for diagnosis and may provide prognostic and predictive information.

### Purpose of test

This test identifies any of 174 specific gene fusions known to be present in paediatric solid tumours and sarcomas using NanoString nCounter technology. This test does not identify gene fusions other than these 174 specific gene fusions.

| SN | Sarcoma type | Fusion | Variants, n |
|---|---|---|---|
| 1 | Alveolar soft part sarcoma | *ASPSCR1/TFE3* | 2 |
| 2 | Alveolar rhabdomyosarcoma | *PAX3/FOXO1, PAX7/FOXO1, PAX3/FOXO4, PAX3/NCOA1, PAX3/NCOA2, PAX3/AFX, PAX3/INO80D* | 7 |
| 3 | Aneurysmal bone cyst | *CDH11/USP6, COL1A1/USP6, OMD/USP6, TRAP150/USP6, ZNF9/USP6* | 9 |
| 4 | Angiomatoid fibrous histiocytoma, Clear cell sarcoma | *EWSR1/ATF1, EWSR1/CREB1, EWSR1/CREM, FUS/ATF1* | 8 |
| 5 | Biphenotypic sinonasal sarcoma | *PAX3/MAML3* | 1 |
| 6 | Infantile/Congenital fibrosarcoma | *ETV6/NTRK3, EML4/NTRK3* | 3 |
| 7 | Desmoplastic small round cell tumor (DSRCT) | *EWSR1/ERG, EWSR1/WT1* | 10 |
| 8 | Endometrial stromal sarcoma | *EPC1/PHF1, FN1/ALK, JAZF1/SUZ12, JAZF1/PHF1, MEAF6/PHF1, YWHAE/NUTM2, ZC3H7B/BCOR* | 9 |
| 9 | Epithelioid hemangioendothelioma | *WWTR1/CAMTA1,YAP1/TFE3* | 3 |
| 10 | Ewing sarcoma | *EWSR1/ERG, EWSR1/FLI1, EWSR1/ETV1, EWSR1/ETV4, EWSR1/FEV, FUS/ERG, FUS/FEV* | 31 |
| 11 | Ewing-like sarcoma (rare variants) | *BCOR/CCNB3, EWSR1/NFAT2, EWSR1/SMARCA5, EWSR1/SP3* | 5 |
| 12 | Extraskeletal myxoid chondrosarcoma | *EWSR1/NR4A3, RBP56/NR4A3, TAF15/NR4A3, TCF12/NR4A3, TFG/NR4A3* | 8 |
| 13 | Inflammatory myofibroblastic tumor | *ATIC/ALK, CARS/ALK, CLTC/ALK, PPFIBP1/ALK, RANBP2/ALK, SEC31A/ALK, TPM3/ALK, TPM4/ALK, NAB2/PDGFRB, TFG/ROS1, YWHAE/ROS1, EML4/ALK, PRKAR1A/ALK, LMNA/ALK, TFG/ALK* | 17 |
| 14 | Lipoblastoma | *COL1A2/PLAG1, HAS2/PLAG1* | 2 |
| 15 | Lipoma | *LPP/HMGA2* | 2 |
| 16 | Mesenchymal chondrosarcoma | *HEY1/NCOA2, IRF2BP2/CDX1, NUP107/LGR5* | 3 |
| 17 | Mesothelioma | *EWSR1/YY1* | 1 |
| 18 | Myoepithelial tumor | *EWSR1/POU5F1, EWSR1/PBX1, EWSR1/PBX3, EWSR1/ZNF444, FUS/POU5F1, FUS/KLF17* | 8 |
| 19 | Myxoid liposarcoma | *FUS/DDIT3, EWSR1/DDIT3* | 16 |
| 20 | Nodular fasciitis | *HMGA2/LPP, MYH9/USP6* | 5 |
| 21 | Pericytoma | *ACTB/GLI1* | 5 |
| 22 | Synovial sarcoma | *SS18/SSX1, SS18/SSX2, SS18/SSX4, SS18L1/SSX1, SS18/RESP2/SSX1* | 19 |
| 23 | Tenosynovial giant-cell tumor | *COL6A3/CSF1* | 3 |
| 24 | Undifferentiated small blue round cell tumor | *CIC/DUX4, CIC/FOXO4* | 3 |

### Specimen requirements

10 unstained sections of tumour and a corresponding H&E-stained histological section, OR a paraffin block of tumour.

### Turnaround time

The test is batched and performed every fortnight. The test itself takes 3 working days to complete.

### Caveats

RNA integrity and concentration must meet assay requirements. Low tumour content may result in an inaccurate result. The specific gene fusion present needs to be expressed at high levels.

### Proficiency testing

Exchange with an overseas centre performing the same test.

### Reference

Chang KTE et al. Development and evaluation of a pan-sarcoma fusion gene detection assay using the NanoString nCounter platform. J Mol Diagn 2018; 20: 63-77.

## Test 4

## Gene fusion detection in solid tumours by anchored multiplex PCR (Archer FusionPlex pan-solid assay).

### Background

A proportion of solid tumours including sarcomas have gene fusions. Identification of specific gene fusions are important for diagnosis and may provide prognostic and predictive information.

### Purpose of test

This test identifies the presence of a gene fusion involving any of the 101 listed genes known to be involved in gene fusions in solid tumours of various histological subtypes by next-generation sequencing-based anchored multiplex PCR (Archer FusionPlex). Prior knowledge of the fusion breakpoints and partner genes is not required, and the breakpoints and partner genes are idenotified through their sequences. The target (or 'anchored') genes and their covered exons are as follows:

| | Genes | Covered exons | | Genes | Covered exons | | Genes | Covered exons |
|---|---|---|---|---|---|---|---|---|
| 1 | *AKT1* | 2,3,4,5 | 36 | *FOSB* | 1,2 | 71 | *PDGFB* | 2,3 |
| 2 | *AKT3* | 1,2,3 | 37 | *FOXO1* | 1,2,3 | 72 | *PDGFRA* | 7,10,11,12,13,14,15 |
| 3 | *ALK* | 2,4,6,10,16,17,18,19,20,21,22,23,26 | 38 | *FOXO4* | 2,3 | 73 | *PDGFRB* | 8,9,10,11,12,13,14 |
| 4 | *AR* | 1,2,3,4,5,6,7,8 | 39 | *FUS* | 3,4,5,6,7,8,9,10,11,13,14 | 74 | *PHF1* | 1,2 |
| 5 | *ARHGAP26* | 2,10,11,12 | 40 | *GLI1* | 4,5,6,7 | 75 | *PIK3CA* | 2 |
| 6 | *AXL* | 18,19 | 41 | *GRB7* | 10,11,12 | 76 | *PKN1* | 10,11,12,13 |
| 7 | *BCOR* | 6,7,8,12,14,15 | 42 | *HMGA2* | 1,2,3,4,5 | 77 | *PLAG1* | 1,2,3,4 |
| 8 | *BRAF* | 1,2,3,4,5,7,8,9,10,11,12,13,15,16 | 43 | *INSR* | 12,13,14,15,16,17,18,19,20,21,22 | 78 | *PPARG* | 1,2,3 |
| 9 | *BRD3* | 9,10,11,12 | 44 | *JAK2* | 6,7,8,9,10,11,12,13,14,15,16,17,18,19,20 | 79 | *PRKACA* | 2 |
| 10 | *BRD4* | 10,11 | 45 | *JAK3* | 10,11,12,17,18,19 | 80 | *PRKCA* | 4,5,6,9 |
| 11 | *CAMTA1* | 3,8,9,10 | 46 | *JAZF1* | 2,3,4 | 81 | *PRKCB* | 3 |
| 12 | *CCNB3* | 2,3,4,5,6 | 47 | *MAML2* | 2,3 | 82 | *RAF1* | 4,5,6,7,8,9,10,11,12 |
| 13 | *CCND1* | 1,2,3,4,5 | 48 | *MAST1* | 7,8,9,18,19,20,21 | 83 | *RELA* | 3,4 |
| 14 | *CD274* | 2,3,4,5,7 | 49 | *MAST2* | 2,3,5,6 | 84 | *RET* | 2,4,6,8,9,10,11,12,13,14 |
| 15 | *CIC* | 17,18,19,20 | 50 | *MEAF6* | 4,5 | 85 | *ROS1* | 2,4,7,31,32,33,34,35,36,37 |
| 16 | *CSF1* | 5,6,7,8,9 | 51 | *MET* | 2,4,5,6,13,14,15,16,17,21 | 86 | *RSPO2* | 1,2 |
| 17 | *CSF1R* | 11,12,13 | 52 | *MKL2* | 11,12,13 | 87 | *RSPO3* | 2 |
| 18 | *DNAJB1* | 1,2 | 53 | *MN1* | 1,2 | 88 | *SS18* | 4,5,6,8,9,10,11 |
| 19 | *EGFR* | 1,7,8,9,16,17,18,19,20,24,25 | 54 | *MSMB* | 2,3,4 | 89 | *STAT6* | 1,2,3,4,5,6,7,15,16,17,18,19,20 |
| 20 | *EPC1* | 9,10,11 | 55 | *MUSK* | 7,9,10,12,13,14,15 | 90 | *TAF15* | 5,6,7 |
| 21 | *ERBB2* | 4,5,23,24,25,26 | 56 | *MYB* | 7,8,9,11,12,13,14,15,16 | 91 | *TCF12* | 4,5,6 |
| 22 | *ERBB4* | 2,3,4,14,15,16,17,18,23 | 57 | *MYBL1* | 8,9,10,11,12,13,14,15 | 92 | *TERT* | 2,3,5,7,9,10,11,12,15 |
| 23 | *ERG* | 2,3,4,5,6,7,8,9,10,11 | 58 | *MYOD1* | 1,2 | 93 | *TFE3* | 2,3,4,5,6,7,8 |
| 24 | *ESR1* | 3,4,5,6,7,8 | 59 | *NCOA1* | 12,13,14,15 | 94 | *TFEB* | 1,2,3,4 |
| 25 | *ESRRA* | 2,3 | 60 | *NCOA2* | 11,12,13,14,15,16 | 95 | *TFG* | 3,4,5,6,7 |
| 26 | *ETV1* | 3,4,5,6,7,8,9,10,11,12,13 | 61 | *NOTCH1* | 2,4,24,25,26,27,28,29,30,31 | 96 | *THADA* | 24,25,26,27,28,29,30,36,37 |
| 27 | *ETV4* | 2,4,5,6,7,8,9,10 | 62 | *NOTCH2* | 5,6,7,24,25,26,27,28,29 | 97 | *TMPRSS2* | 1,2,3,4,5,6 |
| 28 | *ETV5* | 2,3,7,8,9 | 63 | *NR4A3* | 3,4 | 98 | *USP6* | 1,2,3 |
| 29 | *ETV6* | 1,2,3,4,5,6,7 | 64 | *NRG1* | 1,2,3,4,5,6,8,10 | 99 | *VGLL2* | 1,2,3,4 |
| 30 | *EWSR1* | 4,5,6,7,8,9,10,11,12,13,14 | 65 | *NTRK1* | 2,4,6,8,10,11,12,13,14 | 100 | *YAP1* | 1,2,5,6,7 |
| 31 | *FGFR1* | 2,3,4,5,6,7,8,9,10,11,12,17 | 66 | *NTRK2* | 5,7,9,11,12,13,14,15,16,17,18 | 101 | *YWHAE* | 5 |
| 32 | *FGFR2* | 2,3,5,6,7,8,9,10,16,17 | 67 | *NTRK3* | 4,7,10,12,13,14,15,16 | | | |
| 33 | *FGFR3* | 3,5,8,9,10,16,17 | 68 | *NUMBL* | 3 | | | |
| 34 | *FGR* | 2 | 69 | *NUTM1* | 3,4,5 | | | |
| 35 | *FOS* | 4 | 70 | *PAX3* | 6,7,8 | | | |

### Specimen requirements

10 unstained sections of tumour and a corresponding H&E-stained histological section, OR a paraffin block of tumour.

### Turnaround time

The test is batched and performed every fortnight. The test itself takes 5 working days to complete.

### Caveats

RNA integrity and concentration must meet assay requirements. Low tumour content may result in an inaccurate result.

### Proficiency testing

College of American Pathologists proficiency testing programme.

## Test 5

## Microsatellite instability (MSI) testing for endometrial cancer.

### Background

In KKH, all patients with endometrial cancer are screened for Lynch syndrome by both MSI testing and immunohistochemistry. MSI testing screens for the phenotype of microsatellite instability to identify patients who require further genetics referral and testing so that appropriate care can be given to affected patients to reduce the risks of a second malignancy.

### Purpose of test

The MSI test compares the allelic profiles of five mononucleotide microstatellite markers (NR-21, BAT-26, BAT-25, NR-24 and MONO-27) generated by amplification of DNA from matching tumour and normal samples, usually from a hysterectomy specimen and using a commercial MSI PCR kit (MSI Analysis System v1.2, Promega, Madison, WI, USA). Tumours are classified as MSI-high, MSI-low or MSS (microsatellite stable).

### Specimen requirements

10 unstained sections of tumour and normal tissue each with corresponding H&E-stained histological sections, OR a paraffin block (or two if necessary) of tumour and normal tissue.

### Turnaround time

The test is batched and performed every Thursday. The test itself takes 3 working days to complete.

### Caveats

DNA integrity and concentration must meet assay requirements. Low tumour content may result in an inaccurate result. MSI PCR testing serves to identify the phenotype of microsatellite instability. MSI PCR does not identify the specific mismatch repair genes which are mutated. An MSI-high tumour does not equate to Lynch syndrome. MSH6-mutated tumours may not be MSI-high and such tumours will therefore not be identified by MSI testing alone. MSI-high tumours with retained MMR protein expression may have a *POLE* gene mutation.

### Proficiency testing

College of American Pathologists proficiency testing programme.

### Reference

McMeekin DS et al. Clinicopathologic significance of mismatch repair defects in endometrial cancer: An NRG Oncology/ Gynecologic Oncology Group Study. J Clin Oncol 2016; 34(25): 3062-8.

## Test 6

## MLH1 promoter methylation analysis.

### Background

Endometrial cancers with loss of the DNA mismatch protein MLH1 by immunohistochemistry require further testing to determine the methylation status of the *MLH1* gene promoter. A positive result for *MLH1* promoter methylation will in most situations be consistent with a sporadic MSI-high tumour. A negative result for MLH1 promoter methylation necessitates further genetics referral and assessment.

### Purpose of test

This test assesses the methylation status of the 3' clinically significant region of the *MLH1* gene promoter by methylation-specific melting curve analysis.

### Specimen requirements

10 unstained sections of tumour and a corresponding H&E-stained histological section, OR a paraffin block of tumour.

### Turnaround time

The test is batched and performed every Monday. The test itself takes 3 working days to complete.

### Caveats

DNA integrity and concentration must meet assay requirements. Low tumour content may result in an inaccurate result. Rare cases of Lynch syndrome may have methylation of *MLH1* as the second hit in Lynch syndrome tumorigenesis. Mutations to the MLH1 promoter sequence unrelated to methylation will result in amplicons that exhibit a different melt curve compared to the methylated and unmethylated wildtype promoter samples. Identification of the exact mutations present in the sequence of the MLH1 promoter amplicon requires additional sequencing studies which is not offered as part of this test.

### Proficiency testing

College of American Pathologists proficiency testing programme.

### Reference

Wong A, Ngeow J. Hereditary syndromes manifesting as endometrial carcinoma: how can pathological features aid risk assessment? Biomed Res Int 2015; 2015: 219012.

## Test 7

## Molecular genotyping of hydatidiform mole by comparative STR analysis.

### Background

Accurate diagnosis of molar pregnancies as partial or complete hydatidiform moles is important for determining risk of subsequent gestational trophoblastic neoplasia and appropriate patient follow-up.

### Purpose of test

The test compares the short tandem repeat (STR) profile of maternal (decidual) and placental (villous) tissue. This is achieved by PCR amplification of multiple STR loci using fluorescently labeled PCR primers from the Powerplex21 STR kit (Promega, Madison, WI, USA) followed by sizing of the PCR products by capillary electrophoresis to determine the genotype of the pregnancy.

### Specimen requirements

10 unstained sections of separate villous and decidual tissue and the corresponding H&E-stained histological section, OR a paraffin block (or two if necessary) of villous and decidual tissue.

### Turnaround time

The test is batched and performed every Monday. The test itself takes 3 working days to complete.

### Caveats

DNA integrity and concentration must meet assay requirements. Cross contamination of maternal and placental tissue may result in inaccurate results. The results of this test must be correlated with histological findings and any other relevant ancillary investigations (e.g. p57 immunohistochemical staining) and clinical findings (e.g. presence of a fetus and exclusion of multiple pregnancies). Mosaic conceptions may generate complicated genotyping results which can be challenging to interpret. Chromosomal trisomies may confound interpretation if the number of informative loci is inadequate. The genotyping result of biparental diploidy may be misinterpreted as nonmolar if morphological features and p57 results are not correlated with.

### Proficiency testing

Internal quality assurance programme.

### Reference

Lipata F et al. Precise DNA genotyping diagnosis of hydatidiform mole. Obstet Gynecol 2010; 115: 784-94.

## Test 8

## *MYOD1* c.365 T>G p.Leu122Arg (p.L122R) mutational analysis by Sanger sequencing.

### Background

A proportion of spindle cell/sclerosing rhabdomyosarcomas harbour a recurrent somatic point mutation of the *MYOD1* gene c.365T>G p.Leu122Arg resulting in the mutated MyoD1 protein having MYC-like properties. *MYOD1*-mutated spindle cell/sclerosing RMS have an aggressive clinical course.

### Purpose of test

The test identifies the specific *MYOD1* point mutation c.365 T>G p.Leu122Arg (p.L122R).

### Specimen requirements

10 unstained sections of tumour and a corresponding H&E-stained histological section, OR a paraffin block of tumour.

### Turnaround time

5 working days.

### Caveats

DNA integrity and concentration must meet assay requirements. Low tumour content (<20%) may result in a false negative result.

### Proficiency testing

Internal quality assurance programme.

### Reference

Kohsaka S et al. A recurrent neomorphic mutation in MYOD1 defines a clinically aggressive subset of embryonal rhabdomyosarcoma associated with PI3K-AKT pathway mutations. Nat Genet 2014; 46: 595-600.

## Test 9

## *POLE* exons 9, 13 and 14 mutational analysis by Sanger sequencing for endometrial carcinomas.

### Background
Endometrial carcinomas can be stratified into four prognostic groups based on molecular features. The "ultramutated" group harbours *POLE* exonuclease domain mutations (EDM) and is associated with favourable progression-free survival even though histological grade is often high.

### Purpose of test
The test identifies mutations affecting exons 9, 13 and 14 of the *POLE* gene.

### Specimen requirements
10 unstained sections of tumour and a corresponding H&E-stained histological section, OR a paraffin block of tumour.

### Turnaround time
5 working days.

### Caveats
DNA integrity and concentration must meet assay requirements. Low tumour content (<20%) may result in an inaccurate result.

### Proficiency testing
Internal quality assurance programme.

### Reference
Wong A et al. Mutation spectrum of POLE and POLD1 mutations in South East Asian women presenting with grade 3 endometrioid endometrial carcinomas. Gynecol Oncol 2016; 141:113-20.

## Test 10
## SNP microarray for cancer FFPE specimens (OncoScan)

### Background
This SNP microarray-based assay interrogates the whole genome to detect copy number changes and loss of heterozygosity (LOH) in FFPE tumour specimens. Specific chromosomal copy number changes and LOHs may be useful for characterizing certain tumours e.g. identifying gene or chromosomal segmental copy number changes for subtype determination of medulloblastoma subgroups and amplification of MYCN, loss of 1p (including LOH) and gain of 11q in neuroblastomas. Additional chromosomal copy number changes may also have clinical significance to other tumour types.

### Purpose of test
Microarray testing for cancer is helpful in identifying genome-wide chromosomal alterations not practically identified by fluorescence in-situ hybridisation (FISH) testing and may help in diagnosis, prognosis and therapeutic decisions.

### Specimen requirements
10 unstained sections of tumour and a corresponding H&E-stained histological section, OR a paraffin block of tumour.

### Turnaround time
The test is batched with a turnaround time of 2-3 weeks.

### Caveats
DNA integrity and concentration must meet assay requirements. Low tumour content (<20%) may result in an inaccurate result. Specimens fixed or processed in alternative fixatives other than buffered formalin is unacceptable. This test does not detect balanced chromosomal rearrangements and its positional information. The results of this test may reveal incidental findings, including constitutional abnormalities, unrelated to the original reason for referral.

### Proficiency testing
College of American Pathologists proficiency testing programme.

### References
Foster JM et al. Cross-laboratory validation of Oncoscan FFPE Assay, a multiplex tool for whole genome tumour profiling. BMC Med genomics 2015; 8:5

Jung HS et al. Utitilization of the Oncoscan microarray assay in cancer diagnosis. Applied Cancer Research 2017; 37:1

Rustin JG et al. Utility of Oncoscan array testing to further characterize eleven medulloblastoma cases. Cancer Genet 2016; 6:293

Pinto N et al. Segmental chromosomal aberrations in localised neuroblastoma can be detected in formalin-fixed paraffin-embedded tissue samples and are associated with recurrence. Pediatric Blood Cancer. 2016; 63(6):1019-23