KK Women's and Children's Hospital
SingHealth

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UEN: 198904227G

Please affix patient identification label or complete the following fields.
NAME:
NRIC/ID NO.:
ACCOUNT NO.:
DATE OF BIRTH:
GENDER: ☐ Male ☐ Female

MOLECULAR PATHOLOGY REQUEST FORM
DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE

CLINICAL HISTORY:	**SPECIMEN TYPE: (please tick)**
Tumour location: Working diagnosis: Relevant findings (e.g. FISH, IHC results):	☐ Paraffin block of tumour ☐ Unstained sections* *Note: 20 unstained sections must be submitted for Test 9 **SPECIMEN IDENTIFICATION NUMBER:**
REQUESTING PHYSICIAN: Signature: Name: MCR: Telephone number: Email address:	**PURPOSE OF TESTING: (please tick all applicable statements)** ☐ This is a current clinical case and the results will be utilized for patient care ☐ This test is performed for research / publication purposes ☐ This is performed for verification of an existing molecular result

☐ Test 1: Medulloblastoma group determination by NanoString nCounter gene expression profiling
☐ Test 2: Gene fusion detection in solid tumours by anchored multiplex PCR (Archer FUSIONPlex pan solid V2 assay)
☐ Test 3: Microsatellite instability (MSI) testing for endometrial cancer
☐ Test 4: *MLH1* promoter methylation analysis
☐ Test 5: Molecular genotyping for identification of molar pregnancies
☐ Test 6: *MYOD1* p.L122R mutational analysis for spindle cell/sclerosing rhabdomyosarcomas
☐ Test 7: *FOXL2* p.C134W mutational analysis for juvenile/adult granulosa-cell tumour of ovary
☐ Test 8: OncoScan SNP microarray FFPE tumour analysis
☐ Test 9: Targeted genomic profiling of solid tumours by AmpliSeq Childhood Cancer Panel (informed consent required)

Test 1
Medulloblastoma group determination by NanoString nCounter gene expression profiling

Background
The World Health Organization Classification of Tumours of the Central Nervous System recognizes genetically-defined medulloblastoma groups – 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 group 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
5 working days.

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 groups.

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 solid tumours by anchored multiplex PCR (Archer FUSIONPlex pan solid V2 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 129 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 identified through their sequences. The target (or 'anchored') genes and their covered exons are as follows:

Genes	Exons	Genes	Exons	Genes	Exons
ACVR2A	1,2,3	*FOXR2*	2,3	*PAX8*	3
AKT1	2,3,4,5	*FUS*	3,4,5,6,7,8,9,10,11,13,14	*PDGFB*	2,3
AKT2	2,5,11	*GLI1*	4,5,6,7	*PDGFD*	5,6,7
AKT3	2,3,4,9	*GRB7*	10,11,12	*PDGFRA*	7,10,11,12,13,14,15
ALK	2,4,6,8,10,12,14,16,17,18,19,20,21,22,23,26	*HMGA2*	1,2,3,4,5	*PDGFRB*	8,9,10,11,12,13,14
AR	1,2,3,4,5,6,7,8	*IGF1R*	13,14,15	*PHF1*	1,2,10,11,12
ARHGAP26	2,10,11,12	*INSR*	2,12,13,14,15,16,17,18,19,20,21,22	*PHKB*	4
ARHGAP6	2	*JAK2*	6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,22	*PIK3CA*	2,15
AXL	11, 18,19, 20	*JAK3*	10,11,12,17,18,19	*PKN1*	10,11,12,13
BCOR	2, 4,6,7,10,12,14,15	*JAZF1*	2,3,4	*PLAG1*	1,2,3,4
BRAF	1,2,3,4,5,7,8,9,10,11,12,13,14,15,16,18	*KIT*	1	*PPARG*	1,2,3
BRD3	9,10,11,12	*MAML2*	2,3	*PRDM10*	13,14
BRD4	2,10,11,12,13,14	*MAP2K1*	2	*PRKACA*	2
CAMTA1	3,8,9,10	*MAST1*	7,8,9,18,19,20,21	*PRKACB*	2,3,4
CCNB3	2,3,4,5,6,7	*MAST2*	2,3,5,6,15,16,17	*PRKCA*	4,5,6,9,15
CCND1	1,2,3,4,5	*MBTD1*	3,15,16,17	*PRKCB*	1,3,7,8,9
CIC	14,15,16,17,18,19,20	*MDM2*	2,4,5,6,8,9,10	*PRKCD*	9,10,11,12,15,18
CRTC1	1,2,3,4	*MEAF6*	4,5	*PRKD1*	2,10,11,12,13
CSF1	2,3,4,5,6,7,8,9	*MET*	2,13	*PRKD2*	10,11,12,13
CSF1R	11,12,13	*MGEA5*	4,5,6,7,8,9,12,13,14,15	*PRKD3*	10,11,12,13
DNAJB1	1,2	*MKL2*	11,12,13	*RADS1B*	3,4,5,6,7,8,9
EGF	16,17,18,19	*MN1*	1,2	*RAF1*	2,4,5,6,7,8,9,10,11,12
EGFR	1,7,8,9,14,15,16,17,18,19,20, 24,25,26	*MSMB*	2,3,4	*RELA*	1,2,3,4,11
EPC1	9,10,11	*MUSK*	7,9,10,12,13,14,15	*RET*	2,4,6,8,9,10,11,12,13,14
ERBB2	4,5,13,15,17,23,24,25,26	*MYB*	7,8,9,11,12,13,14,15,16	*ROS1*	2,4,7,31,32,33,34,35,36,37
ERBB4	2,3,4,14,15,16,17,18,23	*MYBL1*	8,9, 10,11,12,13,14,15	*RSPO2*	1,2,3
ERG	2,3,4,5,6,7,8,9,10,11	*MYC*	1,2,3	*RSPO3*	2
ESR1	1,2,3,4,5,6,7,8	*NCOA1*	11,12,13,14,15	*SS18*	2,3,4,5,6,8,9,10,11
ESRRA	2,3	*NCOA2*	11,12,13,14,15,16	*SS18L1*	1,2,3,8,9,10
ETV1	3,4,5,6,7,8,9,10,11,12,13	*NCOA3*	2,13,14,15,16,20	*STAT6*	1,2,3,4,5,6,7,15,16,17,18,19,20
ETV4	2,3,4,5,6,7,8,9,10	*NFATC2*	2,3,9,10	*TAF15*	5,6,7,9
ETV5	2,3,7,8,9	*NFE2L2*	1,2,3,4,5	*TCF12*	4,5,6
ETV6	1,2,3,4,5,6,7	*NFIB*	2,9,10,11	*TERT*	2,3,5,7,9,10,11,12,15
EWSR1	4,5,6,7,8,9,10,11,12,13,14	*NOTCH1*	2,4,5,24,25,26,27,28,29,30,31	*TFE3*	2,3,4,5,6,7,8
FGF1	2	*NOTCH2*	5,6,7,24,25,26,27,28,29	*TFEB*	2,3,4,5,6,9,10
FGFR1	2,3,4,5,6,7,8,9,10,11,12,17	*NR4A3*	2,3,4,5,7,9	*TFG*	3,4,5,6,7,8
FGFR2	2,3,5,6,7,8,9,10,16,17,18	*NRG1*	1,2,3,4,5,6	*THADA*	24,25,26,27,28,29,30,31,36,37
FGFR3	3,5,8,9,10,11,12,13,14,16,17,18	*NTRK1*	1,2,3,4,5,6,7,8,9,10,11,12,13,14	*TMPRSS2*	1,2,3,4,5,6
FGR	2,3	*NTRK2*	4,5,6,7,8,9,10,11,12,13,14,15,16,17,18	*USP6*	1,2,3
FOS	4	*NTRK3*	3,4,5,6,7,8,9,10,11,12,13,14,15,16,17	*VGLL2*	1,2,3,4
FOSB	1,2	*NUMBL*	2,3	*WWTR1*	3,4
FOXO1	1,2,3	*NUTM1*	2,3,4,5,6	*YAP1*	1,2,3,4,8,9
FOXO4	2,3	*PAX3*	2,3,4,5,6,7,8	*YWHAE*	5

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 weekly. The test itself takes 5 working days to complete.

Caveats
RNA integrity and concentration must meet assay requirements. Low tumour content (<50%) may result in an inaccurate result.

Proficiency testing
College of American Pathologists proficiency testing programme.

Test 3
Microsatellite instability (MSI) testing for endometrial cancer

Background
Patients with endometrial cancer can be screened for Lynch syndrome by 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 microsatellite markers (NR-21, BAT-26, BAT-25, NR-24 and MONO-27) generated by amplification of DNA from matching tumour and normal samples 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 (<30%) 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/Gynaecologic Oncology Group Study. J Clin Oncol 2016; 34(25): 3062-8.

Test 4
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 (<30%) 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 wild-type 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 5
Molecular genotyping for identification of molar pregnancies

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 labelled PCR primers from the GenePrint-24 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 Tuesday. 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
Alternative performance assessment programme.

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

Test 6
MYOD1 p.L122R mutational analysis for spindle cell/sclerosing rhabdomyosarcomas

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.365T>G p.Leu122Arg (p.L122R) by Sanger sequencing.

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 (<50%) may result in a false negative result.

Proficiency testing
Alternative performance assessment 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 7
FOXL2 p.C134W mutational analysis for juvenile/adult granulosa-cell tumour of ovary

Background
Adult-type granulosa cell tumours of the ovary (aGCTs) are rare tumours that represent 2–5% of ovarian malignancies. The prognosis of this tumour is favourable, and it is characterized by slow progression. 10–30% of these tumours recur after 4–7 years of the primary surgery and the 5-year survival rate from the first recurrence is 55%, for the incompletely resected patients. Heterozygous point mutation of c.402C>G in the *FOXL2* gene can be found in 97% of cases of aGCT.

Purpose of test
The test identifies the specific *FOXL2* point mutation c.402C>G p.Cys134Trp (p.C134W) by Sanger sequencing.

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 (<50%) may result in a false negative result.

Proficiency testing
Alternative performance assessment programme.

Reference
Schrader KA et al The specificity of the *FOXL2* c.402C>G somatic mutation: a survey of solid tumors PLoS One. 2009 Nov 24;4(11):e7988

Kommoss S et al *FOXL2* molecular testing in ovarian neoplasms: diagnostic approach and procedural guidelines. Mod Pathol. 2013 Jun;26(6):860-7

Shah SP et al Mutation of *FOXL2* in granulosa-cell tumors of the ovary. N Engl J Med. 2009 Jun 25;360(26):2719-29

Test 8
OncoScan SNP microarray FFPE tumour analysis

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

Test 9
Targeted genomic profiling of solid tumours by AmpliSeq Childhood Cancer Panel

Background
Cancers harbour gene mutations. Precision medicine seeks to identify mutations to improve diagnostic accuracy, prognostication and identification of mutations that may be targeted by drugs to improve the treatment outcome.

Purpose of test
The AmpliSeq Childhood Cancer Panel is a next-generation sequencing test that uses high-throughput amplicon sequencing for the identification of somatic single nucleotide variants (SNVs), copy number variants (CNVs) and gene fusions in genes commonly affected in childhood and childhood-type solid tumours.

Genes tested for hotspot mutations:
ABL1, ABL2, ALK, ACVR1, AKT1, ASXL1, ASXL2, BRAF, CALR, CBL, CCND1, CCND3, CCR5, CDK4, CIC, CREBBP, CRLF2, CSF1R, CSF3R, CTNNB1, DAXX, DNMT3A, EGFR, EP300, ERBB2, ERBB3, ERBB4, ESR1, EZH2, FASLG, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GATA2, GNA11, GNAQ, H3F3A, HDAC9, HIST1H3B, HRAS, IDH1, IDH2, IL7R, JAK1, JAK2, JAK3, KDM4C, KDR, KIT, KRAS, MAP2K1, MAP2K2, MET, MPL, MSH6, MTOR, MYC, MYCN, NCOR2, NOTCH1, NPM1, NRAS, NT5C2, PAX5, PDGFRA, PDGFRB, PIK3CA , PIK3R1, PPM1D, PTPN11, RAF1, RET, RHOA, SETBP1, SETD2, SH2B3, SH2D1A, SMO, STAT3, STAT5B, TPMT, USP7, ZMYM3.
Genes tested for mutations in all exons:
APC, ARID1A, ARID1B, ATRX, CDKN2A, CDKN2B, CEBPA, CHD7, CRLF1, DDX3X, DICER1, EBF1, EED, FAS, GATA1, GATA3, GNA13, ID3, IKZF1, KDM6A, KMT2D, MYOD1, NF1, NF2, PHF6, PRPS1, PSMB5, PTCH1, PTEN, RB1, RUNX1, SMARCA4, SMARCB1, SOCS2, SUFU, SUZ12, TCF3, TET2, TP53, TSC1, TSC2, WHSC1, WT1, XIAP.
Genes tested for CNVs:
ALK, BRAF, CCND1, CDK4, CDK6, EGFR, ERBB2, ERBB3, FGFR1, FGFR2, FGFR3, FGFR4, GLI1, GLI2, IGF1R, KIT, KRAS, MDM2, MDM4, MET, MYC, MYCN, PDGFRA, PIK3CA.
Genes tested for RNA fusions (1706 specific fusion variants only):
*ABL1, ABL2, AFF3, ALK, BCL11B, BCOR, BCR, BRAF, CAMTA1, CCND1, CIC, CREBBP, CRLF2, CSF1R, DUSP22, *EGFR, ETV6, EWSR1,FGFR1, FGFR2, FGFR3, FLT3, FOSB, FUS, GLI1, GLIS2, HMGA2, JAK2, KAT6A, KMT2A, KMT2B, KMT2C, KMT2D, LMO2, MAML2, MAN2B1, MECOM, MEF2D, MET, MKL1, MLLT10, MN1, MYB, MYBL1, MYH11, MYH9, NCOA2, NCOR1, NOTCH1, NOTCH2, NOTCH4, NPM1,NR4A3, NTRK1, NTRK2, NTRK3, NUP214, NUP98, NUTM1, NUTM2B, PAX3, PAX5, PAX7, PDGFB, PDGFRA, PDGFRB, PLAG1, RAF1, RANBP17, RARA, RECK, RELA, RET, ROS1, RUNX1, SS18, SSBP2, STAG2, STAT6, TAL1, TCF3, TFE3, TP63, TSLP, TSPAN4, UBTF, USP6, WHSC1, YAP1, ZMYND11, ZNF384.* ***EGFRvIII fusion variant is excluded from the reportable range of this test.**

Specimen requirements
20 unstained sections of tumour and corresponding H&E-stained section, OR paraffin block of tumour.

Turnaround time
The test is batched with a turnaround time of 4-6 weeks.

Consent
Signed informed consent is required.

Caveats
Tumour content must be >50%. DNA and RNA quality and concentrations must meet assay requirements.

The DNA assay component does not detect variants occurring at allele frequency of less than 10%, exon deletions, and variants in regions for which sequencing coverage is less than 100x. The RNA assay component detects 1706 specific gene fusion variants only (available on request). The test does not detect splice variants, variants located in regions with pseudogene interference and variant types not included in validation.

This test is validated for somatic variants only. Variants identified may include germline variants even though these are not specifically tested for. For this reason, a suitably-qualified physician must be responsible for obtaining signed informed consent, appropriately counselling the patient on the nature of the test, and interpreting and explaining test results to the patient with appropriate clinico-pathological correlation.

Proficiency testing
College of American Pathologists proficiency testing programme.

Reference
Hiemenz MC et al, A Comprehensive Next-Generation Sequencing Panel for Pediatric Malignancies. J Mol Diagn 2018, 20: 765e776