KK Women's and Children's Hospital
SingHealth

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

KKH DPLM MOLECULAR HISTOPATHOLOGY REQUEST FORM

All fields must be completed

CLINICAL HISTORY:	**SPECIMEN TYPE: (please tick)**
Tumour location: Working diagnosis: Relevant findings (e.g. FISH, IHC results):	☐Snap frozen tumour ☐Paraffin block of tumour ☐Unstained sections (x10) with corresponding H&E-stained section Specimen identification number:
REQUESTING PHYSICIAN:	**PURPOSE OF TESTING: (please tick all applicable statements)**
Signature: Name: MCR No.: Contact No.:	☐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

GYNAECOLOGICAL/OBSTETRICAL SPECIMENS (please tick)		
ENDOMETRIAL CARCINOMA	1	☐ Microsatellite Instability (MSI) Testing [Fragment Analysis]
	2	☐ *MLH1* Promoter Methylation Analysis [PCR-Melt Curve Analysis]
	3	☐ *POLE* Full Exons 9,13 and14 Mutational Analysis [Sanger Sequencing]
ENDOMETRIAL STROMAL SARCOMA	4	☐ *JAZF1-SUZ12* t(7;17) Gene Fusion Detection [RTPCR]
	5	☐ *YWHAE-NUTM2* t(10;17) Gene Fusion Detection [RTPCR]
	6	☐ *ZC3H7B-BCOR* t(X;22) Gene Fusion Detection [RTPCR]
MOLAR PREGNANCY/ PRODUCTS OF CONCEPTION	7	☐ Molecular Genotyping of Hydatidiform Mole [STR-based Fragment Analysis]
OVARIAN TUMOURS	8	☐ *FOXL2* p.C134W Mutational Analysis
	9	☐ *TERT* promoter C228T Mutational Analysis
PAEDIATRIC SPECIMENS (please tick)		
CLEAR CELL SARCOMA OF KIDNEY/ PRIMITIVE MALIGNANT MESENCHYMAL TUMOUR OF INFANCY	10	☐ *BCOR* Exon 15 Internal Tandem Duplication [Sanger Sequencing]
EWING SARCOMA	11	☐ *EWSR1-FLI1* t(11; 22) Types 1 and 2 Fusion Gene Detection
INFANTILE FIBROSARCOMA/ CONGENITAL MESOBLASTIC NEPHROMA	12	☐ *ETV6-NTRK3* t(12:15) Fusion Gene Detection [RTPCR]
MEDULLOBLASTOMA	13	☐ Medulloblastoma Subtyping - WNT, SHH, Groups 3 and 4 [NanoString®]
NEUROBLASTOMA	14	☐ *ALK* p.F1174L and p.R1275Q Mutational Analysis [Sanger Sequencing]
	15	☐ *MYCN*, 1p and 11q Copy Number Evaluation [FISH]
PAEDIATRIC LOW-GRADE GLIOMA	16	☐ *KIAA1549-BRAF* Fusion Gene Detection (Variants 16-9, 15-9, 16-11) [RTPCR]
	17	☐ *BRAF* p.V600E [Immunohistochemical Stain]
RHABDOMYOSARCOMA	18	☐ *MYOD1* p. L122R Mutational Analysis [Sanger Sequencing]
	19	☐ *PAX3-FOXO1* t(2;13) and *PAX7-FOXO1* t(1;13) Gene Fusion Detection [RTPCR]
SARCOMAS/ PAEDIATRIC SOLID TUMOURS	20	☐ Sarcoma/ Paediatric Solid Tumour Gene Fusion Detection (see note overleaf)

Note for Test 20: Testing for sarcoma/ paediatric solid tumour gene fusions will be performed using either or both of the following molecular platforms. Where applicable, results will be validated orthogonally, usually by RTPCR.

1. **The NanoString® nCounter assay®** is a high-throughput hybridization technique using target-specific probes that can be customized to test for numerous fusion transcripts in a single assay using RNA from formalin-fixed, paraffin-embedded material (Chang KTE et al. Development and evaluation of a pan-sarcoma fusion gene detection assay using the NanoString nCounter platform. J Mol Diagn 2017). The NanoString assay targets 174 unique fusion junctions in 25 sarcoma types as follows:

Table 1 Summary of the Sarcoma Fusion CodeSet Coverage

Sarcoma type	Fusion	Variants, *n*
Alveolar soft part sarcoma	*ASPSCR1-TFE3*	2
Alveolar rhabdomyosarcoma	*PAX3-FOXO1, PAX7-FOXO1, PAX3-FOXO4, PAX3-NCOA1, PAX3-NCOA2, PAX3-AFX, PAX3-INO80D*	9
Aneurysmal bone cyst	*CDH11-USP6, COL1A1-USP6, OMD-USP6, TRAP150-USP6, ZNF9-USP6*	9
Angiomatoid fibrous histiocytoma	*EWSR1-ATF1, EWSR1-CREB1, EWSR1-CREM, FUS-ATF1*	1
BCOR-rearranged sarcoma	*BCOR-CCNB3, ZC3H7B-BCOR*	2
Biphenotypic sinonasal sarcoma	*PAX3-MAML3*	1
CIC-rearranged sarcoma	*CIC-DUX4, CIC-FOXO4*	3
Clear-cell sarcoma	*EWSR1-ATF1, EWSR1-CREB1, EWSR1-CREM, FUS-CREM*	10
Congenital fibrosarcoma	*ETV6-NTRK3, EML4-NTRK3*	3
Dermatofibrosarcoma protuberans (tested using preliminary CodeSet)	*COL1A1-PDGFB*	23
Desmoplastic small round cell tumor	*EWSR1-WT1*	10
Epithelioid hemangioendothelioma	*WWTR1-CAMTA1, YAP1-TFE3*	3
Ewing sarcoma	*EWSR1-ERG, EWSR1-FLI1, EWSR1-ETV1, EWSR1-ETV4, EWSR1-FEV, FUS-ERG, FUS-FEV*	31
Ewing-like sarcoma (rare variants)	*EWSR1-NFAT2, EWSR1-SMARCA5, EWSR1-SP3*	5
Extraskeletal myxoid chondrosarcoma	*EWSR1-NR4A3, RBP56-NR4A3, TAF15-NR4A3, TCF12-NR4A3, TFG-NR4A3*	8
High-grade endometrial stromal sarcoma	*YWHAE-NUTM2, ZC3H7B-BCOR*	2
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
Lipoblastoma	*COL1A2-PLAG1, HAS2-PLAG1*	2
Low-grade endometrial stromal sarcoma	*JAZF1-SUZ12, JAZF1-PHF1, MEAF6-PHF1, EPC1-PHF1*	7
Mesenchymal chondrosarcoma	*HEY1-NCOA2, IRF2BP2-CDX1, NUPIO7-LRG5*	3
Myoepithelial tumor of soft tissue	*EWSR1-POU5F1, EWSR1-PBX1, EWSR1-ZNF444*	4
Myxoid liposarcoma	*FUS-DDIT3, EWSR1-DDIT3*	16
Nodular fasciitis	*HMGA2-LPP, MYH9-USP6*	5
Pericytoma of bone	*ACTB-GLI1*	5
Synovial sarcoma	*SS18-SSX1, SS18-SSX2, SS18-SSX4, SS18L1-SSX1, SS18-RESP2-SSX1*	17
Tenosynovial giant-cell tumor	*COL6A3-CSF1*	3

2. **Archer® FusionPlex® Sarcoma Assay** is a targeted next-generation sequencing-based assay that can simultaneously detect and identify fusions involving any of 26 genes associated with soft tissue tumours without prior knowledge of fusion partners or breakpoints.

SN	Gene	Target Exon	Direction	SN	Gene	Target Exon	Direction
1	*ALK*	19,20,21,22	5'	15	*NTRK3*	13,14,15,16	5'
2	*CAMTA1*	8,9,10	5'		*NTRK3*	13,14,15	3'
3	*CCNB3*	2,3,4,5,6	5'	16	*PDGFB*	2,3	5'
4	*CIC*	19,20	3'	17	*PLAG1*	1,2,3,4	5'
5	*EPC1*	9,10,11	3'	18	*ROS1*	31,32,33,34,35,36,37	5'
6	*EWSR1*	4,5,6,7,8,9,10,11,12,13	3'	19	*SS18*	10,11	5'
7	*FOXO1*	1,2,3	5'		*SS18*	4,5,6,8,9,10	3'
	FOXO1	1,2,3	3'	20	*STAT6*	1,2,3,4,5,6,7,16,17,18,19	5'
8	*FUS*	4,5,6,7,8,9, 10,11,14	3'	21	*TAF15*	6,7	5'
9	*GLI1*	4,5,6,7	5'		*TAF15*	5,6,7	3'
	GLI1	4,5,6,7	3'	22	*TCF12*	4,5,6	3'
10	*HMGA2*	1,2,3,4,5	3'	23	*TFE3*	3,4,5,6	5'
11	*JAZF1*	2,3,4	3'	24	*TFG*	4,5,6,7	3'
12	*MEAF6*	4,5	3'		*TFG*	6	5'
13	*MKL2*	11,12,13	5'	25	*USP6*	1,2,3	5'
14	*NCOA2*	11,12,13,14	5'	26	*YWHAE*	5	3'