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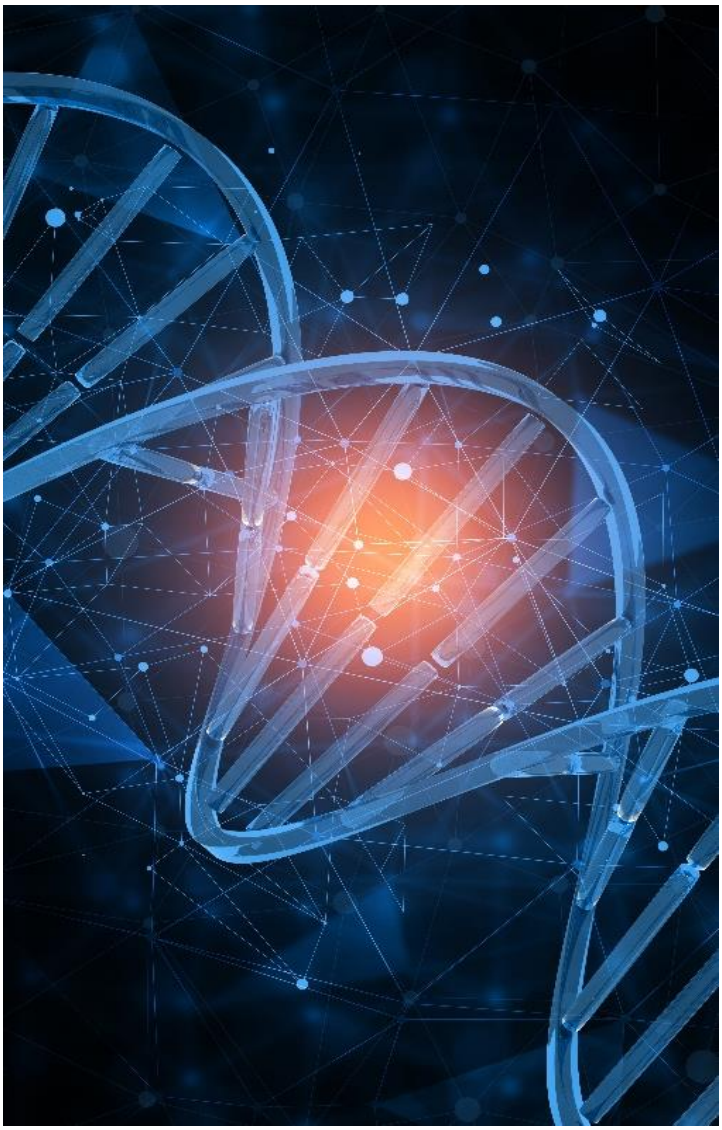

BIOMARKERS AND MOLECULAR PATHOLOGY TESTS FOR CENTRAL NERVOUS SYSTEM TUMOURS

SEPTEMBER 2022

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Content

Biomarkers and tests by tumour type

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Tumours of the sellar region
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Craniopharyngiomas: • Beta-catenin immunohistochemistry • BRAF p.V600E immunohistochemistry or Sanger sequencing

Immunohistochemistry

No.	Antibodies	Corresponding genes/ surrogates/ lineage	Staining pattern in the tumour cells	Availability	
				NUH	KKH
1.	**IDH1 p.R132H**	*IDH1* R132H	Cytoplasmic, nuclear	✓	
2.	**ATRX**	*ATRX*	Loss of nuclear staining	✓	✓
3.	**P53**	*TP53*	Nuclear	✓	✓
4.	**BRAF p.V600E**	*BRAF* V600E	Cytoplasmic		✓
5.	**H3K27M**	Histone 3	Nuclear	✓	✓
6.	**H3K27me3**	Histone 3 (trimethyl K27)	Loss of nuclear staining	✓	✓
7.	**EZHIP**	*EZHIP*	Nuclear		✓
8.	**H3G34R**	Histone 3	Nuclear		✓
9.	**L1CAM**	*RELA*	Membranous		✓
10.	**P65**	*RELA*	Nuclear		✓
11.	**LIN28A**	*C19MC*	Cytoplasmic	✓	✓
12.	**INI1**	*SMARCB1*	Loss of nuclear staining	✓	✓
13.	**BRG1**	*SMARCA4*	Loss of nuclear staining		✓
14.	**BCOR**	*BCOR*	Nuclear		✓
15.	**STAT6**	*STAT6*	Nuclear	✓	
16.	**PIT1**	PIT1 lineage	Nuclear	✓	
17.	**SF1**	SF1-lineage	Nuclear	✓	
18.	**T-PIT**	T-PIT lineage	Nuclear	✓	
19.	**Prolactin**	Lactotroph	Cytoplasmic	✓	
20.	**Growth hormone**	Somatotroph	Cytoplasmic	✓	
21.	**ACTH**	Corticotroph	Cytoplasmic	✓	
22.	**Beta-catenin**	*CTNNB1*	Nuclear	✓	

Specimen requirements

One unstained coated section of tumour for each antibody, accompanied by 1 unstained coated section of tumour for negative control for each request.

Turnaround time

1 day.

Caveats

As the mutant-specific antibody only identifies the specific mutation isoform, a negative staining does not exclude the presence of an alternative mutation. In this circumstance, sequencing may be necessary.

CNS-M01: *IDH1* and *IDH2* mutations by Sanger sequencing

Background
IDH1/2 mutational status is critical for the classification of adult-type diffuse gliomas. The presence of *IDH1* or *IDH2* mutation is associated with substantially improved prognosis in astrocytoma and is a crucial finding in oligodendroglioma.

Purpose of test
IDH sequencing enables the detection of the rarer variants of *IDH1* and *IDH2* mutations at codons 132 and 172, respectively, which will not be identified by IDH1 R132H immunohistochemistry.

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

Turnaround time
1 week.

Caveats
DNA integrity and concentration must meet assay requirements. Low tumour content may result in an inaccurate result. Note that an alternative method to determine *IDH1* and *IDH2* mutational status is by test CNS-M12 Ampliseq Childhood Cancer NGS Panel.

CNS-M02: Chromosomal 1p and 19q co-deletion by fluorescent in-situ hybridization (FISH)

Background
The presence of combined 1p and 19q whole chromosomal arm losses in the presence of *IDH* mutation defines oligodendroglioma. 1p deletion or 1p/19q codeletion in the absence of *IDH* mutation and a concurrent *KIAA1549-BRAF* gene fusion are also frequently observed in diffuse leptomeningeal glioneuronal tumour.

Purpose of test
To detect the presence of 1p and 19q deletion.

Specimen requirements
4 paraffin sections, unstained, each 4 μm thick, mounted onto positively charged/ coated slides and a corresponding H&E-stained histological section of the tumour, OR, a paraffin block of the tumour.

Turnaround time
1 week.

Caveats
A solid cellular tumour region is preferred to the infiltrating edge of the tumour. As FISH probes only target the telomeric ends of chromosome 1p and 19q at 1p36 and 19q13 respectively, partial deletion of chromosomal 1p and 19q cannot be excluded by this test. Partial 1p and 19q deletion may be seen in cases with wildtype *IDH*. If segmental chromosomal alterations such as partial deletion of 1p and 19q are possibilities, or if definitive identification of full length chromosomal 1p and 19q deletion is required, please order OncoScan SNP microarray test (CNS-M11).

CNS-M03: *CDKN2A* deletion by FISH

Background
CDKN2A/B homozygous loss has been identified as a marker of poor prognosis in patients with *IDH*-mutant astrocytomas and meningiomas. *CDKN2A/B* homozygous loss upgrades an otherwise histologically grade 2 or 3 *IDH*-mutant astrocytoma to an astrocytoma, *IDH*-mutant, CNS WHO grade 4, or an otherwise histologically low grade meningioma to an anaplastic meningioma, CNS WHO grade 3.

Purpose of test
To identify *CDKN2A* loss. The FISH probe also targets the *CDKN2B* gene.

Specimen requirements
2 paraffin sections, unstained, each 4 μm thick, mounted onto positively charged/ coated slides and a corresponding H&E-stained histological section of the tumour, OR, a paraffin block of the tumour.

Turnaround time
1 week

References
Brat DJ, et al. cIMPACT-NOW update 5: recommended grading criteria and terminologies for IDH-mutant astrocytomas. Acta Neuropathol. 2020; 139(3): 603-608.

CNS-M04: *EGFR* amplification, chromosome 7 and 10 status by OncoScan SNP microarray

Background
EGFR amplification, whole chromosomal 7 gain and 10 loss have been identified as diagnostic biomarkers for glioblastoma, *IDH*-wildtype. In a histologically low-grade diffuse astrocytoma that is *IDH*-wildtype, the presence of either *EGFR* amplification, or, combined whole chromosomal 7 gain and 10 loss will upgrade the tumour to a glioblastoma, CNS WHO grade 4.

Purpose of test
To identify whole chromosomal 7 gain and 10 loss or *EGFR* amplification in an otherwise histologically low grade-appearing, *IDH*-wildtype adult-type diffuse glioma.

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

References
Stichel D, et al. Distribution of EGFR amplification, combined chromosome 7 gain and chromosome 10 loss, and TERT promoter mutation in brain tumors and their potential for the reclassification of IDHwt astrocytoma to glioblastoma. Acta Neuropathol. 2018; 136: 793-803. Brat DJ, et al. cIMPACT-NOW update 3: recommended diagnostic criteria for "Diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV". Acta Neuropathol. 2018; 136: 805-810.

CNS-M05: *MGMT* promoter methylation by methylation-specific polymerase chain reaction (PCR)

Background
Epigenetic silencing of the O6-methylguanine-methyltransferanse (*MGMT*) DNA repair gene by promoter methylation has been associated with better response to treatment and improved overall survival in glioma patients receiving alkylating agent in addition to radiotherapy. Methylation-specific polymerase chain reaction provides a qualitative result for the *MGMT* promoter methylation status.

Purpose of test
Methylation-specific polymerase chain reaction provides a qualitative result for the *MGMT* promoter methylation status.

Specimen requirements
One H&E and five unstained sections of 10µm thickness of the tumour.

Turnaround time
The test is batched and performed every fortnight with a turnaround time of 21 days. The test is performed in Molecular Diagnosis Centre, NUH.

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

CNS-M06: *BRAF* p.V600 mutation by Sanger sequencing

Background
A proportion of paediatric low grade glial and glioneuronal tumours, such as pilocytic astrocytoma, pleomorphic xanthoastrocytoma and ganglioglioma, may harbour a *BRAF* p.V600E point mutation. This finding may be relevant for targeted therapeutics.

Purpose of test
BRAF V600 sequencing enables the detection of the common p.V600E and the rarer variants of mutation involving the *BRAF* gene at codon 600.

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

Turnaround time
1 week.

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

CNS-M07: *MYCN* amplification by fluorescent in-situ hybridization (FISH)

Background
Spinal cord ependymoma with *MYCN* amplification is associated with frequent dissemination and poor outcome.

Purpose of test
To detect *MYCN* amplification in spinal cord ependymoma.

Specimen requirements
4 paraffin sections, unstained, each 4 μm thick, mounted onto positively charged/ coated slides AND a corresponding H&E-stained histological section of the tumour, OR, a paraffin block of the tumour.

Turnaround time
1 week.

References
Ghasemi DR, et al. MYCN amplification drives an aggressive form of spinal ependymoma. Acta Neuropatho 2019; 138: 1075-1089. Swanson AA, et al. Spinal cord ependymomas with MYCN amplification show aggressive clinical behaviour. J Neuropathol Exp Neurol 2019;78: 791-797.

CNS-M08: Chromosome 1q gain by OncoScan SNP microarray

Background
Chromosome 1q gain has been identified as an independent predictor of both event-free and overall survival in posterior fossa ependymomas of childhood.

Purpose of test
To identify whole chromosomal 1q gain in posterior fossa ependymomas of childhood.

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

References
Mendrzyk F, et al. Identification of gains on 1q and epidermal growth factor receptor overexpression as independent prognostic markers in intracranial ependymoma. Clin Cancer Res. 2016; 12 (7): 2070-9. Junger ST, et al. Improved risk-stratification for posterior fossa ependymoma of childhood considering clinical, histological and genetic features – a retrospective analysis of the HIT ependymoma trial cohort. Acta Neuropathol. 2019; 7(1): 181.

CNS-M09: Medulloblastoma molecular group determination by NanoString nCounter gene expression profiling

Background
The World Health Organization Classification of Tumours of the Central Nervous System recognises 4 principal molecular groups in medulloblastoma – 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 the 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 molecular groups.

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

CNS-M10: Solid tumours gene fusion detection by anchored multiplex PCR (Archer FusionPlex pan-solid V2 assay)

Background

Some CNS tumours and intracranial mesenchymal tumours are characterised by gene fusions. Identification of specific gene fusions are important for diagnosis and may provide diagnostic and prognostic 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.

Target (or 'anchored') genes and their covered exons

	Genes	Covered exons		Genes	Covered exons		Genes	Covered exons
1	*ACVR2A*	1,2,3	44	*FOXR2*	2,3	87	*PIK3CA*	2,15
2	*AKT1*	2,3,4,5	45	*FUS*	3,4,5,6,7,8,9,10,11,13,14	88	*PKN1*	10,11,12,13
3	*AKT2*	2,5,11	46	*GLI1*	4,5,6,7	89	*PLAG1*	1,2,3,4
4	*AKT3*	2,3,4,9	47	*GRB7*	10,11,12	90	*PPARG*	1,2,3
5	*ALK*	2,4,6,8,10,12,14,16,17,18,19,20,21,22,23,26	48	*HMGA2*	1,2,3,4,5	91	*PRKACA*	2
6	*AR*	1,2,3,4,5,6,7,8	49	*IGF1R*	13,14,15	92	*PRKCA*	4,5,6,9,15
7	*ARHGAP26*	2,10,11,12	50	*INSR*	2,12,13,14,15,16,17,18,19,20,21,22	93	*PRKCB*	1,3,7,8,9
8	*ARHGAP6*	2	51	*JAK2*	6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,22	94	*RAF1*	2,4,5,6,7,8,9,10,11,12
9	*AXL*	11,18,19,20	52	*JAK3*	10,11,12,17,18,19	95	*RELA*	1,2,3,4,11
10	*BCOR*	2,4,6,7,10,12,14,15	53	*JAZF1*	2,3,4	96	*RET*	2,4,6,8,9,10,11,12,13,14
11	*BRAF*	1,2,3,4,5,7,8,9,10,11,12,13,14,15,16,18	54	*KIT1*	1	97	*ROS1*	2,4,7,31,32,33,34,35,36,37
12	*BRD3*	9,10,11,12	55	*MAML2*	2,3	98	*RSPO2*	1,2,3
13	*BRD4*	2,10,11,12,13,14	56	*MAP2K1*	2	99	*RSPO3*	2
14	*CAMTA1*	3,8,9,10	57	*MAST1*	7,8,9,18,19,20,21	100	*SS18*	2,3,4,5,6,8,9,10,11
15	*CCNB3*	2,3,4,5,6,7	58	*MAST2*	2,3,5,6,15,16,17	101	*STAT6*	1,2,3,4,5,6,7,15,16,17,18,19,20
16	*CCND1*	1,2,3,4,5	59	*MBTD1*	3,5,16,17	102	*TAF15*	5,6,7,9
17	*CIC*	14,15,16,17,18,19,20	60	*MDM2*	2,4,5,6,8,9,10	103	*TCF12*	4,5,6
18	*CRTC1*	1,2,3,4	61	*MEAF6*	4,5	104	*TERT*	2,3,5,7,9,10,11,12,15
19	*CSF1*	2,3,4,5,6,7,8,9	62	*MET*	2,13	105	*TFE3*	2,3,4,5,6,7,8
20	*CSF1R*	11,12,13	63	*MGEA5*	4,5,6,7,8,9,12,13,14,15	106	*TFEB*	2,3,4,5,6,9,10
21	*DNAJB1*	1,2	64	*MKL2*	11,12,13	107	*TFG*	3,4,5,6,7,8
22	*EGF*	16,17,18,19	65	*MN1*	1,2	108	*THADA*	24,25,26,27,28,29,30,31,36,37
23	*EGFR*	1,7,8,9,14,15,16,17,18,19,20,24,25,26	66	*MSMB*	2,3,4	109	*TMPRSS2*	1,2,3,4,5,6
24	*EPC1*	9,10,11	67	*MUSK*	7,9,10,12,13,14,15	110	*USP6*	1,2,3
25	*ERBB2*	4,5,13,15,17,23,24,25,26	68	*MYB*	7,8,9,11,12,13,14,15,16	111	*VGLL2*	1,2,4
26	*ERBB4*	2,3,4,14,15,16,17,18,23	69	*MYBL1*	8,9,10,11,12,13,14,15	112	*YAP1*	1,2,3,4,8,9
27	*ERG*	2,3,4,5,6,7,8,9,10,11	70	*MYC*	1,2,3	113	*YWHAE*	5
28	*ESR1*	1,2,3,4,5,6,7,8	71	*NCOA1*	11,12,13,14,15	114	*NCOA3*	2,13,14,15,16,20
29	*ESRRA*	2,3	72	*NCOA2*	11,12,13,14,15,16	115	*NFATC2*	2,3,9,10
30	*ETV1*	3,4,5,6,7,8,9,10,11,12,13	73	*NOTCH1*	2,4,5,24,25,26,27,28,29,30,31	116	*NFE2L2*	1,2,3,4,5
31	*ETV4*	2,3,4,5,6,7,8,9,10	74	*NOTCH2*	5,6,7,24,25,26,27,28,29	117	*NFIB*	2,9,10,11
32	*ETV5*	2,3,7,8,9	75	*NR4A3*	2,3,4,5,7,9	118	*PAX8*	3
33	*ETV6*	1,2,3,4,5,6,7	76	*NRG1*	1,2,3,4,5,6	119	*PDGFD*	5,6,7
34	*EWSR1*	4,5,6,7,8,9,10,11,12,13,14	77	*NTRK1*	1,2,3,4,5,6,7,8,9,10,11,12,13,14	120	*PHKB*	4
35	*FGF1*	2	78	*NTRK2*	4,5,6,7,8,9,10,11,12,13,14,15,16,17,18	121	*PRDM10*	13,14
36	*FGFR1*	2,3,4,5,6,7,8,9,10,11,12,17	79	*NTRK3*	3,4,5,6,7,8,9,10,11,12,13,14,15,16,17	122	*PRKACB*	2,3,4
37	*FGFR2*	2,3,5,6,7,8,9,10,16,17,18	80	*NUMBL*	2,3	123	*PRKCD*	9,10,11,12,15,18
38	*FGFR3*	3,5,8,9,10,11,12,13,14,16,17,18	81	*NUTM1*	2,3,4,5,6	124	*PRKD1*	2,10,11,12,13
39	*FGR*	2,3	82	*PAX3*	2,3,4,5,6,7,8	125	*PRKD2*	10,11,12,13
40	*FOS*	4	83	*PDGFB*	2,3	126	*PRKD3*	10,11,12,13
41	*FOSB*	1,2	84	*PDGFRA*	7,10,11,12,13,14,15	127	*RAD51B*	3,4,5,6,7,8,9
42	*FOXO1*	1,2,3	85	*PDGFRB*	8,9,10,11,12,13,14	128	*SS18L1*	1,2,3,8,9,10
43	*FOXO4*	2,3	86	*PHF1*	1,2,10,11,12	129	*WWTR1*	3,4

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

CNS-M11: Genome wide copy number profiling by OncoScan SNP microarray for FFPE specimens

Background
This SNP microarray-based assay interrogates the whole genome to detect copy number changes and loss of heterozygosity (LOH) in FFPE tumour specimens.

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.

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.

CNS-M12: Comprehensive Genomic Profiling by Ampliseq Childhood Cancer NGS Panel

Background

The Ampliseq Childhood Cancer NGS panel is a next-generation sequencing-based targeted gene panel used to identify somatic single nucleotide variants (SNV), copy number variants (CNV) and gene fusions affecting genes primarily relevant in paediatric and paediatric-type solid tumours occurring in older patients. The DNA assay detects SNV from hotspots of 86 genes, full exons of 44 genes, and copy number variants (CNV) from 28 genes. The RNA assay can detect more than 1700 fusion isoform variants involving 91 genes. Mutations relevant to brain tumours are covered by this assay. Signed informed patient consent is required (see Consent form, page 22).

Purpose of test

This assay is important for the diagnosis of tumours that have SNV and gene fusions which define the tumour type, and which may provide critical information for prognosis and targeted therapy. The purpose is to improve diagnostic accuracy for solid tumours, prognostication and identification of potential therapeutic targets.

Hotspots		
ABL1	*FBXW7*	*NCOR2*
ABL2	*FGFR1*	*NOTCH1*
ALK	*FGFR2*	*NPM1*
ACVR1	*FGFR3*	*NRAS*
AKT1	*FLT3*	*NT5C2*
ASXL1	*GATA2*	*PAX5*
ASXL2	*GNA11*	*PDGFRA*
BRAF	*GNAQ*	*PDGFRB*
CALR	*H3F3A*	*PIK3CA*
CBL	*HDAC9*	*PIK3R1*
CCND1	*HIST1H3B*	*PPM1D*
CCND3	*HRAS*	*PTPN11*
CCR5	*IDH1*	*RAF1*
CDK4	*IDH2*	*RET*
CIC	*IL7R*	*RHOA*
CREBBP	*JAK1*	*SETBP1*
CRLF2	*JAK2*	*SETD2*
CSF1R	*JAK3*	*SH2B3*
CSF3R	*KDM4C*	*SH2D1A*
CTNNB1	*KDR,*	*SMO*
DAXX	*KIT*	*STAT3*
DNMT3A	*KRAS*	*STAT5B*
EGFR	*MAP2K1*	*TERT*
EP300	*MAP2K2*	*TPMT*
ERBB2	*MET*	*USP7*
ERBB3	*MPL*	*ZMYM3*
ERBB4	*MSH6*	
ESR1	*MTOR*	
EZH2	*MYC*	
FASLG	*MYCN*	

CNV
ALK
ABL2
BRAF
CCND1
CDK4
CDK6
EGFR
ERBB2
ERBB3
FGFR1
FGFR2
FGFR3
FGFR4
GLI1
GLI2
IGF1R
JAK1
JAK2
JAK3
KIT
KRAS
MDM2
MDM4
MET
MYC
MYCN
PDGFRA
PIK3CA

Full Genes	
APC	*NF1*
ARID1A	*NF2*
ARID1B	*PHF6*
ATRX	*PRPS1*
CDKN2A	*PSMB5*
CDKN2B	*PTCH1*
CEBPA	*PTEN*
CHD7	*RB1*
CRLF1	*RUNX1*
DDX3X	*SMARCA4*
DICER1	*SMARCB1*
EBF1	*SOCS2*
EED	*SUFU*
FAS	*SUZ12*
GATA1	*TCF3*
GATA3	*TET2*
GNA13	*TP53*
ID3	*TSC1*
IKZF1	*TSC2*
KDM6A	*WHSC1*
KMT2D	*WT1*
MYOD1	*XIAP*

Fusion		
ABL1	*KMT2C*	*PAX5*
ABL2	*KMT2D*	*PAX7*
AFF3	*LMO2*	*PDGFB*
ALK	*MAML2*	*PDGFRA*
BCL11B	*MAN2B1*	*PDGFRB*
BCOR	*MECOM*	*PLAG1*
BCR	*MEF2D*	*RAF1*
BRAF	*MET*	*RANBP17*
CAMTA1	*MKL1*	*RARA*
CCND1	*MLLT10*	*RECK*
CIC	*MN1*	*RELA*
CREBBP	*MYB,*	*RET*
CRLF2	*MYBL1*	*ROS1*
CSF1R	*MYH11*	*RUNX1*
DUSP22	*MYH9*	*SS18*
EGFR	*NCOA2*	*SSBP2*
ETV6	*NCOR1*	*STAG2*
EWSR1	*NOTCH1*	*STAT6*
FGFR1	*NOTCH2*	*TAL1*
FGFR2	*NOTCH4*	*TCF3*
FGFR3	*NPM1*	*TFE3*
FLT3	*NR4A3*	*TP63*
FOSB	*NTRK1*	*TSLP*
FUS	*NTRK2*	*TSPAN4*
GLI1	*NTRK3*	*UBTF*
GLIS2	*NUP214*	*USP6*
HMGA2	*NUP98*	*WHSC1*
JAK2	*NUTM1*	*YAP1*
KAT6A	*NUTM2B*	*ZMYND11*
KMT2A	*PAX3*	*ZNF384*
KMT2B		

Specimen requirements
20 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 an expected 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 produce a whole genome copy number result. We provide tumour-only (somatic) testing. No germline testing will be performed.

User guide

Purpose of this booklet
This booklet summarises and illustrates the biomarkers and molecular tests available for CNS tumours offered by the Department of Pathology and Molecular Diagnosis Centre, NUH, and the Department of Pathology and Laboratory Medicine, KKH. This booklet will be updated regularly to keep you informed of the new biomarkers and molecular tests we are offering. The aim is to provide a seamless one-stop ordering system for users including pathologists and oncologists and other clinicians involved in the care of patients with CNS tumours to request for a pathology consultation or specific biomarker or molecular testing as covered in this booklet.

How to order the test?
If you have a CNS tumour case that will benefit from testing covered by this booklet, please submit your request to either NUH or KKH laboratories (addresses and contact details given in the next page). Even if the testing required for your case needs to be performed at both laboratories, it is only necessary to submit your request to one laboratory (either NUH or KKH), and we will take care of any inter-laboratory transfers. Please use the seamless request form in page 21 and please contact us if you have a question.

Enquiries and request forms

Department of Pathology, National University Hospital, Singapore	
Department of Pathology, NUH (For local institutions only)	**Contact:** ☎ +65-6772 2332/ 2330 **Department of Pathology** 5 Lower Kent Ridge Road Level 3, Main Building National University Hospital Singapore 119074 **Click the PDF icon for the request form:**  Adobe Acrobat Document
NUH Referral Laboratory (NRL) Services (Local or overseas)	**Contact:** ☎ +65-6778 5171 ✉ nrl@nuhs.edu.sg **NUH Referral Laboratories Pte Ltd** 5 Lower Kent Ridge Road Level 1, Main Building National University Hospital Singapore 119074 **Click the PDF icon for the request form:**  Adobe Acrobat Document

Department of Pathology and Laboratory Medicine, KK Women's and Children's Hospital, Singapore	
Molecular Histopathology Laboratory	**Contact:** ☎ +65-6394 1402/ 1377 ✉ molhisto@kkh.com.sg **Molecular Histopathology Laboratory** **Department of Pathology and Laboratory Medicine** Basement 1, Children's Tower KK Women's and Children's Hospital 100 Bukit Timah Road Singapore 229899 **Click the PDF icon for the request form:**  Adobe Acrobat Document

Consent

Reminder

Signed informed consent from the patient or the legal guardian must be obtained by a qualified physician for CNS-M12: Ampliseq Childhood Cancer NGS panel. The signed consent form must accompany the seamless request form at the time of test ordering.

Click the PDF icon for the consent form:

Adobe Acrobat Document

Disclaimers

The tests offered in this booklet are laboratory-developed and their performance characteristics are determined by the Department of Pathology and Molecular Diagnosis Centre, NUH, and the Department of Pathology and Laboratory Medicine, KKH. These tests have been validated for clinical use. Our laboratories are accredited by the College of American Pathologists and participate in appropriate proficiency testing programmes.

*** The End ***