THE PATHCARE NEWS

PATHCARE SEND AWAY SERVICE TO MOLECULAR NEUROPATHOLOGY DIVISION, UNIVERSITY COLLEGE LONDON HOSPITALS

PathCare has recently set up an efficient send away service for neuropathology molecular requests. This is to improve our test menu for this rapidly growing and specialised field as well as to improve the turn around time of tests such as MGMT promoter methylation. A brief description of the current test menu and their cost:

MGMT promoter methylation

MGMT is an enzyme that is responsible for DNA repair following alkylating-agent chemotherapy. In the course of tumour development, the **MGMT gene may be silenced by methylation of its promoter,** thereby preventing repair of DNA damage and **increasing the potential effectiveness of alkylating agent chemotherapy (temozolomide)**. In a large randomized trial comparing radiation alone with radiation plus concomitant and adjuvant temozolomide, the two-year overall survival for patients treated with combined radiation and chemotherapy was 49 percent for those with MGMT-methylated tumours and 15 percent for those with unmethylated tumours; moreover, those with a methylated MGMT promotor derived a greater degree of benefit from the addition of temozolomide to radiation than those with an unmethylated promotor.

Multiple clinical studies have confirmed that **MGMT promoter methylation is prognostic of improved survival,** independent of established clinical factors. In a meta-analysis of 11 studies examining the prognostic value of MGMT promotor status, a methylated MGMT promotor was associated with an improvement in both progression-free survival and overall survival.

Ordering information:

Specimen requirements	• **Histology block OR 4 slides (4µm) and 8-10 slides (10µm)**
Method	Methylation sensitive High-Resolution Melting (MS-HRM) analysis
Turnaround time	3-4 weeks
Billing	**Payment must be made upfront** before the specimen will be sent. Patient will be invoiced directly and may subsequently try to claim this from the medical aid. **R 7 970.40***

Glial tumour panel

Includes 1p/19q, 7p (EGFR), CDKN2A/B, IDH1, IDH2, H3F3A (histone H3.3 K27M & G34), TERT, BRAF, MGMT methylation analysis.

The backbone of glioma classification is light microscopy, aided by immunohistochemistry and molecular testing.
Traditional histologic grading criteria do not necessarily provide prognostic power when IDH gene status is taken into account, and certain molecular markers are more powerful and have been incorporated into grading.

IDH1/IDH2 mutation — Mutations in isocitrate dehydrogenase type 1 (IDH1) and, less commonly, type 2 (IDH2) are a defining feature of the majority of World Health Organization (WHO) grade 2 and 3 diffuse astrocytic and oligodendroglial tumors and confer **significantly improved prognosis** compared with IDH-wildtype tumours.

Immunohistochemical staining for the most common mutant form of IDH1 (R132) should be performed on all diffuse glioma specimens for diagnostic purposes. **Less common mutations** in IDH1 and all IDH2 mutations will not be identified using this antibody but can be detected **using DNA sequencing approaches.**

1p/19q codeletion — Whole-arm deletion of 1p and 19q due to an unbalanced translocation between chromosomes 1 and 19 is a defining **feature of oligodendroglial tumours and a powerful predictor of favourable therapeutic response and survival** among patients with **diffuse gliomas.** Testing for 1p/19q-codeletion status should be performed on all tumours with oligodendroglial differentiation but is **not necessary in more astrocytic IDH-mutant tumours that have clear evidence of TP53 or ATRX mutations.**

CDKN2A/B deletion — In IDH-mutant diffuse gliomas, homozygous deletion of cyclin-dependent kinase inhibitor 2A/B (CDKN2A/B) is a **negative prognostic marker,** and its presence helps to establish an **integrated diagnosis** in two specific cases:

• In **oligodendrogliomas** with histologic features that are borderline between grade 2 and 3, the presence of a CDKN2A homozygous deletion is consistent with a central nervous system (CNS) **WHO grade 3 tumour.**

• In **IDH-mutant diffuse astrocytomas,** the presence of a CDKN2A/B deletion establishes the tumour as CNS **WHO grade 4,** even in the absence of high-grade histology.

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H3 K27M mutation — H3 K27M mutations in H3F3A (one of two genes encoding the histone H3.3 variant) is present in the majority of **diffuse gliomas in the pons and other midline locations** (eg, thalamus, spinal cord), most commonly in children. They occur rarely in other glial tumors, including ependymomas.

H3 G34 mutation — Missense mutations in the H3F3A gene can result in G34R or G34V mutations. These alterations are a defining feature of **diffuse hemispheric glioma, H3 G34-mutant,** a newly recognized tumour type in the 2021 WHO classification.

EGFR, TERT — For **IDH-wildtype, H3-wildtype** astrocytic tumours without high-grade histologic features, the presence of any one of the following molecular features **establishes a diagnosis of glioblastoma,** even in the absence of microvascular proliferation or necrosis:
- Epidermal growth factor receptor (EGFR) amplification
- Telomerase reverse transcriptase (TERT) promotor mutation

BRAF alterations — Alterations in BRAF characterize specific subsets of gliomas:
- KIAA1549-BRAF fusion – Tandem duplication of chromosome 7q34 resulting in fusion of the BRAF and KIAA1549 genes is observed in 60 to 80 percent of **sporadic pilocytic astrocytomas.**
- BRAF V600E mutation – V600E point mutations in the BRAF gene are present in a variety of glioma subsets, including approximately two-thirds of **pleomorphic xanthoastrocytomas,** 20 percent of **gangliogliomas,** and 10 percent of **pilocytic astrocytomas. Occasional diffuse gliomas** may also have BRAF V600E mutations.

Ordering information:

Specimen requirements	• **Histology block OR 4 slides (4µm) and 8-10 slides (10µm)**
Method	qPCR, Sequencing, HRM-PCR
Turnaround time	4-6 weeks
Billing	**Payment must be made upfront** before the specimen will be sent.
	R 12 260.70*

Methylation array

Methylome profiling uses arrays to determine DNA methylation patterns across the genome. Although not yet widely available, it is now recommended as an **effective method for classification** when used alongside standard technologies. The Illumina 850k EPIC array assesses the DNA methylation status of a small proportion of the 850,000 individual CpG sites on the array. An algorithm, developed at the DKFZ in Heidelberg, Germany, is able to distinguish brain tumour entities based on their methylation profile. It is currently based on over 10,000 reference brain tumour cases and is periodically updated. Circumstances in which methylome profiling may be most useful to pathologists include:
- **Diagnostically challenging neoplasms,** including some rare tumour types and subtypes that can only be diagnosed by methylome profiling.
- **Small biopsy samples**, which may be limiting for standard technologies.

Methylome profiling may also be used as a surrogate marker for genetic events, for example, when a methylome signature is characteristic of an IDH-wildtype glioblastoma in the absence of IDH mutation testing.

Ordering information:

Specimen requirements	• **Histology block**
Method	Methylation array, analysis with classifier algorithm, developed at DKFZ in Heidelberg
Turnaround time	4-6 weeks
Billing	**Payment must be made upfront** before the specimen will be sent.
	R 19 541.20*

If you require any additional information, please email molecularoncology@pathcare.co.za or contact the following:

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***All prices subject to change. Contact drsystems@pathcare.co.za for current pricing information**