Bristol Genetics Laboratory

North Bristol NHS Trust

Glioma Service

Contact details:
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Sample Required:
Paraffin embedded tumour tissue:
MGMT/ BRAF* p.(Val600Glu)/ *IDH/Histones

- **>50% tumour:** 5 x 10µm sections in a clean universal
- **<50% tumour**: 10 x 5µm slide mounted sections along with H&E with regions of >30% tumour highlighted

1p/19q co-deletion

- 4 x 4µm sections on 'APES' or 'sticky' slides along with H&E with the appropriate tumour rich area(s) marked.

***BRAF* Fusion**

- 10 x 5µm slide mounted sections along with H&E with regions of >30% tumour highlighted

Samples should be accompanied by a fully completed Neuropathology request form (available as download at www.nbt.nhs.uk/genetics or from the laboratory).

Consent and DNA Storage:
All genetic testing requires consent. It is the responsibility of the referring clinician to ensure that appropriate consent has been obtained.

DNA is stored from **all** patients unless consent for this is specifically denied.

Stored samples may be used for quality assurance purposes and may be used anonymously for the development of new tests for the disorder in question.

Clinical Background

Gliomas are the most common group of brain tumours and are classified by cell type, grade, location and molecular markers according to WHO 2016 classification and include oligodendroglioma, astrocytoma and glioblastoma.

Services offered:

1p and 19q co-deletion analysis
Co-deletion of chromosome regions 1p36 and 19q13 is diagnostic for oligodendroglioma and is a statistically significant predictor of chemosensitivity and longer recurrence free survival. 1p and 19q analysis is carried out by Fluorescence *in situ* Hybridisation (FISH) using the CE marked, Abbott Molecular 1p36/1q25 and 19q13/19p13 probe kit.

***MGMT* promoter methylation analysis**
Around 50% of glioblastoma and astrocytoma patients will respond to alkylating chemotherapy agents such as temozolomide. Patients who will respond to this treatment have methylated CpG islands in the promoter region of the O6 methylguanine DNA methyltransferase gene (*MGMT*). *MGMT* promoter methylation is determined by methylation sensitive pyrosequencing after bisulphite conversion of DNA extracted from tumour tissue.

***IDH1* and *IDH2* analysis**
Isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) are key metabolic enzymes in the citric acid cycle. *IDH* mutations are found in the majority of low-grade gliomas (>80%) and also in secondary glioblastomas, and confer a favourable prognosis. *IDH1* codon 132 and *IDH2* codon 172 analysis is carried out by Sanger Sequencing, with a sensitivity of 15% mutant DNA in a background of wild-type DNA.

***BRAF* analysis**
Pilocytic astrocytomas are the most common paediatric form of glioma. *KIAA1549-BRAF* fusions are detected in 60-80% pilocytic astrocytomas, but are rare in other tumour types. Detection of this fusion aids differentiation from other low grade tumour types. *BRAF* fusion analysis is determined by real-time PCR to detect the most common *KIAA1549-BRAF* fusions (*16-9, 15-9, 16-11*). FISH is offered as a second line test if rtPCR is negative.

The c.1799T>A p.(Val600Glu) *BRAF* mutation is present in a proportion of *KIAA1549-BRAF* fusion negative pilocytic astrocytomas. This mutation can also be associated with paediatric high grade glioma, epithelioid and giant cell glioblastoma. *BRAF* codon 600 analysis is carried out by pyrosequencing and can detect a mutation with a sensitivity of 5% mutant DNA in a background of wild-type DNA.

Histone analysis
WHO 2016 CNS guidelines classify a distinct entity of paediatric diffuse gliomas with a midline location with K27M mutations (p.Lys28Met in HGVS nomenclature) in the histone H3.3 gene *H3F3A*, or less commonly in the related histone H3.1 gene *HIST1H3B.* In addition, mutations at G34 (p.Gly35 in HGVS nomenclature) of *H3F3A* have been reported in cerebral hemisphere paediatric high-grade astrocytic tumours (WHO grade III/IV). Analysis for these hotspots is carried out by Sanger Sequencing, with a sensitivity of 15% mutant DNA in a background of wild-type DNA.

Referrals:

Referrals are accepted from Consultant Neuropathologists.

Quality:

BGL participates in the GenQA schemes for these services.

Target reporting time: 14 days

Clinical advice: We would recommend contact with Professor Kathreena **Kurian** Consultant Neuropathologist, Southmead Hospital, Bristol (Tel: 0117 4142402)

Laboratory contact: For enquiries/requesting contact: claire.faulkner@nbt.nhs.uk

Reference:

Louis *et al* 2016. WHO Classification of Tumours of the Central Nervous System. Acta Neuropathol. Jun;131(6):803-20

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