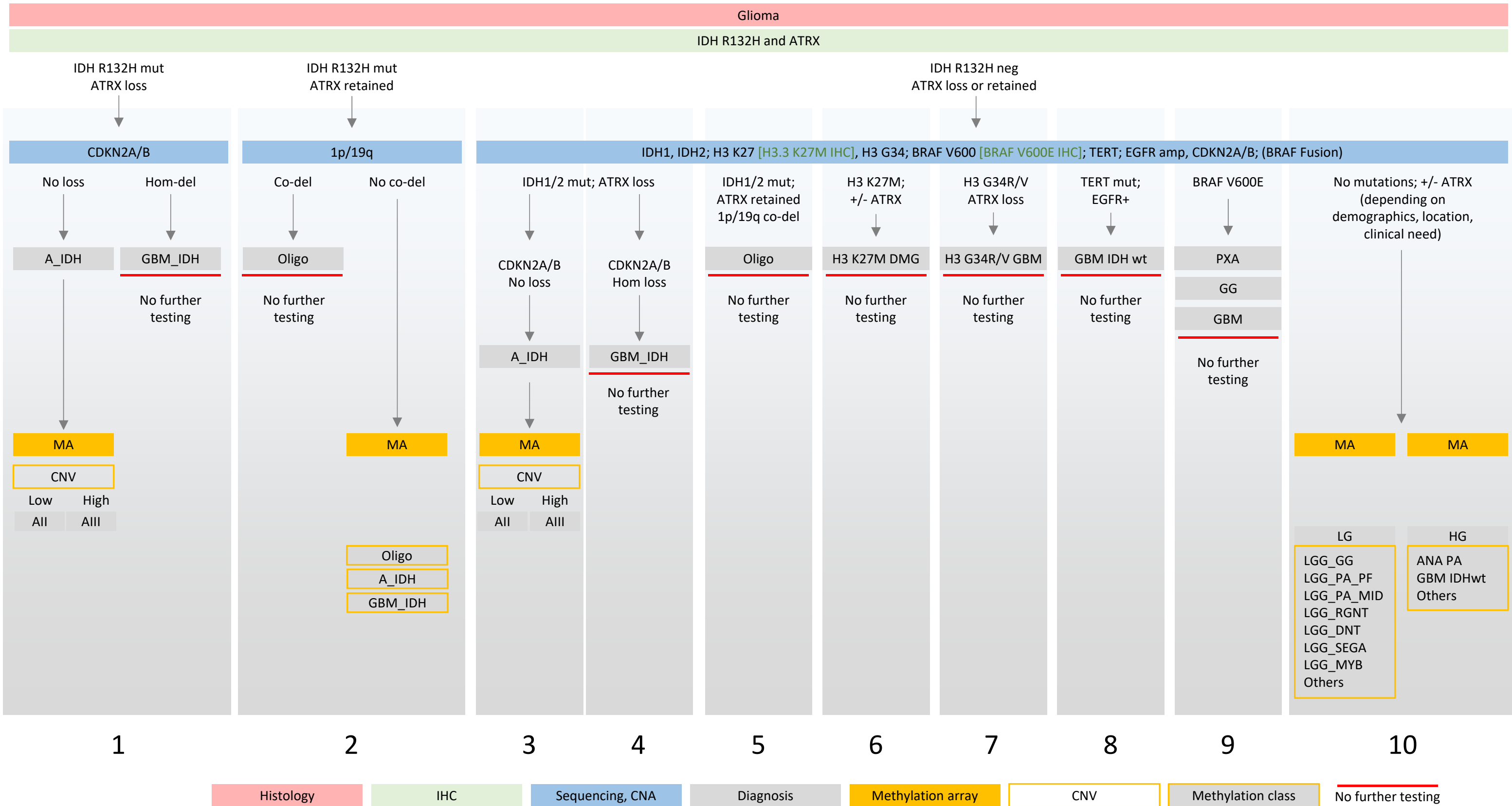


# Diagnostic algorithm adult gliomas



Diagnostic testing algorithm for gliomas in adults as applied in our practice in the Division of Neuropathology.

**The first layer is the histological assessment.** The histological identification of a glial tumour is **followed by the standard application of the antibodies IDH1 (R132H) and ATRX.** This identifies a majority of IDH-mutant gliomas (column 1, 2).

**Column 1:** IDH- mutant astrocytomas with ATRX loss are further tested for CDKN2A/B homozygous deletion to stratify high risk from lower risk astrocytomas. Lower risk IDH-mutant astrocytomas are also assessed for copy number variation, a suggested prognostic factor. This is achieved by the readout of the copy number variation (CNV) component of the methylation arrays.

**Column 2:** IDH-mutant gliomas with retained ATRX expression are further tested for 1p/19q co-deletion with a conventional copy number assay (in our practice combined with TERT promoter mutation analysis). Those IDH-mutant tumours which have retained ATRX expression and either no co-deletion or an ambiguous copy number result, are further tested with methylation array. This helps to differentiate IDH-mutant oligodendrogliomas from IDH-mutant astrocytomas or glioblastomas with retained ATRX protein expression.

**Columns 3-5:** Gliomas which are negative for IDH1 R132H are further tested for a panel of biomarkers: IDH1, IDH2, H3 K27 and G34, BRAF, TERT promoter, EGFR and CDKN2A/B. IDH-mutant gliomas are shown.

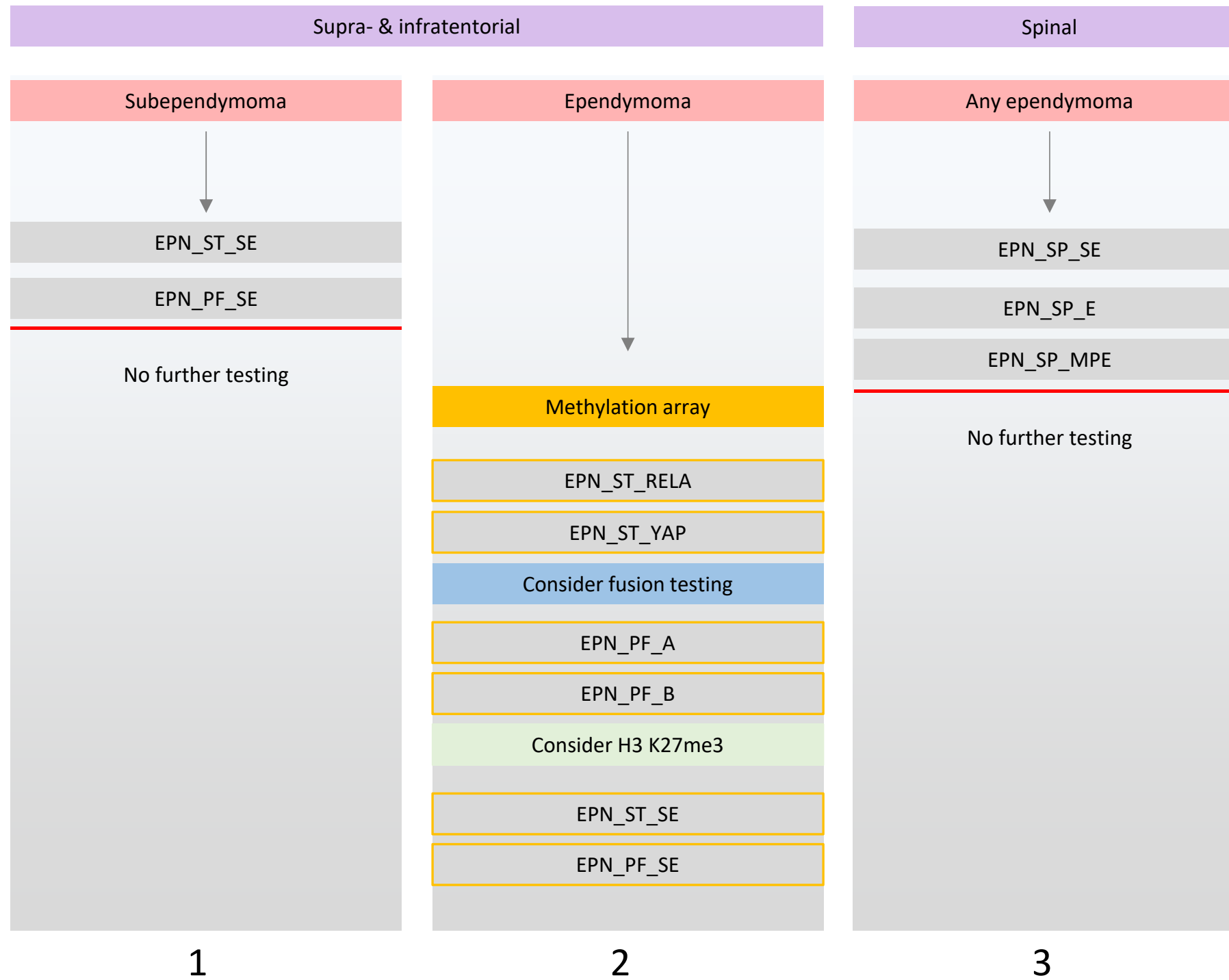
**Columns 6-7:** The outcomes from histone mutation testing.

**Column 8:** A significant proportion of IDH-wildtype, EGFR-amplified and TERT promoter mutant glioblastomas are represented. These molecular entities do not require further testing at present.

**Column 9:** The detection of a BRAF V600E mutation usually does not require further methylation array analysis. Those glial tumours with unequivocal histology (e.g. DNET, RGNT, ganglioglioma, IDH-wildtype GBM) are usually not further tested.

**Columns 10:** Tumours with non-characteristic and non-specific low-grade or high-grade histology and inconclusive molecular profile undergo methylation array analysis to inform of the methylation class which may also suggest candidate mutations that can be further tested for subsequent validation, such as rare mutations in histone variant encoding genes other than H3F3A. Often the methylation analysis also serves as a risk stratifier.

# Diagnostic algorithm ependymomas



Location	Histology	IHC	PCR, FISH
Diagnosis	Methylation array	Methylation class	No further testing

Diagnostic algorithm for ependymomas. In our diagnostic practice ependymomas in adults are infrequent.

**We first stratify the tumours by location and histological appearance.**

**Column 1:** Identification of **subependymomas** is histologically straightforward and these tumours undergo no further testing.

**Column 2:** Supra- and infra-tentorial ependymomas are directly tested with methylation array. RELA and YAP fusions may be further tested depending on the Classifier result. In our practice, EPN\_PF\_B are practically non-existent in the adult population, but H3 K27me3 expression status is technically straightforward and affordable and can be tested for completeness. A small proportion of supratentorial ependymomas with “classical” histology may be reclassified as subependymoma.

**Column 3:** Most spinal tumours are clinically low risk (with exception of MYC amplified rare forms) and their outcome is mainly determined by the extent of the surgical removal. Unless there is a specific clinical need or unusual histology, spinal tumours are not further tested with methylation arrays.