

Assessment Run 13 2005

Glial Fibrillary Acidic Protein (GFAP)

The slide to be stained for Glial Fibrillary Acidic Protein (GFAP) comprised:
 1: Brain (autopsy material fixed approximately 6 weeks), 2: Astrocytoma,
 3: Glioblastoma, 4: Meningioma, and 5: Parotid gland. All tissues were fixed in
 10% neutral buffered formalin.



Criteria for assessing a GFAP staining as optimal included: A strong and distinct cytoplasmic staining of the normal astrocytes in the brain and some myoepithelial cells in the parotid gland as well as the astrocytoma and glioblastoma. All other cells should be negative.

53 laboratories participated. At the assessment 11 achieved optimal (21 %), 27 good (51 %), 13 borderline (25 %) and 2 (4 %) poor marks.

The following GFAP antibodies were used:

mAb clone **6F2** (DakoCytomation, n=13; Monosan, n=1).
 mAb clone **GA-5** (BioGenex, n=2; NeoMarkers, n=1; NovoCastra, n=1).
 mAb clone **SP2/AG14** (Laboserv, n=1)
 pAbs **Z0334** (DakoCytomation, n=35) and **760-2516** (Ventana, n=2).

Optimal staining in this assessment was obtained with the mAb clone 6F2 (6/14), GA-5 (1/4) and the pAb Z0334 (4/35).

Using clone 6F2 an optimal staining was achieved with HIER in either Tris-EDTA/EGTA pH 9 as the buffer (optimal in 5 out of 6) or TRS low pH (S1699 DakoCytomation) (optimal in 1 out of 2).

Using pAb Z0334 an optimal staining was achieved with HIER in Tris-EDTA/EGTA pH 9 (optimal in 3 out of 15) and with proteolytic epitope retrieval with Proteinase K (1 out of 12).

Using clone GA-5, the optimal staining was based on HIER using TRS low pH (S1699 DakoCytomation).

The mAb clone 6F2 was used in the range of 1:100 – 500, the pAb Z0334 1:500 – 5.000 and the mAb clone GA-5 1:100.

The most frequent causes of insufficient staining were:

- Omission of epitope retrieval
- Too diluted or too concentrated primary antibody
- Excessive proteolytic retrieval

The prevalent feature of an insufficient staining was a too weak staining of the neoplastic cells in the astrocytoma and glioblastoma. This was typical found in the protocols omitting epitope retrieval and/or using a too dilute concentration of the primary antibody. The majority of the laboratories were able to detect GFAP in the normal astrocytes in the brain specimens. Another frequent feature of the insufficient staining was excessive proteolytic retrieval causing severe impairment of the morphology and extraction of the cytoplasm, thus giving a false negative reaction. Also a too high concentration of the primary Ab, especially the pAb Z0334 resulted in an unspecific reaction of the meningioma.

In the assessment a distinct detection of GFAP in the myoepithelial cells in the parotid gland was generally seen in the optimal staining. However, as the number of these cells was relative low in the material, the parotid gland is not always reliable as control tissue for GFAP demonstration.

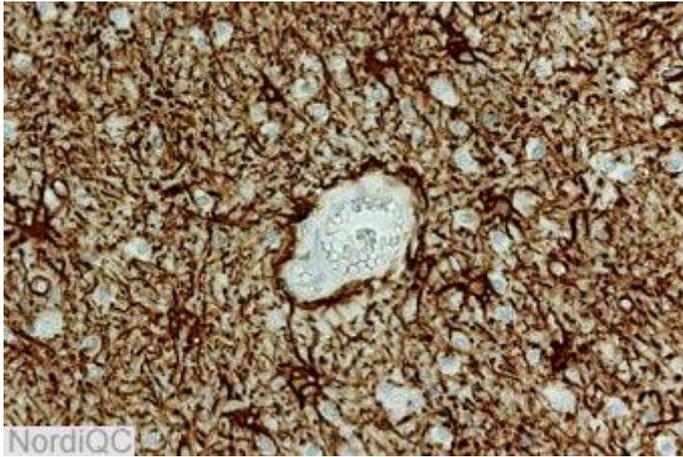


Fig. 1a
Optimal staining for GFAP in the brain. The astrocytes show a strong and distinct cytoplasmic staining.

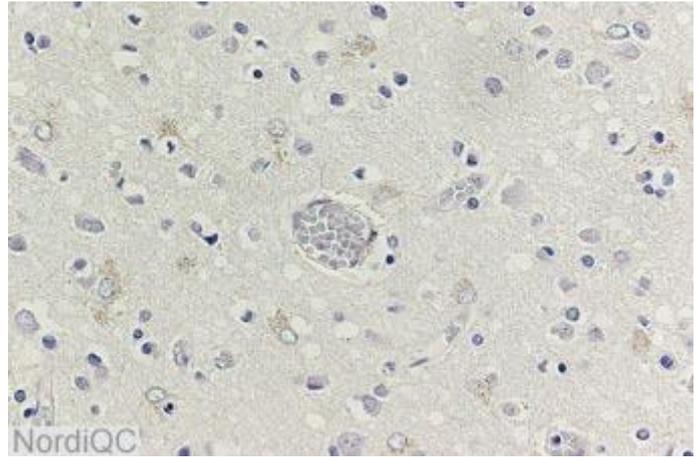


Fig. 1b
Insufficient staining for GFAP in the brain. The astrocytes are virtually negative (same field as in fig. 1a.)

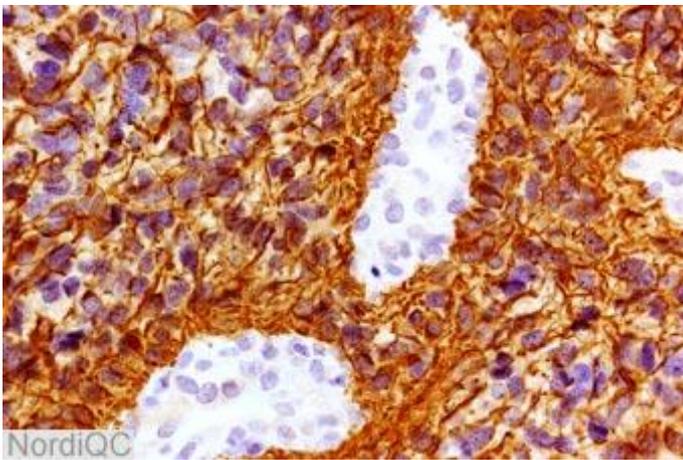


Fig. 2a
Optimal staining for GFAP in the glioblastoma. The neoplastic cells show a strong and distinct cytoplasmic staining with no staining of the endothelial cells.

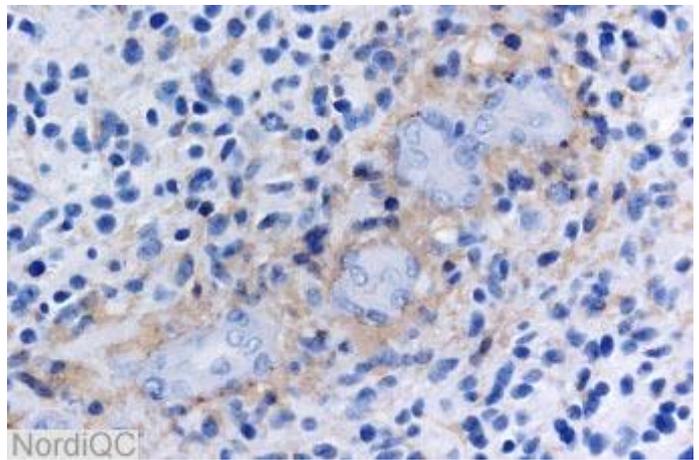


Fig. 2b
Insufficient staining for GFAP in the glioblastoma (same field as in fig. 2a). Only a few of the neoplastic cells are weakly stained (same protocol as fig. 1b).

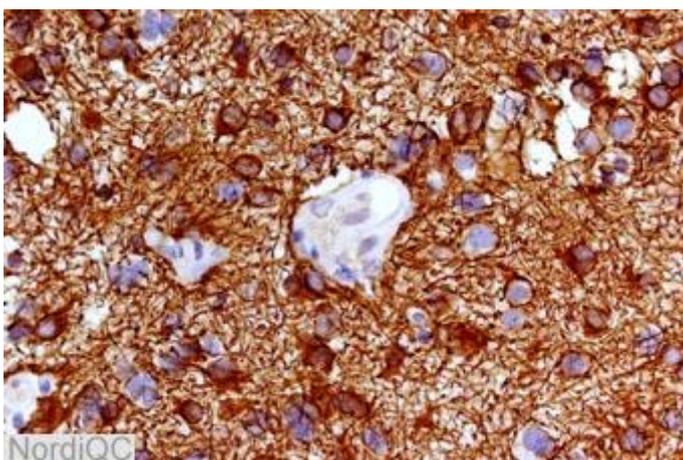


Fig. 3a
Optimal staining for GFAP in the astrocytoma. The neoplastic cells show a strong and distinct cytoplasmic staining.

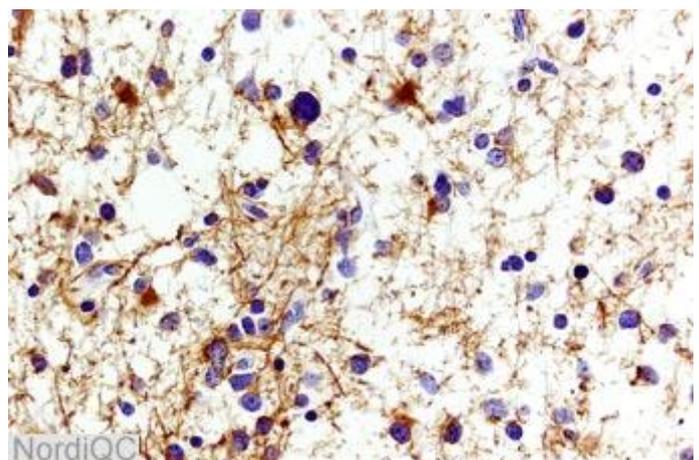


Fig. 3b
Insufficient staining for GFAP in the astrocytoma (same field as in Fig. 3a). The neoplastic cells show an impaired morphology and only remnants of the cytoplasm are demonstrated. This staining pattern was frequently observed with usage of (excessive) proteolysis.

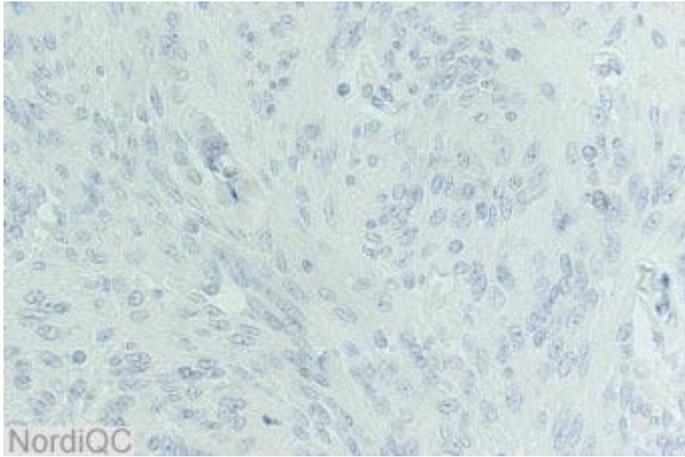


Fig. 4a
Optimal staining for GFAP in the meningioma. The neoplastic cells are negative.

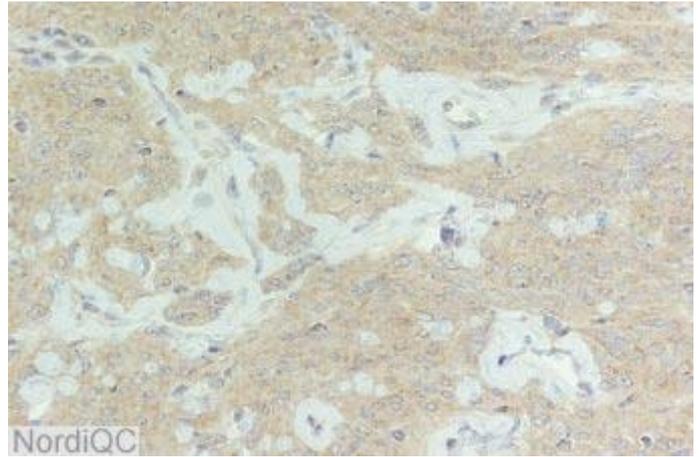


Fig. 4b
Insufficient staining for GFAP in the meningioma (same field as in fig. 4a). The neoplastic cells show a false cytoplasmic staining. This pattern was frequently observed with the use of a too concentrated primary Ab, especially the polyclonal Ab Z0334. Also compare the staining in fig. 5b, same protocol.

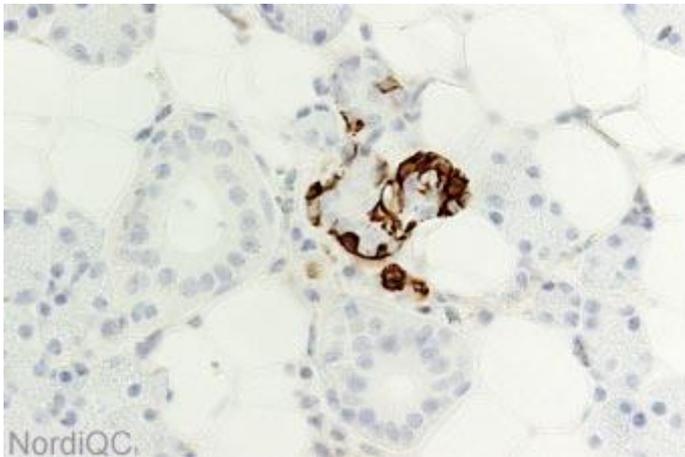


Fig. 5a
Optimal staining for GFAP in the parotid gland. The myoepithelial cells are stained, whereas the epithelial cells are negative.

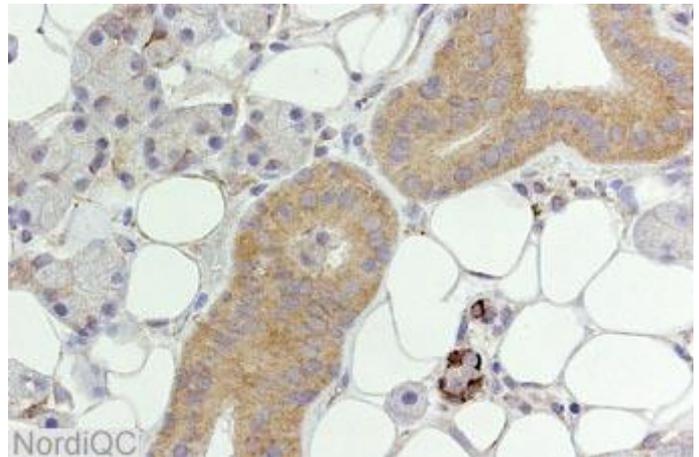


Fig. 5b
Insufficient staining for GFAP in the parotid gland (same field as in fig. 5a). The epithelial cells of the ducts show a false cytoplasmic staining (same protocol as fig. 4b.).

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