

# Application of laser microdissection and real-time Q-PCR on immunocytochemically identified neurons of the human brain: results from post-mortem material.

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## OBJECTIVE

A brain homogenate includes information from all neuronal and non-neuronal cell populations making it difficult to make cell specific conclusions. Therefore, it is necessary to study the changes in expression profile at the single cell level in specific cell populations. The purpose of our study was to examine how different processing of the postmortem brain tissue could influence the availability of total RNA of tyrosine hydroxylase (TH) immunocytochemically-identified neurons isolated by laser microdissection from human brain sections. We investigated the effect of fixation and immunocytochemical (ICC) procedure on RNA availability using real-time Q-PCR results, without prior RNA amplification steps.

## MATERIAL AND METHODS

Our material consisted of two different brain areas, hypothalamus and locus coeruleus, which were either paraffin-embedded or fresh frozen, from two male control subjects aged from 75-78 years, with post mortem delay 4-7 hours. Fresh-frozen sections were post fixed with cold acetone for 4 minutes.

### Modification of the classical peroxidase-antiperoxidase (PAP) ICC procedure:

- 1hr incubation with TH antiserum at room temperature (RT) [1:1000 in incubation buffer-1X TBS with 0.25% gelatin and 0.5% Triton X-100, pH 7.6-containing Protector RNase Inhibitor (40U/μl, Roche) ]
- 30min incubation with goat anti-rabbit serum in incubation buffer at RT [1:100]
- 30min incubation with PAP in incubation buffer at RT [1:1000]
- Visualization with 0.5mg/ml 3',3' diaminobenzidine containing 0.01% H<sub>2</sub>O<sub>2</sub> for maximum 10 minutes

### Microdissection

We used the P.A.L.M. Microlaser System (PALM Microlaser Technologies). 100 ICC neurons were dissected and catapulted directly in the cap of an eppendorf tube containing mineral oil.

### RNA extraction

Carrier: 1μl LPA (1μg/μl)  
250μl Trizol  
50μl isopropanol-precipitation on dry ice (30min-1hr)  
\*Paraffin tissue: 1hr incubation with proteinase K at 53°C  
Resuspension in 5 μl st. H<sub>2</sub>O

### 1<sup>st</sup> strand cDNA synthesis

0.5μl random hexamers (10X, Roche)  
65°C for 10 minutes, 25°C for 5 minutes

1μl Superscript II (200U/μl, Invitrogen)  
50°C for 1hr

phenol/chloroform extraction-precipitation  
with 100% ethanol  
Resuspension in 20μl st. H<sub>2</sub>O

### Q-PCR

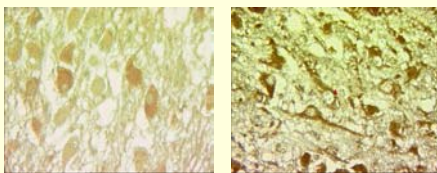
Primers for Elongation Factor 1a (EIF1a), Cytochrome c oxidase (Cox 1), Arginine Vasopressin (AVP) and TH were designed using Primer Express 2.0.

RNA extracted from IMR32 neuroblastoma cell line was used as control RNA

Efficiency was calculated using LinRegPCR 7.4 (Ramakers et al, 2003)

## RESULTS

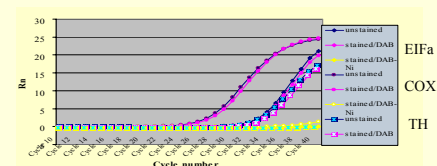
### 1. Immunocytochemistry



Non-coverslipped paraffin-embedded human SON section stained for TH

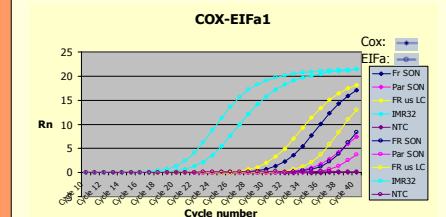
Non-coverslipped fresh-frozen human SON section stained for TH

### 2. Comparison between unstained and stained human locus coeruleus sections



The immunocytochemical procedure does not affect the Q-PCR results. The addition of Ni in the DAB seems to reduce the signal (>6 cycles).

### 3. Comparison between paraffin-embedded and fresh frozen human supraoptic nucleus

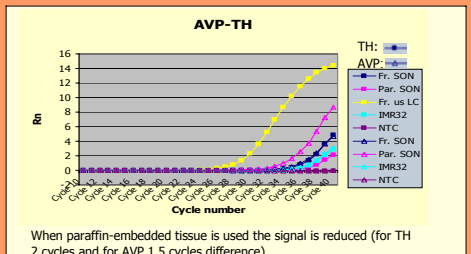


When paraffin-embedded tissue is used the signal is reduced (for COX 5 cycles and for EIFa 2 cycles difference)

## CONCLUSIONS

**Single neuron Q-PCR can be applied without prior amplification on postmortem human brain tissue using laser microdissection on immunocytochemically-identified sections.**

- Both paraffin-embedded and fresh frozen sections of postmortem human brain can be used for laser microdissection and Q-PCR.
- Small difference on the Ct value between stained and non-stained sections. Intensification with Ni reduces the signal.
- For all genes studied, the signal appeared in lower cycle threshold -range 2-5 cycles- when total RNA was extracted from frozen, as compared with paraffin embedded material.



When paraffin-embedded tissue is used the signal is reduced (for TH 2 cycles and for AVP 1.5 cycles difference)

## AKNOWLEDGMENTS

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