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Protocol for preparing post-mortem tissue for intracellular neuromelanin quantification in H&E-stained sections.

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ABSTRACT

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We use this protocol and it's working

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Tissue Processing

1 Formalin-fixed paraffin-embedded tissue blocks from the pontine and midbrain regions were sectioned at 5µm, collected onto adhesive microscope slides and allowed to dry at room temperature for 24 hours.

2 Slides were incubated in the oven at 60°C for 30 minutes to melt the paraffin.

3 To remove the paraffin, sections were submerged in Xylene (Panreac Applichem 211769.2714) for 3 x 3 minutes, followed by rehydration in decreasing ethanol concentrations (100% ethanol for 2 x 5 minutes, 95% ethanol for 2 x 5, 70% ethanol for 2x5 minutes) and distilled H_2O for 5 minutes.

4 Standard hematoxylin-eosin (H&E) staining.

Image Acquisition

5 Sections were scanned using 20x objective (NA=0.8) with pre-set focusing and exposure parameters for optimal neuromelanin signal quality with an automated Slide Scanner Olympus (SLIDEVIEW VS200, Tokyo,

Japan).

6 Identical parameters were applied to each scanned section to ensure consistency in capturing neuromelanin.