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Olfactory mucosa sampling and processing

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ABSTRACT

This protocol describes the olfactory mucosa sampling and processing for RT-QulC assays and was adapted from A. Stefani et al. (doi:10.1093/brain/awab005).

EXTERNAL LINK

<https://pubmed.ncbi.nlm.nih.gov/33855335/>

PROTOCOL CITATION

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Stefani A, Iranzo A, Holzknecht E, Perra D, Bongianni M, Gaig C, et al. Alpha-synuclein seeds in olfactory mucosa of patients with isolated REM sleep behaviour disorder. Brain. Oxford University Press; 2021;144:1118–26.

KEYWORDS

olfactory, mucosa, nasal, sample, RT-QulC, SAA

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MATERIALS TEXT

- benchtop centrifuge with cooling function
- waterbath sonicator
- regular lab equipment (pipetts, tips, tubes)
- nylon flocked swabs (like FLOQBrush®; CopanItalia Spa)
- 1µl plastic inoculation loops, disposable, sterile
- NaCl 0.9% (w/v) aqueous solution
- PBS

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Olfactory mucosa sampling

- 1 Two olfactory mucosa samples are collected per person, one nasal swabbing from each nostril.

A sterile, disposable swab is inserted in the nasal cavity, (similar to a covid test but more towards the top) to reach the olfactory and not the epithelial mucosa.
The swab is gently rolled on the mucosal surface, removed, and the tip is cut and placed in a 15-ml conical centrifuge tube with 0.9% NaCl solution.

Olfactory mucosa processing

- 2 Olfactory mucosa cells are extracted from the swab by vortexing the tube for 1 min at room temperature.
- 3 Swab is removed from the tube and the cell suspension is centrifuged at 2000 x g for 20 mins at 4 °C.
- 4 Supernatant is removed and the pellet is directly processed or frozen at -80 °C for processing later.
- 5 An inoculation loop is used to collect equal amounts of pellet (~ 2 µl) which are then dipped into a tube containing 120 µl PBS.
- 6 Pellets are dissolved by sonicating the tube in a bath sonicator at 120 W for at least 1 min.
- 7 Samples can be directly used for RT-QulC assay.