Sample treatment for rabies diagnosis

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ANSES - Nancy Laboratory for Rabies and Wildlife

European Union Reference Laboratory for Rabies

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2. FOREWORD

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3. INTRODUCTION

This Standard Operating Procedure (SOP) describes the preparation of brain samples for the detection of lyssavirus antigen (by the FAT) or rabies virus (by the RTCIT) for diagnostic purpose that fulfills the requirements of the ISO/IEC 17025.

This protocol presents one possible way to collect brain samples. Alternative methods for brain samples collection (occipital foramen as an example) may be used by laboratories.

4. PURPOSE AND SCOPE

Post-mortem rabies diagnosis is carry out on brain tissues collected from suspect mammals. This preparation requires the necropsy of the suspected animal and the removal of the brain so that to collect different areas of interest to optimize the confidence of the rabies diagnosis.

5. REFERENCE DOCUMENTS

- Rupprecht, C.E., Fooks, A.R., Abela-Rider, B. (Eds.), 2018. Laboratory Techniques in Rabies, fifth edition, World Health Organization, Geneva.
- OIE, 2018, Manual of standards for diagnostic tests and vaccines, Rabies Chapter, 578-612.

6. ABBREVIATIONS

- FAT Fluorescent Antibody Test
- PBS Phosphate Buffered Saline
- RABV Rabies Virus

7. EQUIPMENT AND MATERIALS

- Necropsy table
- Hammer and blades/knives (or necropsy oscillating saw)
- Scissors, scalpels, forceps, spoons, dissection instruments
- Bio hazardous waste containers
- Refrigerated chambers: -20°C (± 5°C), +5°C (± 3°C), <-65°C (long term storage of specimens)
- Labcoat, face mask, over-boots, goggles and gloves

8. PROCEDURE

8.1. BRAIN REMOVAL

All dissection instruments and surfaces (necropsy table, benches) must be clean and properly disinfected.

- Head must be firmly held using a forceps clamped in the eye sockets (second operator) or using a bench vise.
- Cut the skin with a scalpel along the top median axis and recline the skin
- Remove the temporal muscles.
- A craniotomy is carried out using hammer/blades (or saw), large enough to properly expose the brain and to remove it.

- Remove *dura mater* with a scalpel and narrow dissection pliers.
- Remove the brain with a spoon insuring to remove the cerebellum and the lower half of the brainstem (*medulla oblongata*) as well, and place it in a plate.

Note: dissection instruments must be changed for each necropsied head

8.2. BRAIN SPECIMENS SELECTION

A qualitative evaluation of the brain condition is necessary before selecting brain specimens. Deteriorated, highly putrefied, liquefied brains may lead to inconclusive diagnostic results and may be rejected according to the rules set up in the laboratory.
- two sets of cortex specimens are dissected.
- two sets of cerebellum specimens are dissected (cross-section through the two cerebellar hemispheres).
- two sets of brainstem specimens are dissected (a cross section is carried out in the lower part to include the *medulla oblongata*).
- two sets of the Ammon's horn (*hippocampus*) are dissected (*hippocampus* are located deep in the medial temporal lobe of each cerebral hemisphere).

One set of each brain specimens, freshly dissected, is used to carry out rabies diagnosis (preparation of slides for FAT as example). The other sets are reserved and stored at -20°C (± 5°C), in case of test repetition. In tested positive, reserved brain specimens are stored at <65°C.

Note: For small mammals such as mouse or bats, the whole brain is removed after craniotomy. A transversal section is preferably carried-out so that to the tissue cut includes cortex, cerebellum and brainstem. This tissue cut is used to prepare slide impressions for FAT.