Detection of Lyssavirus antigen by the Fluorescent Antibody Test (FAT)

Version 01

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ANSES - Nancy Laboratory for Rabies and Wildlife

European Union Reference Laboratory for Rabies

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2. FOREWORD

This procedure has been validated by ANSES - Nancy Laboratory for Rabies and Wildlife
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3. INTRODUCTION

This Standard Operating Procedure (SOP) describes the detection of lyssavirus antigen by the FAT for diagnostic purpose that fulfills the requirements of the ISO/IEC 17025.

This protocol should not be considered transferable as it stands. It shall be adapted by each user for its working conditions and shall be validated with reference materials or samples of known status (as performed in the frame of rabies diagnosis proficiency testing by example).

The assay validation should meet the OIE Standard for validation (Chapter 1.1.6. "Principles and methods of validation of diagnostic assays for infectious diseases") and should be able to detect a broad spectrum of globally circulating rabies virus strain (Chapter 3.1.17: "Rabies infection with rabies virus and other lyssaviruses)".

4. PURPOSE AND SCOPE

This *in-vitro* test detects rabies antigens in brain specimens and may be used as a primary diagnostic test for clinical suspicions of rabies disease in mammals.

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

- CVS Challenge Virus Standard
- EBLV European Bat Lyssavirus
- FAT Fluorescent Antibody Test
- FITC Fluorescein isothiocyanate
- PBS Phosphate Buffered Saline
- RABV Rabies Virus

7. PRINCIPLE OF THE METHOD

This microscopic test relies on the *in vitro* detection of rabies virus antigens present in the cerebral tissue of infected animals. The procedure consists in incubating a labeled antibody (fluorescein isothiocyanante) with rabies brain tissue. If present, rabies antigens will bind with the labeled antibody and produce a fluorescent apple-green signal that can be visualized with a fluorescence microscope.
Fresh, frozen or glycerolated brain material may be examined. This procedure is intended to the technician staffs who are qualified for this technique.

7.1. REAGENTS

- Sterile PBS
- Sterile distilled water
- Undiluted cold acetone
- Glycerol based mounting medium
- Fluorescent conjugate
- Brain of mice/others mammals infected with RABV/CVS-27/EBLV (positive control)
- Brain of uninfected mice/other mammals (negative control)

7.2. EQUIPMENT AND MATERIALS

- Slides and coverslips

- Wooden tongue-depressor
- Single-channel pipette/dispenser and appropriate sterile tips suited to the volumes
- Box slide folder
- Fluorescent microscope (excitation filter: 492 nm; stop filter: 510 nm)
- 37°C± 2°C incubator and humidified tray
- Class II laminar flow hood
- Bio hazardous waste containers
- Refrigerated chambers: -20°C (± 5°C), +5°C (± 3°C), -80°C (long term storage of specimens)
- Labcoat, face mask, goggles and gloves

8. PROCEDURE

8.1. PREPARATION OF IMPRESSIONS

- Prepare brain impressions from small sections of Ammon's Horn, cortex, brain stem and cerebellum (impressions are prepared by pressing the slide against a clean wooden tongue-depressor, with just enough pressure to create a slight spread of brain areas on each field).
- Air-dry slides at room temperature from 15 to 30 minutes.
- Prepare positive and negative controls slides as the same time than test slides. Positive control slide(s) may be prepared from brain tissue of a mammal experimentally/naturally infected with RABV/CVS-27/EBLV. The negative control slide is prepared from the from brain tissue brain of a healthy mammal.

For rabies diagnosis of bats, slides are prepared from the totality of the brain.

8.2. FIXATION, STAINING AND MOUNTING

- Once dried, immerse slides in a jar filled with pure chilled acetone and fix at -20°C for 30 minutes.
- Remove slides and air-dry.
- Distribute 50 µl of rabies conjugate on each impression and incubate slides at 37°C for 30 minutes in a humidified tray/chamber to prevent from drying.
- Wash slides once in PBS, and once with distilled and air-dry.
- Add one drop of mounting medium on each slide and add coverslip.

NB: The FITC anti-rabies conjugate is directed against the nucleoprotein of the virus. It must be prepared according to the leaflet provided by the manufacturer (if commercial product). A working dilution must be established upon reception.

Acetone bath can be re-used during 1 month if negative controls remain negative.

8.3. MICROSCOPE READING

- Observe the slides under fluorescent microscope at magnification x200 and x400 (reading is carried-out by two qualified persons, independently).

Rabies antigens will appear in positive control slide and test slides as fluorescent-apple-green intracytoplasmic inclusions (dust-like particles to large, round/oval pinhead shape).

8.3.1. VALIDATION

A diagnosis session is considered valid provided that:

- Fluorescence is detected in positive controls
- No staining is detected in the negative control

8.3.2. INTERPRETATION

Results for a test sample is reported as positive or negative for rabies based on observed staining in test sample and provided that the test is validated:

- The sample is positive if specific fluorescence is detected in the sample.
- The sample is negative if no specific staining is detected in the sample.