WORK PROGRAMME of EURL for RABIES

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EURL for Rabies
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INTRODUCTION

The ANSES Nancy Laboratory for Rabies and Wildlife has been nominated as European Union Reference Laboratory (EURL) for rabies on 1st July 2008.


The main objective of the EURL for rabies is to coordinate the network of National Reference Laboratories (NRLs) for Rabies to obtain high quality results by the provision of reference methods, evaluation of new techniques or reagents, proficiency testing schemes and training to laboratory staff. They therefore support the creation of a well-performing disease surveillance network throughout the European Union.
Regulation (EU) 625/2017 Art 94(2):
European Union reference laboratories designated in accordance with Article 93(1) shall be responsible for the following tasks insofar as they are included in the reference laboratories’ annual or multiannual work programmes that have been established in conformity with the objectives and priorities of the relevant work programmes adopted by the Commission in accordance with Article 36 of Regulation (EU) No 652/2014:
(taking into account Art 147 of (EU) 2017)

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- **Art. 94.2.a** Providing national reference laboratories with details and guidance on the methods of laboratory analysis, testing or diagnosis, including reference methods.

- **Art. 94.2.b** Providing reference materials to national reference laboratories

- **Art. 94.2.c** Coordinating the application by the national reference laboratories and, if necessary, by other official laboratories of the methods referred to in point (a), in particular, by organising regular inter-laboratory comparative testing or proficiency tests and by ensuring appropriate follow-up of such comparative testing or proficiency tests in accordance, where available, with internationally accepted protocols, and informing the Commission and the Member States of the results and follow-up to the inter-laboratory comparative testing or proficiency tests.

- **Art. 94.2.l** Where relevant for their area of competence, cooperate among themselves and with the Commission, as appropriate, to develop methods of analysis, testing or diagnosis of high standards.

Sub-activity 1.1 (Update of EURL procedures)

**Objectives:** To provide the NRL network with the most up-to-date detailed techniques of laboratory analysis.
**EURL for Rabies**

**Description:** The EURL reference procedures will be revised and updated according to the international reference standards (OIE guidelines). All the documentation will be available on the EURL website for consultation and download.

**Expected Output:** To ensure the dissemination of up-to-date versions of reference procedures and consequently helping in maintaining a high-quality level of harmonised techniques within the network.

**Duration:** 2018-2020 (annual activity)

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**Sub-activity 1.2 (Inter-laboratory test antigen detection using dRIT)**

**Objectives:**
The EURL wishes to organise an inter-laboratory (ILT) assay to evaluate laboratories in the handling of the Direct Rapid Immunohistochemical Test (DRIT) which should be recognised in 2018 as a new OIE reference method for rabies diagnosis.

**Description:**
The DRIT was developed at the CDC in the 2000’s. Like the Direct Fluorescent Antibody (DFA) test, the DRIT detects viral antigens in the central nervous system of mammals (RABV and other lyssaviruses). This method is a simplified version of the standard avidin-biotin complex immunohistochemical diagnostic assay. It is already in routine use in North America for support of oral wildlife rabies vaccination programs. This technique is as specific and sensitive as the DFA. Because of its next inclusion in the Terrestrial Manual chapter on rabies (expected for May 2018), the EURL for rabies aims at organising an inter-laboratory assay to evaluate the performance of laboratories with this new method. To that end, a panel of 10 lyophilised brain samples will be prepared and distributed to all participants. The proficiency test in planned for 2019. Before the implementation of the proficiency test, and for an appropriate transfer of the technique into the EURL, an internal evaluation will be necessary (planned for 2018). Thus, the activities planned for 2018 do not consist in the realization of the ILT itself but in the internal preparation for this technique and in the starting of batch virus production. The EURL diagnostic team will be trained by the developer of the technique and internal evaluation will be performed. The objective of the internal evaluation is therefore to evaluate accuracy, fidelity and reproducibility to ensure that the EURL masters the technique before launching the ILT. This technique requires the purchase of monoclonal antibodies including a step of labelling and purification.

**Expected Output:**
Expected Output: Although the DRIT is more particularly indicated for laboratories with limited resources (in Africa and Asia), this inter-laboratory test will contribute to determine whether or not the DRIT provides similar performances when used by different national reference laboratories for rabies and to evaluate the performance of the monoclonal antibodies cocktail that will be selected for this study. The results should contribute to have a clearer picture of the overall performance of the DRIT and determine the ease of use of the DRIT by unexperienced laboratories. During 2018, internal evaluation will be assessed and four batches of rabies virus will be produced to start to prepare the ILT panel test.

**Duration:** 2018-2019
**Sub-activity 1.3 (Annual review of the NRLs data)**

**Objectives:** The objective will be to supply the NRLs with an annual review on tests and analysis performed in the frame of rabies diagnosis carried out for surveillance and rabies control within the network. Assessment of the number of tests performed in EU Member States and in certain bordering countries for diagnosis, typing, virus titration, serology, tetracycline detection and age determination and to report their results and their trend at the European level will provide a better knowledge of the network, the techniques used and the needs for the future.

**Description:** An online questionnaire survey will be sent to each NRL from EU and certain bordering countries in February of each year to collate the data of the previous year. The data will be then analysed, maps and tables will be built and the report will be sent to all NRLs in June of the same year.

**Expected Output:** Production of an annual report of tests and analysis performed in the frame of rabies surveillance and control within the EU and certain bordering countries. Better information-sharing within the network. This annual survey and report allow the centralization of rabies laboratories data and to share and compare results of rabies programs between NRLs. All laboratory results (diagnosis, serology, tetracycline detection, etc.) are reviewed at national level, providing tables and maps that give a good picture of the overall situation at EU level.

**Duration:** 2018-2019-2020 (annual activity)

**Sub-activity 1.4 (Evaluation of a rapid diagnostic kit)**

**Objectives:** RIDT, a rapid diagnostic test, is a medical diagnostic test easy and quick to perform. The principal advantages of such tests are their suitability for preliminary or emergency medical screening and for use in facilities with limited resources while they provide results within only few minutes. The EURL wishes to organise an inter-laboratory assay to evaluate the repeatability and the reproducibility of a rapid immunodiagnostic test (RIDT) that has been extensively described and evaluated in the recent years by individual laboratories.

**Description:** The adaptation of Lateral Flow Assays (LFA) for rabies was first described in the mid-2000s. During the 10 past years, more than 11 publications relating the evaluation of LFA for rabies diagnosis have been published, demonstrating the growing interest for these immunochromatographic technologies. One of these LFA, the Rapid ImmunoDiagnostic Test (RIDT), has been extensively evaluated by individual laboratories and has provided promising results when compared to the gold standard Fluorescent Antibody Test. The EURL for rabies will organise an inter-laboratory assay to evaluate the repeatability and the reproducibility of the RIDT using a panel of 10 lyophilised brain samples (as prepared for the current proficiency tests for rabies diagnosis). Two different batches of RIDT will be investigated to detect potential performance variations between batches. The RIDT kits will be purchased by the EURL and dispatched to all laboratories to ensure that each participant evaluates the same batches.
**EURL for Rabies**

**Expected Output:** Although the RIDT is not intended (for the time being) to be a reference test for rabies diagnosis, nor to replace the current FAT and RTCIT, this inter-laboratory test will contribute to determine whether or not the RIDT provides similar performances when used by different laboratories and to evaluate batch-to-batch consistency of such immunochromatographic tests. The information obtained from the repeatability and reproducibility assessments, together with the sensitivity and specificity performances obtained in previous studies, will contribute to have a clearer picture of the overall performance of the RIDT and make it potentially suitable as a complementary test for rabies diagnosis. This comparative trial will be the only one that will be launched by the EURL in 2018 (A.1.2 is a preparation activity for 2019).

Rapid diagnostic kits are booming techniques developed for many pathogens. They do not require any special equipment (apart from protective equipment such as blouses, gloves, etc.) and are therefore promising techniques for easy animal disease screening.
TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- Art. 94.2.d Coordinating practical arrangements necessary to apply new methods of laboratory analysis, testing or diagnosis, and informing national reference laboratories of advances in this field.
- Art. 94.2.e Conducting training courses for staff from national reference laboratories and, if needed, from other official laboratories, as well as of experts from third countries.
- Art. 94.2.g Providing information on relevant national, Union and international research activities to national reference laboratories.

Sub-activity 2.1 (Training activities and scientific consulting)

**Objectives:** To provide full technical and scientific assistance to NRLs from EU MSs and bordering countries on laboratory analysis related to rabies control and surveillance. Training and consulting will be provided on request, and depending on the outcomes of proficiency tests.

**Description:** The Laboratory Lyssavirus Unit staff includes 15 people. The Unit is composed of 4 teams, each headed by an experienced scientist who can provide expertise, scientific and technical support under the rabies EURL mandate. The areas of expertise are diagnosis, molecular biology, virology, virus titration, biomarker determination, vaccinology and epidemiology.

Upon NRL requests, the EURL will organise training sessions on:
- rabies diagnosis,
- molecular biology, typing,
- rabies virus production (*in vivo* and *in vitro*), rabies virus titration,
- biomarker detection in teeth,
- antibody detection in wildlife (ELISA test).

The training will take place in the EURL (column “training”) or in the facilities of the trained laboratories (column “mission” for the EURL staff).

**Expected Output:** To maintain an harmonised and high level laboratory analysis capacities within the EU.

**Duration:** 2018-2019-2020 (annual activity)
Sub-activity 2.2 (Website management and newsletter)

**Objectives:** To facilitate and accelerate the dissemination of information.

**Description:** The EURL for Rabies has established an internet website dedicated to the NRLs network. The website is hosted at https://eurl-rabies.anses.fr and allows consultation of news and events dealing with rabies in the EU, the NRL network presentation, the EURL activities and reports, workshop presentations, including the work programmes and technical reports. Each NRL has received a login and a password giving an access to the documentation, training list, reagent catalogue, etc...

The website will be regularly updated with the news of the network and a newsletter will be prepared every two months and sent to the NRL network to facilitate the diffusion of these updates. The newsletter will include the news of the EURL activities, the news of the NRL network, the agenda of forthcoming events linked to rabies, the rabies notifications and a selection of recent publications on rabies laboratory techniques and epidemiology in Europe.

**Expected Output:** Improvement of rabies knowledge dissemination. EURL procedures available for NRLs in the website. The list of NRLs (in public access) and the list of reference reagents and their supplier will be updated.

**Duration:** 2018-2019-2020 (annual activity)

Sub-activity 2.3 (Organising annual Workshop for Rabies)

**Objectives:** To facilitate the networking as well as the sharing of information within the NRLs network.

**Description:** On an annual basis, the EURL for rabies will organise a workshop for gathering all EU National Reference Laboratories for rabies and several laboratories from certain third countries after consultation and agreement of the EC. The workshop is the opportunity to share information on rabies actualities and on the work that has been carried out during the year. Participants might be invited to deliver a presentation especially for participants from countries where rabies still occurs.

**Expected Output:** Satisfaction of the participants (assessed through a survey questionnaire) and high number of NRLs attending the meeting.

**Duration:** 2018-2019-2020 (annual activity)

Sub-activity 2.4 (Participation in meetings or congress)

**Objectives:** Keeping abreast of development in surveillance, epidemiology and prevention of rabies throughout the world.

**Description:** One expert of the EURL will attend the annual international rabies conference (RITA) that will be held in Mexico to be informed on rabies scientific advances on epidemiology, vaccination and laboratory techniques. Such conference participation will also be the opportunity for the EURL staff to share its experience and to present results on the ongoing projects to the worldwide rabies scientific community.
Expected Output: Maintaining strong skills of the EURL scientists.

Duration: 2018-2019-2020 (annual activity)

TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO THE EUROPEAN COMMISSION AND OTHER ORGANISATIONS

Please, provide activities related to Regulation (EU) 2017/625: (Number of Sub-activity boxes can be adjusted by EURL)

- Art. 94.2.f Providing scientific and technical assistance to the Commission within the scope of their mission.

- Art. 94.2.h Collaborating within the scope of their mission with laboratories in third countries and with the European Food Safety Authority (EFSA), the European Medicines Agency (EMA) and the European Centre for Disease Prevention and Control (ECDC).

- Art. 94.2.i Assisting actively in the diagnosis of outbreaks in Member States of foodborne, zoonotic or animal diseases, or of pests of plants, by carrying out confirmatory diagnosis, characterisation and taxonomic or epizootic studies on pathogen isolates or pest specimens.

Sub-activity 3.1 (Scientific consulting)

Objectives: To offer full scientific assistance to the Commission and to other European institutions (EFSA, EMA, ECDC).

Description: At request, the EURL staff composed of five scientists of whom each has its own expertise in a defined rabies area (Diagnosis, vaccinology, virology, serology, molecular biology, epidemiology and oral vaccination control) will provide assistance to the European institutions.

Expected Output: Providing skilled scientific and technical advices.

Duration: 2018-2019-2020 (annual activity)

Sub-activity 3.2 (Diagnosis confirmation and/or typing)

Objectives: To provide rabies diagnosis confirmation, virus isolation and typing at request.
**EURL for Rabies**

**Description:** In case of unexpected outbreaks, of questionable results, and of any unexpected results within a NRL, the EURL will provide case confirmation. Once sent by the NRL, the EURL will examine the submitted sample(s) using FAT, RTCIT and molecular biology techniques. In case of positive diagnosis, sequencing of the partial N genome will be performed. In the situation of the detection of a vaccine-induced case, or a newly identified species, a full genome analysis (NGS) will have to be undertaken for an accurate genetic characterisation. FTA® papers, for the isolation, purification and storage of nucleic acids, will be offered to NRLs to simplify and reduce the cost of sample shipping (only if partial gene sequencing is needed as NGS techniques require to obtain organic tissue, which requires a specialised transport in dry ice).

**Expected Output:** Assisting actively NRLs in the diagnosis of outbreaks or unexpected or unexplained diagnosis results.

**Duration:** 2018-2019-2020 (annual activity)

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**Sub-activity 3.3 (Phylogeographic study of rabies in Romania)**

**Objectives:** To analyse the spreading pattern of rabies in Romania.

**Description:** Phylogeography is the study of the processes responsible for the geographic spread of a disease. In the frame of rabies, despite the fact that the European Union managed to quasi-eliminate the disease from its area, no recent tools have been used to characterise the dispersal movement of the virus over time. Using population genetic diversity analysis could provide more insights in the virus historical/biogeographic processes. The study will include around 100 samples, sampled in the laboratory archive, from different period and different representative areas. Virus samples will be firstly tested in the NRL by referenced diagnosis techniques and then analysed by molecular biology methods in the EURL before their propagation by MIT (mouse inoculation test). Extracted rabies RNA will be amplified by NGS at the ANSES-Ploufragan National Genomics Platform (France). Full genome sequences will be analysed for determining the rabies variant in the country as well as included in the phylogeography analysis for studying the dispersal of strains throughout the studied zone. As stipulated in the rabies EURL mandate (Regulation No 415/2013 Annex 1, point 1.g) the EURL will thus “characterize the rabies virus by the most modern methods to allow a better understanding of the epidemiology of this disease”. This project is therefore part of this context as it involves analyzing the strains of Romania with the latest molecular tools and then using the latest techniques in bioinformatics. This will make it possible to trace the route of the different rabies strains in an UE country, study that, to our knowledges, has not yet been done.

**Expected Output:** Acquisition of further knowledge on the geographical spread of the rabies virus in Romania, to forecast its responses to biogeographic barriers and to gain unique insights of its interactions with the environment.

**Duration:** 2018-2019-2020
Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- Art. 94.2.j Coordinating or performing tests for the verification of the quality of reagents and lots of reagents used for the diagnosis of foodborne, zoonotic or animal diseases and pests of plants.
- Art. 94.2.k Where relevant for their area of competence, establishing and maintaining:
  i. reference collections of pests of plants and/or reference strains of pathogenic agents;
  ii. reference collections of materials intended to come into contact with food used to calibrate analytical equipment and provide samples thereof to national reference laboratories;
  iii. up-to-date lists of available reference substances and reagents and of manufacturers and suppliers of such substances and reagents.

Sub-activity 4.1 (Development and maintenance of the EU rabies virus collection)

**Objectives**: To maintain and develop the EU rabies virus collection.

**Description**: The choice of new rabies virus strains to be included in the virus collection will depend on outbreaks and collaboration opportunities. Approximately 4 new strains will be included in the collection each year. New strains will be collected, produced in vivo on mice and stored in liquid nitrogen. The batch will be validated using FAT (Fluorescent Antibody test), RTCIT (Rabies Tissue Culture Infection test) and molecular biology techniques (Real-time PCR test). Each new strain will also be sequenced for its genetic characterisation.

**Expected Output**: Variability enlargement of strains available for the benefits of the NRL network.

**Duration**: 2018-2019-2020 (annual activity)
## REQUIREMENTS RELATED TO OTHER LEGISLATION

Please specify applicable legislation: Nil

### Sub-activity 5.1 *(name of Sub-activity)* Nil

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### Sub-activity 5.2 *(name of Sub-activity)*

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### Sub-activity 5.3 *(name of Sub-activity)*

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### Sub-activity 5.x *(name of Sub-activity)*

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No remarks