European Union Reference Laboratory for Rabies

WORK PROGRAMME 2016-2017

I. Legal duties

The functions and duties of the European Union Reference laboratory (EURL) for rabies are described in the Commission Regulation (EU) No 415/2013 of 6 May 2013 laying down additional responsibilities and tasks for the EU reference laboratories for rabies, bovine tuberculosis and bee health and amending Regulation (EC) No 737/2008 designating the EURL for crustacean diseases, rabies and bovine tuberculosis. The Commission Regulation (EU) No 737/2008 also amends Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council.

II. Objectives for the period January 2106 - December 2017

The objectives of the work programme 2016-2017 are based on those given in the Regulation (EC) N°882/2004 (Article 32 (7)) and in the Regulation (EU) 652/2014 laying down provisions for the management of expenditure relating to the food chain, animal health and animal welfare, and relating to plant health and plant reproductive material. In this latter Regulation, the general objective of EC through EURLs is to contribute to a high level of health in humans, animals and plants, ensuring a high level of protection for consumers and the environment, whiles favouring competitiveness and creation of jobs.

Four operational objectives of the EC with indicators and expected results were previously provided:

- Objective 1. To ensure the development and use of high quality analytical methods across the EURL framework
- Objective 2. To maintain appropriate level of proficiency testing ensuring efficiency of control analysis methods
- Objective 3. To ensure the availability of scientific and technical assistance provided by the EURLs
- Objective 4. To ensure a sound and efficient management of EURL funding cycle.

This work programme has been established considering those four objectives for which the expected results and indicators are mentioned according to the different activities undertaken.

Activity 1: Technical support

The EURL will provide full assistance to the NRLs concerning their requests as regards laboratory techniques related to rabies diagnosis, typing and follow-up of oral rabies vaccination (ORV) campaigns.

This activity is in compliance with the objective 3. The overall indicator is the satisfaction degree provided by the NRLs, the expected results are that the EURL for rabies respond timely and adequately to all the assistance requests (both are assessed in the annual survey of NRLs satisfaction).

Sub Activity 1.1:
Technical support: producing, controlling, storing and supplying biological materials and virus collection

The EURL rabies virus collection will be maintained (storage in liquid nitrogen). Depending on outbreaks and opportunities, new rabies virus strains will be produced and stored to enlarge the rabies virus collection.

The biological materials that will be available for rabies diagnosis to the NRLs are:

- Positive controls infected with RABV, EBLV-1, EBLV-2, ABLV, DUVV, BBLV species (strains available in the laboratory and subject to the consent of the owner of the strain) and negative controls for rabies diagnosis and for typing;
- Lyophilised preparations of fixed reference viruses (CVS 11 for *in vitro* tests and CVS 27 for *in vivo* tests).

The biological materials and facilities that will be available for follow-up of oral vaccination campaigns are:

- CD-ROM describing the operating procedure for determining tetracycline presence;
- Fox teeth samples (positive and negative controls for determining tetracycline presence);
- Wildlife blood samples.

Other technical support available to the NRLs:

- Experimental station capacities with mice, cats, dogs, foxes and raccoon dogs: support to laboratories willing to obtain strains of certain rabies viruses produced on animals.

Sub Activity 1.2:
Technical support: confirmatory tests for diagnosis, follow-up of oral vaccination campaigns and typing

The EURL will receive, examine and report on samples submitted by EU Member States and type strains from NRLs upon request. FTA® papers will be offered to NRLs to simplify and reduce the cost of shipping samples.

This activity is part of the objective 1 and should allow the performance of high quality methods with reliable results throughout the NRLs network.

Activity 2: Training activities

The Lyssavirus Unit of the laboratory is headed by Dr Florence Cliquet. The Unit is composed of 4 teams represented by 15 agents. Each team is 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, serology, virology, virus titration and epidemiology.

Upon NRL requests, the EURL will organise training sessions on

- rabies diagnosis techniques,
- typing of rabies isolates,
- rabies virus titration,
- biomarker determination,
- serological analyses for wildlife.

The trainings will take place in the EURL (column “training”) or will take place in the facilities of the trained laboratories (column “mission” for the EURL staff) according to the needs outlined by the inter-laboratory test results.

This activity is fully responding to the objectives 1 and 3.

Activity 3: Inter-laboratory tests, data collection

The objective 2 is assessed through this activity. It is expected that all NRLs complete testing successfully.

Sub Activity 3.1: Inter-laboratory tests to evaluate rabies diagnostic tests (FAT, RTCIT, Real Time PCR, RT-PCR)

To follow-up the performance of NRLs on rabies diagnosis, an inter-laboratory test on the fluorescent antibody test (FAT), rabies tissue culture inoculation test (RTCIT) and on the molecular biology techniques (RT-PCR and real time PCR) will be conducted annually, in 2016 and in 2017.

The different steps of the trials are the followings:

- Contacting all NRLs EU laboratories (and possibly some from third countries after consultation and approval of the EC) to establish a list of interested laboratories;
- Producing positive and negative reference materials (ten new batches will be produced for the need of the trial. A minimum of one month is necessary to produce and validate a new batch of virus *in vivo*);
- Testing validity, stability, homogeneity of the constituted panel;
- Distributing a panel of characterised samples for inter-laboratory comparison and validation;
- Interpreting all results of participating laboratories, then writing and dispatching a synthesis report.

Sub Activity 3.2: Collecting and analysing data and information on the methods of rabies diagnosis used by laboratories

In parallel to their participation in the inert-laboratory test, the procedures used by Member States for rabies diagnosis (FAT, RTCIT, RT-PCR, Real Time RT-PCR) will be collected via questionnaires on the techniques employed. Each step of the protocols will be analysed for all laboratories and compared to the OIE or/and WHO reference tests. On the basis of the inter-laboratory test results and the synthesis of procedures used in Member States, recommendations on key points to consider in each step of the procedures will be included in the inter-laboratory report. The objective of the EURL is to ensure a satisfactory level of rabies diagnosis performance into the EU and to propose recommendations in case of detection of critical gap in the technique used.

Sub Activity 3.3: Inter-laboratory test to evaluate serological tests used by NRLs for the follow-up of ORV campaigns

The EURL will organize a proficiency test on serological techniques performed by the NRLs by using wildlife samples collected in the field. The main objective of this proficiency test will be to have a global overview of the performances of the techniques and protocols undertaken by the NRLs to titrate the rabies antibodies in wildlife samples. Considering the number of different techniques in use in the EU (seroneutralisation tests and ELISA tests), an ultimate objective for next years would be to try to get a better harmonization of results obtained by the NRLs.

Sub Activity 3.4: Inter-laboratory tests to evaluate tetracycline and age determination techniques

The technique of tetracycline (TTC) and age determination is widely used within the EU in the frame of oral vaccination follow-up. Most of vaccine baits include tetracycline to provide a life-long marking of bones and teeth of the bait consumers. When applying oral rabies vaccination, international institutions (WHO, OIE, EC) recommend controlling the vaccination effectiveness by notably analysing the presence of fluorescence in fox and raccoon dog teeth. To evaluate the performance of NRLs following the first (2010), second (2012) and third inter-laboratory test (2014), a third inter-laboratory test will be conducted.

The different steps of the trials are the followings:

- Contacting all EU laboratories (and possibly some from third countries after consultation and agreement of the EC) to establish a list of interested laboratories;
- Collecting positive and negative reference materials (red fox jaws issued from vaccinated areas);
- Testing half jaws to characterize the sample (positive, negative for TTC, age determination);
- Constituting a panel with the remaining half jaws;
- Distributing a panel of characterised samples for inter-laboratory comparison and validation;
- Collecting the methods used by participating laboratories using an online technical questionnaire;
- Interpreting all results of participating laboratories and analysis of the techniques used, then writing and dispatching a synthesis report.

Sub Activity 3.5: Collecting and analysing data on tests carried out in the EC

Every year, the EURL will organize an annual survey on tests and analysis performed in each NRL. This will help to evaluate the number of tests performed in EU Member States for diagnosis, typing, virus

titration, serology, tetracycline detection and age determination and to report their results at an European level.

Activity 4: Workshop, meetings and network management

Sub-activity 4.1: Organising an annual workshop for NRLs

On an annual basis, the EURL for rabies organises 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.

In 2016, the workshop will take place in France in April 2016 (date yet to be determined). The workshop will focus on:

- Proficiency tests for rabies diagnosis: We will present the last NRLs inter-laboratory tests for rabies diagnosis (2015 session). For the first time, the lowest proportion of laboratories with discordant results was found in Real Time PCR and not in the FAT (gold standard technique). Laboratories are more and more performant in molecular biology techniques and definitely more performant with these novel tools than with the RTCIT (which is recommended as a confirmatory test by both OIE and WHO while molecular techniques are still not We would like to open a discussion on such results, the possible change in the designation of reference techniques and their implication in the routine diagnosis of rabies.
- Rabies conjugates: During last summer, the laboratories had to face a shortage of anti-rabies conjugate (Fujirebio conjugate). The new produced batches received by laboratories were not able to detect EBLV strains properly. The information has been shared within the network. We would like to discuss on the impact and the different measures taken in the different NLRs following this incident.
- Presentations from NRLs: *Lithuania*: After being declared rabies free country according to the OIE criteria, one case has been detected in last October and a second case this month. We will invite the NRL to give an update of the rabies surveillance situation in Lithuania. *Norway*: We will invite the NRL from Norway to discuss on the first detection of a bat rabies strain on their territory (EBLV-2 isolated in 2015, confirmed by the EURL). *Romania*: Romanian NRL has isolated a rabies vaccine induced case in a cow (case confirmed by the EURL). This is the first identification on such case on a domestic animal (cow). We would like to invite our Romanian colleague to discuss on this particular case. *France*: Two significant events have occurred in 2015 on the French territory. The coming workshop will be the opportunity to share information on these two events: rabies detection in a dog in Guyana and rabies case importation from Algeria (via Hungary).

The progress in the evaluation of the rabies qPCR techniques performed in the EURL in 2015 will also be presented. Recent rabies activities (laboratory techniques and rabies surveillance) will also be presented by some participating laboratories and discussed within the network.

In spring 2017, the workshop will take place in Spain and will focus on:

- Proficiency tests for rabies serology: In 2016 will be held the first inter-laboratory test on the serological techniques used for the follow-up of oral rabies vaccination (ORV) campaigns. Currently, a wide range of techniques and material are used to assess the herd immunity level in

the ORV targeted population. The 2017 workshop will be the opportunity to conclude on this study and on the comparability of the techniques. Further steps to investigate, if any, will be debated with all NRLs.

- Proficiency tests for rabies diagnosis: WHO is currently opening a discussion to potentially include the real Time PCR as a reference technique (WHO meeting of 09 December 2015). We would like to discuss on this matter and to share on WHO expert meeting outcomes in 2017.
  Presentations from NRLs: In the 2017 workshop will be discussed the key points of the rabies laboratory activities and the significant rabies events of 2016.
- Work programme 2018-2019: Because it will be the end of the two year program of the EURL, including the end of the real time PCR evaluation project, 2017 workshop would be the opportunity to discuss on the new challenges in rabies diagnosis and techniques of ORV follow-up, needs and collaborative study opportunities of the laboratory network.

Sub-activity 4.2: Keeping abreast of development in surveillance, epidemiology and prevention of rabies throughout the world

The EURL will attend and participate in the annual international rabies conference in 2016 and in 2017 (RITA congress) to share its experience in epidemiology and virology in regards to rabies.
The EURL will also provide the European Commission and NRLs with scientific advice and technical assistance at his request.

Sub-activity 4.3: Website management

A new internet website dedicated to the NRLs network went online in 2014. 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 password giving an access to the documentation, training list, reagent catalogue, etc...
The website will be regularly updated and a monthly newsletter including the news of the website will be prepared and sent to the NRL network.

Activity 5: Evaluation and development of techniques

Sub Activity 5.1: Comparison and evaluation of the different Real Time PCR methods

The EURL demonstrated in 2015 an identical sensitivity of detection for six one-step TaqMan qRT-PCR kits currently used in NRLs, regardless of the machine used. We found a limit of detection at 95% (LOD95%) of approximately 20 copies/µl of RNA for the 6 tested kits, using validated rabies primers and TaqMan probe. Based on these previous results, we propose to continue the comparison of real-time PCR methods with the evaluation of four published primers/labelled probes currently used in laboratories for the amplification of specific RABV. In a first step, we will determine the performance of the method (LOD95%) using two synthetic RNA controls (a Greek fox isolate and the fixed rabies strain CVS-27), the four selected couples of primers/TaqMan probes and the most efficient one-step Taqman kit (RNA UltraSense). Following this preliminary study, the specificity of the four couples of primers/probes will be

undertaken on a collection of rabies virus held in the EURL laboratory. An additional collection of rabies virus fixed on FTA paper will be performed and will complete the specificity study.

The overall objective of this activity is to ensure the development of new high quality/state of art analytical methods for possible use in a next future for routine rabies diagnosis, as such methods are still not recommended as reference tests.