European Union Reference Laboratory for Rabies

WORK PROGRAMME 2015

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 - December 2015

The objectives of the work programme 2015 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 helath 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 are provided in Annex 1 of the Regulation relating to work programme 2015:

- 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 mentionned 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 vaccination 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 (multi-annual)

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).

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 (multi-annual)

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 obtention of high quality methods through 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, virology, virus titration and epidemiology.

Upon NRL requests, the EURL will organise training sessions on

- rabies diagnosis,
- typing virus isolates,
- rabies virus titration,
- biomarker determination.

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).

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

Activity 3: Inter-laboratory tests, data collection and technique evaluation

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) (annual)

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 in 2015.

The different steps of the trials are the followings:

- Contacting all European laboratories (and possibly some from third countries after consultation and agreement 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 (annual)

The procedures used by Member States for rabies diagnosis techniques (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: Collecting and analysing data on tests carried out in the EC (annual)

The EURL will request an annual report from 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.

Activity 4: Meetings, workshop and network management

Sub-activity 4.1: Organising an annual workshop for NRLs (annual)

On an annual basis, the EURL for rabies organise a workshop for gathering all European National Reference Laboratories for rabies and several laboratories from certain third countries after consultation and agreement of the EC. The meeting 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 2015, the workshop will take place in Croatia, Zagreb, on 27-28 May 2015. This workshop location has been chosen to celebrate the entry of the Croatia into the European Union and therefore the entry of the laboratory into the network of European National Reference Laboratories for Rabies. The workshop will focus on the last inter-laboratory tests results for diagnosis and on biomarker (Tetracycline) assessment in red fox jaw. The progress in the evaluation of the rabies qPCR techniques performed in the EURL in 2014 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.

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

The EURL will attend and participate in meetings and conferences (RITA congress expected in 2015) in epidemiology and virology in regards to rabies (with prior Commission' agreement, cost of 3000 euros expected) and will also provide the European Commission with scientific advice and technical assistance at his request.

The EURL could also organize a sharing experience meeting with some specialists of the Center for Disease Control (USA) in Nancy. The objective will be to share the EU and United State experience in the management of laboratory network for the harmonisation of rabies diagnosis techniques. This could help in developing or keeping up to date a high quality harmonisation programme (expecting cost for the meeting 5000 euros).

Sub-activity 4.3: Website management

An Internet website on the EURL's activities went online in 2010. The website is hosted at http://www.ansespro.fr/eurl-rabies and allows consultation of the network presentation, EURL 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, strain database, etc...
A new more dynamic version of the current EURL website should be online in 2014. The EURL wishes to extend the use of this communication tool to facilitate the share of information regarding rabies and the associated laboratories activities actuality.

Activity 5: Research Activities

Sub Activity 5.1: Comparison and evaluation of the different Real Time PCR methods (multi-annual)

The EURL demonstrated in 2014 (publication under submission) that the choice of master mix reagents, the concentration of primers, the analytical platforms linked to the real-time detection platform and the methodology used (one-step vs. two-step systems) can impact the sensitivity of results for SYBR GREEN real-time RT-PCR assays showing that PCR protocols should be systematically optimized and validated for different instruments. We found a better sensitivity of detection for optimized one-step PCR assays compared to optimized two-step assays whatever the machine used.

Based on these previous results, we propose to undertake in 2015 a cross-platform evaluation of TaqMan one-step real time RT-PCR master mixes kits. The TaqMan one-step RT-PCR, which is commonly used in NRLs, differs of the SYBR Green real time RT-PCR, due to the presence of primers/labeled probes leading to a more specific result. The selected master mixes will be those used in NRLs techniques (n=7) and the machines used will be the ones of the laboratory showing different throughput capacities and real-time detection platforms. The study will be focused on pan-Lyssavirus primers and TaqMan probes currently used in the NRLs to amplify specifically the rabies virus. Following this study, the evaluation of published primers/labeled probes allowing the specific rabies virus amplification will be undertaken on the more efficient one-step kits. This activity will consequently need to purchase probes and primers (subcontracting).

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.