European Union Reference Laboratory for Rabies

WORK PROGRAMME 2014

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 2014

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.

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.

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 training 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).

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

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, real time PCR) will be conducted in 2014.
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: Inter-laboratory tests to evaluate tetracycline and age determination techniques

The technique of tetracycline (TTC) and age determination is widely used within Europe 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) and second inter-laboratory test (2012), a third inter-laboratory test will be conducted.

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;
- 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;
- Interpreting all results of participating laboratories, then writing and dispatching a synthesis report.

Sub Activity 3.3: 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 recommendations is to obtain, as far as necessary and possible, the standardisation of these methods within Europe. Harmonized Standard Operating Procedures will be created or updated according to the inter-laboratory test and questionnaires analysis conclusions.

Sub Activity 3.4: Collecting and analysing data and information on the methods of tetracycline and age determination used by laboratories (annual)

The procedures used within EU Member States for Tetracycline and Age determination on red fox tooth will be collected via technical questionnaires. Each step of the protocols will be analysed for all laboratories. A report will be established with a synthesis for all procedures; special attention will be given to technical points that are different or adapted from the existing test.

Sub Activity 3.5: 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.

Sub Activity 3.6: Comparison and evaluation of the different Real Time PCR methods (multi-annual)

The EURL will continue to perform the comparison of the Real Time RT-qPCR using one step and two step RT-PCR kits on several species of rabies virus held by the EURL. The "in-house" methods will be finally compared to commercially available kits for the determination of the analytical sensitivity (LOD) and performance of tests. While 2013 focused on SYBR Green® comparison techniques, 2014 will start to assess the comparison of the TaqMan® techniques. The final purpose of this activity is to assess the sensitivity and specificity of the various qPCR techniques that will act as support for the qPCR standardisation.

Activity 4: Meetings and workshop

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 2014, the workshop will focus on a reference technique for rabies diagnosis using cell culture and on the harmonisation of the molecular biology methods for rabies diagnosis.

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 2014) in epidemiology and virology in regards to rabies (with prior Commission' agreement) and will also provide the European Commission with scientific advice and technical assistance at his request.

Activity 5: Website management (multi-annual)

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 all EURL reports, including the work programmes and technical reports. Each NRL has received a login and password giving an access to the documentation, reagent catalogue, strain database, etc...
A new EURL website version is under preparation for 2014.
The EURL website presenting the EURL's aptitudes and activities, a list of the NRLs, news of the laboratory network and agenda of EURL activities will be updated regularly.

Activity 6: Research Activities

Research Activities: Molecular epidemiology of rabies in Baltic countries

The EURL will continue to carry out the collaboration with NRLs from Baltic countries to study the phylogeny and surveillance of rabies in Baltic countries. The work planned will not require expenses of reagents and equipment. The study is intended to gather and analyse all available results of rabies virus sequencing and laboratory results including rabies surveillance data in each of the 3 Baltic countries.