EUROPEAN HEALTH AND DIGITAL EXECUTIVE
AGENCY (HADEA)
Department A Health and Food Unit A2 EU4Health/SMP

Single Market Programme (SMP Food)

SMP-FOOD-2023-EURL-EURC-AG-IBA
Activities of the EU reference laboratories and EU reference centres in 2023-2024

SUBMISSION FORM: DESCRIPTION OF THE ACTION
(Annex 1 – Description of the action (part B))

SMP-FOOD-2023-EURL-EURC-AG-IBA

Activities of the EU reference laboratories and EU reference centres in 2023-2024

Applicant shall provide information on each question contained in the Form. The information filled in the Form, shall be clear, concise, consistent and complete.

For questions on the information requested in this Form, please contact: please contact: HADEA-EURL@ec.europa.eu.

For questions on the eGRANTS Portal Submission System, please contact the IT Helpdesk.

Applicant - COORDINATOR (Name of EURL)	EURL for Rabies
Topic	**Activities and programme of the EURL for Rabies**
Implementation period	**1/1/2023 – 31/12/2024**

CONTACT PERSON for the programme :

Name	Emmanuelle Robardet
Function	Director of the EURL for Rabies
e-mail	emmanuelle.robardet@anses.fr

ASSOCIATED PARTNER 1 (Name)	/
Contact person :	/
e-mail :	/

Contents

1. LIST OF ABBREVIATIONS AND KEY WORDS

Ct	cycle threshold
dPCR	Digital PCR
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked Immuno Assay
EMA	European Medicines Agency
EU	European Union
EURL	European Union Reference Laboratory
FAT	Fluorescent Antibody Test
FTA	Flinders Technology Associates
ILT	Inter-Laboratory Test
LD	Limit of Detection
LFA	Lateral Flow Assays
LFD	Lateral Flow Device
LOD	Limit of Detection at 95%
MTA	Mutual Transfer Agreement
NGS	Next Generation Sequencing
NRL	National Reference Laboratory
ORV	Oral Rabies Vaccination
PTL	Proficiency Testing Laboratory
RABV	Classical Rabies Virus named also *Rabies Lyssavirus*
RTCIT	Rabies Tissue Culture Infection Test
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
RT-qPCR	Real Time Reverse Transcriptase Polymerase Chain Reaction
WOAH	World Organization for Animal Health

2. INTRODUCTION

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 16 of Regulation (EU) No 2021/690:

(taking into account Art 147 of (EU) 625/2017)

The ANSES Nancy Laboratory for Rabies and Wildlife has been nominated as European Union Reference Laboratory (EURL) for rabies on 1st July 2008 (Regulation (EC) No 737/2008). 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. 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. These activities support the maintenance of a well-performing disease surveillance network throughout the European Union.

3. ACTIVITIES

3.1. TO ENSURE AVAILABILITY AND USE OF HIGH QUALITY METHODS AND TO ENSURE HIGH QUALITY PERFORMANCE BY NRLs

- *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 *(Work Package)* 1.1 *(Annual Review of NRLs Rabies data))*

Objectives: The objective will be to supply the NRLs with an annual review on tests and analyses performed in the frame of rabies surveillance (rabies diagnosis) and on tests performed in the frame of rabies control (serological and bait uptake survey performed after ORV). Assessment of the number of tests performed and results, trends, in EU Member States and in some bordering will be reported. This overview will provide a better knowledge of the laboratory network, the techniques used and potential needs for the future.

Description: An online-questionnaire survey will be sent each year in February to each NRL from the EU and certain bordering countries 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 analyses performed in the frame of rabies surveillance and control within the EU and some bordering countries. Better information-sharing within the network. This annual survey and report allows the centralisation 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: Annual activity

Sub-activity *(Work Package)* 1.2 *(Proficiency test on rabies diagnosis)*

Objectives: Comparability of surveillance data and, as a result, comparability of laboratory diagnosis capabilities is a critical element for a precise estimation of the epidemiological situation over large areas. Proficiency testing on rabies diagnosis will evaluate the individual performance of NRLs from the EU and certain bordering third countries on the current rabies diagnosis reference techniques and will monitor laboratories' continuing performance on their ability to draw an accurate rabies diagnosis conclusion. Participants will have to mimic their routine diagnosis process as much as possible.

Description: Performance of NRLs on rabies diagnosis will be assessed by conducting an inter-laboratory test on rabies diagnosis conclusion assessed on homogenates of brain samples to mimic as much as possible standard rabies diagnosis. Such a study will provide an estimation of rabies diagnosis performance at network level.

The different steps of the trials are the followings:

- Contacting all NRL of EU and some from third countries to establish a list of interested laboratories. Ten laboratories from third countries will be invited to take part for free. The list of expected third countries will be Albania, Bosnia and Herzegovina, Kosovo, Moldavia, Montenegro, North Macedonia, Serbia, Turkey, Ukraine. All third countries are neighbouring countries of EU where cooperation is critical for rabies elimination in EU. Indeed, most of them are or have been involved in control and monitoring programmes co-founded by the EC. Other countries like EU countries without designated NRL, Norway, Switzerland, will have the opportunity to take part but at their own expenses.

- Producing positive and negative reference materials. Four new batches will be produced per year for the need of the trial. A minimum of one month is necessary to produce and validate one batch of rabies 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.

Expected Output: Performance evaluation of NRLs from EU and some of the EU's neighbouring countries to maintain an adequate quality level of rabies diagnosis capacity within the network. In case of discrepant results, technical recommendations based on the technical questionnaire analysis will be established.

Duration:

Proficiency test for rabies diagnosis is organised every two years

In 2023 will occur the PTL evaluation.

Batch production will be performed in 2023 and 2024 for the 2025 PTL.

Sub-activity *(Work Package)* 1.3 *(Digital PCR)*

Objectives: To evaluate the digital PCR as a new molecular biology tool for routine rabies diagnostic method.

Description: Rabies diagnosis in animals relies on postmortem laboratory tests, as recommended by the World Organization for Animal Health (WOAH). The WOAH recommended diagnosis tests for clinical rabies case confirmation in animals are the Direct Fluorescent Antibody Test, the Direct Rapid Immunohistochemical Test, the Rabies Tissue Culture Infection Test, the Pan-Lyssavirus real–time and/or conventional PCR assays.

The detection of all known Lyssavirus species can be achieved by two types of molecular approaches, that are the SYBR Green RT-qPCR based on universal pan-Lyssavirus primers (described by Hayman et al., in 2011) and/or the pan-Lyssavirus TaqMan RT-qPCR (LN34 assay) described by Wadwha et al. in 2017.

Both approaches present advantages and limits. The EURL for rabies evaluated deeply the SYBR Green RT-qPCR in intra-laboratory condition and according to the NF U47-600 standard. We observed a detection limit of PCR of 50 copies/µL, 100% for the diagnosis sensitivity and 100% for the diagnosis specificity. Although pan-Lyssavirus SYBR Green RT-qPCR is sensitive, specific and allows the detection of all known Lyssavirus species, a dissociation curve analysis must be undertaken at the end of the real-time PCR to confirm the specificity of the test result. The dissociation curve analysis can sometimes be challenging, especially when samples are autolyzed or in the presence of PCR interferences. Similarly, in the case of degraded or mummified sample, non-specific amplification can take place and yield an erroneous result that is very difficult to interpret. Compared with the SYBR Green method, the TaqMan multiplexe probe RT-qPCR (LN34 assay) is based on the combination of degenerate primers and two probes to achieve high sensitivity and specificity. The TaqMan RT-PCR LN34 assay has been shown by Wadwha et al. (2017) to detect a wide range of RABV variants and other lyssaviruses as well as a limit of detection > 1 copy/µL. Moreover, the LN34 assay is described as able to eliminate the non specific amplifications from the non specific binding of the degenerated primers and/or primer dimers, making the implementation/adaptation of the LN34 assay easier in the laboratories equipped with real-time PCR for the detection of rabies virus RNA than the SYBR Green RT-PCR.

PCR-based techniques has been developed and extensively used over the last two decades for research. Since the 2000s, numerous RT-qPCR methods have been developed and published for the detection of lyssavirus RNA, either by conventional RT-PCR and/or by real-time RT-qPCR. Digital PCR is a new generation of traditional quantitative PCR which can be used for the absolute quantification of target nucleic acids. The digital PCR (dPCR) provides several advantages over real-time PCR, including more precise measurements and absolute quantification without the need for a standard curve. Since the 2010s, the dPCR has been performed for the detection of a variety of viruses, including human immunodeficiency virus, cytomegalovirus, human herpes virus and recently the virus SARS COV-2. The dPCR that relies on the detection of end point PCR products is less susceptible than qPCR to inefficient amplification, which can occurs due to primer and/or probe mismatches or inhibitors in samples. The dPCR is known as more reliable than qPCR for inhibition-prone samples. A good correlation has also been shown by Pavšič et al. (2016) between the dPCR and accurate viral load in clinical samples.

The EURL for rabies laboratory will be equipped with a digital PCR at the end of 2022, in the frame of collaborative research studies on SARS-COV-2 pandemy.Based on the ongoing validation study undertaken on the LN34 assay by real-time RT-qPCR, we propose to deeply evaluate the dPCR real-

time cycler by using the LN34 assay for a sensitive and rapid detection of RABV variants in Europe Union additionally to bat lyssaviruses.

In a first step, we will develop the LN34 assay on the dPCR real-time cycler . PCR parameters will be evaluated according to the NF U47-600 standard including the limit of detection of the PCR and of the method, as well as the cut off of the method for interpreting results. The inclusivity of the method, the diagnosis sensitivity and the diagnosis specificity will be performed in a second step in 2025.

Expected Output: The overall objective will be to evaluate this new high quality/state of art analytical method in an European rabies context for a possible use in a next future for routine rabies diagnosis.

Duration: 2023-2024

Sub-activity *(Work Package)* 1.4 *(Molecular biology inter-laboratory study)*

Objectives: The EURL wishes to organize an inter-laboratory test (ILT) to precise some method characteristics (sensitivity and limit of detection) of the real-time RT-PCR (SYBR Green RT-PCR, Pan-Lyssavirus (LN34) TaqMan RT-PCR and TaqMan RABV RT-PCR) in the context of detection of classical rabies virus (RABV). The evaluation will be done in two parts: one the PCR only and one the full method (extraction, reserve transcription and PCR).

Description: Real-time RT-PCR that presents the advantage to be rapid, specific and sensitive is increasingly used by NRLs for the confirmation of rabies diagnosis in animals. The ILT organized in 2022, based on stabilised RNA diluted in biological quality water, showed a variability in the sensitivity of the real-time RT-PCR in the participating laboratories for detecting low traces of RABV genome with highest discrepancies frequencies noted for weak positive RABV samples (28<Ct≤35) harbouring 20 – 30 % of discrepant results when using TaqMan RABV RT-PCR or SYBR Green RT-PCR, respectively.

The EURL wishes to organize in 2024 an ILT to evaluate more precisely the real-time RT-PCR for the specific detection of RABV by determining the limit of detection at 95% of the PCR (LOD95% of PCR) in NRLs. In conjunction to the determination of the LD PCR, the ILT will be developed to evaluate also the limit of detection of the full method (i.e. method= RNA extraction followed by real-time RT-PCR). Detailed instructions will be provided with the operating procedure that must be carried out. The instructions will be established with reference to the AFNOR NF-U-47-600 standard.

Briefly, the panel will be constituted by serial dilutions of a positive RABV RNA sample varying between 10^4 copies/µL to 1 copie/µL for determining the LOD95% of PCR and serial dilutions of a supernatant of brain homogenate spiked with different concentrations of RABV RNA (10^5 copies/µL to 1 copie/µL) for the determination of the LD Method. RNAs will be extracted from mouse brain samples (as prepared for the current proficiency tests for rabies diagnosis), validated and stored at -80°C before sending to the participating laboratories. All batches of RNAs will be validated for their homogeneity and stability at-80°C prior to sending. RNA samples being highly susceptible to

degradation, each panel will be shipped on dry ice (UN1845) to ensure that they remain frozen until the test is performed. The panel that will be provided will be used for the evaluation of only one method (the one routinely used by the lab).

Expected Output: This inter-laboratory test dedicated to the real-time RT-PCR will determine precisely the inter-laboratory sensitivity of this molecular method which is increasingly used in NRLs in replacement of the cell isolation test. This inter-laboratory test will also help to assess in the different laboratories the limit of detection of RABV genomes.

Duration: 2024

Sub-activity *(Work Package)* 1.5 *(ELISA kits results using various vaccines: inter-laboratory evaluation)*

Objectives: Two different ELISA kits are currently used within EU to assess anti-body presence in wildlife following ORV campaigns performed with several oral vaccines. This study will attempt to assess inter-laboratory variations when using ELISA kits on samples of animals vaccinated by new generation vaccines.

Description: In 2016-2017 and in 2019-2020, we organised two inter-laboratory studies on the rabies antibody detection in wildlife samples collected in orally vaccinated areas in Europe to overview the performances of the techniques currently used by the National Reference Laboratories (NRLs).

The first study demonstrated that two commercial ELISA have been mainly used by NRLs and satisfactory results were obtained with both kits whatever the origin and quality of the tested samples. However, only strong and mean positive samples as well as negative samples were tested in the first study. That is the reason why, we initiated a second study to have much more precise information regarding the performances of these two ELISA with samples having titers around the threshold of positivity.

Through this second study, we collected data and showed that the limit of detection obtained with the BioPro ELISA was lower than the one obtained with the Bio-Rad ELISA. We also underlined that the limit of detection varied for the same ELISA between participating laboratories.

However, these results cannot fully explain the huge discrepancies observed for several years, when monitoring the oral rabies vaccination (ORV) campaigns, between the results of the tetracycline detection and the level of rabies antibodies.

For these two studies, only the SAG2 oral vaccine (Virbac, France) was used to immunize animals, but the manufacture of this vaccine was stopped at the end of 2017 and is no longer used since then during ORV campaigns.

In the quite same time, new generations of oral vaccine have been developed by CEVA (IDT) (France) and Bioveta (Czech Republik). These oral vaccines have been used in the ORV campaigns done in European countries since 2018 and 2019 respectively.

The two ELISA kits, used to detect rabies antibodies in wildlife samples, were developed over 10 years ago. Therefore, the question of sensitivity of the current ELISA kits to detect antibodies elicited when using new generation of oral vaccines could be raised.

To try to answer this question, we propose to organize a new inter-laboratory study to assess the performances of these ELISA kits to detect rabies antibodies elicited specifically by these new oral vaccine generations in wildlife.

Expected Output: Evaluation of the influence of two ELISA kits on anti-body detection in animals vaccinated by different oral vaccines.

Duration: Start in 2024

3.2. TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

- *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 *(Work Package)* 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 testing.

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 need is estimated at two trainings activities per year, one at the EURL (column “training” of the budget) and one in the facilities of the trained laboratory (column “mission” of the budget).

Expected Output: To maintain a harmonised and high-level laboratory analysis capacity within the EU.

Duration: Annual activity

Sub-activity *(Work Package)* 2.2 *(Website management)*

Objectives: To facilitate the dissemination of information within the EU rabies network.

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.

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: Annual activity

Sub-activity *(Work Package)* 2.3 *(Organising annual Workshop for Rabies)*

Objectives: To consolidate the networking between NRLs as well as the sharing of information within the EU rabies network.

Description: On an annual basis, the EURL for rabies will organise a workshop for gathering all EU National Reference Laboratories for rabies and 10 laboratories from third countries as approved by the Commission Implementing Regulation (EU) No 135/2013. The list of expected third countries countries will be Albania, Bosnia and Herzegovina (two laboratories), Kosovo, Moldavia, Montenegro, North Macedonia, Serbia, Turkey and Ukraine. All third countries are neighbouring countries of EU where cooperation is critical for rabies elimination in EU. Indeed, most of them are or have been involved in control and monitoring programmes co-founded by the EC. Other countries like EU countries without designated NRL, Norway, Switzerland, Morocco, Tunisia and Algeria (North African countries are countries important to keep in collaboration with the network for rabies control within EU), will have the opportunity to take part in the meeting at their own expenses. The workshop gives the opportunity to share information on rabies actualities and on the work that has been carried out during the past 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: Annual activity

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

- *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 *(Work Package)* 3.1 *(Scientific consulting)*

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

Description: At request, the EURL staff composed of four 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 EU institutions (EUOHZ data and scientific validation by example).

Expected Output: Providing skilled scientific and technical advices.

Duration: Annual activity

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

Objectives: To provide rabies diagnosis confirmation, virus isolation and typing at request.

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 shipment (only if partial gene sequencing is needed as NGS techniques need to obtain organic tissue, which requires a specialised transport in dry ice). Support for phylogenetic studies of new cases will be offered.

Expected Output: Actively assisting NRLs in the diagnosis of outbreaks or investigation of unexpected or unexplained diagnosis results and in phylogenetic studies.

Duration: Annual activity

Sub-activity *(Work Package)* 3.3 *(Participation in meetings or congress)*

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

Description: Two experts of the EURL will attend annually the international rabies conference (RITA Rabies In the Americas) 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 European ongoing projects to the worldwide rabies scientific community. One expert will also attend another conference on Virology, Wildlife disease or Veterinary epidemiology depending on the opportunities (in animal virology and/or animal epidemiology conferences, depending on the costs).

Each network provides unique opportunities to share lessons learnt and challenges faced. The European Union being the only part of the world where rabies elimination in wildlife and domestic animals has been almost achieved, the participation of the EURL and sharing of the EU experience in such meeting could potentially be helpful for the rabies community. Also as specified in the EURL for rabies responsibilities, keeping abreast of development in surveillance, epidemiology and prevention of animal disease throughout the world is of utmost importance and such congress are unique opportunities to update such information.

Expected Output: Maintaining strong skills of the EURL scientists.

Duration: Annual activity

3.4. REAGENTS AND REFERENCE COLLECTIONS

- *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 *(Work Package)* 4.1 *(Development and maintenance of the EU rabies virus collection)*

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

Description: The EURL as constituted since his designation a rabies virus collection in long term storage (-150°C). New strains will be included in the collection each year. The choice of new rabies virus strains to be included in the virus collection will depend on outbreaks and collaboration opportunities. New collected strains will be produced in vivo on mice to impact as little as possible the concerned strain (virulence, genetic characteristics, etc.). 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 and made available to NRLs upon request and with original strain supplier agreement. For this, a MTA (Anses Mutual Transfer Agreement) will be conducted between the EURL and each parties (the supplier and the requester).

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

Duration: Annual activity

Sub-activity *(Work Package)* 4.2 *(Evaluation of rabies diagnosis related kits)*

Objectives: To evaluate new rabies diagnosis products found on the market.

Description: The objective of this activity will be to conduct a monitoring (in web markets by example) to detect new products available to European laboratories for rabies diagnosis.

The material could be constituted of Lateral flow assays (LFAs), also called immunochromatographic tests, or Lateral Flow Device (LFDs). 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.

Other type of kits that could be evaluated are commercial real-time RT-PCR kits used for rabies or RABV RNA detection. These molecular biology techniques include multiple steps all using specific reagents and kits that could have an impact on the performance of the technique. Newly identified

reagents will so be evaluated. To evaluate diagnosis sensitivity and specificity of kits on naturally infected samples, import of positive samples from endemic countries could be organised by the EURL.

Expected Output: Evaluation of new kit (rapid test, molecular biology kit).

Duration: Annual activity

Sub-activity *(Work Package)* 4.3 *(name of Sub-activity)*

Objectives:

Description:

Expected Output:

Duration:

3.5. REQUIREMENTS RELATED TO OTHER LEGISLATION

Sub-activity *(Work Package)* 5.1 *(name of Sub-activity)*

Objectives:

Description:

Expected Output:

Duration:

Sub-activity *(Work Package)* 5.2. *(name of Sub-activity)*

Objectives:

Description:

Expected Output:

Duration:

Sub-activity *(Work Package)* 5.3. *(name of Sub-activity)*

Objectives:

Description: Expected Output: Duration:

4. REMARKS

Previous budgets allocated to the EURL for Rabies since 2016 are the following: 2016-2017 -> 554,500 2018 -> 269,000 2019-2020 -> 555,000 2021-2022 -> 516,493 Thus, our budget is almost the same since years with an amount of 270-280,000 euros per year with the exception of the 2021-2022 period. 2021-2022 expected budget was really particular as submitted in 2022, one year after the start of the working period (2021). Following the COVID health crisis, missions (workshop, conference trips and training) did not restart on site (physically) and at a normal frequency until mid-2022. In consequence, when we submitted in 2022, we deleted from the expected budget all the usual cost for mission of 2021 to be as close as possible to the real expenses of this period, that was considerably reduced compared to previous years. In consequence, to establish the present program and budget (2023-2024), we referred to previous data with annual grants of 270-280,000 euros.