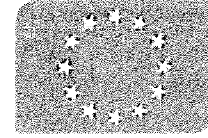

MAISONS-ALFORT LABORATORY FOR ANIMAL HEALTH

AND

DOZULÉ LABORATORY FOR EQUINE DISEASES

EU REFERENCE LABORATORY FOR EQUINE DISEASES

2014 Scientific program of the European Union Reference Laboratory for Equine Diseases

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RÉPUBLIQUE FRANÇAISE

This document describes the 2014 scientific program of the European Union reference Laboratory (EU-RL) for equine diseases. The described program is established in accordance with seven main activities which are listed as follows:

Activity 1: Equine Viral Arteritis (EVA), Equine Infectious Anemia (EIA) and Equine Herpes Viruses (EHV)
Activity 2: West Nile and other exotic encephalitis and Vesicular stomatitis
Activity 3: Dourine
Activity 4: Contagious equine metritis (CEM)
Activity 5: Glanders
Activity 6: Training
Activity 7: Coordination and management

Activity 1 : Equine Viral Arteritis (EVA) , Equine Infectious Anemia (EIA) and Equine Herpes Viruses (EHV)

Sub-activity 1 : Development and validation of diagnostic methods

Objectives: Equine Infectious Anemia (EIA)

✓ *Improvement of a molecular diagnostic test for EIA*

EIAV is a retrovirus related to the Human Immunodeficiency Virus (HIV). EIAV is a RNA viruse exhibiting a high rate of mutation. To date the only official test to diagnose a case is the Agar Gel Immuno-Diffusion (AGID) test that allow to detect antibodies anti-EIAV. This serological method is rather specific but not sensitive. Moreover, antibodies appeared 30days following the infection. During this incubation period the infected horse is infectious and can potentially transmit the virus to equids around. Moreover, some PCR and RT-PCR have been developed over the year but they do not detect a broad range of EIAV strain. In order, to shorten the time of quarantine and to identify a case quicker we have started a project to develop a molecular diagnostic tool that will allow toidentify EIA viruses from different origin. The research program "Set up a real-time RT-PCR for EIA diagnosis" started in 2012 will be continued and should be done by the end of 2014 in collaboration (Covetlab organization) with our colleagues Dr. R Bouwstra (CVI, Netherlands), Dr. F. Steinbach (AHVLA, UK), Dr. M. Jurelmam (SVA, Sweden).

✓ *Improvement of a serological diagnostic test for EIA*

The official test used to identify an EIA case in equid is the AGID test, also known as the Coggins test. This test is specific but lack of sensitivity. Some ELISA tests, faster to perform and more sensitive to the AGID have been developed over the past few years. Since ELISA tests are more sensitive and less specific compared to the AGID test, each positive sample with ELISA have to be confirmed in AGID. A case is identify and declared only when AGID test is positive. Sometimes, samples may be positive in ELISA and negative with AGID. AGID tests and ELISA tests allow the detection of antibodies anti-p26 only. The only serological method that is able to confirm an infection is the Western blot (WB). Indeed, this method allows the detection of antibodies recognizing different viral proteins. In order to improve the serological test available in Europe that will help to determine the status of such samples (positive in ELISA and negative in AGID), we propose to develop and validate a WB protocol that could be used by NRLs. First of all, EIAV viruses will have to be isolated and amplify on horse PBMC. These viral particles will be used as antigen for the WB.

✓ **Expected outputs** :

- Development and validation of a PCR/RT-PCR for the detection of EIA
- Implementation and validation of a the WB diagnostic tools for EIA

✓ **Performance indicators :**

- PCR/RT-PCR SOP available to NRLs
- WB SOPs available to NRLs

Sub-activity 2 : *Training and support to NRLs*

Objectives: Equine Viral Arteritis (EVA), Equine Infectious Anemia (EIA and Equine Herpes Viruses (EHVs)

- Positive cases will be confirmed according to NRLs requirements
- Biological samples (Viral strains, cell lines, sera, blood) will be provided upon NRLs requests.
- With reference to EVA and EIA PT results, training sessions will be organized for NRLs who faced sensitivity or specificity issues with their diagnosis tools.

✓ **Expected outputs :**
- Positive and doubtful cases will be confirmed or unvalidated
- Training of scientists and technicians from NRLs to varied diagnosis tools

✓ **Performance indicators :**

- *Number of confirmed and unvalidated cases*
- Supply of biological samples to NRLs.
- Number of scientist/technicians trained

Sub-activity 3 : Epidemiological and specific study

Objectives: Equine Viral Arteritis (EVA)

✓ *Construction of an infectious clone (Master/PhD Student)*

EVA belongs to the *Arteriviridae* family and infects equids only. He can be transmitted by the respiratory and veneral routes. Following the primary infection, the virus may persist in the reproductive tract of some stallions. Those stallions shed the virus in their semen and act as viral reservoir. Viral persistence in the reproductive tract of stallions seems to be testosterone-dependent but the molecular events associated to this persistence are poorly understood. In order to better understand those events, we proposed to construct an EAV infectious clone from the highly pathogenic EAV strains isolated in 2007 in Normandy, France that caused death of foals and multiple abortions. This project will be done in several years in collaboration with Dr. U Balasuriya from the Gluck Equine Research Center, Lexington, KY, USA.

✓ *Epidemiology and EAV characterization in Serbia*

A collaboration with the Scientific Veterinary Institute "Novi Sad" of Republic of Serbia (Dr. Diana Lupulovic) will start in 2014. The aim of this collaboration is to determine the seroprevalence, performing the virus neutralization test (VNT) of EAV in the Serbian stallions population as well as characterize virus that would be isolated from shedder stallions.

✓ **Expected outputs** :
- Cloning the full length genome of the EAV 2007 strain isolated in Normandy in a plasmid
- Sero and molecular epidemiology of EAV in Serbia

✓ **Performance indicators :**
- Obtain a clone of EAV 2007 strain
- Number of seropositive stallions and number of shedders stallions in republic of Serbia
-
- EAV characterization

Objectives: Equine Infectious Anemia (EIA)

✓ *EIAV characterization*

Molecular characterization of EIAV strains circulating in Europe should be done. This project is achieved in collaboration with the NRLs from Belgium, Italy, Germany and Romania. The aim of this study is to better understand and describe the different viral population of EIA viruses circulating in Europe. (project will be on several years)

✓ **Expected outputs** :
- EIAV characterization

✓ **Performance indicators :**
- Number of EIAV characterized

Sub-activity 4 : EIA Proficiency test and Workshop

Organization in 2014 of a proficiency test for EIA serological diagnosis using Agar Gel Immuno-Diffusion test (AGID).
This PT will be followed later during the fall season by a workshop

✓ **Expected outputs** :
- Success of NRLs in the organized EIA ring trial

✓ **Performance indicators :**
- Number of participants and average rate of success in PT test

Activity 2: West Nile, other exotic encephalitis and vesicular stomatitis

Sub-activity 1: Development and validation of diagnostic tools

Objectives: West Nile Virus (WNV) and other *flaviviruses* causing meningo-encephalitis in equids

✓ *Production of reference sera*

Reference samples (horse sera positive for IgG and/or IgM and EDTA blood from viremic horses) were obtained in 2013 through experimental infection of horses by the subcutaneous route with different flaviviruses, including lineage 1 and 2 West Nile viruses and Japanese Encephalitis virus. 5 horses were infected in BSL3 facilities (INRA PFIE platform, Nouzilly, France) and monitored for 2 months after infection. For easier shipment and longer lifespan, the above-mentioned reference sera will be lyophilised in 2014. In the first place, we will evaluate whether the lyophilisation process modifies or not neutralizing, IgG and IgM antibody titers in the reference sera. If not, sera will be lyophilised; alternatively, sera will be stored frozen. Reference sera will be shipped to NRLs upon request.

Moreover, viremia level and duration in reference EDTA blood will be evaluated and compared in a unique real-time RT-PCR assay (as the panflavivirus real-time RT-PCR protocol described by Patel et al, 2013), necessitating prior implementation and internal validation of this new assay. Upon results validation, the corresponding operating procedure will be published on the EU-RL website and reference EDTA blood will be inactivated and made available upon NRLs request.

Patel P, Landt O, Kaiser M, Faye O, Koppe T, Lass U, Sall AA, Niedrig M. 2013. Development of one-step quantitative reverse transcription PCR for the rapid detection of flaviviruses. Virol J. 10:58. doi: 10.1186/1743-422X-10-58.

✓ *Novel diagnostic tools = Implementation of Luminex assays for the multiplex detection of flavivirus antibodies*

The microsphere immunoassay multiplex technology (Luminex technology) has been implemented in 2013 at the ANSES laboratory for WNV serological diagnosis. The difficulty of flaviviruses serology is the antibody cross reactivity observed among members of the JEV serocomplex, as well as to a lesser extent between flaviviruses belonging to different serocomplexes. In such a context, the Luminex would offer possibility of multiplexing different flavivirus antigens and of comparing the antibody response against different flaviviruses in a single run. Recombinant envelope Domain III of different

flaviviruses (WNV, JEV, TBEV and USUTUV), which differ between close and divergent flaviviruses, have been produced (collaborative work with P. Desprès, Institut Pasteur) and used for the validation of novel indirect IgG ELISAs. From mid 2013, they will be used as flavivirus antigens for the development of a Luminex protocol helping in the differentiation of flavivirus infections, in comparison to the reference virus neutralisation tests (Project planned on several years, 2013-2015).

Objectives: equine exotic encephalitis viruses

✓ *Improvement of diagnostic tests for equine exotic encephalitis viruses*

Concerning exotic encephalitis alphaviruses (E, W and VEEV), and although recombinant E2 proteins have been obtained (collaborative work with P. Desprès, Institut Pasteur) and indirect IgG ELISA developed, the validation process of these new ELISA tools cannot be completed due to the lack of positive field sera. Consequently our priority for 2014 will be to develop collaborations with American and Asian laboratories and to collect field sera.

✓ **Expected outputs** :

- Lyophilized horse reference sera positive for antibodies against different flaviviruses present in Europe (WNV, USUV, TBEV) or considered as a threat (JEV), shipped on demand of the NRLs
- Horse reference EDTA blood positive for flavivirus RNA, shipped on demand of the NRLs
- Implementation of a multiplex luminex tool for the serological diagnosis of flavivirus infections

✓ **Performance indicators :**

- Number of positive reference sera and EDTA blood for flaviviruses
- The operating procedure for real-time panflavivirus RT-PCR will be published on the EU-RL website
- A new operating procedure for the serological diagnosis of flavivirus infections by Luminex will be disseminated to NRLs (2015).

Objectives: Vesicular stomatitis virus (VSV)

✓ *Improvement of diagnostic tests for Vesicular stomatitis virus (VSV)*

A one-step duplex real time RT-PCR was developed and implemented for detection and typing of VSV. A SOP is under writing and will be published on the EU-RL website. Validation of this method is in progress. This method was based on previously published primers and probes (Hole 2006) and may not detect all circulating VSV strains. New primer and probe sets allowing a larger detection of VSV strains will be designed by bioinformatic analysis of available sequences. These primers and probes will be tested in the one step duplex method and validated.

Production of recombinant proteins from VS will be continued using baculovirus expression system. Once purified, these proteins will be used as antigen for both luminex assay and Elisa applications.

✓ **Expected outputs :**

- Validation of a one-step rtRT PCR method for VS detection and typing of a larger panel of VSV strains
- Production of VSV protein
- Prototype of Luminex immunoassay for VSV antibodies detection

✓ **Performance indicators :**

- 1 newly one step rt RTPCR method for VS disseminated to NRLs by the way of website

Sub-activity 2: Training and support to NRLs

Objectives : West Nile Virus WNV and VSV

- Support to NRLs in confirming or invalidating positive and doubtful cases upon NRLs request
- Biological samples (reference and field strains, cell lines, sera, blood) will be provided upon NRLs requests.
- With reference to WNV and VSV PT results, training sessions will be organized for NRLs who encountered sensitivity or specificity issues with their diagnosis tools.

✓ **Expected outputs :**
  - Positive and doubtful cases will be confirmed or invalidated;
  - Training of scientists and technicians from NRLs to varied diagnosis tools

✓ **Performance indicators :**
  - Number of confirmed and invalidated cases
  - Supply of biological samples to NRLs.
  - Number of trained NRLs

Sub-activity 3: Epidemiological and specific studies

Objectives: West Nile Virus (WNV)

Epidemiological studies: a collaboration with the University of Agriculture of Pakistan, Faisalabad (Dr. M. Saqib) has been initiated in 2013 as regards flavivirus circulation in Pakistan. About 300 horse sera positive for flavivirus antibodies have been sent to the EU-RL. These sera will be tested in 2014 by WNV and JEV neutralization tests with the aim to evaluate the seroprevalence of WNV and JEV infections in horses in Pakistan and to get JEV positive field horse sera to validate our new diagnostic tools (ELISA and luminex technology. See sub-activity 1).
A new flavivirus, close to a tick-borne flavivirus, Meaban, initially isolated from gulls in Brittany, France, has been isolated from ticks sampled recently in Medes Island, Spain (collaborative work with Thierry Boulinier, Centre d'Ecologie Fonctionnelle et Evolutive and Elsa Jourdain, INRA) (Arnal et al, manuscript in preparation). Its genetic evolution along the years and among geographic areas (France, Spain) will be assessed, as well as its virulence potential for mammals and birds.

Specific study: WNV and JEV can cause severe meningo-encephalitis in a fraction of infected horses. Effective vaccines, either inactivated or recombinant vaccines, have been developed against these 2 flaviviruses, however they are sparsely used (WNV vaccines) or not used (JEV vaccine, not authorized in Europe). Consequently the development of antiviral drugs efficient at reducing neurological symptoms and preventing virus replication in horses after WNV or JEV infection would be of great value.
A collaborative research program with Pasteur Institute, Paris (PO. Vidalain) aims at identifying small molecules with a broad antiviral spectrum, expected to be active against viruses belonging to different virus families and genus. The Normandy Centre for Drug Studies and Research (CERMN, Caen) has identified a promising molecule in its chemical library (sr1057). The antiviral activity of sr1057 against WNV and JEV will be tested in horse cell lines to evaluate the *in vitro* efficacy and safety of the molecule. Thereafter the pharmacokinetics and antiviral activities of sr1057 will be evaluated *in vivo* in mice. For this latter purpose, a murine model of JEV infection will be developed (WNV models, in Balb/c and C57Bl/6J, are already available in our laboratory). Finally, the ultimate goal will be to evaluate this molecule in infected horses to prove its safety and efficacy (in 2015 or 2016).

We are also pursuing our efforts towards the characterization of WNV virulence determinants. A reverse genetics system for the production of *de novo* recombinant WNV particles has been obtained in 2011 in our laboratory (see Bahuon et al, 2012) and we would like to address the impact of specific point mutations observed in emerging European WNV strains for WNV

virulence in incidental (mammalian) and reservoir (avian) hosts. For this purpose, mutant viruses have already been generated. Their growth kinetics will be evaluated in cell-culture systems, their pathogenicity will be evaluated in Balb/cJ mice (mammalian model) after peripheric and intracerebral inolulations, as well as in birds (corvids and hens, in collaboration with B. Lambrecht, CERVA CODA).

Bahuon C, Desprès P, Pardigon N, Panthier JJ, Cordonnier N, Lowenski S, Richardson J, Zientara S, Lecollinet S. 2012. IS-98-ST1 West Nile virus derived from an infectious cDNA clone retains neuroinvasiveness and neurovirulence properties of the original virus. PLoS One;7(10):e47666.

✓ **Expected outputs :**

- Horse positive sera well characterized for WNV and JEV neutralizing antibodies
- Bird positive sera for Meaban neutralizing antibodies, widely reactive in WNV competition ELISA, as well as Meaban virus positive tick samples
- Disease risk for mammals associated to Meaban-like flavivirus circulation in Spain and Europe
- Demonstration of the safety and efficacy against WNV and JEV of the sr1057 molecule *in vitro* and *in vivo* in mice
- Molecular markers of WNV virulence for European WNV strains

✓ **Performance indicators :**

- Number of positive WNV and JEV sera received
- Number of positive samples for Meaban antibodies or RNA

Objectives : Vesicular stomatitis virus (VSV)

Specific study : development of a recombinant VSV- Canine Adenovirus based vaccine
VSV-G protein coding gene will be cloned into a non-replicative canine Adenovirus vector previously developed in our laboratory. Production of recombinant VSV-G protein will be assessed *in vitro* after transfection of the recombinant Adenovirus vector into canine cells. Immunogenicity will be evaluated by Elisa method. The ability of the recombinant virus to induce VSV neutralizing antibodies will be tested in a murine model. In positive case, the evaluation of this candidate vaccine in horse will be planed (in 2015 or 2016)

✓ **Expected outputs :**

- Production of a recombinant non replicative VSV-Canine Adenovirus

✓ **Performance indicators :**

- Success in induction of VSV neutralizing antibodies in a murine model

Sub-activity 4: VSV Proficiency test

Organization in 2014 of a proficiency test on molecular detection and typing of VSV (matrix: RNA)

✓ **Expected outputs :**

- Success of NRLs in the organized VSV ring trial

✓ **Performance indicators :**

- Number of participants and average rate of success in PT test

Activity 3 : Program in Parasitology (Dourine)

Sub-activity 1 : Development and validation of diagnostic methods and tools

Because of the lack of identified and characterized biological dourine samples for the optimization of the diagnostic methods, a priority will be for 2014 to use every opportunity to get serums or strains, as explained in three following points:

- The discussion of the 2012 European workshop on dourine (Caen France) organized by the EU-RL conclude that method and reagents for dourine serology had to be optimized. Based on these conclusions, a European network on equine trypanosomiasis was created, including the EU-RL, two dourine NRLs (Germany and Italy) and three international research teams. The goal of this network is to define a global optimization strategy, to share biological samples and working reagents in order to propose appropriate modifications of dourine serological diagnosis for the next revision of the dedicated OIE chapter of the terrestrial manual. The first meeting was organized on February 2013. One meeting is planned in 2014, organized by the EU-RL.

- Difficulties have been encountered in order to collaborate and work with the Moscow OIE reference laboratory for dourine. To solve the situation, a working meeting will be planned in Moscow with the scientists from the All-Russian Research Institute for Experimental Veterinary Medicine (VIEV) and the EU-RL (the director of the EU-RL Dr Stephan Zientara and the scientist in charge of the dourine Dr Julien Cauchard). Dr Touratier, from the Non Tsetse Transmitted Animal Trypanosomosis (NTTAT) will be associated to this working meeting. This opportunity should also allow the EU-RL to exchange biological samples, since Russia is the country declaring most of the clinical cases of dourine at the OIE.

- Collaboration is being organized with Dr Allan Guthrie from the University of Pretoria (South Africa) in order to sample horses and donkeys identified as clinically sick from dourine.

The work on the sequencing and analyses of the *Trypanosoma equiperdum* and *Trypanosoma evansi* whole genomes will continue through the annotation of the genomes and the sequencing of one or two new strains (collected in 2014 as described above) in order to provide as much information as possible on the classification of the two parasites in the trypanozoon subgenus.

The first stage of the optimization of the serodiagnostic tools (2014 target) will be to compare and characterize the strains currently available by studying their protein expression profiles. The targeted proteins will be the minor surface proteins including ISG (invariant surface of glycoproteins). The genomes of *T. equiperdum* and *T. evansi* will allow us to have the sequence of the proteins of interest.

✓ **Expected outputs** :
  - Gathering as many biological reagents as possible;
  - Identification of proteins of interest that could be used for serological diagnostic.

✓ **Performance indicators :**
  - Organization of a meeting for the European network on equine trypanosomosis;
  - Number of reagents and samples exchanged.

Sub-activity 2 : Training and support to NRLs

✓ **Expected outputs :**

- Training sessions will be organized according to NRLs requirements;
- Positive and doubtful cases will be confirmed according to NRLs requirements;
- Supplying of reagents to the NRLs will continue ; Especially sera and antigens produced and validated in 2013 from the *Trypanosoma equiperdum* OVI strain (the choice of the strain is based on consensus established during the workshop in 2012) will be provided to the NRLs.

✓ **Performance indicators:**

- Involvement in the confirmation of cases;
- Supply of biological reagents to the NRLs.

Activity 4: Contagious equine metritis (CEM)

Sub-activity 1: Production and validation of diagnostic methods and tools

The research program started in 2013 "Improvement of CEM bacteriological diagnosis and development of an infection model for the comparison of the pathogenicity of CEM organism and *T. asinigenitalis*" will be continued in collaboration with the AES chemunex company (manufacturer of culture medium). Concerning the development of a new culture medium to improve the performance of the culture method, nutritional requirements of *Taylorella* genus will be studied using data from genomic sequences recently obtained for *Taylorella equigenitalis* and *Taylorella asinigenitalis*.

✓ **Expected outputs:**

- Development of a new culture medium to increase the sensitivity of the CEM diagnosis.

✓ **Performance indicators:**

- Testing at least one new composition of culture medium.

Sub-activity 2: Support to NRLs and epidemiological study

✓ Training sessions for the NRLs will be planed according to NRLs requirements;

✓ Biological elements (reference and field strains) will be provided according to NRLs requirements;

✓ CEM positive cases will be confirmed according to NRLs requirements;

✓ Molecular diversity of CEM isolates circulating in Europe will be analyzed in collaboration with the NRLs. The aim of the study is to better understand and describe the different *Taylorella equigenitalis* population circulating in Europe (and if it is possible *Taylorella asinigenitalis*). The NRLs could perform themselves the MLST method previously developed by the EU-RL or send to the EU-RL their *Taylorella* strains (this project will be on several years).

✓ **Expected outputs:**

- Better knowledge of *Taylorella equigenitalis* (and *Taylorella asinigenitalis*) strains circulating in Europe.

✓ **Performance indicators:**

- Number of *Taylorella equigenitalis* (and *Taylorella asinigenitalis*) strains received and typed by MLST.

Activity 5 : Glanders

Sub-activity 1: *Development and validation of diagnostic methods and tools*

- Constitution of a lyophilized reference serum for Glanders.

Collaboration with LANAGRO of Brazil, Recife (Dr V. Lucia) has been initiated in 2013. About 1L of serum from a naturally infected horse has been collected and should be sent to EU-RL in order to constitute a reference serum. This one will be filtered, re-tested by CFT then lyophilized. This serum will be used as reference serum for CFT and ELISA analysis. Other contacts have been established with other Brazilian scientists in order to collect more sera from naturally infected horses (*via* Dr V. Lucia).

- Development and validation of an indirect ELISA for Glanders.

A prototype ELISA has been developed for the detection of *B. mallei*. Briefly, a crude antigenic fraction has been prepared from the ATCC 23344 *B. mallei* strain. Thanks to a collaboration initiated with the University of Agriculture of Pakistan, Faisalabad (Dr M. Saqib), about 100 sera have been sent to the EU-RL. Pakistani sera from truly infected (n=48) and potentially exposed (n=49) equines (horses and mules) have been tested. A good correlation between CFT and ELISA results were obtained. Negative European sera (n=120) as well as sera with doubtful or (false) positive CFT results (n=9, including 2 paired sera) were also included in the panel. Except one paired sera which gave CFT positive results, all sera were negative by ELISA. This prototype needs further validations (sensitivity, specificity, stability...) but already appears as an interesting alternative test when positive or doubtful results are obtained by CFT. This work will be continued. Sera from naturally infected animals from other endemic areas (at least from Brazil) will be tested with this prototype. Negative sera from the whole part of Europe will be also collected from NRLs for specificity determination.

An anti-horse conjugate is currently used in this prototype. Trials will be performed with protein G in order to allow detection of antibodies in all equines, without any restriction (including donkeys).

- Collect of tissues from naturally infected horses.

In collaboration with LANAGRO (Brazil), tissues have been collected by Dr V. Lucia from an euthanized diseased horse. Other samples have been requested from future equine cases. Once received by EU-RL, these samples will be used for the validation of the detection of *B. mallei* by real-time PCR in naturally infected tissues.

B. mallei and *B. pseudomallei* were recently added to the French list of "classified" pathogens. An authorization from the French administration is now required for the manipulation of these bacteria and any associated material (DNA, biological samples from infected animals). An official document has been prepared and forwarded to the authorities in July 2012, and an official inspection took place in our laboratory on 16 and 17 June 2013. Once this authorization is given (expected in September), biological samples collected from our Brazilian partner (LANAGRO) will be transferred to the EU-RL and used for the PCR validation.

✓ **Expected outputs:**

- Validated indirect ELISA for the serological diagnosis of glanders;
- Lyophilized sera from naturally infected horses;
- Validated real-time PCR on naturally infected tissues

✓ **Performance indicators**

- Implementation of an indirect ELISA test for the serological diagnosis of glanders;
- Lyophilized reference sera from *B. mallei* naturally infected horses, sent on request to NRLs.

Sub-activity 2: Training and support to NRLs

✓ **Expected outputs :**

- Training sessions will be organized according to NRLs requirements;
- Positive and doubtful cases will be confirmed or invalidated according to NRLs requirements;
- Reagents and/or support will be supplied to the NRLs upon request.

✓ **Performance indicators :**

- Number of trained NRLs;
- Number of confirmed and invalidated cases;
- Supply of biological reagents to the NRLs.

Activity 6 : Training

Training sessions for the NRLs will be planed. A priority will be accorded on the NRLs who have failed WNV or EVA proficiency tests but other trainings could be proposed by the EU-RL in function of remaining budget and on demand of NRLs.

✓ **Expected outputs :**

- Improve the diagnostic of equine diseases in the NRLs

✓ **Performance indicators :**

- Number of training organized

Activity 7 : Coordination and management

Sub-activity 1 : Coordination, management and communication

✓ General coordination by the EU-RL of the NRL network (dispatch of documents, coordination of the scientific and technical support to NRLs...)

✓ Assist Commission with scientific and technical advice

✓ In house follow-up of EU-RL activities, expenses, support to laboratory units involved in EU RL activities

✓ Preparation of the activity report, workshop reports, scientific program and performance indicator reports

✓ Improvement and up to date of the EU RL website

  ✓ An regular up to date of NRL contacts database

  ✓ A scientific monitoring of the equine diseases managed by EU-RL and by the updating of parts "news" or "documentation" as necessary

  ✓ The addition of all next workshops, proficiencies tests, training sessions and documentation

  ✓ The addition of SOPs for each equine diseases diagnostic methods

✓ ***Expected outputs :***

- Good communication between the EU-RL and the NRLs network

- To assist the Commission in case of specific request and to have trained personnel available for emergency situations occurring within the Union

✓ **Performance indicators**

- Number of website visits

- Positive feedback of the project officer concerning the equine diseases EU RL

Sub-activity 2 : annual NRLs workshops

On 2014, two workshops on one day will be organized in ANSES Maisons-Alfort and will be focused on EIA and glanders diseases. Results and analysis of the ring trials for EIA and glanders will be discussed as well as diagnosis improvement and epidemiological situations of the two diseases. Following the two workshops, final reports and all presentations will be published on the website.

✓ **Expected outputs :**

- High participation of NRLs for the two workshops.

- Good quality of Proficiency tests restitution and good quality of debates for the two workshops

✓ **Performance indicators**

- Number of participating NRLs in the two workshops

- Number of positive satisfaction surveys for the two workshops.