



MAISONS-ALFORT LABORATORY FOR
ANIMAL HEALTH

AND

DOZULÉ LABORATORY FOR EQUINE
DISEASES



***EU REFERENCE LABORATORY
FOR
EQUINE DISEASES***

2013 Scientific program of the European Union Reference Laboratory for Equine Diseases

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This document describes the 2013 scientific program of the European Union reference Laboratory (EU-RL) for equine diseases. The described program is established in accordance with six 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: 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

The research program started last year "Set up a real-time RT-PCR for EVA and EIA diagnosis" will be continued in collaboration (Covetlab organization) with our colleagues from the CVI (Netherlands), VLA (UK), SVA (Sweden) and the DTU (Denmark).

✓ **Expected outputs :**

- Validation of a one-step qRT-PCR method for EAV
- At least 1 reference serum will be available for NRLs

✓ **Performance indicators**

- Development of one step qRT-PCR method that will be provided to NRLs
- 1 reference serum available for NRLs

Sub-activity 2 : Organization of proficiency tests

- EVA : a proficiency testing including NRLs will be organized

✓ **Expected outputs :** 2 ring trials organized for EVA (1 serology and 1 virology PT) and high success of NRLs.

✓ **Performance indicators :** Number of ring trial organized and average rates of NRLs success

Sub-activity 3 : Training and support to NRLs

- EVA : a workshop will be organized in 2013
- EHV : a workshop session is planned in 2013
- EVA, EIA and EHV : training session for the NRLs will be planned
- Positive cases of EVA, EIA and EHV will be confirmed according to NRLs requirements.

✓ **Expected outputs :**

- Improvement of the EVA, EHV diagnostic methods

- Reduction in the number of NRLs failing PT test
- ✓ **Performance indicators :**
 - Average rates of NRL success for next PT test in 2016 higher than 2013

Sub-activity 4 : Epidemiological investigation

- Molecular characterization of EIAV strains circulating in Europe. 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 :**
 - Better knowledge of strains circulating in Europe and trace their origin
- ✓ **Performance indicators :**
 - Number of sequences received and characterized

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

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

- ✓ *Production of reference sera*
 - Production of reference samples (sera positive for IgG and/or IgM and EDTA blood from viremic horses) through experimental infection of horses by the subcutaneous route with different flaviviruses , including lineage 1 and 2 West Nile viruses. 5 horses will be infected in BSL3 facilities (INRA PFIE platform) and monitored for 2 months after infection. For transport and for longer lifespan, the lyophilisation of those sera will be planned in 2014. At first, it will be checked whether lyophilisation steps modify antibody titer in sera. Then, lyophilized sera samples will be sent to some other NRLs
- ✓ *Novel diagnostic tools = Implementation of luminex assays for the multiplex detection of flavivirus antibodies*
 - A luminex protocol for the multiplex detection of flavivirus antibodies in humans and animals (horses in particular) has been developed by JC Manuguerra (Institut Pasteur). This protocol is being validated with well characterized IgG and IgM positive sera at Institut Pasteur and could be subsequently implemented at ANSES (project will be on several years).
- ✓ **Expected outputs :**
 - To have 5 reference samples (Sera positive for IgG and IgM and EDTA blood from viremic horses) for 5 flaviviruses (WNV lineage 1 and 2, JEV, TBEV and Usutu), to use them for the next NRLs PT tests and to sent them to NRLs when requested
 - Implementation of Luminex method at Anses for WNV
- ✓ **Performance indicators :**

- Numbers of flaviviruses reference samples collected (lyophilization in a next step).
- ✓ *Improvement of diagnostic tests for equine exotic encephalitis*
 - Conclusion of the development and validation steps for the 3 IgG ELISA tests with E2 glycoproteins from alphaviruses (Eastern, Western and Venezuelan equine encephalitis viruses; EEEV, WEEV and VEEV respectively) by finding and testing enough positive equine encephalitis sera
 - Development of a multiplex serological diagnostic test based on luminex liquid array technology in collaboration with Pasteur Institute (project will be on several years).
 - Continuation of the development and validation of ELISAs based on ectodomain III (EDIII) of the envelop glycoprotein for the detection of IgG and IgM antibodies directed against different flaviviruses (West Nile, Japanese encephalitis, Usutu viruses) in varied species.
- ✓ **Expected outputs :**
 - Validation of 3 IgG ELISA tests with E2 glycoproteins for 3 alphaviruses (EEEV, VEEV and WEEV)
 - Implementation of Luminex method at Anses for exotic encephalitis viruses
- ✓ **Performance indicators :**
 - Newly ELISA available diagnostic/ method disseminated to NRLs by the way of website
 - 1 publication on this method in peer-reviewed journal
- ✓ *Improvement of diagnostic tests for vesicular stomatitis (VS)*
 - Recombinant protein from VS will be produced and introduced as antigen in the Luminex liquid array test developed.
 - Development of one-step real time RT-PCR will be continued by improving the specificity and the sensitivity of the test.
- ✓ **Expected outputs :**
 - Validation of a one-step rRT PCR method for VS
 - Establishment of luminex test for serological diagnostic of VS
- ✓ **Performance indicators :**
 - 1 newly one step rt RTPCR method for VS disseminated to NRLs by the way of website
 - Protocol of luminex test available for VS

Sub-activity 2 : Organization of proficiency tests

- ✓ Organization in 2013 of a proficiency test on diagnostic methods for the detection of WNV nucleic acids (matrix : infected and inactivated samples) and/or antibodies (matrix : horse sera)
- ✓ **Expected outputs :**
 - 2 ring trials organized for WNV (1 serology and 1 virology PT) and high success of NRLs
- ✓ **Performance indicators :**
 - Number of ring trial organized and average rates of NRL success

Sub-activity 3 : Training and support to NRLs

- Organization in 2013 of one training session on diagnostic methods for the detection of WNV nucleic acids and/or antibodies, on demand of NRLs
 - Organization in 2013 of a workshop on equine encephalitis viruses
 - Support will be provided to NRLs for implementation of VS real time RT-PCR and VN tests.
- ✓ **Expected outputs :**
 - Improvement of diagnostic methods in NRLs for WN and reduction in the number of NRLs failing PT test.
 - ✓ **Performance indicators :**
 - Average rates of NRL success for next WN PT test in 2016 higher than 2013

Sub-activity 4 : Epidemiological investigation

- *Epidemiological investigations* on WNV prevalence in horses and bird species in Romania (Danube delta, Veterinary College of Iasi), in Tunisia (IRVT, Tunis) and more widely around the Mediterranean basin will continue.
- ✓ **Expected outputs :**
 - Better knowledge of strains circulating around the Mediterranean basin
 - ✓ **Performance indicators :**
 - Number of WNV received and characterized

Activity 3 : Program in Parasitology (Dourine)

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

- ✓ *Research and reference activities*
 - Depending on the results obtained by analysis of *Trypanosoma equiperdum* and *Trypanosoma evansi* genomes, two analysis will be used, in silico analysis or chromatography profil of antigens.

- The scientific network will be extended in order to provide the field clinical samples that are missing for research and validation of methods.
 - An experimental infection of horses is planned with a control group and a treated group. The first goal of this experimentation is to prove that dourine can be efficiently treated and that slaughtering described in the terrestrial code of the OIE can be avoided. The second goal is to get biological samples for research and development.
 - Production of reference reagents, serum and antigens, will be validated.
- ✓ **Expected outputs :**
- One new *T. equiperdum* supposed strain to be studied
 - At least two new clinically characterized serum samples in our serum bank
 - Antigens produced and available for NRLs
 - Positive reference serum available for NRLs
- ✓ **Performance indicators :**
- Number of samples available in our bank
 - Number of reagents available for NRLs

Sub-activity 2 : Training, meeting and support to NRLs

- Training sessions will be organized regarding proficiency testing results
 - The EU RL will participate to the organization of the “non tsetse transmitted animal trypanosomosis” (NTTAT) meeting in Paris – June. This is the opportunity to meet scientists we can't see in classical congress, like scientists from Mongolia or other third countries.
 - Positive and doubtful cases will be confirmed according to NRLs requirements
 - Supplying of reagents to the NRLs will continue
- ✓ **Expected outputs :**
- To carry out a training session for each applicant NRL
- ✓ **Performance indicators :**
- Number of training organized

Activity 4: Contagious equine metritis (CEM)

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

- Using data from genomic sequences recently obtained, nutritional requirements of *Taylorella* genus will be studied to improve culture media used for the isolation and the

characterization of the CEM agent, and thus to increase the sensitivity of the CEM diagnosis;

✓ **Expected outputs:**

- Development of a new culture medium to improve the performance of the culture method and beginning of its validation phase

✓ **Performance indicators:**

- Initiation of the validation phase of the newly developed medium

Sub-activity 2: Specific studies

- An infection model not requiring animal experimentation will be under consideration to compare the pathogenicity of the CEM organism and *T. asinigenitalis*. In parallel, mutagenesis tools could be developed in order to identify in longer-term genetic markers of virulence within and between subspecies.

✓ **Expected outputs:**

- Test and comparison of different infection models (not requiring animal experimentation) for the study and the comparison of the CEM organism and *T. asinigenitalis* pathogenicity

✓ **Performance indicators:**

- At least 1 presentation or poster or publication in a peer-reviewed journal on the development of an infection model

Sub-activity 3: Training and support to NRLs

- Training sessions for the NRLs will be planned according to NRLs requirements;
- Positive cases of CEM will be confirmed according to NRLs requirements;
- NRLs could send their *Taylorella* strains (*T. equigenitalis* and *T. asinigenitalis*) isolated in 2013 or previously to analyze their molecular diversity by the MLST technique which has just been developed by the EU-RL.

✓ **Expected outputs:**

- To carry out a training session for each applicant NRL
- Provision of scientific and technical support for each request (confirmation of CEM case or strain typing by MLST) by the NRLs

✓ **Performance indicators:**

- Number of training session (one per NRL)
- Number of confirmed CEM cases and number of typed strains by MLST

Activity 5 : Glanders

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

- A 2nd horse immunization will be scheduled in 2013 with mixed *B. mallei* strains to improve the immunogenicity and possibly to induce a more sustainable seroconversion than for the 1st immunization. Sera will be used for the preparation of a European glanders horse candidate standard serum;
 - Development of a sensitive and specific ELISA test should be continued for an improved direct diagnostic;
 - Implementation of CFT alternative serological tests in the EU-RL laboratory (Western blot, commercial competitive ELISA kit).
 - Standardization of real time PCR methods at EU level and optimization of multiplex assays that discriminate *B. mallei* and *B. pseudomallei* will be continued.
 - The validation of real time PCR for genome detection of *B. mallei* and *B. pseudomallei* will be performed on biological samples artificially contaminated samples because naturally infected samples are not available.
- ✓ **Expected outputs:**
- Improvement of Glanders diagnostic methods
 - Positive serum available for NRLs
 - Validated methods available for NRLs
- ✓ **Performance indicators**
- Number of samples available for NRLs
 - Good average rates of NRL success for next PT tests

Sub-activity 2 : Training and support to NRLs

- CFT training sessions for the NRLs will be planed;
- ✓ **Expected outputs :**
- Improvement of the Glanders serological diagnostic.
 - Provision of scientific and technical support
- ✓ **Performance indicators :**
- Good average rates of NRL success for next PT tests

Activity 6 : 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, work shop reports, scientific program and performance indicator reports
- Improvement and up to date of the EU RL website by :
 - ✓ Improvement of the ergonomics of the website ;
 - ✓ Development of a database on the website with the collection of strains in the different NRLs and EU RL
 - ✓ 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 feed back of the project officer concerning the equine diseases EU RL

Sub-activity 2 : annual NRLs workshops

- Two workshops on one day will be organized in ANSES Maisons-Alfort and will be focused on EVA and WN diseases. Results and analysis of the ring trials for WNV and EVA will be discussed as well as diagnosis improvement and epidemiological situations of the two diseases

- Following the two workshops, the final report and all the presentations will be made and 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.