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EUROPEAN UNION REFERENCE LABORATORY (EU-RL) FOR BOVINE TUBERCULOSIS

WORK PROGRAMME 2016-2017

Version 5

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The purpose of the work programme is to cover the objectives (general, specific and operational) defined in the Annex to the Commission Implementing Decision establishing the work programme for the years 2016 and 2017 on financial contribution to the European Union reference laboratories, taking into account the responsibilities and tasks defined in the Annex II to Commission Regulation (EC) No 415/2013 regarding the EU-RL for Bovine Tuberculosis:

1. To coordinate, in consultation with the Commission, the methods employed in the Member States for diagnosing bovine tuberculosis.

2. To facilitate the harmonization of techniques throughout the Union, in particular specifying standard test methodologies.

3. To organize workshops for the benefit of national reference laboratories as agreed in the work programme and estimated budget referred to in Article 2 of Implementing Regulation (EU) No 926/2011, including training of experts from the Member States and, as appropriate, from third countries, in new analytical methodologies.

4. To provide technical assistance to the Commission and, upon its request, to participate in international forums relating to the diagnostic of bovine tuberculosis, concerning in particular the standardization of analytical methods and their implementation.

5. To perform research activities and, whenever possible, coordinate research activities directed towards the improved control and eradication of bovine tuberculosis.

A. MAIN ACTIVITIES OF THE EU-RL FOR BOVINE TUBERCULOSIS FOR 2016-2017.

1. Immunological diagnosis of bovine tuberculosis.

1.1. Single Intradermal Tuberculin test.

Operational objective 1: To ensure the development and use of high quality analytical methods across the EU-RL network.

Description: One of the main activities for the EU-RL for Bovine Tuberculosis is to test the biological potency of the Purified Protein Derivatives (PPD or tuberculin) in sensitized guinea pigs since they are the reagents for the official *in vivo* (skin test) and *in vitro* (IFN-γ) diagnostic assays based on cell-mediated immune response for the diagnosis of bovine tuberculosis. Nowadays there are several PPD manufactures all over Europe and the potency testing protocols between NRLs are not harmonized. Moreover, the technical capacity to perform the skin test must be guaranteed trough accreditation of the protocol.

Objectives: To define a harmonized protocol for guinea pig potency testing (GPPT) to be implemented in each NRL. During 2016 and beginning of 2017, the EU-RL will test the European Pharmacopeia protocol and from March 2017 the protocol based on inactivated *M. bovis* strains (OIE protocol) will be set up in the EU-RL. Moreover, the EU-RL will start the process to validate the skin test protocol in the field.

Expected outputs: a) Standard protocol for biological potency testing in guinea pigs to be set up in the NRLs; b) Skin test protocol accreditation.

1.2. Use of IFN-γ in the EU.

Operational objective 1: To ensure the development and use of high quality analytical methods across the EU-RL network.

Description: The IFN-γ test is used together with the skin test to detect a higher number of infected animals. During 2012, a Scientific Opinion on the use of gamma interferon test for the diagnosis of bovine tuberculosis [EFSA Journal 2012;10(12):2975] was published and consequently, the EU-RL has been working to produce a harmonized protocol with a recommendation to be used as an alternative to the tuberculin skin test for the establishment and maintenance of an officially tuberculosis-free herd status and for certification for intra-Union trade in bovine animals. In this sense, the EU-RL together with other NRLs (France, Italy, United Kingdom and Ireland) has produced an exhaustive working document that compiles the scientific information regarding the IFN protocol. This protocol will be presented in the 2015 workshop to all the NRLs to reach consensus regarding harmonization of critical steps (bovine PPD concentration, origin

PPDs, time between collection and stimulation in the laboratory, duplicates, mitogen, plasma conservation and interpretation criteria and cut-off point).

Objectives: To reach consensus regarding the harmonization IFN protocol among NRLs. The standardized methodology will be validated (2016) and accreditated (2017) to be included in the IFN-γ comparative test in 2017. The protocol will be sent to NRLs in 2017 and data about the implementation of the recommended protocol based on the EFSA opinion will be compiled if used by any of the Member States.

Expected outputs: To define the harmonized IFN-γ protocol between all the NRLs.

2. Setting up an alternative protocol for bacteriological culture: molecular detection of *Mycobacterium tuberculosis* complex in animal tissues.

Operational objective 1: To ensure the development and use of high quality analytical methods across the EU-RL network.

Description: During the last two years, the EU-RL has developed an extraction protocol combined to a Real Time PCR assay for the detection of members of the *Mycobacterium tuberculosis* complex (MTBC) in animal tissues. This protocol, when validated could replace the bacteriological culture.

Objectives: Work will be performed to increase the sensitivity of the direct extraction technique. Moreover, the optimized direct extraction protocol will be compared with the culture protocol. A set of samples (800 total) will be analyzed in parallel to be able to perform a Bayesian study. The main objective is the identification of mycobacteria to also enhance the molecular characterization performed directly from clinical samples. The Standard Operation Procedure (SOP) will be validated during 2017 and several NRLs (UK, Ireland, Italy, France, Austria, Estonia, etc.) will be involved in the process. Finally, a protocol will be sent to the European Commission for consideration as an official testing for bovine tuberculosis.

Expected outputs: To create a robust and efficient molecular protocol for the detection and molecular characterization of members of the MTBC in animal tissues.

3. Molecular database.

Operational objective 1 and 3: To ensure the development and use of high quality analytical methods across the EU-RL network; and to ensure the availability of scientific and technical assistance provided by the EU-RLs.

Description: DVR-spoligotyping is still considered as the routine molecular characterization protocol for members of the MTBC. Two main databases are available: the SITVIT Database (Public Health, Demay *et al.* 2012) and the mbovis.org (Animal Health, Smith *et al.* 2012). In 2015, the mbovis.org database has been migrated to the

VISAVET server. During 2016-2017, a specific European database will be designed (mbovisEU.org) within the mbovis.org. For this objective, the EU-RL will contact the NRLs to collect all the characterization data regarding *M. bovis*/*M. caprae* isolates in Europe. This will allow the registration of the isolates identified in each Member State and therefore facilitate future epidemiological studies between Member States. The last step (scheduled 2017-2018) will be to include the *M. bovis*/*M. caprae* isolates identified in human through the Public Health NRLs.

Objectives: a) To maintain the mbovis.org database in the VISAVET server (EU-RL website); and b) to create a specific European database (mbovisEU.org) to generate epidemiological data between European countries.

Expected outputs: Maintenance of a molecular database (workdwide-mbovis.org and European-mbovisEU.org) to guarantee the standardization of nomenclature for DVR-spoligotyping profiles allowing epidemiological studies.

4. Comparative tests.

Operational objective 1 and 2: To ensure the development and use of high quality analytical methods across the EU-RL network; and to maintain appropriate level of proficiency testing ensuring efficiency of control analysis methods.

Description: The EU-RL has the responsibility of organising periodical comparative tests of diagnostic procedures and operating laboratory proficiency test of NRLs to ensure high quality and harmonization of laboratory testing of animal diseases across the European Union. The ring trials organized by the EU-RL are focused in three main topics: 1) Bacteriological culture and direct extraction; 2) Identification and molecular characterization; and 3) Immunological diagnosis. The EU-RL will organize a total of four ring trials in 2016 and 2017. The topics will be discussed with the NRLs in the workshop that will be held in Madrid at the end of 2015, although the EU-RL will propose the following ring trials: bacteriological culture & direct extraction, histopathological diagnosis, detection of IFN-γ and PPDs potency testing.

Objectives: To organize four ring trials for all the NRLs. The proposed comparative tests will be bacteriological culture & direct extraction, histopathological diagnosis, detection of IFN-γ and PPDs potency testing.

Expected outputs: To ensure high quality and harmonization of laboratory protocols of bovine tuberculosis in European Member States.

5. Missions.

Operational objective 1, 2 and 3: To ensure the development and use of high quality analytical methods across the EU-RL network; to maintain appropriate level of

proficiency testing ensuring efficiency of control analysis methods; and to ensure the availability of scientific and technical assistance provided by the EU-RLs.

Description: If requested by the EC or under specific circumstances, the EU-RL staff will visit the European Commission or NRLs. Moreover, to perform the studies regarding tuberculosis testing (*in* vivo and *in vitro*) the EU-RL staff visits: a) cattle farms to collect blood samples for the EU-RL samples bank; b) slaughterhouses to collect tissue samples (lymph nodes and organs); and c) CVO offices to discuss sampling of cattle farms. Another objective of the EU-RL is to keep abreast of developments mainly in diagnosis and epidemiology of tuberculosis and therefore the EU-RL staff attend congresses, workshops, training courses and they are updated through reports from experts, legislation, scientific papers, etc.

Objectives: a) To assist the EC/NRLs; b) To collect biological samples (farm/slaughterhouse); and c) To attend conferences and training courses.

Expected outputs: a) Technical advice/collaboration with EC/NRLs; b) Collection of samples for field studies included in the WP; and c) Scientific training of the EU-RL staff.

6. Training of personnel

Operational objective 1, 2 and 3: To ensure the development and use of high quality analytical methods across the EU-RL network; to maintain appropriate level of proficiency testing ensuring efficiency of control analysis methods; and to ensure the availability of scientific and technical assistance provided by the EU-RLs.

Description: As included in the Annex II of the Commission Regulation (EC) No 415/2013, the EU-RL must train experts from the Member States. These training mobilities are focused in laboratory protocols (bacteriological culture, direct extraction, identification by PCR, molecular characterization, detection of IFN-γ) as well as accreditation process and the workflow in a Biosafety Laboratory Level 3.

Objectives: Short visits (2-3 days) for two National Reference Laboratories per year to allow the establishment of laboratory protocols and techniques in their laboratory of origin. The trainee will present the activities of his/her NRL to the EU-RL and will submit a brief report after the visit.

Expected outputs: Training of NRL staff in mycobacteria protocols (culture, direct extraction, PCR, DVR-spoligotyping, MIRU-VNTR, IFN-γ test) and accreditation system.

7. Workshop.

Operational objective 1, 2 and 3: To ensure the development and use of high quality analytical methods across the EU-RL network; to maintain appropriate level of proficiency testing ensuring efficiency of control analysis methods; and to ensure the availability of scientific and technical assistance provided by the EU-RLs.

Description: A workshop is an annual meeting for information and coordination for all National Reference Laboratories.

Objectives: To organize a workshop in 2017 with all the NRLs to present the information regarding the activities performed by the EU-RL in the previous years and to find out the priorities and necessities regarding bovine tuberculosis diagnosis of the different NRLs.

Expected outputs: Annual meeting in 2017 to share information regarding EU-RL activities with the NRLs.

8. Expert Meeting.

Operational objective 1, 2 and 3: To ensure the development and use of high quality analytical methods across the EU-RL network; to maintain appropriate level of proficiency testing ensuring efficiency of control analysis methods; and to ensure the availability of scientific and technical assistance provided by the EU-RLs.

Description: The Guinea Pig Potency Test (GPPT) is the protocol defined in the legislation for determination of the biological potency of the PPDs. Determination of the biological potency is relevant for the correct performance of the skin test protocol in bovine tuberculosis diagnosis. In this sense, Dr. Bakker has a broad experience in this assay since he has been in charge for several years in the Dutch NRL. The EU-RL, considers his participation important to reach a general consensus regarding not only the protocol but also the interpretation of the results.

Objectives: To organize a meeting with an expert in Guinea Pig Potency Test (GPPT).

Expected outputs: Standard protocol and interpretation criteria for biological potency testing in guinea pigs.

B. OTHER ACTIVITIES OF THE EU-RL FOR BOVINE TUBERCULOSIS FOR 2016-2017.

Operational objective 1, 2, 3 and 4: To ensure the development and use of high quality analytical methods across the EU-RL network; to maintain appropriate level of proficiency testing ensuring efficiency of control analysis methods; to ensure the availability of scientific and technical assistance provided by the EU-RLs; and to ensure a sound and efficient management of EU-RL funding cycle.

The following tasks will remain permanent activities of the EU-RL for 2016-2017.

1. Preparation, control and supply of laboratory material (including in house spoligotyping membranes) and protocols.

2. Collection of representative samples (tissue samples, strains, DNA, serum/plasma).

3. Isolation, identification and typing of mycobacteria.

4. World Wide Web page update.

5. Technical assistance to the Commission and NRLs and participation with EFSA and international organizations (bovine tuberculosis subgroup of the Task Force, OIE).

6. Dissemination (presentations at international and national congresses or conferences, and publication in international and national journals).

7. Keeping abreast of developments (papers, conferences, training courses, reports, legislation, etc.) and research activities (collaboration with NRLs, participation in research projects, etc.).

8. Technical and financial management of the activities included in the work programme.