WORK PROGRAMME of EURL for BOVINE TUBERCULOSIS

PERIOD: 2019/2020

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CONTACT DETAILS

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SUMMARY

INTRODUCTION

(Regarding relevant regulations and functions)

The purpose of the work programme is to cover the objectives (general, specific and operational) and the priorities defined in the Annex to the Commission Implementing decision of 17th October 2017 on the adoption of the work programmes of the Commission for the year 2018, 2019 and 2020. Moreover, the responsibilities and tasks defined in the article 94 Regulation (EU) 2017/625 and Annex II to the Commission Regulation (EC) No 415/2013 regarding the EU-RL for Bovine Tuberculosis will be also taken into account in the work-programme.

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 36 of Regulation (EU) No 652/2014:
(taking into account Art 147 of (EU) 625/2017)

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

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *Art. 94.2.a* ***Providing national reference laboratories with details and guidance on the methods of laboratory analysis, testing or diagnosis, including reference methods.***
  Sub-activity 1.1. Database with recommended protocols (BT Protocols Database).
- *Art. 94.2.b* ***Providing reference materials to national reference laboratories***
  Sub-activity 1.2. Reference material for mycobacteria protocols.
  Sub-activity 1.3. Replacement of the International Standard Bovine Tuberculin (ISBT).
- *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.***
  Sub-activity 1.4. Comparative 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 1.5. Optimization and improvement of official techniques for the diagnosis of bovine tuberculosis.
  Sub-activity 1.6. Evaluation of novel methodologies for diagnosis of bovine tuberculosis.
  Sub-activity 1.7. Biological Potency of PPDs.

Sub-activity 1.1. *Database with recommended protocols (BT Protocols Database)* (Art. 94.2.a).

Objectives: Create and maintain a database with recommended protocols and instructions for carrying out the official and complementary tests for bovine tuberculosis diagnosis.

Description: Creation and mainteinance of a dedicated space in the EU-RL website (EU-RL Databases, BT Protocols Database) where Member States can find a collection of the recommended methods for the diagnosis of bovine tuberculosis (culture, direct detection of MTBC from tissue samples, identification by PCR, DVR-spoligotyping, detection of gamma-interferon, histopathology, guinea pig and cattle potency tests), protocols for carrying out the techniques and useful information on the critical points and inherent difficulties of the methods. In each protocol, information regarding accreditation will be also included.

Expected Output: Creation of a collection of protocols and useful information for the implementation of laboratory methods for the detection of bovine tuberculosis.

Duration: 2019-2020.

Sub-activity 1.2. *Reference material for mycobacteria protocols* (Art. 94.2.b).

Objectives: Create reference material to be used as quality controls for the performance of the standardized protocols and to test new techniques.

Description: The EU-RL will collect biological samples from healthy and naturally infected animals to be tested in the EU-RL laboratory with the standardized procedures. The material will be divided in several aliquots and properly stored in the laboratory's equipment. The EU-RL will conduct studies in order to define the main properties of the material (homogeneity, repeatability, reproducibility and stability) in a short and long term, based on the protocol used. The reference material will include, for instance, homogenized tissue samples for bacteriological culture and direct extraction, live isolates belonging to MTBC, DNA from MTBC members for PCR and molecular characterization techniques, or plasma/serum samples from different animal species. In addition, the preparation of the spoligotyping membrane will be included as reference material and also the whole genome sequence (WGS) of some MTBC isolates will be available. The reference material will be included and available to all NRLs through the EU-RL website (EU-RL Databases, BT Reference Material Database).

Expected Output: Creation of a reference material for the quality control of the protocols implemented to detect MTBC and/or diagnose tuberculosis in animals.

Duration: 2019-2020.

Sub-activity 1.3. *Replacement of the International Standard Bovine Tuberculin (ISBT)* (Art. 94.2.b).

Objectives: To participate in the collaborative study organized by the OIE in order to replace the International Standard Bovine Tuberculin (ISBT).

Description: Since 1986 there is an International Standard Bovine Tuberculin (ISBT) that nowadays is stored at -20ºC in the Medicines and Healthcare products Regulatory Agency-National Institute for Biological Standards and Control (MHRA-NIBSC). The ISBT is the reference reagent in the biological potency tests of PPDs performed in guinea pigs and cattle. Since the stock of this reagent is declining, the OIE has created and *ad hoc* group to set up a timetable and the protocols in order to replace the actual ISBT. In summary, the scheduled activities include: a) Definition of selection criteria for bulk material; b) Request to manufacturers of the bulk material; c) Selection of ISBT candidates; d) Preliminary fill of ampoules (NIBSC); e) Review of protocols by *ad hoc* OIE group and statistician; f) Evaluation of preliminary fill material (OIE-RL); g) Main fill of 5,000 ampoules (NIBSC); h) International

Collaborative Study to test two candidates; and i) Data analysis, report and repository for new ISBT in NIBSC. The EU-RL participates actively in the *ad hoc* meetings organized by the OIE for reviewing the protocols, the International Collaborative Study (ICS) and the evaluation of the results. During 2018, the EU-RL has participated in the revision of protocols, and due a delay in producing the ampoules by the NIBSC, the ICS has been delayed for 2019. In this ICS, the EU-RL will test the new ISBT (ISBT-2) in guinea pigs with the live and heat-inactivated protocols and in natural reactors in cattle.

Expected Output: Participation in the ICS (guinea pigs and cattle) to select and store an ISBT-2 to be distributed by NISBC, under request, to all laboratories and manufacturers worldwide for biological potency testing.

Duration: 2019.

Sub-activity 1.4*. Comparative tests* (Art. 94.2.c).

Objectives: Organize two comparative tests per year.

Description: The EU-RL has the responsibility of organising periodical comparative tests of diagnostic procedures to ensure high quality and harmonization of laboratory testing of bovine tuberculosis across the European Union. The EU-RL ring trials are focused in four main topics: 1) Bacteriological culture and direct detection; 2) Identification and molecular typing; 3) Histopathology; and 4) Immunological diagnosis. The EU-RL will organize two ring trials each year. The call to participate, submission of results and reporting will be carried out through the EU-RL website (Ring Trial Application).

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

Duration: 2019-2020.

Sub-activity 1.5*. Optimization and improvement of official techniques for the diagnosis of bovine tuberculosis* (Art. 94.2.l).

Objectives: Perform studies to optimize and improve the intradermal tuberculin test and the interferon-gamma assay for the diagnosis of bovine tuberculosis.

Description: The official tests for bovine tuberculosis are the skin test and the interferon-gamma assay (Council Directive 64/432). Both protocols have several critical points and the main objective of this sub-activitiy is to study the effect of some of these factors in the performance of both tests. The studies that could be performed within this activity would be:

a) Characterization of the diagnostic interference caused by non-tuberculous mycobacteria in the diagnosis of bovine tuberculosis. The antigenic similarity between mycobacteria has been postulated as the possible origin of diagnostic interferences. Therefore, infection or co-infection with non-tuberculous mycobacteria may cause false positive or negative reactions. To evaluate this effect, laboratory animals will be experimentally infected with non-tuberculous mycobacteria prevalent in cattle (field isolates) and then official diagnostic tests will be applied to characterize the response.

b) Evaluation of the tuberculin injection syringes as a cause of non-specific reactions that can be interpreted as inconclusive/positive reactions in the intradermal tuberculin test regardless the infection status of the animals. Currently in the EU, different models of syringes (Dermojet, McLintock, Hauptner, Henke and Muto) are used for the intradermal injection of tuberculins. The most commonly used systems (Dermojet and McLintock) have been proven to be accurate and reliable tools. The EU-RL will test the Hauptner system used for PPD inoculation in other Member States to determine the existence or not of a significant number of non-specific reactions associated with the syringe.

c) Evaluation of the fraudulent use of corticosteroids to modify the results of the official diagnostic tests. Since the implementation of official campaigns for the eradication of TB in animals, frauds aimed

at modifying the results of official diagnostic tests have been described. In certain TB-farms, the use of corticosteroids prior the interpretation of the skin test has been suspected in order to reduce the reaction at the point of inoculation, which may lead to false negative results. Studies to elucidate the effect of these corticosteroids in the read out of the skin test will be evaluated as well as methodology to detect the corticosteroids in the skin/hair to demonstrate the possible fraud.
All the activities performed during 2019-2020 will be informed to all NRLs in order to keep them up-to-date on the tasks carried out by the EU-RL. The objective would be to submit a brief report with information derived from the activities carried out annually by the EU-RL as well as the most important results and possible derived scientific publications.

Expected output: Optimize and improve the official diagnostic tests for the diagnosis of bovine tuberculosis through the study of several critical factors that could affect their performance.

Duration: 2019-2020.

Sub-activity 1.6*. Evaluation of novel methodologies for diagnosis of bovine tuberculosis* (Art. 94.2.l).

Objectives: To evaluate novel methodologies for diagnosis of bovine tuberculosis in order to coordinate practical arrangements necessary to apply them in each National Reference Laboratory.

Description: The Regulation (EU) 2017/625 defines the responsibilities and task of the EU-RL indicating that it has to coordinate new methodology of laboratory analysis, testing or diagnosis. In this sense, the EU-RL will work in new methodology or developing existing one in order to make it available to all NRLs. In this sense, within this working programme the EU-RL is planning to perform studies in:
a) Evaluation and optimization of Real Time PCRs for the identification of *M. bovis* and *M. caprae*, the main causative agents of bovine tuberculosis. The detection and identification of MTBC has been the priority of the EU-RL (IS*6110* target). However, few Real Time PCRs have been described and the identification of the MTBC species is more precise than the identification at complex level. The implementation of these PCRs will allow NRLs to identify fast and quickly the MTBC members.
b) Evaluation and optimization of alternative DNA direct extraction methods from tissue samples. The EU-RL has implemented a standardized protocol for the DNA extraction and detection of mycobacteria from lymph nodes samples. However, other technologies have been used in order to increase the number of samples tested at the same time, and therefore, to provide quick results to detect MTBC. For instance, the KingFisher™ Flex Magnetic Purification System (Thermofisher Scientific) offers highly versatile, automated magnetic-particle processing for DNA from any source with an excellent reproducibility and quality.
During 2019-2020, the EU-RL will validate any other new methodology available to the Scientific Community prior EC approval. As described for sub-activity 1.5., the NRLs will be informed about the activities related to new protocols performed by the EU-RL.

Expected Output: Evaluate new methodology as an alternative or complementary diagnostic tool for diagnosis of bovine tuberculosis.

Duration: 2019-2020.

Sub-activity 1.7. *Biological Potency of PPDs* (Art. 94.2.l).

Objectives: To define a harmonized protocol for guinea pig potency testing (GPPT) to be implemented in the NRLs.

Description: One of the main activities of the EU-RL for Bovine Tuberculosis is to test the biological potency of the Purified Protein Derivatives (PPD or tuberculin) in 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. The EU-RL is currently working on the

definition of a standardized protocol for potency testing in order to fulfil the requirements defined by the European Commission, European Pharmacopoeia, OIE and Member States. Moreover, during 2019, the OIE International Collaborative Study (Sub-activity 1.3.) will be performed and the results will throw valuable information in order to define the critical factors as well as the best suitable protocol (live *vs.* inactivated) for GPPT. The EU-RL will gather all the available information to recommend a standard protocol.

Expected Output: Standard protocol for biological potency testing in guinea pigs to be set up in the NRLs.

Duration: 2019-2020.

2 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *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.***

  Sub-activity 2.1. EU-RL website.
  See also Sub-activity 1.6. Evaluation of novel methodologies for diagnosis of bovine tuberculosis (Art. 94.2.I).
- *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.***

  Sub-activity 2.2. Training courses.
- *Art. 94.2.g* ***Providing information on relevant national, Union and international research activities to national reference laboratories.***

  Sub-activity 2.3. EU-RL Databases.

Sub-activity 2.1. *EU-RL website* (Art. 94.2.d).

Objectives: To inform the NRLs and the European Commission of the advances in bovine tuberculosis through the EU-RL website.

Description: The EU-RL for Bovine Tuberculosis website has several sections to share information with Member States and the European Commission: a) Bovine tuberculosis section with updated information regarding etiology, diagnosis, epidemiology and eradication; b) EU-RL activities section with information regarding the Work Programme, Workshops, Ring Trials, Training mobility, Visits, Meetings and Missions; and c) Documents section including EU-RL and Bovine Tuberculosis (legislation, Manuals, Working Documents, Reports, Scientific Opinions, etc.). As detailed in Sub-activity 1.5. (Optimization and improvement of official techniques for the diagnosis of bovine tuberculosis) and Sub-activity 1.6. (Evaluation of novel methodologies for diagnosis of bovine tuberculosis), the EU-RL will inform the NRLs regarding the studies performed and the practical arrangements necessary to apply standard or new methodology. This information will be included in the EU-RL website intranet.

Expected Output: To maintain and update the EU-RL website in order to provide updated information of all the relevant aspects regarding bovine tuberculosis (new protocols, ring trials, reference materials, etc.).

Duration: 2019-2020.

Sub-activity 2.2. *Training courses* (Art. 94.2.e).

Objectives: Short visits (2-4 days) for three National Reference Laboratories per year to allow the establishment of laboratory protocols and techniques in their laboratory of origin. To facilitate the implementation of the protocols in the NRLs, training material will be produced and will be available through the EU-RL website.

Description: As defined in article 94 of the Commission Regulation (EC) No 2017/625 the EU-RL must conduct training courses for staff from NRLs and, if needed, from other official laboratories, as well as experts from third countries. The training mobility is focused in laboratory protocols (bacteriological culture, direct extraction, identification by PCR, molecular characterization, histopathology, and detection of IFN-γ) as well as accreditation process and the workflow in a BSL-3. The trainee will present the activities of his/her NRL to the EU-RL and will submit a brief report after the visit. Moreover, learning material will be produced with the aim to assist laboratories in the set up of protocols in their laboratories (EU-RL website, BT Learning Material).

Expected Output: a) Training of three NRLs staff per year in protocols (culture, direct extraction, PCR, DVR-spoligotyping, VNTR, histopathology, IFN-γ test) and accreditation system; b) Learning Material.

Duration: 2019-2020.

Sub-activity 2.3. *EU-RL databases* (Art. 94.2.q).

Objectives: To maintain and update the specific EU-RL databases to provide relevant information to National Reference Laboratories.

Description: The EU-RL database section (EU-RL website) contains several databases with relevant information for National Reference Laboratories regarding:
a) Molecular information. The mycoDB.eu includes characterization data of *M. bovis/M. caprae* isolates identified in each Member State by DVR-Spoligotyping and therefore facilitates future epidemiological studies between Member States.
b) Scientific publications. Every three months the BT Publications Database publishes a newsletter with the main scientific papers regarding culture, extraction and identification, molecular characterization, immunology and other relevant aspects.
c) Laboratory protocols. See Sub-activity 1.1. (BT Protocols Database).
d) Reference material. See Sub-activity 1.2. (BT Reference Material Database).
e) Learning material. The objective of this database is to compile basic information to perform laboratory protocols (BT Learning Material).
f) Images. The BT Images Database will include pictures of laboratory protocols (equipments, reagents, colonies, culture media, etc.) to facilitate the performance/understanding of several protocols.

Expected Output: Maintenance and update the EU-RL Database (mycoDB.eu, BT Publications, BT Protocols, BT Reference Material, BT Learning Materials and BT Images Databases).

Duration: 2019-2020.

Sub-activity 2.4. *Workshop* (Art. 94.2.q).

Objectives: To organize a workshop in 2019 with all the NRLs and the EC desk officer to present the activities performed by the EU-RL in the previous years.

Description: The EU-RL for Bovine Tuberculosis organizes a workshop every two years. During the one and a half day workshop, there are presentations mainly from the EU-RL (EU-RL main activities, comparative test, databases, etc.) and NRLs (relevant activities related with bovine tuberculosis diagnosis). The workshop also includes time for discussion in order to determine the main priorities/problems encountered by the NRLs. All the presentations, agenda and pictures are available in the website (EU-RL Activites).

Expected Output: Annual meeting in 2019 to share information regarding EU-RL activities with the NRLs/EC.

Duration: 2019.

3 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO THE EUROPEAN COMMISSION AND OTHER ORGANISATIONS

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *Art. 94.2.f* ***Providing scientific and technical assistance to the Commission within the scope of their mission***
  Subactivity 3.1. Missions.
- *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).***
  Subactivity 3.2. Collaboration with other countries and International Agencies.
- *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.***
  Subactivity 3.3. Isolation, identification and typing of mycobacteria.

Sub-activity 3.1. *Missions* (Art. 64.2.f).

Objectives: Provide scientific and technical assistance to the Commission (and NRLs) in bovine tuberculosis.

Description: As defined in the article 94 (Commission Regulation 2017/625) the EU-RL should provide scientific and technical assistance to the Commission. The EU-RL system to inform the European Commission is based on: a) Telephone or e-mail communication; b) Information downloaded in the website (BT Databases, Ring Trials, etc.); and c) Visit to the European Commission/NRL if necessary.

Expected Output: To provide reliable and updated information to the European Commission (and Member States).

Duration: 2019-2020.

Sub-activity 3.2. *Collaboration with other countries and International Agencies* (Art. 94.2.h).

Objectives: Collaborate with laboratories in third countries and International Agencies such as EFSA, EMA, ECDC and OIE.

Description: The EU-RL will inform third countries (ie. REMESA Laboratories) regarding the organization of comparative tests (Sub-activity 1.4.), training courses (Sub-activity 2.2.) and workshop (Sub-activity 2.4.). Moreover, the EU-RL will remain at the disposal of the main Organizations (EFSA, EMA, ECDC, OIE, European Pharmacopoeia, etc.) in order to provide scientific and technical information. In this context the EU-RL collaborates, through Sub-activity 1.3. (Replacement of the International Standard Bovine Tuberculin, ISBT), with the OIE.

Expected Output: Collaboration with Third Countries Laboratories as well as International Organizations to share scientific and technical information.

Duration: 2019-2020.

Sub-activity 3.3*. Isolation, identification and typing mycobacteria* (Art. 94.2.h).

Objectives: To assist the NRLs in the diagnosis of outbreaks in Member States by carrying out isolation, identification by PCR and molecular characterization (DVR-Spoligoyting, VNTR analysis, WGS).

Description: Not all the NRLs of the Member States have all the protocols for bovine tuberculosis set up in their laboratories. This fact is more obvious in Officially Tuberculosis Free Member States. In the other hand, the EU-RL has all the methodology implemented and accredited (UNE-EN ISO/IEC 17025:2005). In this sense, the EU-RL will collaborate with the NRLs that need to apply the whole set of protocols in the eventuality of an outbreak.

Expected Output: Assist NRLs regarding isolation, identification and molecular characterization of mycobacteria.

Duration: 2019-2020.

4 REAGENTS AND REFERENCE COLLECTIONS

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *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.***

  See sub-activities 1.2 (Reference material for mycobacteria protocols) and 1.3. (Replacement of the ISBT).

- *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;***

    Sub-activity 4.1. Creation of a reference collection of MTBC strains characterized by Whole Genome Sequencing.
  - ***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.***

    See sub-activities 1.1. (Database with recommended protocols), 1.2. (Reference material for mycobacteria protocols), and 2.1. (EU-RL website).

Sub-activity 4.1. ***Creation of a reference collection of MTBC strains characterized by Whole Genome Sequencing*** **(Art. 94.2.k.i).**

Objectives: Creation of a collection of strains of MTBC characterized by WGS.

Description: Among the tasks of the EU-RL is the application of molecular typing methods for the characterization of the isolates and the conduction of epidemiological studies. The transition from the traditional molecular typing methods (DVR-Spoligotyping and VNTR anlysis) towards whole genome sequencing (WGS) techniques is expected in the coming years. The EU-RL plans to gradually create a collection of fully sequenced strains. Strains will be sequenced by WGS techniques. The assembled genomes of these strains will be used to create a database that will be administrated by EU-RL. This collection of strains and the corresponding database with the assembled genomes will be a valuable tool for conducting epidemiological studies based on WGS.

Expected Output: Collection of fully sequenced strains of MTBC and of a database containing the assembled genomes.

Duration: 2019-2020.

5 REQUIREMENTS RELATED TO OTHER LEGISLATION

Please specify applicable legislation:
(Number of Sub-activity boxes can be adjusted)

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

Objectives: Description: Expected Output: Duration:

REMARKS

(if necessary)