WORK PROGRAMME of EURL for BOVINE TUBERCULOSIS

PERIOD: 1/1/2021 – 31/12/2022

Version 1.0
(date 14/09/2021

CONTACT DETAILS

Contact person:	Dr. Lucía de Juan (EU-RL Director)
Address:	VISAVET Health Surveillance Centre Universidad Complutense de Madrid Avda. Puerta de Hierro s/n 28040 Madrid, Spain
Phone number:	+34 913944300
Fax number:	+34 913943795
E-mail address:	dejuan@visavet.ucm.es

SUMMARY

INTRODUCTION

(Regarding relevant regulations and functions)
A work programme for two years (2021-2022) is presented to follow the Regulation (EU) 2021/690 of the European Parliament and of the Council of 28th April 2021 and the Commission Implementing Decision of 6th April 2021. 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 16 of Regulation (EU) No 2021/690:
(taking into account Art 147 of (EU) 625/2017)

NOTE: The level of compliance of some of the activities will depend on the COVID situation.

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 tuberculosis.
  Sub-activity 1.6. Evaluation of other methodologies for diagnosis of tuberculosis.

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

Objectives: Database with protocols according to regulation 2016/429 and supplementing legislation.

Description: The Regulation (EU) 2016/429 ("Animal Health Law") and its supplementing legislation (2018/1882, 2018/1629, 2020/688 and 202/689) became applicable on the 21st of April 2021. In Article 6 of Commission Delegated Regulation (EU) 2020/689 it is specified the cascade that should be followed regarding diagnostic methods, indicating that the specific legislation and the relevant details and guidance must be available on the websites of the European Union Reference Laboratories. This information should include collection of samples, techniques, validation and interpretation. During 2021 and 2022, protocols will be downloaded in the EU-RL BT Protocols Database website.

Expected Output: Collection of protocols and useful information for the implementation of laboratory methods for the diagnosis of tuberculosis.

Duration: 2021-2022.

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 into 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 *M. tuberculosis* complex (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: 2021-2022.

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

Objectives: To give advice 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 created and *ad hoc* group to set up a timetable and the protocols in order to replace the actual ISBT. During 2020, the first collaborative study finished and, after revision of the results, it was decided to perform an extra analysis with the live *M. bovis* sensitization protocol by the Argentinian OIE Laboratory. The EU-RL will give technical and scientific advice for this second trial and will be involved in the interpretation of the results.

Expected Output: Scientific and technical advice to the OIE *ad hoc* group 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: 2021-2022.

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

Objectives: Organize three comparative tests.

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 one ring trial in 2021 and two ring trials in 2022. 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: 2021-2022.

Sub-activity 1.5*. Definition of official techniques for the diagnosis of tuberculosis* (Art. 94.2.l).

Objectives: Perform field studies or literature review to define the diagnostic tests for the infection with MTBC (*M. bovis, M. caprae* and *M. tuberculosis*).

Description: The Regulation (EU) 2016/429 ("Animal Health Law") and its supplementing legislation (2018/1882, 2018/1629, 2020/688 and 202/689) are in force since the 21st of April 2021. Delegation Regulation 2020/688 lays down requirements for testing of bovine animals (Annex I, Part 2) and of caprine, camelid and cervid animals and Delegation Regulation 2020/689 specifies the rules for surveillance, eradication programmes and disease-free status. The EU-RL should perform field studies and literature review to make available the test protocols for all the species (bovine, caprine, camelid and cervid animals) together with assistance to the EC.

Expected output: Define the diagnostic tests for the infection with *Mycobacterium tuberculosis* complex (*M. bovis, M. caprae* and *M. tuberculosis*).

Duration: 2021-2022.

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

Objectives: To evaluate other methodologies for diagnosis of 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 may work in new methodology, developing existing one (PCRs, DNA extraction, serological tests, etc.) or testing new commercial kits in order to make them available to all NRLs. The NRLs will be informed about the activities related to new protocols performed or tested by the EU-RL.

Expected Output: Evaluate new methodology as an alternative or complementary diagnostic tool for diagnosis of the infection with *M. tuberculosis* complex.

Duration: 2021-2022.

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.l).
- *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 2.4. Workshop.

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

Objectives: To inform the NRLs and the European Commission of the advances in 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) 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 Tuberculosis (legislation, Manuals, Working Documents, Reports, Scientific Opinions, etc.). As detailed in Sub-activities 1.5. and 1.6., 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. During 2021 and 2022, the focus will be the update of the Protocols Database (see Sub-activity 1.1.) to fulfil Regulation (EU) 2016/429 ("Animal Health Law") and its supplementing legislation.

Expected Output: To maintain and update the EU-RL website (mainly Protocols Database) in order to provide updated information of all the relevant aspects regarding tuberculosis (new standard operating procedures, guidelines, ring trials, reference materials, etc.).

Duration: 2021-2022.

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.

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 focuses 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.

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

Duration: 2021-2022.

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) Laboratory protocols. See Sub-activity 1.1. (Protocols Database).
c) Scientific publications. Every three months the Publications Database publishes a newsletter with the most relevant scientific papers regarding culture, extraction and identification, molecular characterization, immunology and other aspects.
d) 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.
e) Reference material. See Sub-activity 1.2. (Reference Material Database).
f) Learning material. See Sub-activity 2.2. The objective of this database is to compile basic information to perform laboratory protocols (Learning Material).

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

Duration: 2021-2022.

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

Objectives: To organize a workshop in 2021 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 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). Due to the COVID-19 pandemic, the workshop will be online.

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

Duration: 2021.

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***

  Sub-activity 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).***

  Sub-activity 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.***

  Sub-activity 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 animal 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 uploaded in the website (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: 2021-2022.

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

Objectives: Collaborate with laboratories in non Member States and International Agencies such as EFSA, EMA, ECDC and OIE.

Description: The EU-RL will inform candidate countries, potential candidate counties, European Free Trade Association (EFTA) countries and 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 with the OIE in Sub-activity 1.3. (Replacement of the International Standard Bovine Tuberculin, ISBT) and also to update the Mammalian Tuberculosis Chapter.

Expected Output: Collaboration with non Member States Laboratories as well as International Organizations to share scientific and technical information.

Duration: 2021-2022.

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 17025:2017). 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: 2021-2022.

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;***

    See sub-activity 1.2. (Reference material for mycobacteria protocols).
  - ***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).

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)