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

WORK PROGRAMME 2013 – PROPOSAL – VERSION 1

VISAVET

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The objective of the work programme is to cover the five points of Annex II to Commission Regulation (EC) No 737/2008:

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

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

3. To organize workshops for the benefit of national reference laboratories as agreed in the work programme and annual budget referred to in Articles 2 to 4 of Regulation (EC) No 156/2004, 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 for a 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, co-ordinate research activities directed towards the improved control and eradication of bovine tuberculosis.

A. MAIN ACTIVITIES OF THE EU-RL FOR BOVINE TUBERCULOSIS FOR 2013

1. Potency test of tuberculins.

Description: A key task of the EU-RL for the last years has been the potency testing studies of all tuberculins (Purified Protein Derivative, PPD) and antigens submitted by National Reference Laboratories (NRLs). These reagents are essential for *in vivo* and *in vitro* diagnostic assays based on cell-mediated immune response. Single intradermal tuberculin (SIT) or single intradermal cervical comparative tuberculin (SICCT) test, are the main techniques used worldwide as diagnostic tests in the tuberculosis eradication campaigns. The potency of a tuberculin is estimated by comparing the size of the reaction elicited by an intradermal inoculation and comparison to the size of the reactions of a "standard" tuberculin of known potency (CVI, Lelystad) in naturally infected cattle.

Objectives: Evaluation of the results obtained in cattle in the EU-RL and in guinea pigs in the NRLs or manufacturers to elaborate a final report regarding the potency of tuberculins in Europe. The evaluation (cattle *vs.* guinea pigs) would be done with the data obtained in the previously years. Moreover, the EU-RL will develop preliminary studies in guinea pigs (infected *vs.* sensitized) to determine the potency test of tuberculins in this animal species in order to define the necessity of testing the tuberculins in cattle.

Expected outputs: a) Final report of the potency studies of the tuberculins and future recommendations; and b) Preliminary results studying and comparing the biological potency of the tuberculins in guinea pigs (infected *vs.* sensitized).

Performance indicators: AH.PT.3.

2. Set up of a direct extraction technique from tissue samples.

Description: The Council Directive 64/432/EEC specifies that the bacteriological culture must be used for confirmation of the infection in an animal although this protocol has the drawback that is time consuming. During the last two years, the EU-RL has tested different protocols for direct extraction of DNA from tissue samples to select the best one and a ring trial for direct extraction has been organized.

Objectives: To perform a comprehensive study of the selected protocol for direct extraction from tissue samples mainly focusing in the homogenization step.

Expected outputs: To define the best homogenization system to obtain an adequate sensitivity of the direct extraction technique considering the bacteriological culture as the gold standard.

Performance indicators: AH.PT.2, AH.PT.3, AH.ANA.1, AH.ANA.2, AH.ANA.QI.

3. Comparative tests

Description: A main activity of the EU-RL for bovine tuberculosis is the organisation of periodic ring trials for standardization and setting up of techniques, and to assure the quality of the results offered by the NRLs.

Objectives: To organize two ring trials for all the NRLs: a) Direct extraction and microbiological culture from naturally tissue samples; and b) Determination of the analytical sensitivity of the PCR for the identification of members of the *Mycobacterium tuberculosis* complex.

Expected outputs: a) Evaluation of the methodology for the direct extraction in comparison with the bacteriological culture; and b) selection of the most sensitive PCR for the detection of *M. tuberculosis* complex isolates. Recommendation of the use of a protocol based on the results.

Performance indicators: AH.PT.1, AH.PT.2, AH.PT.3, AH.PT.4, AH.PT.5, AH.PT.6, AH.ANA.1, AH.ANA.2, AH.ANA.QI.

4. Mycobacteria recovery from different culture media.

Description: The EU-RL has collected information regarding the different media (solid/liquid) used in the NRLs for primary isolation or subculture of members of the *Mycobacterium tuberculosis* complex and there are more than 10 manufacturers and media available.

Objectives: To evaluate the sensitivity of the culture media used in the NRLs.

Expected outputs: To define the sensitivity of the culture media to be able to recommend the media that should be used for isolation of Mycobacteria in the Member States.

Performance indicators: AH.PT.R, AH.ANA.1, AH.ANA.2, AH.ANA.Q1.

5. Assessment of IFN-gamma kits.

Description: In the eradication campaigns for bovine tuberculosis, the IFN-γ test is used for detection of the IFN-gamma against *M. bovis* and *M. avium* antigens. This technique has a higher sensitivity than the skin test and therefore its use is recommended together with the skin test to detect a higher number of infected animals. Nowadays, there are available different commercial kits to detect the IFN-gamma.

Objectives: To evaluate the IFN-gamma kits available in the market to define their suitability for tuberculosis diagnosis. For these objectives several kits will be tested with a set of samples to determine the sensitivity and specificity.

Expected outputs: To define the sensitivity and specificity of the IFN-gamma kits available in the market for tuberculosis diagnosis.

Performance indicators: AH.PT.2, AH.ANA.2, AH.ANA.QI.

6. Reference reagents.

6.1. European Standard.

Description: The European Pharmacopoeia recognized several years ago the WHO international standard (IS) for bovine tuberculin. The stock of the actual international standard is limited and long-standing and it is used as a control in the *in vivo* testing of the tuberculins. During 2012, preservation studies have been carried out to define the best conservation system.

Objectives: The main objective would be the production of an European Standard tuberculin with similar potency to the IS to be distributed to the stakeholders for their potency testing studies. During 2013, potency tests in guinea pigs will be carried out to test the potency and define the suitability of the European Standard to perform the potency studies.

Expected outputs: To test the European Standard *in vivo* (guinea pigs) to guarantee its suitability as an internal control in the potency test studies.

6.2. Other reference reagents.

Description: As defined in the Annex II of the Commission Regulation (EC) No 737/2008 one of the tasks of the EU-RL is to prepare, control and supply reference

reagents to the National Reference Laboratories in order to standardise the tests and reagents used in the Member States.

Objectives: a) To set up a Mycobacteria strain collection to distribute to the NRLs as internal controls for different protocols (culture, PCRs); and b) To collect positive and negative lymph nodes and ADNs for the organization of ring trials.

Expected outputs: Mycobacteria strain collection and reference reagents to organize the ring trials.

Performance indicators: AHPT2.

7. World Wide Web page

Description: The website is a tool for dissemination the roles and tasks of the EU-RL. This website includes the list of NRLs, activities, resources, legislation, EU-RL contact details and links. During 2012, the EU-RL for Bovine Tuberculosis has designed and set up the website (www.bovinetuberculosis.eu) including the BT Protocols database too. The goal during the following years is to focus in each section of the website to improve it or include new ones depending on the queries derived from the NRLs or the European Commission.

Objectives: a) Maintenance of the website; b) To develop an on-line system within the website to register and report the results in the comparative tests organized by the EU-RL.

Expected outputs: On-line system for the registration and participation in the comparative tests.

Performance indicators: AH.R&D.1, AH.ANA.1, AH.PT.1.

8. Missions

Description: The EU-RL staff visits farms to perform field studies (potency testing) and collect samples (blood) and also slaughterhouses to collect tissue samples to perform the bacteriological culture and/or to be included in the sample reference bank. Moreover, the staff of the laboratory must keep abreast of developments in surveillance, epidemiology and prevention of tuberculosis through attendance to congresses, workshops, training courses, reports from experts, legislation, scientific papers, etc.

Objectives: a) To collect biological samples (farm/slaughterhouse); and b) To attend conferences (ie. 6th International Conference on *M. bovis*, United Kingdom; 16th International Symposium of the World Association of Veterinary Laboratory Diagnosticians, Germany) and training courses (ie. programs for statistical analysis of data and training to work with field/laboratory animals).

Expected outputs: Collection of samples for diagnosis activities and organization of comparative tests and scientific training of the EU-RL staff (conferences and training courses).

Performance indicators: AH.NRL.1, AH.NRL.2, AH.NRL.3, AH.NRL.QI.

9. Training of personnel

Description: The short training mobilities are designed to facilitate the inter-laboratory comparison of techniques and to provide training in specific methodologies.

Objectives: Short visits for two National Reference Laboratories to allow the establishment of new protocols and techniques in their laboratory of origin.

Expected outputs: Training of NRL staff in mycobacteria protocols (culture, PCR, spoligotyping, IFN-g test), and accreditation. The trainee will submit a brief report after the visit.

Performance indicators: AH.PT.QI, AH.ANA.1, AH.NRL.4, AH.NRL.QI.

10. Workshop.

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

Objectives: To organize a workshop with all the NRLs to present the information regarding the potency studies of the tuberculins and the results of the ring trials.

Expected outputs: Annual meeting to share information with the NRLs together with the elaboration of a workshop report for all the attendees.

Performance indicators: AH.NRL.1, AH.NRL.2, AH.NRL.3, AH.NRL.QI.

B. OTHER ACTIVITIES OF THE EU-RL FOR BOVINE TUBERCULOSIS FOR 2013.

The following tasks will remain permanent activities of the EU-RL for 2013.

1. Preparation, control and supply of reference reagents, and protocols.

Performance indicators: AH.PT.2, AH.PT.Q1, AH.ANA.1.

2. Collection of representative samples of *Mycobacterium* spp.

Performance indicators: AH.PT.2.

3. Collection of representative serum/plasma recovered from infected animals.

Performance indicators: AH.PT.2.

4. Isolation, identification and typing of *Mycobacterium* spp.

Performance indicators: AH.PT.2, AH.PT.Q1.

5. Supply of the home-made spoligotyping membranes.

Performance indicators: AH.PT.2.

6. Technical assistance to the Commission and NRLs and participation in the bovine tuberculosis subgroup of the Task Force.

Performance indicators: AH.COM.1, AH.COM.2, AH.COM.Q1, AH.OIE.1, AH.OIE.QI.

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

Performance indicators: AH.R&D.1.

8. Keeping abreast of developments (papers, conferences, training courses, reports, legislation, etc.).

Performance indicators: AH.COM.Q1, AH.OIE.1, AH.R&D.1.

9. Research activities (collaboration with NRLs, participation in research projects, etc.).

Performance indicators: AH.COM.Q1, AH.OIE.1, AH.R&D.1.