European Union Reference Laboratory for Bee Health

WORK PROGRAMME 2016-2017

I. Legal duties

The functions and duties of the European Union Reference Laboratory (EU RL) for bee health are described in Commission Regulation (EU) No 415/2013 of 6 May 2013 laying down additional responsibilities and tasks for that laboratory and amending in Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council and repealing Regulation (EU) No 87/2011.

II. Objectives for 2016-2017

Summary:

In 2016-2017, the main activities of the EU RL should focus on:

- the animation of the National reference laboratory network on bee diseases through the organisation of the annual workshop,
- ensuring scientific and technical support for the establishment of uniform practices by transfer and support for adoption of EURL reference methods, inter-laboratory comparative testing and trainings;
- the statistical data analysis from the two years of EPILOBEE programme and the production of scientific publications;
- the support to the European commission, upon request, in the drafting of an epidemiosurveillance project;
- the cooperation on working group and projects with EFSA and international organisations, upon request.

Activity	Description	Objectives	Expected outputs
1.	**Coordination of the network of NRLs for honeybee health**		
1.1	Organisation of events and interactions with the NRLs	➢ To coordinate and give support to the NRL network	- Organisation of the annual workshops (one per year) - Production of the annual workshop minutes - Production of supporting documents and presentations for the workshops - Organisation of trainings for the harmonization of diagnostic techniques: on pesticide residues (2016) and on a molecular method (2017) - Production of the trainings minutes - Production of supporting documents and presentations for the trainings - Daily surveillance of the EURL email box by several scientists and administrative people - Production of leaflets on honeybee diseases (e.g. European Foulbrood and Nosemosis) - Announcements of EURL events on the website - Regular updates on important news regarding honeybee health that might happen in European Union (e.g. SHB dissemination, regular update of the website) (The ring trials are described in part 3.Honeybee diseases and part 4. Pesticides)
1.2	Collating information from the NRLs, for having an overview on the evolution of the NRL analytical capacities	➢ To gather information about the NRL network analytical capacities	- Production of a questionnaire on the NRL activities every year - Production of a report on the NRL activities every year

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2.	**Epidemiology**		
2.1	Data analysis and publication	➢ To finalise the statistical analysis of EPILOBEE data ➢ To publish the EPILOBEE results ➢ To present the EPILOBEE results at international conferences	- Production of scientific publications in international journals for the scientific valorisation of the Epilobee programme (results and lessons to be learnt) - Production and analysis in 2016 of a questionnaire for evaluating the EPILOBEE project (implementation / management / outcomes) to send to the 17 MSs - Submission of oral presentations at international meetings (EurBee (2016), APIMONDIA (2017))
2.2	Support and technical assistance to the EC and the MS – collaboration with EFSA and international organisations	➢ To ensure support/ technical assistance and/or collaboration related to SHB (SHB: small hive beetle) following its detection in Italy ➢ To ensure support/ technical assistance and/or collaboration related to future projects on honeybee colony mortality surveillance	- Participation to meetings with EC and MS or with EFSA upon request - Exchanges and coordination with MS if needed - Production of documents (notes, action papers, protocols), advices and guidance to the EC, MS or other international organisations if requested

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3.	**Honeybee diseases**		
3.0	**Multi-thematic (disease) activities- Support to the NRLs**		
	Maintain and increase the internal stock-culture collections	➢ To maintain and feed the internal stock-culture collections of pathogens with: - the etiological agents of AFB and EFB, with bacteria genetically close to these agents and with bacteria present in honeybee colony - the principal parasites of honeybee (especially *Nosema* and *Acarapis spp*) - the principal arthropods of interest to honeybees	- Production of an updated reference list with strains and isolates available in the EURL stock culture collection (number of strains and isolates in the stock culture collection)
	Ensuring dissemination of analytical and reference methods and materials from the EURL to the NRLs	➢ To ensure the dissemination of reference methods and materials to the NRLs	- Reference methods and materials provided to the NRLs
	Test of automation for nucleic acid extraction	➢ To implement an automated RNA extractions method tested to purify nucleic acids from bee samples. ➢ To increase analytical capabilities and to improve reproducibility ➢ To reduce the risk of cross-contamination between highly positive samples (DNA or RNA target) and negative samples	- Draft method describing the automated method for nucleic acids extraction firstly for the diagnosis of viral diseases.

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3.1	**Bacteriology**		
	Development and validation of the first quantitative method for *Paenibacillus larvae,* aetiological agent of American foulbrood, in the laboratory on honeybee larvae	➢ To validate the real-time PCR quantification method for the identification and quantification of *Paenibacillus larvae* (American foulbrood, AFB) on honeybee larvae ➢ To submit the new developed method and corresponding validation study for publication	- Finalisation of the writing up (procedures and associated documents) for the validation of the real-time PCR method (in accordance with the criteria of the French Standard NF U47-600 for animal health analysis methods – PCR) - Writing up of a protocol to be distributed to the NRL network - Submission of a scientific paper on the validation of the real-time PCR quantification method
	Development of the real-time PCR quantification method for the identification and quantification of *Paenibacillus larvae* on adult honeybees (Adult bees are the most accurate matrix for early detection of AFB in the colony).	➢ To develop a real-time PCR quantification method for the identification and quantification of *Paenibacillus larvae* on adult honeybees ➢ To develop and use a high quality analytical method	- First assays for the method development, according to the French Standard NF U47-600 for animal health analysis methods – PCR) - Writing up of the first steps of the method
	Development and validation of the first quantitative diagnosis method for European foulbrood in the laboratory on honeybee larvae	➢ To validate the real-time PCR quantification method for the identification and quantification of *Melissococcus plutonius* (European foulbrood, EFB) on honeybee larvae ➢ To develop and use a high quality analytical method	- Finalisation of the validation of the real-time PCR quantification method for the identification and quantification of *Melissococcus plutonius* on honeybee larvae. The method should be validated in accordance with the criteria of the French Standard NF U47-600 for animal health analysis methods - PCR

	Project to sequence the complete genome of *Paenibacillus larvae* European isolates (EuroPLarva, European *Paenibacillus larvae* variability) The final objective of EuroPLarva is to identify potential targets for future diagnosis tools.	➢ To pursue the EuroPLarva project launched in 2015 on the typing of isolates, of the main bacteriological agent affecting honeybees, coming from various origins to facilitate and ensure reliability of the diagnosis service in the Union.	- Finalisation of DNA library preparation - Sequencing of the genome of *P. larvae* isolates (performed by the Anses sequencing Platform) - First steps of sequence cleaning and analysis of the various sequences obtained

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3.2	**Parasitology**		
	Bring out the first inter-laboratory comparative testing on molecular based methods for the identification of *Nosema* species organised by the EURL in 2015	➢ To value the inter-laboratory comparative testing organised in 2015 on the capacity of the NRLs to identify *Nosema* species by molecular-based methods	- Production and submission of a scientific article with the participation of all NRLs taking part in the ring trial
	2016: Feasibility study for the preparation of a ring trial on *Nosema* spore quantification	➢ To prepare materials for an inter-laboratory comparative testing on Nosema spore quantification	- Production of reference materials with purified *Nosema* spores from experimental infected bees
	2017: Organisation of the inter-laboratory comparative testing on *Nosema* spore quantification	➢ To evaluate methods used by the NRLs for standardization.	- Production of a panel of samples for the inter-laboratory comparative testing following the 17043 guidelines - Production of the individual and final reports related to the comparative laboratory test on *Nosema* spore quantification
	The detection of *Acarapis woodi* by dissection of the adult honeybee trachea is highly time consuming. The availability of a specific molecular tool should increase the diagnostic potential	➢ To finalise the characterisation of the conventional PCR assay based on the published work of Kojima, *et al.* (2011)[1]. ➢ To support NRLs in the identification of the pathogenic agent (recurrent requests during the last few years) ➢ To develop and use a high quality analytical method on acariosis[2]	- Production of the first steps for the validation of the method (molecular detection of *Acarapis woodi)*

[1] KOJIMA Y., YOSHIYAMA M., KIMURA K. and KADOWAKI T. (2011) PCR-based detection of a tracheal mite of the honey bee *Acarapis woodi*, *Journal of Invertebrate Pathology* **108,** 135–137

[2] disease listed in the list of diseases for which national programmes may be recognized under the Directive 92/65/EEC

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3.3	**Entomology and exotic arthropod**		
	The real time PCR used for the identification of SHB, *Aethina tumida* (adult form and larva) has been validated in 2015. Scientific and technical assistance provided by the EURL to the NRLs for the adoption of this method	➢ To disseminate the molecular method to confirm the identification of *Aethina tumida* in order to improve the standardization of the tools in the NRLs ➢ To support the NRLs in the adoption of the method	- Accreditation in concordance with NF 47-600 guidelines (2016) - Production of the protocol in English for the adult and larva (2016) for diffusion - Sending of the method and the reference material (2016) to NRLs for method adoption - Exchanges with the NRLs for method adoption - Analyse of the NRLs performance (2016/2017) by the evaluation of the LOD_{PCR} and LOD_{method}
	Implementation of molecular method to identify *Aethina tumida* on eggs and hive debris This is essential to have an early diagnosis and to have a test for monitoring free apiaries	➢ To increase the diagnostic potential of the real time PCR by the validation on eggs and hive debris ➢ To develop and use of high quality analytical method	- Collection of coleopteran eggs - Production of the validation steps for eggs and hive debris (LOD_{PCR} and LOD_{method})
	Sequencing of genome from different specimens of *Aethina tumida* (SHB). The final objective is to identify potential targets for future diagnosis tools	➢ To increase knowledge of the molecular structure in order to develop adapted molecular detection tools (e.g. to follow SHB dissemination) ➢ To type of coleopteran specimens coming from various origins to facilitate and ensure reliability of the diagnosis service in the Union	- Preparation of *Aethina tumida*DNA libraries - Sequencing of libraries - Production of *A. tumida* raw sequence data - Production of the *A. tumida* draft genome assembly (contigs)(2017)

	Evaluation of PCR methods for the identification of *Tropilaelaps* species	➢ To disseminate a robust method to the NRLs	- Finalisation of the validation documents for the *Tropilaelaps* spp. molecular identification - Production of the protocol in English to disseminate to the NRLs
	Validation of the morphological identification of *Aethina tumida* The method is based on the OIE method and has been consolidated and improved in 2015. Its performances should be better evaluated	➢ To finalise the validation of the method (evaluation of specificity in particular) and formalisation. (Note: this work will depend on the availability of specimens)	- Production of validation documents for morphological identification of SHB
	To make available the method for morphological identification of *Tropilaelaps* spp.	➢ To submit the method at the international level with a view to suggest improvement to the OIE "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals"	- Publication of the method to make it available for the reference laboratories and to improve the OIE Manual for the reference methods

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3.4	**Virology**		
	Development, standardisation and validation of methods for the detection and the quantification of bee viruses	➢ To produce for the NRLs reference materials (reference samples for standard curves) and methods for the diagnosis of viral diseases ➢ To provide support to the NRLs in the detection of honeybee viruses	- Finalisation of a common protocol for the detection and the quantification of (Acute bee paralysis virus), SBV (Sacbrood virus), DWV (Deformed wing virus) and BQCV (Black queen cell virus) by real time RT-PCR; - Dissemination of the protocol
	Assessment of the specificity of the real-time RT-PCR method for the detection and quantification of CBPV following the proficiency test performed in 2015	➢ To evaluate the specificity of the real-time RT-PCR for the detection and the quantification of the CBPV in bee by a complementary proficiency-test. This test will be restricted to this performance criterion	- Collection of new bee samples free of CBPV - Preparation and shipment of samples to the participants - Analysis of the participant results - Production of the proficiency-test report
	Investigate the possible use of a honeybee cell line (AmE-711line) for *in vitro* production of bee viruses	➢ To request from the authors to acquire this recently established cell line (Goblirsch *et al.*, 2013)[3] and try to subculture it	- Acquisition of AmE-711 line - Production of reagents and subculture trials These outputs will depend on the answer from the authors These outputs will be useful for the production of reference materials, essentially viral purification for the NRLs and inter-laboratory comparative testing organisation
	Support the NRLs for the adoption and use of methods for viral disease diagnosis (by quantitative RT-PCR).	➢ To provide reference materials (Plasmid clones) with a known target load to establish calibration curves ➢ To produce bee samples naturally or experimentally infected with viruses ➢ To organise an inter-laboratory test	- Production of reference material (Plasmid clones) with a known target load for the NRLs to autonomously establish calibration curves - Production of certified reference materials for the NRLs to periodically check the accuracy of their trials - Production of the inter-laboratory test report

[3] Goblirsch MJ, Spivak MS, Kurtti TJ (2013) A Cell Line Resource Derived from Honey Bee (Apis mellifera) Embryonic Tissues. PLoS ONE 8(7): e69831. doi:10.1371/journal.pone.0069831

		to assign values (virus load and its uncertainty) to these reference materials.	
	First steps in the organisation of an inter-laboratory proficiency testing for the diagnosis of DWV infection scheduled for 2018.	➢ To collect samples (positive and negative samples) in the frame of this project	- Collection of bee samples with deformed wings and without clinical signs of DWV infection - Detection and quantification of DWV by real time RT-PCR - Sequencing of detected viruses

Activity	Description	Objectives	Expected outputs
4.	**Pesticides**		
4.1	**Multiresidue methods**		
	Publication of multiresidue methods (prepared in 2015)	➢ To disseminate new analytical methodology on bees and bee products	- Finalisation and publication in a scientific journal
	To ensure the development and use of high quality analytical method through the mass spectrometer detector (GC-MS/MS) purchased in 2015	➢ To adapt and enhance the existing multiresidue methods to the GC-MS/MS instrument (purchased in 2015) ➢ To validate and disseminate new analytical methodology on bees and bee products ➢ To provide support to the NRLs	- Improvement of the quantification and identification of co-eluted analytes for the existing multiresidue methods - Enhancement of the identification and determination of the pesticides in the sample extracts of different matrices
4.2	**Detection and quantification of neonicotinoids**		
	Dissemination of a neonicotinoid method to the NRL network Training on neonicotinoid pesticide analysis will be organised	➢ To disseminate new analytical method to the NRLs for the harmonization of the method of analysis ➢ To support the NRLs in the implementation of the method	- Dissemination of the protocol for the analytical method on neonicotinoid residues - Organisation of a training on neonicotinoid pesticide analysis - Production of a satisfactory questionnaire
	Evaluation of criteria for the preparation of reference materials (in 2016) before organisation of a inter-laboratory comparative testing on a matrix of bee origin	➢ To validate the preparation of reference materials by testing homogeneity and stability of the pesticides studied	- Production of protocols and procedures to prepare reference materials for a group of pesticides (neonicotinoids) - Evaluation of the criteria (homogeneity and stability) following the quality procedure (ISO 17043)
	Organisation of a first inter-laboratory comparative testing on neonicotinoids in bee-related materials (matrix to be defined) (in 2017)	➢ To organize the first inter-laboratory comparative testing on neonicotinoids in a matrix of bee origin	- Production of reference materials for the inter-laboratory comparative testing - Sending of the materials and the protocol to the NRLs - Production of the report including performance and evaluation of the trial