WORK PROGRAMME FOR THE EU
REFERENCE LABORATORY FOR MOLLUSC DISEASES

2014

I. LEGAL FUNCTIONS AND DUTIES

The functions and duties of the EU Reference Laboratory for Mollusc Diseases are given in Annex IV, Part 1 of the Council Directive 2006/88/EC.

II. OBJECTIVES FOR THE PERIOD JANUARY – DECEMBER 2014

Main activities	Sub -activities	Description	Objectives	Expected output	Performance indicators	
I. Coordinate the methods employed for diagnosing diseases	I-1) Organise an Inter-Laboratory Comparison (ILC) test for the detection of some mollusc pathogens by histology and cytology	- Preparation and checking of the required material - Establishing the reference results - Planning the sending and reading of slides - Sending of material In 2015 : - Analysing results - Preparation of the report of the ILC - Presentation of the results of the ILC during the 2015 Annual Meeting	To test the competency of participants regarding the detection of some mollusc pathogens by histology and cytology	Each participant is expected to have more than 60% of good results	AH.PT.1 AH.PT.4	1 60%
	I-2) Additional training for the detection of mollusc pathogens by histology Organise satisfaction survey	When appropriate, specific training (in the EURL and/or by distance using mScope® software) will be proposed to NRLs Satisfaction surveys are sent after the training sessions to participants	To improve the capacity of some NRLs to detect listed mollusc diseases by histology Evaluate the interest of NRLs in the training sessions and improve the organisation of these sessions	All the NRLs should obtain more than 60% of good results in the next ILC test based on histology the EURL	AH.PT.5 AH.PT.6 AH.NRL. 5 & 6	Specific training at distance or in the EURL Minimum expected success rate at the next ILC test based on histology : 60% 75% of positive feedback

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	I-3) Maintain and update the library and collections of mollusc pathogens	The collections of histological material, bacterial strains, ethanol fixed tissue, DNA suspensions are continuously checked and enriched with new reference material from field studies or from outbreaks.	To enrich the different collections	These collections allow the EURL to send reference material to the NRLs on request These collections allow the EURL to prepare material for ILC tests These collections are used for specific or general training especially in histology. These collections are used to develop and validate diagnostic techniques	AH.PT.2	
	I-4) Supply available reference reagents and material to the NRLs on request or when a new diagnostic method is available	Reference material provided to laboratories working on mollusc diseases usually consists of H&E stained histological sections and paraffin blocks when available, bacterial strains as well as DNA suspensions. Considering the increased use of PCR and Real Time PCR in NRLs, the EURL has decided to prepare and monitor under quality management a stock of plasmidic DNA suspensions to be used as positive controls in these PCR assays	To supply reference material to the NRLs	All the NRLs should have: - reference histological slides for the listed mollusc pathogens - positive controls available for the detection of listed pathogens by PCR	AH.PT.2 AH.PT.QI	Per year the EURL sends a minimum of 60 samples The EURL is able to send in less than 3 weeks PCR positive controls for the detection of listed pathogens

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	I-5) Provide the National Reference Laboratories with Standard Operating Procedures (SOPs) for each reference technique (available on the EURL website)	The EURL has written SOPs for molecular biology and histopathology diagnosis. These SOPs are regularly updated following technical improvement or regulation evolution. These SOPs are available under pdf format through the EURL website (http://www.eurl-mollusc.eu/)	To facilitate cooperation between laboratories for the harmonisation of SOPs. To help NRLs in writing the documentation of their Quality Management system.	Updated SOPs for reference methods for the detection of listed mollusc pathogens are available for use by NRLs	AH.PT.3	The EURL will update SOPs already available and if necessary will write new ones (if new reference methods are available or new epidemiological situation occurs)
	I-6) Comparison of PCR assays for the detection of some listed mollusc pathogens	There is no unique PCR assay for the detection of each listed pathogen. In addition to reference technique, NRLs might use their own “in house assay”. A survey carried out in 2013 allowed the EURL to select PCR assays for further comparison and validation works with the NRLs. These comparison and validation works should be initiated in 2014.	To compare and validate most commonly used PCR assays for the detection of listed mollusc diseases	Harmonize the use of PCR assays for the detection of listed mollusc pathogens among the NRLs.	AH.PT.3 and AH.ANA .2	Provide a list of validated and suitable PCR assays for the detection of some listed mollusc pathogens
II- Assist NRLs in the diagnosis of disease outbreaks	II-1) Validation of a Taqman® assay for the detection of *Marteilia refringens* types M and O	The EURL has developed a duplex Taqman® PCR assay for the detection and typing of *Marteilia refringens*. Performance of this assay has been tested on negative populations and infected populations of mussels and oysters.	To establish a SOP for this PCR assay To transfer the technique to all the NRLs	Availability of a new sensitive and specific tool for the detection and typing of *Marteilia refringens*	AH.ANA .1	One new available method for NRLs
	II-2) Development of a multiplex Taqman® PCR assay for the detection of *Bonamia* sp. and *Marteilia refringens*	Infections with *Bonamia* sp. and *Marteilia refringens* are listed endemic diseases affecting flat oysters *Ostrea edulis*. In order to detect both parasites in one step, the EURL has developed a multiplex Taqman® assay including an internal control	To test this PCR assay on more samples for validation To initiate transfer to NRLs	Validation of the assay Dissemination of the PCR assay to several interested NRLs	AH.ANA .2	Validation of the method

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	II-3) Development of a multiplex Taqman® PCR assay and *in situ* hybridization (ISH) assay for the detection of *Bonamia ostreae* and *B. exitiosa*	Infections with *Bonamia ostreae* and *Bonamia exitiosa* are listed diseases affecting flat oysters *Ostrea edulis* in Europe sometimes in same oyster population and even in same oysters. In order to detect both parasites in one step, the EURL would like to develop a multiplex Taqman® assay as well as an ISH assay	To design primers, probes To optimize the Taqman multiplex assay and ISH assay	Optimization of the Taqman multiplex assay and *in situ* hybridization (ISH) assay	AH.ANA .1	Contribution to produce new available PCR and ISH methods for NRLs
	II-4) Comparison of different DNA extraction methods for tissue known to be rich in PCR inhibitors such as digestive gland or clam tissues	Some mollusc tissues are known to be rich in PCR inhibitors and could lead to false negative results in some conditions. In 2013, different DNA extraction methods currently used in NRLs were tested on clam tissue. This comparison approach should be extended to other tissue / species in 2014.	To compare suitability of different commercial DNA extraction kits/methods for some complex tissues	Advice NRLs on the best DNA extraction methods for the detection of pathogens present in complex tissues.	AH.ANA .2	Provide a list of the best methods for DNA extraction in complex tissues.
	II-5) Identify and characterise mollusc pathogen isolates on NRLs' request	The EURL regularly receive samples from NRLs for investigations and diagnosis	To assist NRLs for screening or confirmation diagnosis	Confirm detection of listed or emergent pathogens		Per year the EURL receive a minimum of 30 samples
III- To facilitate the initial and further training	III-1) Organise and prepare the Annual Meeting of the NRLs for mollusc diseases	The EURL will organise the 2014 Annual Meeting for NRLs of mollusc diseases in March or April 2014.	To share information on - the epidemiological situation of EU countries regarding mollusc diseases - diagnosis of mollusc diseases - other mollusc diseases affecting third countries	Establish the situation of EU regarding mollusc diseases in 2013 Training of colleagues from NRLs in mollusc pathology	AH.NRL. 1	One to two representatives from each NRLs for mollusc diseases are expected to attend the 2014 annual meeting (18 to 36 participants) Attendance rate at least 70% of NRL's
	Organize satisfaction survey after the meeting	Organize a satisfaction survey for the annual meeting	To evaluate the interest of NRLs in proposed topics To take into account potential negative feedback to organise next annual meeting	Establish annual meeting agendas which fit with NRLs wishes	AH.NRL. 2 &3	75% of positive feedback

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	III-2) To collect material for and produce a report on the Annual Meeting of NRLs for mollusc diseases	The EURL will produce a report of the meeting including summaries of presentations, discussions and a CDRom of the presentations. The report will circulate among NRLs before its dissemination.	To disseminate information and discussions exchanged during the meeting	Produce and disseminate the report in two months after the annual meeting		
	III-3) Welcome colleagues from NRLs for specific training or visiting NRL for training	Technical staff of the NRLs and other laboratories involved in the diagnosis of mollusc pathogens is regularly welcome for specific training in the EURL. During these periods, the staff of the EURL helps trainees to improve their practice in mollusc disease diagnosis procedures. If necessary and asked by NRLs, staff from the EURL can also visit NRLs for specific training.	To train colleagues from NRLs To improve level of diagnosis and surveillance of mollusc diseases at the EU level	Training of colleagues from NRLs in mollusc pathology	AH.NRL. 4	The EURL regularly welcomes colleagues from NRLs for specific training. At least one colleague should visit us for training in 2014
	III-4) Update mScope®, a tool enabling access to scanned histological slides.	Slides from previous ILC based on histo cytopathology are scanned and can be accessible by NRLs through the EURL website thanks to mScope® software. This tool enables training at distance and self testing. The EURL regularly updates information and material available under mScope®	To give NRLs free access to histological slide collections To train colleagues from NRLs in histo cytopathology	Improve results in the ILCs based on histo-cytology Improve the level of diagnosis for mollusc diseases at the EU level	AH.NRL. 5	mScope® is available at any time by the NRLs through the EURL website
	III-5) Update the internet site of the EU Reference Laboratory (http://www.eurl-mollusc.eu/).	The website is very useful to disseminate information on the main activities of the EURL. This website needs to be regularly updated (reports of last meetings, new updated SOPs, ILC registration results, reports...)	To share updated information on mollusc pathology and EURL activities with other laboratories involved in similar activities especially NRLs	Communication, dissemination of information related to EURL activities		EURL website updated at least once a year

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IV- To have trained personnel available for emergency situations and to assist the commission, EFSA, ...	IV-1) Acquire and maintain competency in screening and confirmatory diagnosis techniques	Organisation of annual competency tests for histo-cytopathology and PCR assays	To have qualified staff available to identify and characterize mollusc pathogen isolates on NRLs' request.	To send results in time (within 3 weeks) especially for first attempt tests and in emergency situation	AH. COM. 1 AH.COM.2	At least one person available for histo cytopathology and one person for real time PCR assays Answer any request related to identification and characterization of mollusc pathogens within 3 weeks
	IV-2) Acquire and maintain competency in surveillance and epidemiology of mollusc diseases	Maintain expertise in mollusc pathology by being involved in several European regional projects	To have qualified staff available to assist the Commission in case of specific requests	To answer any specific request related to mollusc disease surveillance	AH. COM. 1	At least one person available for surveillance and mollusc pathology and one person for epidemiology
	IV-3) Acquire and maintain competency in quality management according to CEN ISO 17025	Regular internal and external training on the CEN ISO 17025	To have qualified staff available to assist NRLs to build their quality management system	To visit and assist NRLs if necessary to establish their quality management system or for audit	AH. COM. 1	At least one person available for quality management according to CEN ISO 17025

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	IV-4) Attend international meetings and conferences	Attendance and participation in International meetings and conferences contribute to maintain competency and to improve our knowledge in specific fields	To participate in the 106th NSA Annual Meeting (29 March-2 April 2014, Jacksonville, Florida, U.S.A.) To participate in the 13th International Congress of Parasitology (10-15 August 2014, Mexico) To participate in the 2nd International Conference on Animal Health Surveillance (7-9 May 2014, La Havana, Cuba)	To improve our knowledge on aquatic diseases and more particularly on parasitic diseases and on animal health surveillance To present results recently obtained by the EURL	AH.COM.1	At least one person will participate
V- Exchange with competent laboratories in third countries	V-1) Collaboration with colleagues from competent laboratories in third country	The EURL collaborates with colleagues from laboratories involved in investigation and surveillance of mollusc diseases including CIBNOR (Baja California, Mexico); VIMS (Virginia, U.S.A.), Aquatic Life Medicine Department (Kusan, Korea) and CSIRO (Victoria, Australia) The EURL is involved in the microcell working group (people working on parasites of the genera *Bonamia* and *Mikrocytos*) The EURL regularly welcomes colleagues from third countries	To exchange information related to mollusc health situation in the world especially on listed pathogens exotic to EU To welcome for training staff from laboratories in third countries	To be recognized as a key laboratory on mollusc diseases diagnosis in the world	AH.OIE.1	Collaboration with colleagues from at least 3 different laboratories from third countries

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VI- Design and conduct epidemiological or experimental studies on the listed diseases in order to improve diagnosis and surveillance at a European level.	VI-1) Determine the geographic distribution and better characterize some members of the genus *Mikrocytos* recently detected in several locations in Europe in infaunal bivalve species	France, Spain and The Netherlands have recently reported mortality of *Donax trunculus* and *Ruditapes philippinarum* associated with the presence of parasites of the genus *Mikrocytos*. However, the distribution of these parasites is not well established and the taxonomic relationships between these different *Mikrocytos* samples need to be investigated.	To alert colleagues from NRLs about the presence of these parasites in Europe and motivate them to get samples when clam mortalities occur To transfer some reference material to facilitate the detection of these parasites by histology To contribute to characterize parasites eventually detected by histology during clam mortality outbreaks	To have European colleagues ready to detect such parasites in order to establish their geographic distribution and to get material to better characterize them	AH. R&D.1	Collaboration with some colleagues from NRLs
	VI-2) Determine the geographic distribution and better characterize bacterial strains of the species *Vibrio aestuarianus* associated with abnormal mortality outbreaks of *Crassostrea gigas* in several locations in Europe	In 2012 and 2013 abnormal mortality events of adults *Crassostrea gigas* have occurred in several oyster producing areas in France. High amount of *Vibrio aestuarianus* is usually detected in moribund oysters. Other European countries including Ireland have reported similar events. However, the distribution of *V. aestuarianus* is not well established and needs to be investigated at the European level.	To alert colleagues from NRLs about the presence of these parasites in Europe and motivate them to get samples when oyster mortalities occur To transfer some reference material and diagnostic technique to facilitate the detection of *V. aestuarianus* To contribute to characterize *V. aestuarianus* eventually detected by PCRduring oyster mortality outbreaks	To have European colleagues ready to detect *Vibrio aestuarianus* in order to establish its geographic distribution	AH. R&D.1	Collaboration with some colleagues from NRLs