

**WORK PROGRAMME FOR THE EU  
REFERENCE LABORATORY FOR MOLLUSC DISEASES**

**2015**

**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 2015**

The following table presents equivalences between activities we have identified and operational objectives proposed in the Commission Work Programme for the year 2015 on financing contribution to the EURLs

OPERATIONAL OBJECTIVES		ACTIVITIES
<b>1</b>	To ensure the development and use of high quality analytical methods across the EU-RL framework	I-4), I-5), I-7) II-1, II-2) III-1), III-2), III-3), III-4) VI-1), VI-2)
<b>2</b>	To maintain appropriate level of proficiency testing ensuring efficiency of control analysis methods	I-1), I-2), I-3), I-4), I-5) III-5)
<b>3</b>	To ensure the availability of scientific and technical assistance provided by the EU-RLs	I-4), I-5), I-6) II-3) III-1), III-2), III-3), III-4), III-5), III-6) IV-1), IV-2), IV-3), IV-4) V-1)
<b>4</b>	To ensure a sound and efficient management of EU-RL funding cycle	VII-1)-

Main activities	Sub - activities	Description	Objectives	Expected output	Performance indicators	
I. Coordinate the methods employed for diagnosing diseases	I-1) Following and ending of the Inter-Laboratory Comparison (2014-ILC-01) test organised in 2014 for the detection of some mollusc pathogens by histology and cytology	Last participants are expected to send their results by April 2015. Once all participants have sent their results: - Analysing results - Preparation of the report of the ILC	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.4	60%
	I-2) Organise an ILC for the detection of the bacteria <i>Vibrio aestuarianus</i> in Pacific cupped oysters <i>Crassostrea gigas</i> by Real Time PCR	-Preparation and checking of the required material -Establishing reference results -Sending samples -Analysing results -Preparation of the report of the ILC	To test the competency of participants regarding the detection of <i>Vibrio aestuarianus</i> by Real Time PCR	About 250 samples are to be prepared  Each participant is expected to have more than 60% of good results	AH.PT.1	1
					AH.PT.2	250
					AH.PT.4	60%
	I-3) Additional training for the detection of mollusc pathogens by histology  Organise satisfaction survey	Based on results obtained during the 2014-ILC-01, 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.NR L.5	At least one specific training organised at distance or in the EURL
					AH.PT.6	Minimum expected success rate at the next ILC test based on histology : 60%
					AH.NRL.6	75% of positive feedback (>85% )

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	I-4) Maintain and update the library and collections of mollusc pathogens	<p>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.</p> <p>In 2015, material will be requested from OIE reference laboratories for listed diseases which are exotic to Europe. Indeed, considering the recent development of molecular tools for the detection of these pathogens, material presently available in the collection of the EURL does not allow providing controls for these PCR tools.</p>	To enrich the different collections	<p>These collections allow the EURL to send reference material to the NRLs on request</p> <p>These collections allow the EURL to prepare material for ILC tests</p> <p>These collections are used for specific or general training especially in histology.</p> <p>These collections are used to develop and validate diagnostic techniques</p>	AH.PT.2	<p>New samples collected through experiments, field studies or confirmatory diagnosis are regularly included in our collections.</p> <p>At least 300 samples are included every year.</p>
	I-5) Supply available reference reagents and material to the NRLs on request or when a new diagnostic method is available	<p>Reference material provided to laboratories working on mollusc diseases usually consists of H&amp;E stained histological sections and paraffin blocks when available, bacterial strains as well as DNA suspensions.</p> <p>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</p>	To supply reference material to the NRLs	<p>All the NRLs should have:</p> <ul style="list-style-type: none"> <li>- reference histological slides for the listed mollusc pathogens</li> <li>- positive controls available for the detection of listed pathogens by PCR</li> </ul>	<p>AH.PT.2</p> <p>AH.PT.Q I</p>	<p>Per year the EURL sends a minimum of 50 samples</p> <p>The EURL is able to send in less than 3 weeks PCR positive controls for the detection of listed pathogens</p>

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	I-6) 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 ( <a href="http://www.eurl-mollusc.eu/">http://www.eurl-mollusc.eu/</a> )	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-7) 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 were initiated in 2014 for the detection of <i>Bonamia</i> sp. and will be continued in 2015.	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
<b>II- Assist NRLs in the diagnosis of disease outbreaks</b>	II-1) Development of a multiplex Taqman® PCR assay for the detection of <i>Bonamia</i> sp. and <i>Marteilia refringens</i>	Infections with <i>Bonamia</i> sp. and <i>Marteilia refringens</i> are listed endemic diseases affecting flat oysters <i>Ostrea edulis</i> . In order to detect both parasites in one step, the EURL has developed a multiplex Taqman® assay including an internal control	To finalize the validation of this PCR assay  To establish a SOP for this PCR assay  To initiate the transfer of the technique to interested NRLs	Availability of a new multiplex tool for the detection of <i>Marteilia refringens</i> and <i>Bonamia</i> sp. in flat oysters	AH.ANA .1  AH.ANA .2	One new available method for NRLs  Validation of the method
	II-2) Development of a multiplex Taqman® PCR assay and <i>in situ</i> hybridization (ISH) assay for the detection of <i>Bonamia ostreae</i> and <i>B. exitiosa</i>	Infections with <i>Bonamia ostreae</i> and <i>Bonamia exitiosa</i> are listed diseases affecting flat oysters <i>Ostrea edulis</i> 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 test these assays on characterized samples  To determine the limit of detection of the techniques	Contribution to the validation of the Taqman multiplex assay and <i>in situ</i> hybridization (ISH) assay	AH.ANA .1	Contribution to produce new available PCR and ISH methods for NRLs

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	II-3) 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
<b>III- To facilitate the initial and further training</b>	III-1) Organise and prepare the Annual Meeting of the NRLs for mollusc diseases  Organize satisfaction survey after the meeting	The EURL will organise the 2015 Annual Meeting for NRLs of mollusc diseases in March or April 2015.  Organize a satisfaction survey for the annual meeting	To share information on - the epidemiological situation of EU countries regarding mollusc diseases - diagnosis of mollusc diseases - other mollusc diseases affecting third countries  To evaluate the interest of NRLs in proposed topics  To take into account potential negative feedback to organise next annual meeting	Establish the situation of EU regarding mollusc diseases in 2014  Training of colleagues from NRLs in mollusc pathology  Establish annual meeting agendas which fit with NRLs wishes	AH.NRL. 1  AH.NRL. 2 &3	One to two representatives from each NRLs for mollusc diseases are expected to attend the 2015 annual meeting (18 to 36 participants) Attendance rate at least 70% of NRL's  75% of positive feedback (>85%)  Agenda of the next Annual meeting will take into account potential negative feedback
	III-2) Organise a Technical Workshop on the diagnosis of some mollusc diseases	The EURL will organise a Technical Workshop combined with the 2015 Annual Meeting. This workshop will include two practical sessions: the first one will be dedicated to the detection of listed mollusc pathogens by histo/cytopathology and the second one will focus on the detection of <i>Vibrio aestuarianus</i>	To train colleagues from NRLs on histology and the detection of <i>Vibrio aestuarianus</i>	Improve diagnostic of mollusc diseases at the EU level	AH.NRL. 5 and 6	One Technical Workshop will be organised in 2015 Attendance rate at least 70% of NRL's
	III-3) To collect material for and produce a report on the Annual Meeting and Technical Workshop of NRLs for mollusc diseases	The EURL will produce a report of both events including summaries of presentations, discussions and a CD Rom of the presentations. The report circulates among NRLs before its dissemination.	To disseminate information and discussions exchanged during these both events	Produce and disseminate the report in two months after the annual meeting		

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	III-4) 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-5) Update mScope®, a tool enabling access to scanned histological slides.	Slides from the EURL collections and 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  At least one at distance training session will be organised in 2015
	III-6) Update the internet site of the EU Reference Laboratory ( <a href="http://www.eurl-mollusc.eu/">http://www.eurl-mollusc.eu/</a> ).	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
<b>IV- To have trained personnel available for emergency situations and to assist the commission, EFSA, ...</b>	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

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	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
	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 17 <sup>th</sup> EAFP meeting (September, 7-11 August 2015, Canary Islands, Spain)	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.CO M.1	At least one person will participate

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<b>V- Exchange with competent laboratories in third countries</b>	V-1) Collaboration with colleagues from competent laboratories in third country	<p>The EURL collaborates with colleagues from laboratories involved in investigation and surveillance of mollusc diseases including VIMS (Virginia, U.S.A.), Aquatic Life Medicine Department (Kusan, Korea),CSIRO (Victoria, Australia) and DFO (MPO, Canada)</p> <p>The EURL is involved in the microcell working group (people working on parasites of the genera <i>Bonamia</i> and <i>Mikrocytos</i>)</p> <p>The EURL regularly welcomes colleagues from third countries</p>	<p>To exchange information related to mollusc health situation in the world especially on listed pathogens exotic to EU</p> <p>To welcome for training staff from laboratories in third countries</p>	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
<b>VI- Design and conduct epidemiological or experimental studies on the listed and emerging diseases in order to improve diagnosis and</b>	VI-1) Determine the geographic distribution and better characterize some members of the genus <i>Mikrocytos</i> recently detected in several locations in Europe in infaunal bivalve species	France, Spain and The Netherlands have recently reported mortality of <i>Donax trunculus</i> and <i>Ruditapes philippinarum</i> associated with the presence of parasites of the genus <i>Mikrocytos</i> . However, the distribution of these parasites is not well established and the taxonomic relationships between these different <i>Mikrocytos</i> samples need to be investigated.	<p>Reference material has been provided to NRLs in 2014 in order for them to detect these parasites by histology</p> <p>In 2015, the EURL will compare parasites of the genus <i>Mikrocytos</i> previously detected during clam mortality outbreaks in Europe. In case new outbreaks occur, the EURL will contribute to characterize these parasites</p>	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



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<b>surveillance at a European level.</b>	VI-2) Determine the geographic distribution and better characterize bacterial strains of the species <i>Vibrio aestuarianus</i> associated with abnormal mortality outbreaks of <i>Crassostrea gigas</i> in several locations in Europe	Since 2012, abnormal mortality events of adults <i>Crassostrea gigas</i> have occurred in several oyster producing areas in France. High amount of <i>Vibrio aestuarianus</i> is usually detected in moribund oysters. Other European countries including Ireland have reported similar events. However, the distribution of <i>V. aestuarianus</i> is not well established and needs to be investigated at the European level.	Reference material was provided to NRLs in 2014 in order for them to detect these parasites by histology In 2015/ - NRLs will be trained to detect the bacteria (technical workshop) - NRLs will be tested regarding their competency to detect the bacteria (ILC). These activities should help NRLs to detect <i>Vibrio aestuarianus</i> in case of <i>C. gigas</i> mortality events. In that context, the EURL will contribute to characterize <i>V. aestuarianus</i> eventually detected by PCR during oyster mortality outbreaks	To have European colleagues ready to detect <i>Vibrio aestuarianus</i> in order to establish its geographic distribution	AH. R&D.1	Collaboration with some colleagues from NRLs
<b>VII- Administrative support</b>	VII-1) Ifremer administrative support to the coordination of the EURL activities	Contribution to establish financial provisional budget Establishment of the financial report Preparation and following of the orders	To contribute to the administrative coordination of the activities of the EURL			