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General aspects of Mediterranean aquaculture diagnostic laboratories

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Country profile and administrative affiliation of laboratories

Of the 75 laboratories to whom the TECAM survey was addressed, 54 replies were received, corresponding to 14 countries.

As regards their affiliation (Table 1) 13 laboratories are from national veterinary services, 14 belong to research institutes, 14 are from the private sector (e.g. feed manufacturing companies, aquaculture companies and consultants), 12 from universities and 2 from other institutions (1 association and 1 interprofessional committee).

Table 1. Administrative affiliation of laboratories by country (question Q.A-2)

Country	Veterinary services	Research institutes	Private sector†	Universities	Others††	Total
Spain	2	3	2	5		12
France	3	4	2		2	10
Greece	1	1	6	2		10
Italy	3			1		4
Portugal		1	2	1		4
Croatia	1	1	1			3
Turkey				3		3
Cyprus	1		1			2
Egypt	1					1
Israel		1				1
Malta		1				1
Morocco		1				1
Romania	1					1
Tunisia		1				1
Total	*13*	*14*	*14*	*12*	*2*	*54*

†Private sector: firm, consultancy, etc.
††Others: association, interprofessional committee.

Regarding the affiliation of the laboratories that have participated in the survey, few comments can be extracted. A clear distribution of affiliations is not observed, either by region or by country. This fact is related to the diversity of the sector and its structure in different countries of the region. It is noteworthy to observe the high number of research centres and universities that carry out diagnostics work. This may be due to several reasons: to the fact that in some countries aquaculture is still a small sector in comparison to other livestock sectors, and the veterinary services still do not attend this demand, or it could also be because the aquaculture productions are growing, and likewise the need for highly qualified research experts is also growing. Furthermore, even in the case of countries such

as Italy where the presence of the veterinary services is noteworthy, knowing that some private companies provide diagnostics services, we did not manage to obtain their participation in the survey. With the publication of the results from this first survey, and the publication on the Internet of the directory of laboratories, we hope to complete the list and the information on the laboratories in the region.

Main species, diseases and techniques worked on in the laboratories

Main species worked on

Of the 54 laboratories that answered the survey 50 are involved in fish diseases (41 of them only in fish diseases), 14 work on mollusc diseases (4 of them only in molluscs diseases), 9 are involved in both mollusc and fish diseases, and 12 make diagnoses of crustacean diseases (Table 2).

Table 2. Species worked on (question Q.A-6)[†]

Fishes	No. labs	Mollusc	No. labs	Crustaceans	No. labs
Seabream and seabass	41	Mussels	12	Shrimp	8
Trout	34	Oysters	12	Freshwater crayfish	1
Eel	21	Clams	9	Other crustaceans	6
Carp	19	Other molluscs	8		
Turbot	16				
Salmon	14				
Other fish species[††]	28				
Total fish	50	Total mollusc	14	Total crustacean	12

[†]From a total of 54 replies.
[††]Other fish species: *Puntazzo puntazzo,* tilapia, mullets, *Acipenser* sp., goldfish, tropical and ornamental fish and catfish.

The finfish species that are most worked with are gilthead seabream, seabass and trout. Second place is held by eel, carp, turbot, salmon and other finfish species including *Puntazzo puntazzo,* tilapia, mullets, *Acipenser* sp., goldfish, tropical and ornamental fish and catfish.

As regards diagnosis of molluscs this is mainly done on mussels, oysters and clams, the main productions in Mediterranean countries.

Finally, there are also some laboratories (12) working on crustaceans, most of them in shrimp (8).

Main diseases worked on

Most laboratories (51 out 54 replies) stated to work on the diagnosis of parasites and bacterial diseases (Table 3). There is a smaller number of laboratories that work on diseases caused by fungi (28) or virus (27). Finally, 21 laboratories are working on nutritional problems, 7 state that they are working on other diseases, such as those related to other problems, including environmental, developmental and tumour-related problems.

The fact that there is a greater number of laboratories that work on parasites and bacteria could have various explanations. For example, that these groups of diseases may have a greater incidence and that many of the diagnostics techniques used for these diseases are more simple and less costly.

In fact, in some non-European countries (for example Egypt, Morocco or Tunisia) laboratories working on virus have not been found. Furthermore, the fact that a laboratory states that it is working on the diagnostics of viral diseases does not necessarily mean that it performs the final diagnosis directly, since this service may be subcontracted.

Table 3. Diseases working on (question Q.A-6)[†]

Diseases	No. labs	No. countries
Parasites	51	13
Bacterial	51	14
Fungal	28	10
Viral	27	9
Nutrition-related	21	10
Others[††]	7	5

[†]From a total of 54 replies.
[††]Others: neoplastic, environmental diseases, disorders of development and "tumour-like" lesions.

Main diagnostic techniques used

Among the diagnostics techniques most frequently used, microscopic observation of fresh samples is worth mentioning, as well as macroscopic study, clinical signs, isolation of bacteria and bacterial biochemical analysis (Table 4).

Table 4. Main diagnostic techniques used (question Q.A-6)[†]

Technique	No. answers
Microscopical observation of fresh samples	53
Macroscopical examination	52
Clinical signs	49
Bacterial isolation	49
Bacterial biochemical identification	42
Histopathology	34
Agglutination	30
Haematological examination	27
ELISA	21
PCR	21
Immunohistochemistry	18
Fluorescent antibody technique	18
Electron microscopy	14
Virus isolation in cell culture	14
Virus seroneutralisation test	13
Immunoblotting	8
Hybridization with DNA probes	8
Immunoelectron microscopy	5
Others	0

[†]Out of a total of 54 surveys, 53 replies.

Used to a lesser degree, but still commonly used, are histopathology, agglutination and haematological analysis. Other techniques are: ELISA, PCR, immunofluorescence and immunohistochemistry.

Less used are electronic microscopy, viral isolation in cell culture as well as seroneutralisation in virus. The diagnostics techniques least used are immunoblotting, hybridisation with DNA probes and immunoelectron microscopy.

Thus, out of 27 laboratories that stated that they worked on virus diagnostics, only 14 (from Croatia, Spain, France, Greece, Italy and Romania) stated that they work on the technique of virus isolation in cell culture.

Another similar case of specialisation occurs with the diagnostics of bacterial diseases. Out of the laboratories that state that they analyse bacterial diseases (51), 49 report that they perform bacteria culture and isolation. The number of laboratories stating that they perform the bacterial biochemical isolation drops to 42, those performing agglutination to 30, and those doing ELISA or PCR to 21.

Regarding the diagnostics of parasite diseases, the case is similar. Out of the 51 laboratories diagnosing parasites, only 34 do histopathology, hence it is probable that a significant number of laboratories only carry out ectoparasite diagnosis.

From these replies one can deduce that some laboratories carry out an initial diagnosis and then do not move on from this phase or send the samples to other laboratories for a corroboration of the diagnosis or a better identification of the pathogen in question.

Sample collection and dispatch

The importance of sampling procedures is stated by the fact that most laboratories, regardless of their affiliation, prefer to work with samples collected by their own personnel or to receive them from farmers (Table 5).

Table 5. Collection and dispatch of samples (question Q.B-1)

	Veterinary services	Research institutes	Private sector	Universities	Total
Taken by own personnel	10 / 13	13 / 13	14 / 14	9 / 12	46 / 52
Sent by farmers	11 / 13	11 / 13	14 / 14	11 / 12	47 / 52
Sent by consultants or feed companies	7 / 13	8 / 13	5 / 14	9 / 12	29 / 52
Sent by other laboratories	9 / 13	6 / 13	2 / 14	7 / 12	24 / 52
Other[†]	4 / 13	2 / 13	0 / 14	1 / 12	7 / 52

[†]Veterinarians, other official services, etc.

Private laboratories (consultants, feed companies, etc.) seem to have a closer relationship with the sector, as all of them stated that they took their own samples or received them from farmers. However, they receive fewer samples from other sources (third laboratories). Although difficult to interpret, there may be several reasons for this. The main one being that private laboratories tend to work as a first step laboratory aiming to provide a fast diagnosis and to propose a prompt treatment, if necessary. On the other hand, the veterinary services, universities and research laboratories receive a higher proportion of samples from third laboratories, which probably means they are more specialized, e.g. identification of pathogens (viruses, etc.).

Aim of laboratory diagnosis

When asked about the aims of the diagnosis made at the different laboratories, all laboratories reported that they worked to diagnose abnormal mortality outbreaks (Table 6).

The diagnosis for the implementation of national regulations is mainly made by the national veterinary services, and laboratories belonging to universities, research institutes and private firms reported this task on a minor scale.

The implementation of epidemiological surveys is made by the national veterinary services (11 answers out of 13), research institutes (9 out of 13) and universities (8 out of 12) but not by private laboratories.

Table 6. Aims of diagnosis made by laboratories (question Q.B-2)

	Veterinary services	Research institutes	Private sector	Universities	Total
To diagnose abnormal mortality outbreaks	13 / 13	13 / 13	14 / 14	12 / 12	52 / 52
As routine controls for commercial purposes	10 / 13	5 / 13	12 / 14	7 / 12	34 / 52
To implement epidemiological surveys	11 / 13	9 / 13	1 / 14	8 / 12	29 / 52
To implement national regulations	11 / 13	6 / 13	4 / 14	3 / 12	24 / 52
Other[†]	0 / 13	2 / 12	0 / 13	1 / 14	3 / 52

[†]Diagnosis made in research projects or for educational purposes.

The diagnosis as a routine control for commercial purposes is mainly made by both private laboratories and laboratories from national veterinary services, and on a minor scale by laboratories from universities and research institutes.

Diagnosis of samples from third countries

Of the 54 laboratories that participated in the survey, 7 laboratories did not answer this questionnaire. As for the rest, 28 stated that they did not make diagnosis of samples from another country, and 19 laboratories (5 private, 4 from veterinary services and 5 from research institutes) did. Most of them received samples for the identification of a pathogen, either virus, bacteria or parasites from other countries of the region but also from outside the region, from another country or even from Asia.

Laboratory diagnosis fee

When the laboratories were asked whether they charged a fee or not for the diagnosis they make, although there was not a clear answer about the procedures followed by the different affiliation of laboratories (Table 7), there are indications that most laboratories charge directly or indirectly for the diagnosis service they provide, either by charging per sample (21), by making the diagnosis under a contract or a project, or in the case of private laboratories (especially the feed companies) by providing this service only for their clients (Table 7).

Table 7. Laboratory diagnosis fee (question Q.B-4)

Charge	Veterinary services	Research institutes	Universities	Private sector	Total
Yes, under contract/project with private firms	6 / 12	7 / 13	6 / 12	4 / 14	23 / 51
Yes, per sample	4 / 12	4 / 13	8 / 12	5 / 14	21 / 51
No	4 / 12	3 / 13	2 / 12	4 / 14	13 / 51
No, but provided only to administration	2 / 12	3 / 13	2 / 12	0 / 14	7 / 51
No, but provided only to clients	1 / 12	0 / 13	0 / 12	6 / 14	7 / 51

Reporting to the administration

When asked if the laboratory reports the diagnosis results to the administration, the answers varied according to the affiliation of the laboratory. Thus:

(i) All laboratories from veterinary services (13) reported that they inform about their results to their

authorities (Ministry of Agriculture, Ministry of Health). Most of them inform about their results on diagnosis and survey concerning notifiable diseases, and others also inform about diagnosis related with outbreaks and epidemiological surveys.

(ii) As for private laboratories (14), 9 out of 12 answers reported not to inform the administration. The other 3 reported they do it, when the disease diagnosed is a notifiable disease.

(iii) As for the laboratories belonging to research institutes (13), it is first to be pointed out that a significant number (9) have agreements with the administration and are recognised as "reference laboratories" for either mollusc or fish diseases, which is why they report to the administration. They report not only notifiable diseases but also disease outbreaks.

(iv) As for laboratories from universities (12), 2 did not answer, 2 answered they did not report and the rest answered that they informed the administration, especially for notifiable diseases.

Production of polyclonal antisera and monoclonal antibodies

Eleven laboratories (Table 8) from 5 countries (France, Greece, Italy, Spain and Turkey) reported the production of polyclonal antisera against fish bacteria (8 laboratories), fish viruses (4 laboratories), fish parasites (1 laboratory) and mollusc pathogens (1 laboratory). All laboratories belong to public institutions: 4 belonging to veterinary services, 4 to universities and 3 to research institutes.

Table 8. Production of reactives and bacterins[†]

	Production of polyclonal antisera	Production of monoclonal antibodies	Production of DNA probes and/or PCR primers	Production of vaccines (bacterins)
Yes	11	0	7	12
No	36	47	40	35
Not answered	7	7	7	7

[†]Out of the 54 surveys.

As for the production of polyclonal antisera against fish viruses they are produced against IPN (4 laboratories), VHS (4 laboratories), IHN (4 laboratories), nodavirus (1 laboratory) and VER (1 laboratory) viruses.

As for the production of polyclonal antisera against bacteria they are produced against the following pathogens: *Vibrio anguillarum* (6 laboratories), *Photobacterium damsela* subsp. *piscicida* (4 laboratories), *Yersinia ruckeri* (4 laboratories), *Aeromonas salmonicida* (2 laboratories), *Hafnia alvei* (1 laboratory), *Flexibacter maritimus* (1 laboratory), *Flavobacterium psychrophilum* (1 laboratory) and *Lactococcus garvieae* (1 laboratory). Some laboratories report to produce antisera but do not inform about the bacteria.

The only laboratory that reports the production of polyclonal antisera against fish parasites is against Myxosporea and *Ichthyophonus* sp. For the mollusc pathogens there is no information.

As for the production of monoclonal antibodies no laboratories (Table 8) have reported their production, which seems to indicate that the development of this reagent is a task for research or for biotechnology companies.

Production of DNA probes and/or primers

Seven laboratories from 3 countries (5 from Spain, 1 from France and 1 from Israel) reported production of DNA probes and/or primers (Table 8).

They are produced against a high number (17) of pathogens (viruses, bacteria and parasites) of

both fish and molluscs, such as: VHSV, IPNV, IHNV, nodavirus, herpes virus, *Yersinia ruckeri*, *Vibrio* spp., *Lactococcus garvieae*, *Pseudomonas anguilliseptica*, *Photobacterium damselae* subsp. *piscicida*, *Hafnia alvei*, *Flavobacterium psychrophilum*, *Flexibacter maritimus*, *Mycobacterium marinum*, *Marteilia refringens*, *Bonamia ostreae* and several Myxosporea.

Production of bacterins

Twelve laboratories from 6 countries (France, Greece, Italy, Morocco, Spain and Turkey) reported the production of bacterins (Table 8). However most of them informed that they did it only for research purposes. Only 1 laboratory declared to be involved in the development of patented vaccines.

Seven laboratories reported the production of bacterins for *Vibrio anguillarum.* Five laboratories reported the production of bacterins for *Yersinia rukeri.* Three laboratories reported the production of bacterins for *Lactococcus garvieae.* Three laboratories reported the production of bacterins for *Photobacterium damselae piscicida.* Two laboratories reported the production of bacterins for *Aeromonas salmonicida.* One laboratory reported the production of bacterins for *Hafnia alvei* and *Flexibacter maritimus.*

Use of commercial diagnostic kits

The use of some type of commercial kits for the diagnosis of bacterial or viral diseases in aquaculture is reported by 34 laboratories corresponding to 14 countries. Thirteen answered they do not use them, and the remaining 7 did not answer this question. Of the 34 positive replies, 5 replies from Morocco, Italy and Greece were not considered because the answers did not specify the kits employed.

It is interesting to comment that a high number of the laboratories that theoretically employ serological kits for the diagnosis of bacterial diseases, do not report their use in the answers included in questionnaire C, which may indicate that the use of these systems is not a routine in those laboratories.

The different kits in use are shown here below according to the disease/group of diseases they are used for. Thus the reader may have an indication of their level of use.

Diagnostic kits/systems used for bacterial fish pathogens

Biochemical identification systems

(i) *API-Systems (Biomerieux, France).* Employed by 21 laboratories from 11 countries distributed as follows: Cyprus (2), Israel (1), Egypt (1), Spain (5), France (1), Greece (3), Italy (2), Malta (1), Portugal (2), Tunisia (1) and Turkey (2).

(ii) *BIOLOG System (USA).* Only employed by 1 laboratory in Israel.

Serological kits

(i) *BIONOR-Agglutination Kits (Mono-Kits) (Norway).* They are utilised by 15 laboratories from 9 countries distributed as follows: Cyprus (2), Spain (3), France (1), Greece (3), Israel (1), Italy (1), Malta (1), Portugal (2) and Turkey (1). These kits are employed mainly for the diagnosis of pasteurellosis (Mono-Pp), vibriosis (Mono-Va) and to a lesser extent for yersiniosis (Mono-Yr) and forunculosis (Mono-As).

(ii) *BIONOR-ELISA based Kits (Aqarapid and AquaEIA kits) (Norway).* These kits are employed by 6 laboratories distributed in 4 countries: Cyprus (1), Spain (1), Greece (2), Portugal (1) and Turkey (1). These kits are employed mainly for the diagnosis of pasteurellosis (Mono-Pp), and to a lesser extent for vibriosis (Mono-Va) and yersiniosis (Mono-Yr).

(iii) *DiagXotics-ELISA based Kit (USA).* This kit is employed only for BKD (*Renibacterium salmoninarum*) diagnosis in 2 laboratories from Greece.

Diagnostic kits used for viral fish pathogens (all are ELISA based kits)

(i) *BIO X (Belgium).* Utilised by 2 laboratories in Spain for the diagnosis of IPNV, VHSV and IHNV (1 laboratory) and SVCV (1 laboratory).

(ii) *TES-LINE (Czech Republic).* Employed by 3 laboratories from 3 countries (Spain, Croatia and Romania) for the diagnosis of IPNV (3 laboratories) and VHSV and SVCV (2 laboratories).

(iii) *DiagXotics (USA).* Employed only by 1 laboratory in Greece for diagnosis of IPNV.

(iv) *TB diagnost*. Used only by 1 laboratory in Spain, but no information is provided for which diagnosis is employed.

Diagnostic kits used for Crustacea

DiagXotics-In situ hybridization kit for shrimp viral diseases (USA). This DNA-based kit is only employed in 1 laboratory of Israel but no specification for which type of virus is reported.

Research needs and technical gaps

With the objective of identifying the common research needs and technical gaps existing in the region, the laboratories were asked about this subject. Although the level of expertise and capability may vary between private and public laboratories and between countries, almost all answers can be grouped in a few areas.

The need for new and faster diagnostic techniques

Fifteen laboratories gave answers related with this subject.

Some answers pointed out the general need for the development of faster and more accurate techniques for the diagnosis of the different pathologies.

A high number of answers (9) pointed out the need to advance in the development of molecular diagnosis methods (PCR and DNA probes mainly) and their applications in the diagnosis of viral and bacterial diseases and also in myxosporean and microsporean parasites of marine species.

The need for the standardisation of diagnostic techniques

Ten laboratories have identified the need for the standardisation of diagnostic methods for the identification of existing pathogens in the region as a high priority.

There were answers for the standardisation bacteriological diagnosis (e.g. vibriosis), or about the validation of reagents, such as primers for PCR or DNA probes to be used in molecular protocols. There was also a mention about the need to agree on reference strains among laboratories.

The need for the production and standardisation of commercial reagents

Eight laboratories pointed out the lack of commercial standardised reactives for routine diagnosis purposes.

Different laboratories mentioned the lack of antispecies polyclonal or monoclonal antibodies, the lack of monoclonal antibodies for viral diseases, or antisera for rapid agglutination tests of various bacterial pathogens.

The need for the development of commercial diagnostic kits

Five laboratories pointed out the necessity for more and better commercial diagnostic kits for the different bacterial, viral and parasitic pathogens.

Two answers were more precise in describing the needs for: (i) biochemical profiling tests (like the API system) tailored to the identification of fish bacteria; and (ii) diagnostic commercial kits for viral diseases without the previous application of cell culture procedures.

The need for the development of more sensitive techniques for the detection of carriers

The need to develop quick, sensitive, non-destructive and reliable techniques for the screening of broodstock and for accurate determination of sub-clinical carrier states has been pointed out by 3 laboratories.

There were specific mentions to several pathogens such as IHNV, VHSV, nodavirus and *Mycobacterium*.