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Design of a new laboratory for quality control of mussel produced in Sagres.

Saber Daoud Abdalla Elwany

Dissertação para a obtenção do grau de Mestre em Química Analítica

Dissertation for obtaining a Master degree in Analytical Chemistry

Mestrado em Qualidade em Análises

(European Master in Quality in Analytical Laboratories)

Trabalho efetuado sob a orientação de:

Work supervised by

Doutora Isabel Maria Palma Antunes Cavaco, University of Algarve, Faculty of Science and Technology and European Master in Quality in Analytical Laboratories (EMQAL).

Doutor John Icely, University of Algarve, Centre for Marine and Environmental Research (CIMA) and Sagremarisco - Viveiros de Marisco, Lda.

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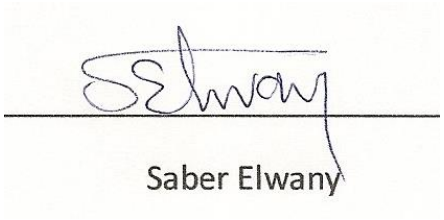
Declaration of Authorship

I declare that I am the author of this work, which is original. The work cites other authors and works, which are adequately referred in the text and are listed in the bibliography.



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Abstract

The growing demand for mussels globally has increased the competition among the producers. Production of high quality mussels demands quality controls in pre- and post-harvesting phases. The main concerns with the mussel industry relate to the pre-harvest stages where monitoring of pollution and management of production.

This present work deals with designing of a laboratory at the Finisterra's aquaculture to control the environmental conditions on the production of mussels focusing on establishing of an efficient quality system. This quality system includes top management commitment, organizational structure and systematically extensive documentation system to comply with the management requirements of the international quality standards. Whilst, the technical part of our quality system covers the environmental conditions of the laboratory, traceability of the results through calibration and quality control activities, and investment in the laboratory personnel. Such quality system will allow the laboratory to be accredited according to ISO/IEC 17025:2005.

This project has been designed with enough flexibility for the Finisterra's management to invest progressively. The base of the laboratory is wet chemistry unit which includes the routine work and preparation for the advanced analysis. Later, Finisterra can complete the work plan by investing in microbiological and advanced analysis.

The key benefits stemming from implementation of this quality system are improved competiveness and reliability, increased quality awareness and teamwork. Furthermore, the laboratory will gain the confidence of the clients, researchers and governmental institutions.

Cost analysis was conducted to estimate the initial and running cost of the quality system. The initial cost is insignificant in comparison to the total laboratory cost whereas running of the quality system increases the total running cost.

Keywords: Quality system, ISO/IEC 17025:2005, Laboratory design, Mussel aquaculture, Quality of shellfish water.

Contents

List of abbreviations

AAS	Atomic Absorption Spectroscopy
ADDIE	Assess, Design, Develop, Implement and Evaluate
BAP	Best Aquaculture Practice
BC	Before Christ
CCC	Coastal Counter Current
CIPP	Context, Input, Process, Product
CIS	Common Implementation Strategy
CVAAS	Cold Vapour Atomic Absorption Spectroscopy
DIN	Dissolved Inorganic Nitrogen
DIP	Dissolved Inorganic Phosphate
EC	European Commission
EPA	Environmental Protection Agency
EQC	External Quality Control
EU	European Union
FAO	Food and Agriculture Organization
FEP	Perfluoro(ethylene/propylene)
GC	Gas Chromatography
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
HPLC	High Performance Liquid Chromatography
HR	Human Resource
HVAC	Heating, Ventilating and Air Conditioning
IEC	International Electro-technical Commission
IPAC	Portuguese accreditition body - Instituto Português de Acreditação

IPO	Input, Process, Output, Outcome
IPQ	Portuguese Institute for Quality - Insituto Português da Qualidade
IQC	Internal Quality Control
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
MPE	Maximum Permissible Error
NA	Not Available
PE	Polyethylene
PP	Polypropylene
PT	Proficiency Test
PTFE	Polytetrafluoroethylene
PVC	Poly(vinyl chloride)
QC	Quality Control
QS	Quality System
RECLARE	Association of Accredited Laboratories in Portugal - Associação de Laboratórios Acreditados de Portugal
SMEWW	Standard Methods for Examination of Water And Wastewater
SPAs	Special Protection Areas
SSTs	Sea Surface Temperatures
SW	South West
TVS	Training Validation System
UV	Ultra Violet
WFD	Water Framework Directive
WHO	World Health Organization

1. Introduction

1.1 Mussel aquaculture

Mussel has been harvested for centuries. Blue mussel shells have been found in kitchen middens dated at 6000 B.C. Until the 19th century, mussels were harvested from wild beds in most European countries for food, fish bait and as a fertilizer. The initial step for mussel aquaculture was based upon storage and relaying fishery products. At the turn of the 1970s, traditional culture was improved by new technological developments using suspended rope culture, longlines (FAO, 2004-2014). Mussel farming is an easy, flexible and realistic measure and can be a cost-effective method to decrease the negative effects of eutrophication in marine waters (Gren et al., 2009). This positive feedback is an important mechanism in shallow ecosystems, that eventually stimulates primary production, hence bivalve food. Thus, mussels are cultured and harvested as a method of water quality management in some areas. Mussels can also be cultured in combination with fish farming, in integrated multitrophic aquaculture (IMTA), where shellfish and seaweed are harvested to compensate for nutrient enrichment through the metabolism of fish feed (European commission, 2012). Numerous of researchers have studied the values of mussel farming in combating the pollution produced through farming of salmon or other finfish (Stirling, 1995; Troell and Norberg, 1998; Whitmarsh et al., 2006; Handå et al., 2012) At the same time, mussel is healthy marine food that produced from a low level of the food chain, and nutrients are recycled from sea to land (Gren et al., 2009).

Marine water aquaculture accounted for about 29.2 percent of world aquaculture production by value in 2010. Global marine-water aquaculture production in 2010 is 18.3 million tonnes consists of marine molluscs (75.5%, 13.9 million tonnes), finfishes (18.7%, 3.4 million tonnes), marine crustaceans 3.8 % and other aquatic animals 2.1%. Figure 1.1 mentions the share of molluscs, mostly bivalves e.g. oysters, mussels, clams, cockles, arkshells and scallops, in the global aquaculture production during the last three decades. A significant part of the global production of marine molluscs, particularly in Europe and America, relies on the widely introduced Japanese carpet shell and Pacific cupped oyster. Moreover, China now produces large quantities of Atlantic bay scallop and Yesso scallop (FAO, 2012).

Among marine molluscs, mussels' production is the third major species group in 2010 (FAO, 2012). In European Union, the production of molluscs and crustaceans accounted about 50 % of the total aquaculture production in 2009 where mussels are dominant among the other species (European commission, 2012).

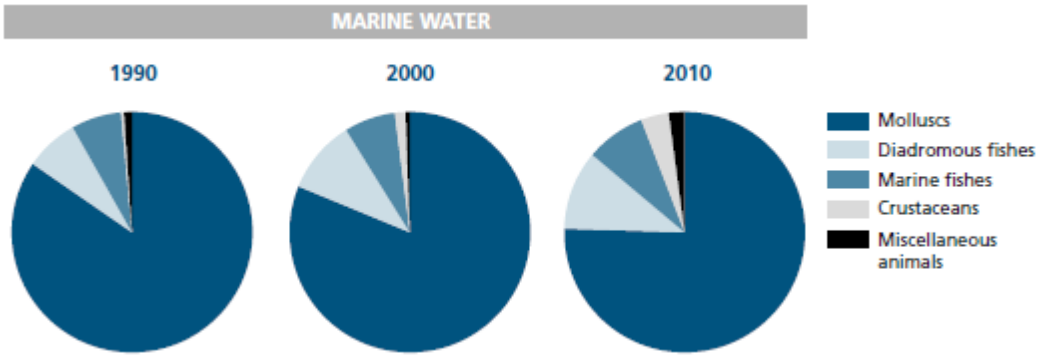


Figure 1.1 World aquaculture production composition in marine water (FAO, 2012).

Three main types of mussel farming are practiced in the EU: shellfish rafts and longlines, intertidal and bottom shellfish culture (European Commission, 2012).

- Shellfish rafts and longlines: Mussel and other shellfish aquaculture in deeper waters, through the use of suspended ropes and longlines from floating rafts, has developed to take advantage of spat fall locations as well as areas of good water quality and food availability. This form of aquaculture has become a particular feature of the Galician coastline of Spain, as well as south, west and northwest of Ireland and some Scottish lochs.
- Inter-tidal shellfish culture is practiced extensively in the Western part of Europe and is one of the older, more traditional forms of aquaculture in the EU. It takes place within the intertidal area, thus benefiting from relatively accessible land-based support as well as the dynamic physical environment of the land/water interface.
- Bottom shellfish culture is a form of shellfish culture where juvenile animals are placed or "re-layed" on a suitable substrate for on-growing. The substrate selected will depend upon the shellfish species being used – mussels and oysters prefer a hard or firm substrate whilst infaunal species such as clams prefer a softer substrate into which they can burrow. This form of aquaculture is often practised in shallow coastal or estuarine areas.

As regards the cultured species, mussels are the main species produced in the EU-27, with two species: the blue mussel *Mytilus edulis* and the Mediterranean mussel *Mytilus galloprovencialis* (European Commission, 2012).

1.2 Laboratory and mussel aquaculture

There is a growing demand for bivalves, not only in historically developed countries, but also in developing regions such as south-east and far-east Asia. The main concerns with the bivalve industry relate to the pre-harvest stages where monitoring of biotoxins, pollution and management of production areas remain problematical, with many producing countries failing to meet the strict requirements imposed by consuming nations. Assistance is needed in improving the pre- and post-harvest practices to produce satisfactory product quality and safety. Thus, the prospects for growing the bivalve industry in the world will depend on the ability to build reliable monitoring and inspection programmes and implement sustainable farming practices (WHO, 2010).

Once the aquaculture sites have been identified for future exploitation or historically exist, the application of monitoring tools is a logical next step. Thus waters should be monitored for multiple contaminants in conjunction with shellfish flesh monitoring for those actual contaminants detected by water quality monitoring (WHO, 2010). A range of natural constituents and potential contaminants can effect on the cultivated mussels depending on the culture area and background water quality as stated in BAP standard. The effects of the pollutants on mussels have been studied, metals (Bebianno et al., 1995), PAHs (Cappello et al., 2013), copper oxide nanoparticles (Gomes et al., 2012), hexavalent chromium (Ciacci et al., 2012), organic compounds (Banni et al., 2010). Whilst Fernández et al (2012) studied the effects of the main marine pollutants, metals, PAHs, PCBs and DDTs in native mussels from the Mediterranean coast of Spain.

Thus, the laboratory has played an important role in mussel aquaculture control by providing the management with the required information. The role of the laboratory is not only to detect the consequences of some pollutants in the aquaculture, but also to prevent some disasters to happen by implementing a quality monitoring program. This program is mentioned in details in regulation (EC) no. 113/2006 of the European Parliament and of the Council, of 12 December 2006. However, the results produced from the laboratory should be reliable, accurate and precise, particularly in case of assessment of compliance with predefined limits.

1.3 Benefits of the quality system

There will be costs in adopting a quality management system but there are compensating benefits (Prichard and Barwick, 2007). One of the biggest benefits of the quality system in analytical laboratory is producing reliable and traceable data. These data have mutual acceptance both nationally and internationally, by manufacturers, regulators, traders and governments (Prichard et al., 1995). Furthermore, accurate and precise results are extremely helpful tool for accepting or rejecting a product according to its specification (Prichard and Barwick, 2007). Hence, ISO/IEC 17025:2005 states that "where applicable information on uncertainty is needed in test reports when the uncertainty affects compliance to a specification limit". Also, regulatory compliance often requires that a measurand, such as the concentration of a toxic substance, be shown to be within particular limits. Measurement uncertainty clearly has implications for interpretation of analytical results as shown in Figure 1.2. A decision rule that is currently widely used is that a result implies non-compliance with an upper limit if the measured value exceeds the limit by the expanded uncertainty. With this decision rule, then only case (i) in Figure 1.2 would imply non-compliance. Similarly, for a decision rule that a result implies compliance only if it is below the limit by the expanded uncertainty, only case (iv) would imply compliance. Another very simple decision rule is that a result equal to or above the upper limit implies noncompliance and a result below the limit implies compliance, provided that uncertainty is below a specified value. This is normally used where the uncertainty is so small compared with the limit that the risk of making a wrong decision is acceptable. To use such a rule without specifying the maximum permitted value of the uncertainty would mean that the probability of making a wrong decision would not be known (EURACHEM, 2007).

In general the decision rules may be more complicated. They may include, for example, that for cases (ii) and (iii) in Figure 1.2, additional measurements should be made, or that manufactured product be compared with an alternative specification to decide on possible sale at a different price (EURACHEM, 2007).

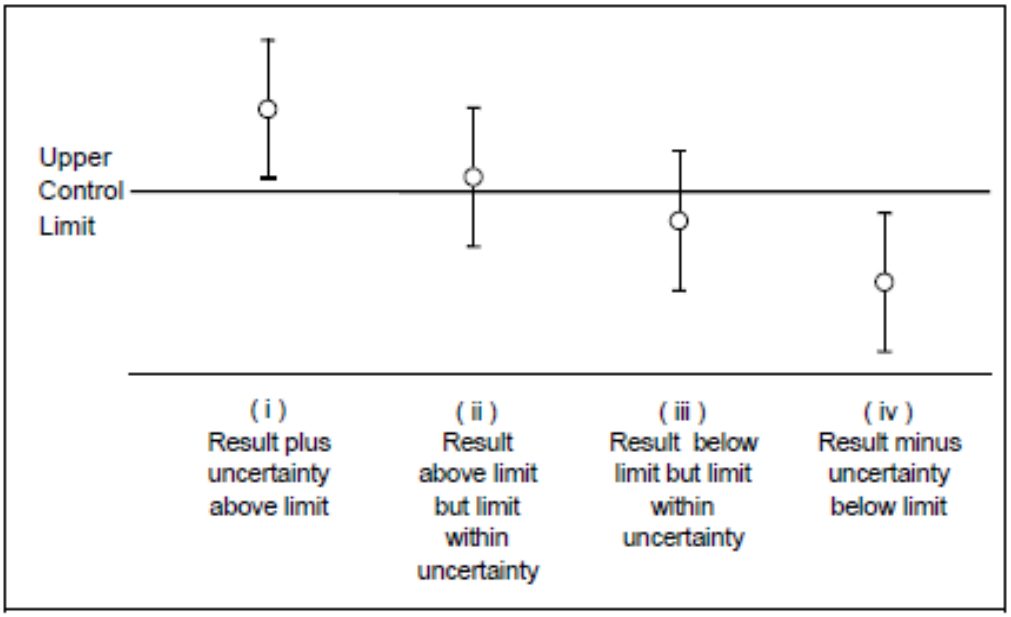


Figure 1.2 Assessment of compliance with an upper limit (EURACHEM, 2007)

On the other hand, producing wrong results has several social and economic impacts in different fields (Prichard and Barwick, 2007):

- In trade, it could lead to the supply of sub-standard goods and the high cost of replacement with subsequent loss of customers.
- In environmental monitoring, mistakes could lead to hazards being undetected or to the identification of unreal hazards.

These mistakes could cause huge costs, both in terms of financial and other resources, and in terms of the distress to individuals and their families. Moreover, such mistakes lead to loss of confidence in the analytical results. At one extreme, loss of confidence puts the future existence of the particular analytical laboratory at risk, but more generally it leads to costly repetition of analyses and, in the area of trade, inhibits the expansion of the world economy (Prichard and Barwick, 2007).

Another benefit of the quality system is the documentation system. Preparation of the documentation is time consuming process and it needs much effort as well. Nevertheless, it is the laboratory's memory and a helpful tool that facilities the communication among different technicians for articulating common principles. In an established business consisting of many laboratories, each operating complex procedures, the more likely it is that misunderstandings and mistakes will have occured and been adopted even though they are bad practice. Even in small laboratories, the

absence of a member of staff who is on holiday or ill can cause confusion. If operating procedures are written down for staff to refer to, as part of the quality management system, the number of such mistakes will be reduced (Prichard and Barwick, 2007).

Therefore, a further positive benefit for laboratories is that the customers of laboratories are increasingly asking for evidence that the laboratory's results are reliable. The easiest way for customers to do this is to insist that an appropriate independent accreditation body accredits any laboratory tendering for their business (Prichard and Barwick, 2007). The benefits of laboratories' accreditation with either ISO/IEC 17025 or ISO 15189 were studied in different countries, Greece (Vlachos et al., 2002), Spain (Bautista-Marín et al., 2012), Turkey (Yanikkaya-Demirel, 2009; Uras, 2009), UK (Maynard, 2003), Canada (Li and Adeli, 2009), Finland (Laitinen, 2009), China (Yang, 2009) and Netherlands (Slagter and Loeber, 2001). The conclusion of those studies is that accreditation of the laboratories increases the quality of the results, motivates the laboratory personnel and is beneficial for all interested bodies. Continuous improvement and dedicated people are the key elements for continuation of the quality assurance in an accredited laboratory. Furthermore, when a laboratory has established its quality management system and has had this assessed and accredited by an external accreditation body, the laboratory can use this recognition of their standards as a positive advertisement for their services (Prichard and Barwick, 2007). Finally, organizations that approach the accreditation process in terms of education and awareness can look forward to a collegial and productive encounter that can verify the high level of quality provided within an organization (Chapman, 2011).

2. Context

2 .1 About Finisterra S.A.

According to work conducted at Finisterra S.A by Márcia Santos (2013), the Finisterra SA performs the cultivation of mussels (*Mytilus* spp. Trade name authorized in Portugal by P n. º587/2006 (I Série-B), de 22 de Junho) using a system of longlines, about 44 ha. This system is installed in the open sea, a mile and a half from the coast, south-west of mainland Portugal, facing the beaches Zavial and Ingrina, located in Sagres (Figure 2.1).

Under the terminology of the European Union (EU) Water Framework Directive (WFD), the Sagres area (SW Portugal) is classified as a mesotidal, moderately exposed Atlantic coastal type, characterized by a narrow continental shelf. It is one of the inter-calibration sites for the North East Atlantic used for the Common Implementation Strategy (CIS) of the WFD (Loureiro et al., 2008). The Sagres area is one of Special Protection Areas (SPAs) belongs to the Natura 2000 ecological network. The Natura 2000 Network aims to protect habitats and species of European interest that are rare or threatened. However it is not a system of strict nature reserves where all human activities are excluded. Instead, it supports the principle of sustainable development. Its aim is to ensure that, within these Natura 2000 sites, human activities, including aquaculture, are undertaken in a way that still allows the site's conservation objectives to be reached (European Commission, 2012).

The Sagres area, off the south west coast of the Iberian Peninsula is affected by northerly winds from May to September (Loureiro et al., 2008). Moreover, this shelf is additionally affected by local westerly winds, that may also induce upwelling episodes, and influenced by the presence of a warm coastal counter current (CCC) originating in the Gulf of Cádiz, that may progress from the southern to the western platform of the peninsular, depending on pressure gradients and south-easterly wind forcing (Relvas and Barton, 2002).The dominance of north winds promotes the occurrence of upwelling events, transporting cold, nutrient rich waters, from the ocean bottom. During these periods, lower SSTs coincide with higher primary production, providing a good environment for culturing bivalves (Fragoso and Icely, 2009).

Figure 2.1 Map of Sagres, Portugal: (A) Location of Finisterra longlines system, (B) Location of Finisterra laboratory.

The production system of Finisterra S.A. is featuring well offshore shellfish farming currently practiced in Portugal. This company produces mussels of the genus *Mytilus* extensively, using a longlines system, with a high potential for offshore aquaculture zone (Santos, 2013). A standard mussel longline consists of individual collectors or sleeves (or one continuous sleeve) suspended from a main line, which is maintained in a horizontal position using floats (Gosling, 2003). The longlines system in Finisterra consists of mounting system, moorings and cement weights 4 and 6 tons (Figure 2.2), and floating system, floating and mooring rafts (Figures 2.2 and 2.3). The suspended ropes are placed with a distance of 50 cm to 1 m from each other (Figure 2.2). In order to maximize the production, longlines of Finisterra S.A. are in an area where the depth varies between 20 m and 30 m and the used culture ropes extend between 2 m and 20 m deep approximately.

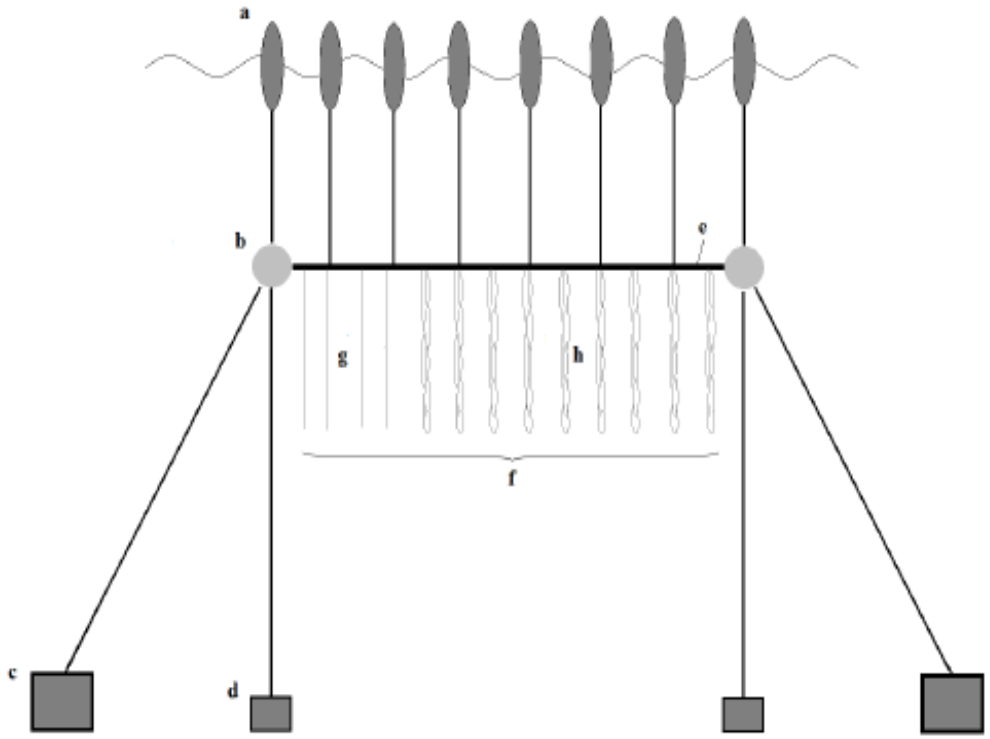


Figure 2.2 Scheme of longlines system used by Finisterra, (a) marker rafts, (b) floating rafts, (c) cement weights 6 tons, (d) cement weights 4 tons, (e) longlines, (f) culture ropes,(g) empty culture ropes for mussel juveniles' growth, (h) culture ropes after mussel growth (Santos, 2013).

Figure 2.3 Different types of floating rafts used in Finisterra longlines system (Santos, 2013).

Juveniles are obtained by simply placing culture ropes, exploiting the natural recruitment in the production process, or collected from natural beds (approximately 5 mm) and fixed to the culture ropes (Figure 2.4a) using biodegradable network (Figure 2.4b). This network is degraded after approximately 10 days which are sufficient to allow attachment of mussels to culture ropes.

Figure 2.4 (a) culture ropes (b) biodegradable network, available from http://www.jjchicolino.es/catalogo.pdf. Accessed in 09 March 2014.

For maintenance of the longlines system and also to transport the mussels to land an adapted vessel for this work is used as mentioned in Figure 2.5.

Figure 2.5 Harvesting of mussel using a vessel at Finisterra (Santos, 2013).

2.2 Laboratory framework

Mussels are bivalve molluscs that feed by filtering a large volume of water and therefore have a capacity to bio-accumulate natural constituents and potential contaminants from the surrounding waters according to BAP standard. Suspended shellfish culture may also have an impact on the water column in both terms of dissolved oxygen levels as well as nutrients. However, the location of this type of system in areas with good water exchange and thus good dispersion of nutrients usually reduces the risk for such effects (European Commission, 2012). Hence, the protection and improvement of the environment necessitate concrete measures to protect waters, including shellfish waters, against pollution as stated in regulation (EC) no. 113/2006 of the European Parliament and of the Council, of 12 December 2006.

BAP standard describes the types of the contaminants affecting the mussel farm, "Many contaminants widely present in trace levels within the marine environment are unlikely to compromise product safety. Other contaminants can be elevated in certain areas due to continuous proximity to direct or indirect sources. Some areas with generally high water quality can be subject to periodic deterioration due to intermittent discharges, pollution spills or even natural events such as harmful algal blooms." Therefore, the laboratory started designing a plan to monitor the water quality at Sagres, this plan consists of three main steps: specification of the monitoring parameters, characterization of the water at Sagres, methods selection. We have to discuss these three steps in details as follows:

1. Specification of the monitoring parameters

   Regarding to regulation (EC) no. 113/2006 of the European Parliament and of the Council, of 12 December 2006., which concerns the quality of shellfish waters and applies to those coastal and brackish waters designated by the EC Member States as needing protection or improvement in order to support shellfish life and growth and thus to contribute to the high quality of shellfish products directly edible by man. The shellfish water quality monitoring programme of the previous directive is mentioned in Table 2.1. Moreover, these parameters are also mentioned by WHO in 2010 as the primary risk factors that affect the shellfish water quality. However, BAP standard recommended physical, chemical and biological parameters to protect both the mussel and the

environment in addition to other parameters to control the mussel production process such as food availability, phytoplankton, which has impacts on the growth and health of molluscs and other organisms.

Table 2.1 The monitoring program of quality of shellfish waters according to regulation (EC) no. 113/2006 of the European Parliament and of the Council, of 12 December 2006

#	parameters	G	I	Reference methods of analysis	Minimum sampling and measuring frequency
1	pH		7-9	Electrometry Measured in situ at the time of sampling	Quarterly
2	Temperature °C	A discharge affecting shellfish waters must not cause the temperature of the waters to exceed by more than 2 °C the temperature of waters not so affected		Thermometry Measured in situ at the time of sampling	Quarterly
3	Coloration (after filtration) mg Pt/l		A discharge affecting shellfish waters must not cause the colour of the waters after filtration to deviate by more than 10 mg Pt/l from the colour of waters not so affected	Filter through a 0·45 µm membrane Photometric method, using the platinum/ cobalt scale	Quarterly
4	Suspended solids mg/l		A discharge affecting shellfish waters must not cause the suspended solid content of the waters to exceed by more than 30 % the content of waters not so affected	- Filtration through a 0·45 µm membrane, drying at 105 °C and weighing - Centrifuging (for at least five minutes, with mean acceleration 2 800 to 3 200 g), drying at 105 °C and weighing	Quarterly
5	Salinity ‰	12 to 38 ‰	≤ 40 ‰ Discharge affecting shellfish waters must not cause their salinity to exceed by more than 10 % the salinityof waters not so affected	Conductimetry	Monthly
6	Dissolved oxygen (Saturation %)	≥ 80 %	≥ 70 % (average value) Should an individual measurement indicate a value lower than 70 %, measurements shall be repeated An individual measurement may not indicate a value of less than 60 % unless there are no harmful consequences for the development of shellfish colonies	- Winkler's method - Electrochemical method	Monthly, with a minimum of one sample representative of low oxygen conditions on the day of sampling. However, where major daily variations are suspected, a minimum of two samples in one day shall be taken

7	Petroleum hydrocarbons		Hydrocarbons must not be present in the shellfish water in such quantities as to: — produce a visible film on the surface of the water and/or a deposit on the shellfish, — have harmful effects on the shellfish	Visual examination	Quarterly
8	Organohalogenated substances	The concentration of each substance in shellfish flesh must be so limited that it contributes, in accordance with Article 1, to the high quality of shellfish products	The concentration of each substance in the shellfish water or in shellfish flesh must not reach or exceed a level which has harmful effects on the shellfish and larvae	Gas chromatograph after extraction with suitable solvents and purification	Half-yearly
9	Metals mg/ Silver Ag Arsenic As Cadmium Cd Chromium Cr Copper Cu Mercury Hg Nickel Ni Lead Pb Zinc Zn	The concentration of each substance in shellfish flesh must be so limited that it contributes in accordance with Article 1, to the high quality of shellfish products	The concentration of each substance in the shellfish water or in the shellfish flesh must not exceed a level which gives rise to harmful effects on the shellfish and their larvae The synergic effects of these metals must be taken into consideration	Spectrometry of atomic absorption preceded, where appropriate, by concentration and/or extraction	Half-yearly
10	Faecal coliforms/100 ml	≤ 300 in the shellfish flesh and intervalvular liquid		Method of dilution with fermentation in liquid substrates in at least three tubes in three dilutions. Subculturing of the positive tubes on a confirmation medium. Count according to MPN (most probable number). Incubation temperature 44 °C ± 0,5 °C	Quarterly
11	Substances affecting the taste of the shellfish		Concentration lower than that liable to impair the taste of the shellfish	Examination of the shellfish by tasting where the presence of one of these substances is presumed	
12	Saxitoxin (produced by dinoflagellates)				

Abbreviations: G = guide and I = mandatory

2. Characterization of the water at Sagres

Before selecting the methods to analyse the monitoring program parameters, it is vital to know the concentration range of each parameter and the type of samples' matrix. Sagres region is influenced by upwelling conditions producing change in the water characterization. The microalgal assemblage peaks during the active upwelling conditions whereas relaxation of upwelling is associated with low nutrient conditions (Loureiro et al., 2005). However, the upwelling introduces near the coast a large flux of comparatively trace metals-poor waters, which prevent the shelf waters from a further dispersal of the land-derived contaminants (Cotte-Krief et al., 2000). The available data were collected as shown in Table 2.2.

Table 2.2 Characterization of the water at Sagres

#	Parameter	Goela et al., 2013	Santos-Echeandía et al., 2012	Loureiro et al., 2005	Cotte-Krief et. al., 2000
1	Temperature	NA	12^{o} C	14.6 - 17.0 ^{o} C	NA
2	Salinity	NA	33 – 35	35.8 – 35.9	36.27 – 36.28
3	pH	NA	NA	NA	NA
4	Alkalinity	NA	NA	NA	NA
5	Dissolved Sulphide	NA	NA	NA	NA
6	Dissolved Oxygen	NA	5.5 mg/L	237 – 258 µM	NA
7	Ammonia	0.55-15.5 µM (DIN)	0.5 – 4.0 µM	0.05 – 0.6 µM	NA
8	Nitrate			4.3 – 19.3 µM	NA
9	Nitrite			0.1 – 0.4 µM	NA
10	Phosphate	0.03-0.40 µM (DIP)	0.1 - 0.5 µM	0.2 – 0.4 µM	NA
11	Silicate	NA	0.06 µM	0.1 – 2.4 µM	NA
12	Suspended Solids	NA	NA	NA	NA
13	Chlorophyll & pigments	NA	NA	NA	NA
14	Coloration	NA	NA	NA	NA
15	Petroleum Hydrocarbons	NA	NA	NA	NA
16	Substances affecting the taste of the shellfish	NA	NA	NA	NA
17	Fecal coliforms	NA	NA	NA	NA
18	Organohalogenated substances	NA	NA	NA	NA
19	Chlorophyll	0.1 - 2.31 µg/L	1.0 µg/L	1.2 – 6.2 µg/L	NA
20	Silver Ag	NA	NA	NA	NA
21	Arsenic As	NA	NA	NA	NA
22	Cadmium Cd	NA	0.15 – 0.25 nM	NA	0.78 – 0.211 nM
23	Chromium Cr	NA	NA	NA	NA
24	Copper Cu	NA	6.0 – 9.0 nM	NA	3.7 – 12.2 nM
25	Mercury Hg	NA	< 8.0 pM	NA	NA
26	Nickel Ni	NA	< 3.0 nM	NA	2.7 – 3.4 nM
27	Lead Pb	NA	< 0.03 nM	NA	7.3 – 29.3 nM
28	Zinc Zn	NA	10.0 – 15.0 nM	NA	NA

NA: Not Available DIN: Dissolved Inorganic Nitrogen DIP: Dissolved Inorganic Phosphate

3. Methods selection

Proper method selection is critical to laboratory performance and must be mentioned under the quality system but in fact there is no simple, established formula (Garfield, 2000). The assessment process may involve a combination of efforts including a literature review, reference to personal experience or the experience of colleagues, consideration of what the instruments are available, the amount of time for analysis, the accuracy and precision required and similar considerations (Garfield, 2000). We established our own criteria to select the suitable methods:

- Legal requirement to use a specific method.
- One of the ISO/IEC 17025:2005 requirements is preference to select methods published in international, regional or national standards.
- Preference to be given to methods that have been applied to the matrix of interest over methods that have been applied in other matrices.
- Methods cover the concentration range of the interest with high accuracy, simple, low cost and rapid are chosen.

Table 2.3 mentions how we applied our criteria to select the suitable standard methods for each parameter.

According to these information, we specified all the parameters that related to environment protection and mussel production process including physical, chemical and microbiological parameters. The available data were used to characterize the water at Sagres and then the suitable standard methods were selected to fit the purpose of our work plan (Table 2.4).

Table 2.3 Selection of the suitable methods

Group	#	Parameter	Standard methods		The selected standard/reasons
			1st	2nd	
Sampling	1	Planning	ISO 5667-1:2006	----------	ISO 5667-1:2006 Guidance on the design of sampling programmes and sampling techniques
	2	Preservation	ISO 5667-3:2012	----------	ISO 5667-3:2012 Preservation and handling of water samples
	3	Sampling	ISO 5667-9:1992	----------	ISO 5667-9:1992 Guidance on sampling from marine waters
Wet-chemistry	1	Water for analytical laboratory use	ISO 3696:1987	----------	ISO 3696:1987 Water for analytical laboratory use
	2	pH	ISO 10523:2008 Glass Electrode	ISO/CD 18191 M-cresol purple	ISO 10523:2008 Glass electrode method is fast, and environmental friendly
	3	Alkalinity	ISO 22719:2008 Potentiometric Titration	----------	ISO 22719:2008 Determination of total alkalinity in sea water using high precision potentiometric titration.
	4	Dissolved Sulphide	ISO 10530:1992 Photometric Method Using Methylene Blue	EPA 9034 Titrimetric Method	ISO 10530:1992
	5	Dissolved Oxygen	ISO 5813:1983 Winkler Method	ISO 5814:2012 Electrochemical Probe	ISO 5814:2012 Electrochemical probe is fast and environmental friendly.
	6	Nitrate	ISO 7890-3:1988 Reduction / Spectrometric Method	SMEWW 4500-$NO3^-$ - B UV Screening Method	SMEWW 4500-$NO3^-$ - B UV screening is fast and easy to work whereas reduction of NO_3^- by Cd/Cu redactor is a complicated method.

Group	#	Parameter	Standard methods		The selected standard/reasons
			1st	2nd	
Wet-chemistry	7	Nitrite	ISO 6777:1984 Spectrometric Method	----------	ISO 6777:1984 Determination of nitrite – Molecular absorption spectrometric method
	8	Phosphate	ISO 6878:2004 Spectrometric Method	----------	ISO 6878:2004 Determination of phosphorus -- Ammonium molybdate spectrometric method
	9	Silicate	ISO 16264:2002 Automated Spectrometric Method	SMFWW, 4500C-SiO_2 Manual Spectrometric Method	SMEWW, 4500C-SiO_2 Molybdosilicate method is the manual version of ISO 16264:2002.
	10	Ammonia	ISO 7150-1:1984 Manual Spectrometric Method	ISO 6778:1984 Ion Selective Electrode	ISO 7150-1:1984 Manual Spectrometric method is more sensitive than Ion Selective Electrode
	11	Suspended solids	ISO 11923:1997 Filtration / Drying	----------	ISO 11923:1997 Determination of suspended solids by filtration through glass fibre filters.
	12	Conductivity, Depth and Temperature	Manufacturer Manual	----------	Manufacturer manual of SEACAT SBE19
	13	Chlorophyll	SMEWW,10200H Spectrometric Method	EPA, 446 Spectrometric Method	EPA, 446 In Vitro Determination of Chlorophylls a, b, c_1 + c_2 and Pheopigments in Marine And Freshwater Algae by Visible Spectrophotometry
	14	Coloration	ISO 7887:2011	----------	ISO 7887:2011 Examination and determination of colour.
	15	Petroleum Hydrocarbons	Visual examination	----------	These methods are recommended by the directive
	16	Substances affecting the taste of the shellfish	By tasting	----------	

Group	#	Parameter	Standard methods		The selected standard/reasons
			1st	2nd	
Microbiology	1	Faecal coliforms	ISO 4831:2006 Most Probable Number	ISO 9308-1:2000 Membrane Filter	ISO 4831:2006 MPN is recommended by the directive
	2	Sampling	ISO 19458:2006	----------	ISO 19458:2006 Sampling for microbiological analysis
	3	General requirements	ISO 7218:2007	----------	ISO 7218:2007 Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations
Advanced analysis	1	Chlorophyll	SMEWW,10200H HPLC method	EPA, 447 HPLC method	EPA, 447 Determination of Chlorophylls a and b and Identification of Other Pigments of Interest in Marine and Freshwater Algae Using High Performance Liquid Chromatography with Visible Wavelength Detection
	2	Organohalogenated substances	ISO 23631:2006 Using GC Or ISO 10301:1997 Using GC	EPA, 8021 Using GC	EPA, 8021 is for determination of Organohalogenated substances generally whereas ISO 23631:2006 is limited to measure haloacetic acid and ISO 10301:1997 is for determination of highly volatile Organohalogenated substances.
	3	Metals: Silver Ag Arsenic As Cadmium Cd Chromium Cr Copper Cu Mercury Hg Nickel Ni Lead Pb Zinc Zn	ISO 15586:2003 Using GFAAS , ISO 12846:2012 Using CVAAS	----------	ISO 15586:2003 includes principles and procedures for the determination of trace levels of: Ag, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, and Zn. ISO 12846:2012 specifies two methods for the determination of mercury in water.

Table 2.4 The concentration range of monitoring parameters and the selected methods.

Group	#	Parameter	Conc. range	Method
Sampling	1	Planning		ISO 5667-1:2006
	2	Preservation		ISO 5667-3:2012
	3	Sampling		ISO 5667-9:1992
Wet-chemistry	1	Water for analytical laboratory use		ISO 3696:1987
	2	Temperature	12 – 17 ^{o} C	Manufacturer Manual
	3	Salinity	33 – 36 %	
	4	pH	NA	ISO 10523:2008
	5	Alkalinity	NA	ISO 22719:2008
	6	Dissolved Sulphide	NA	ISO 10530:1992
	7	Dissolved Oxygen	5.5 mg/L	ISO 5814:2012
	8	Ammonia	0.05 – 0.6 µM	ISO 7150-1:1984
	9	Nitrate	0. 5 – 19.3 µM	SMEWW 4500-NO_3^- -B
	10	Nitrite	0.1 – 0.4 µM	ISO 6777:1984
	11	Phosphate	0.03 – 0.5 µM	ISO 6878:2004
	12	Silicate	0.06 – 2.4 µM	SMEWW 4500-SiO_2- C
	13	Suspended solids	NA	ISO 11923:1997
	14	Chlorophyll and pigments	NA	EPA, 446
	15	Coloration	NA	ISO 7887:2011
	16	Petroleum Hydrocarbons	NA	Visual Examination
	17	Substances affecting the taste of the shellfish	NA	By Tasting
Microbiology	1	Faecal coliforms	NA	ISO 4831:2006
	2	Sampling for microbiology		ISO 19458:2006
	3	General requirements for microbiology		ISO 7218:2007 Amd 1:2013
Advanced analysis	1	Chlorophyll	0.1 – 6.2 µg/L	EPA, 447
	2	Organohalogenated substances	NA	EPA, 8021B
	3	Digestion of the metals		ISO 15587-2
		Mercury Hg	< 8.0 pM	ISO 12846:2012
		Silver Ag	NA	ISO 15586:2003
		Arsenic As	NA	
		Cadmium Cd	0.15 – 0.25 nM	
		Chromium Cr	NA	
		Copper Cu	3.7 – 12.2 nM	
		Nickel Ni	2.7 – 3.4 nM	
		Lead Pb	< 0.03-29.3 nM	
		Zinc Zn	10.0 – 15.0 nM	

NA: Not Available

3. Quality System

3.1 Organization

Finisterra's laboratory is a private laboratory of Portugal, offering quality services in water field to its internal and external customers.

3.1.1 Laboratory's structure

Finisterra's laboratory is specialized in performing standard analysis in water field in order to support research to ensure and improve the quality of the company's product, mussel ssp. The laboratory is designed to perform all the parameters in the laboratory work plan as mentioned in *Chapter 2*. It is specialized in environmental monitoring and has ability to support related research projects by providing the infrastructures for chemical and water microbiological analysis under an accredited quality system. The system will ensure the reliability of results, recognition of the laboratory and confidence of the company form its clients, from the local government and from society in general.

The laboratory consists of three basic units, customer services office and quality department:

- Wet chemistry unit

  This laboratory performs wide range of physical and chemical water analyses such as temperature, salinity, pH, alkalinity, dissolved oxygen, suspended solids, dissolved sulphides, ammonia, nitrite, nitrate, phosphate, coloration in addition to preparation of the samples for advanced analyses, digestion for AAS and CVAAS, filtration for HPLC, and extraction for GC determinations.
- Microbiology unit

  Faecal coliforms are detected in this laboratory, including preparation of the media.
- Advanced analyses unit

  This laboratory includes state of art equipment such as HPLC, GC, AAS and CVAAS.
- Customer services office

  The main activity of this office is to communicate with the internal and external customers.
- Quality department

  This department works to ensure the compliance of the laboratory work with QS.

The laboratory has managerial and technical personnel who have the authority and resources needed to carry out their duties, including the implementation, maintenance and improvement of the quality system. The lines of authority for the laboratory management are shown in the organizational chart (Figure 3.1) , The technical and quality manager for the laboratory have the responsibilities with no conflicting interests may adversely influence the laboratory's compliance with the requirements of the international standards.

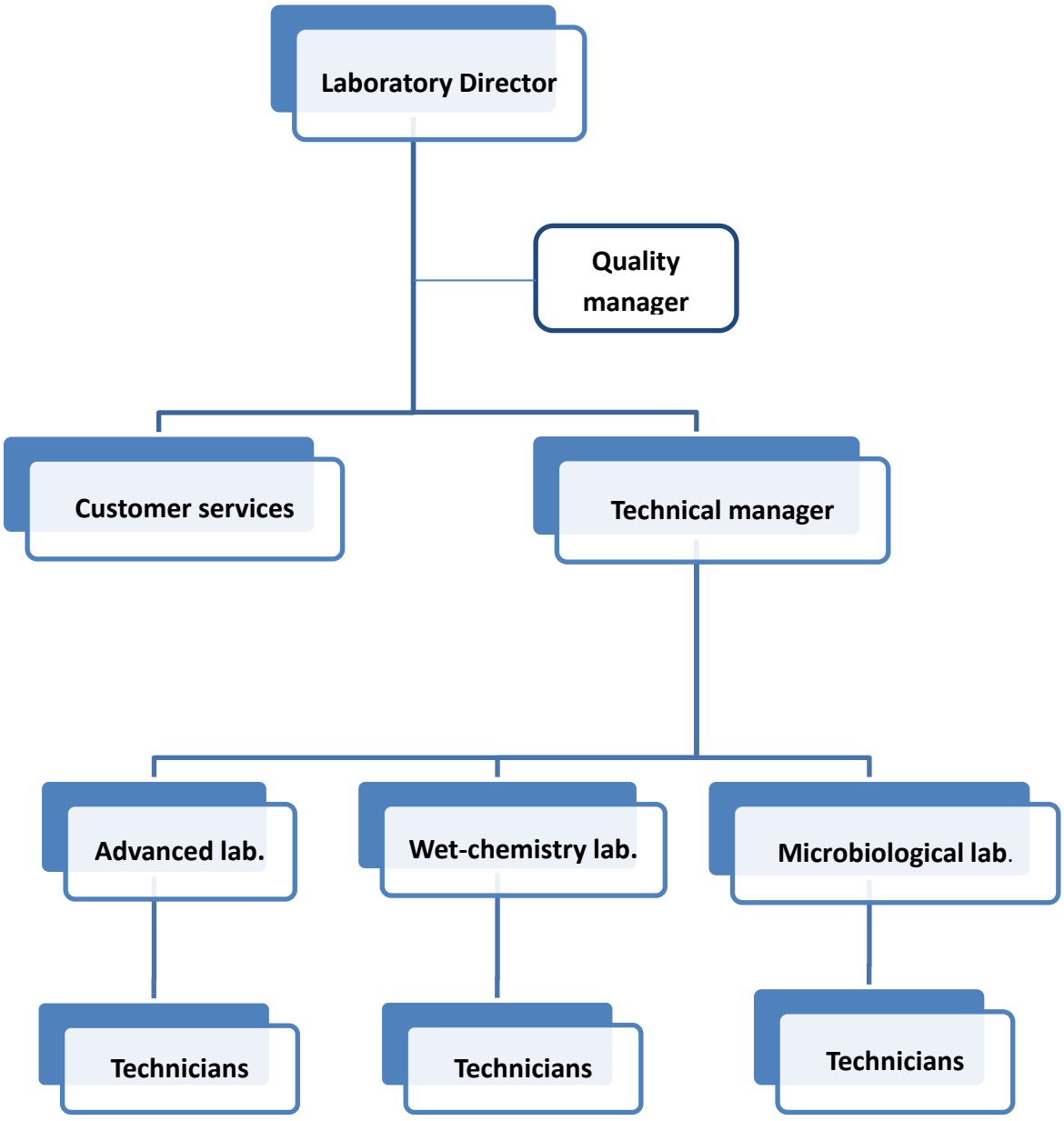


Figure 3.1 Organizational chart of Finisterra's laboratory

3.1.2 Quality policy

Top management is committed to comply with the requirements of ISO/IEC 17025/2005, international standards and relevant legislations providing the integration of organizational structure, procedures and resources needed to fulfil the quality policy.

Mission

Our mission is twofold to fulfil the requirements of regulation (EC) no. 113/2006 of the European Parliament and of the Council, of 12 December 2006 on the quality required of shellfish water and to support research related to the continual improvement of the quality of mussel produced in the Sagres region.

Vision

The laboratory aims to support Finisterra to be one of the internationally leading companies in high quality mussel production, environmental monitoring and water researching field.

Statement and objectives

The commitment of top management is reflected in our laboratory's mission and vision statements, as well as in the following quality objectives:

- To continually monitor the effectiveness of the quality system through periodic internal audits and management reviews considering input from experts and interested parties;
- To provide and maintain the highest reasonable standard of quality in all aspects of measurements.
- Competent and qualified personnel are used to perform the tasks and are familiarized with the quality system documentation in order to implement the policies and procedures.
- To ensure accurate and reliable results using the latest high-tech analytical equipment and best practice.

3.1.3 Documentation

Documentation is very important to ensure that the essential operations for the quality system work effectively and are performed with appropriate and updated documents. All those contributions to the laboratory activities are controlled according to procedures which guaranteed their verification, distribution and revisions. The clear, complete and correct documentation system is the laboratory's credibility and the defensibility of its data (Garfield et al., 2000).

The quality system should be appropriate to the laboratory's activates. Moreover, it should contain all documents, data and instructions to ensure that the work is in accordance with the requirements of ISO/IEC 17025:2005 and Portuguese accreditation body (IPAC). Figure 3.2 shows all the documents of our quality system mentioning the level of each document.

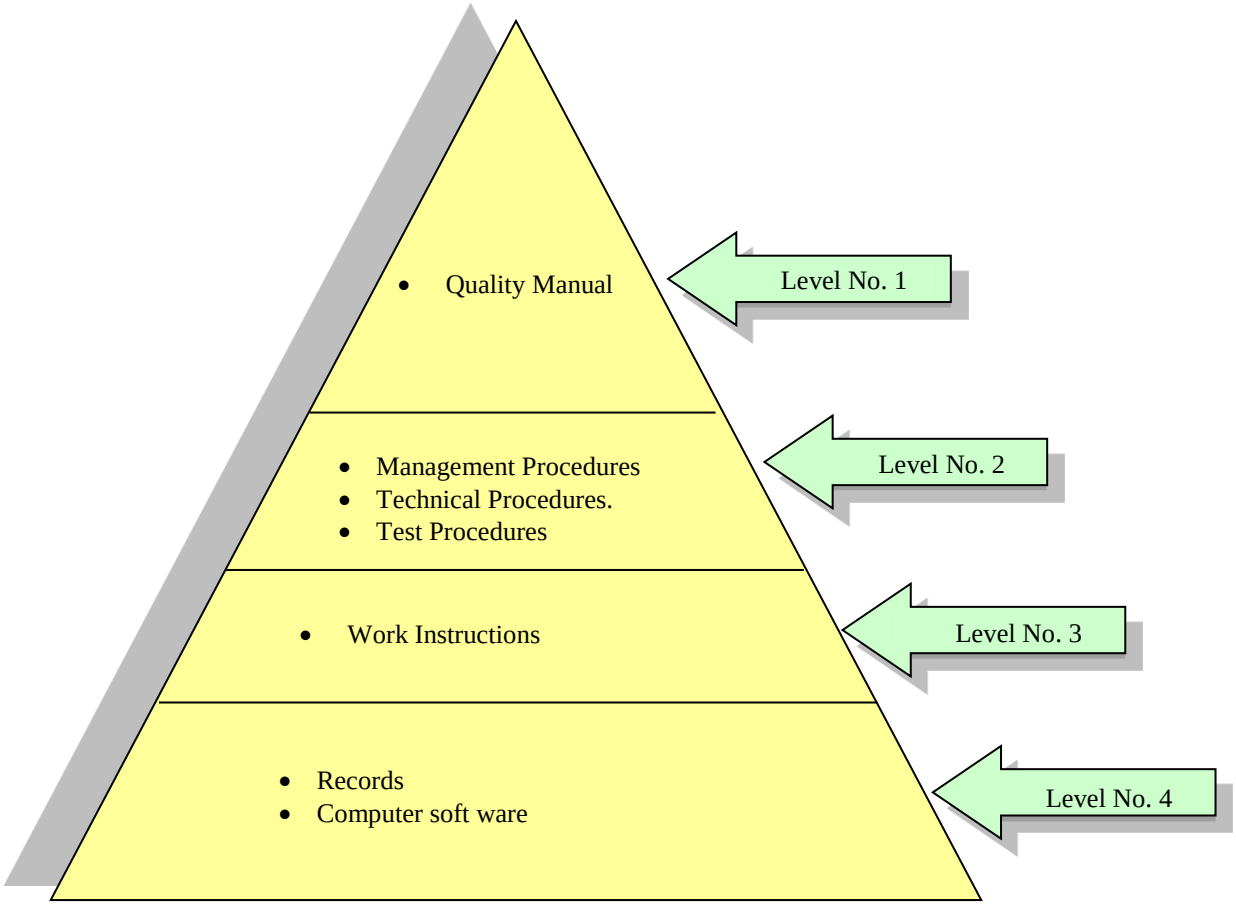


Figure 3.2 Hierarchy of the quality system documents

The main component of the documentation system is the quality manual that sets out the structure of the quality system. Whilst, management and technical documentation sets out how the quality system operates. Testing procedures and work instructions that members of staff have to follow to ensure the quality of results. Associated with the documentation system, a filing system including all the records and formats that the staff members fill during the daily activities. Hence, we designed a frame for the documentation system including all the main documents which the laboratory needs to comply with ISO/IEC 17025:2005. This documentation system includes a quality manual, 8 management procedures, 9 technical procedures, 18 testing procedures and 33 work instructions. The relationship among those documents is shown in Figure 3.3.

During the writing phase of the documentation system, the laboratory may or not need to add or delete some documents. However, the final documentation system should be clear and complete. Also, that system shall be communicated to, understood by, available to, and implemented by the appropriate personnel as stated in ISO/IEC 17025:2005.

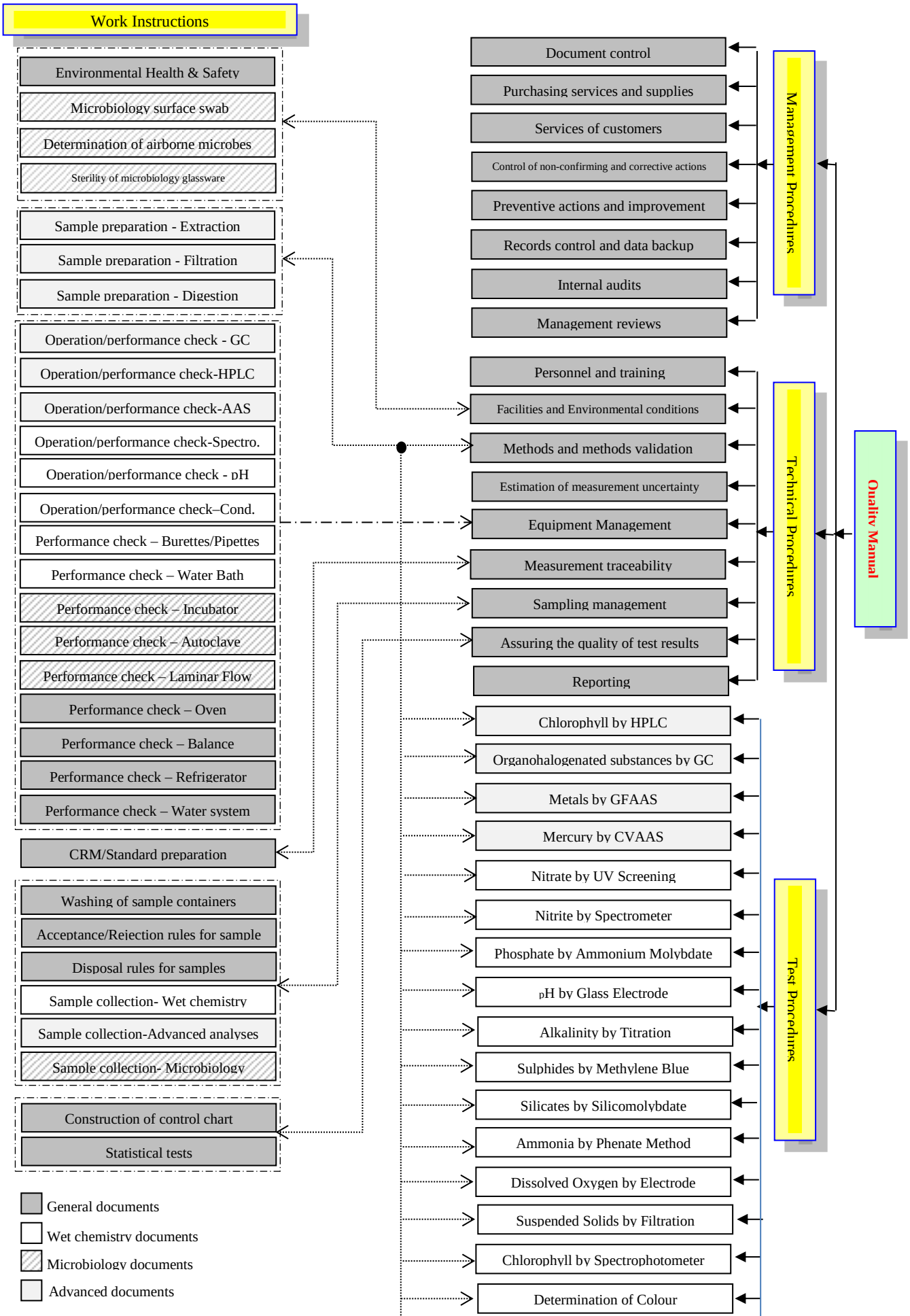


Figure 3.3 The documentation system

3.2 Installation

Where a laboratory is purpose built, it will hopefully be designed in such a way as to minimise any expected problem (Prichard et al., 1995). There are several laboratory building design decisions that can significantly affect building and individual laboratory operating procedures, and make compliance with environmental health and safety regulations more likely and easier to attain (DiBerardinis et al, 2013).However, it can be very expensive to remove some of these problems and laboratory often involves a trade-off between cost and reducing the effects of the problems (Prichard et al., 1995). The laboratory design should be based on data collected from the laboratory management about the work activities, the number and types of the personnel and the interrelationship between the activities and the personnel (DiBerardinis et al, 2013).

3.2.1Building considerations

Finisterra has a plan to build a new building to extend its scope in mussel production activities. At this case, the laboratory will be a part of that building which has unrelated activities. Moreover, the company has a limited area for the laboratory which may be increased at a later time by converting non-laboratory areas such as offices into the laboratory. However, to do this safely, efficiently and cost effectively, advance planning is required to provide a latent reserve capacity to significantly increase the delivery of building systems used for heating, ventilating and air-conditioning, electrical services and piped utilities (DiBerardinis, 2013). Thus, some building considerations should be followed during the design phase to give the laboratory enough flexibility for upgrading. The building may include these space categories (DiBerardinis et al, 2013):

- **Laboratory** is a category of net assignable area in which diverse mechanical services, special supplies and exhaust ventilation devices are available.
- **Laboratory support area** is a category of net assignable area that contains the same services and ventilation facilities as the laboratory; it may adjoin the laboratory unit or may be elsewhere. This area includes glassware and sampling containers store, chemicals store, gas cylinders store, may or not glassware washing room.
- **Administration area** is a category of net assignable area that contains only standard commercial electrical, telecommunication and office ventilation services.

This area includes the laboratory director office, secretarial pool, quality section and customer service office.

- **Personnel support area** is a category of net usable area that is similar in function to administration areas but may contain added mechanical and HVAC services to provide for special needs. This area may include meeting room, toilets and dining facilities.

3.2.2 Laboratory considerations

Many health and safety requirements should be considered in the laboratory design regarding to the work activities. Finisterra work depends on two main disciplines, analytical chemistry and microbiology. The analytical chemistry activities include sample preparation involving digestion, extraction, distillation, mixing, heating, cooling and dilution. The main activity of the analytical laboratory is to analyse water samples using a variety of instruments and analytical techniques, HPLC, GC, AAS, CVAAS and spectrophotometer, some of which produce or utilize some hazardous materials, UV radiations, heating emissions and toxic fumes. Furthermore, hazardous materials always are used including small quantities of toxic chemicals, mineral acids, organic solvent, flammable and compressed gases. On the other hand, the microbiological activities include work with low-to-moderate risk biological agents (Biosafety Level 3). The equipment items found in this laboratory include laminar flow, autoclave, incubator, refrigerator and heating mantel.

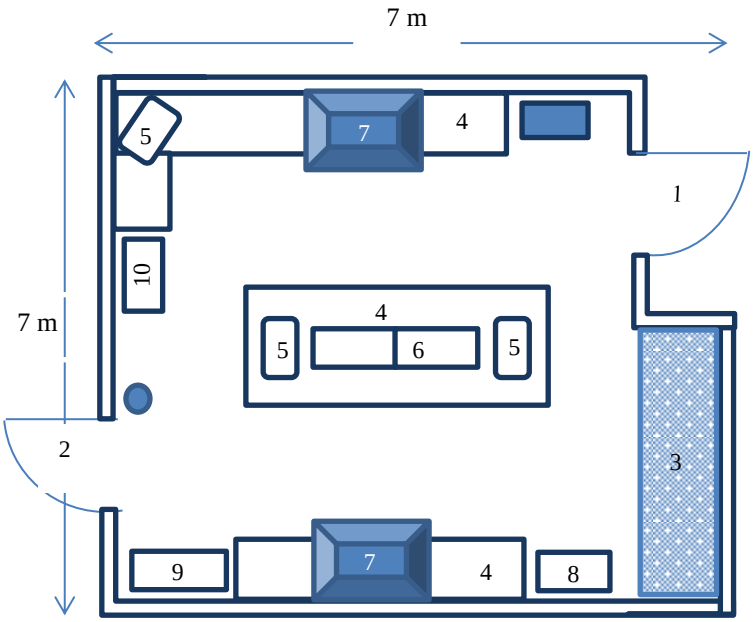
3.2.2.1 Laboratory layout

The laboratory layout is critical for efficient use of space and the safety of laboratory personnel (DiBerardinis et al, 2013). Furthermore, ISO/IEC 17025:2005 states that there shall be effective separation between neighbouring areas in which there are incompatible activities. Regarding to these considerations we designed three separate layouts for the laboratory units, all of them have the same general safety considerations such as primary and emergency entry/egress, suitable aisles between benches, safety showers and eye washers, desks located away from the potential dangerous operations and enough work surface as well as some specific considerations for each layout as the following:

- Wet chemistry layout (Figure 3.4a), which is designed to cover all the wet chemistry parameters in addition to sample preparation for the advanced

analysis. The most important consideration in this layout is safely locating of the chemical fume hoods required. During our design, we dedicated working zones for each activity to guarantee complete separation and to prevent cross contamination as mentioned in Figure 3.4b.

- Advanced analysis layout (Figure 3.5), because HPLC, GC, AAS and CVAAS will be present in this laboratory, special care should be given to their locations relative to the ventilation system and egress routes. Access to the rear of bench mounted analytical instruments is very important for gas, and fluid lines, as well as for data transmission and power connections. Benches and countertops can be split and separated by a safe distance to allow one person at a time access to the back of those instruments (DiBerardinis et al, 2013).
- Microbiology layout (Figure 3.6) is more likely to be a suite of rooms connected through central corridors. It should include washing facilities, sterilizing services and a space for donning and discarding protective garments at entry. In addition to the laboratory should contain class II biosafety cabinet for a single worker. Both the air supply and discharged must pass through HEPA filters for environmental protection. Walls and floors should be monolithic and made of washable and chemically resistant plastic, baked enamel, epoxy or polyester coatings (DiBerardinis et al, 2013).



1. Primary Entry/Exit
2. Emergency Exit
3. Desk
4. Bench
5. Lab. Sink
6. Reagent Shelves
7. Fume hood
8. Balance
9. Water purification unit
10. Refrigerator

Figure 3.4a Layout of wet chemistry laboratory

Safety shower/eye wash Container for waste

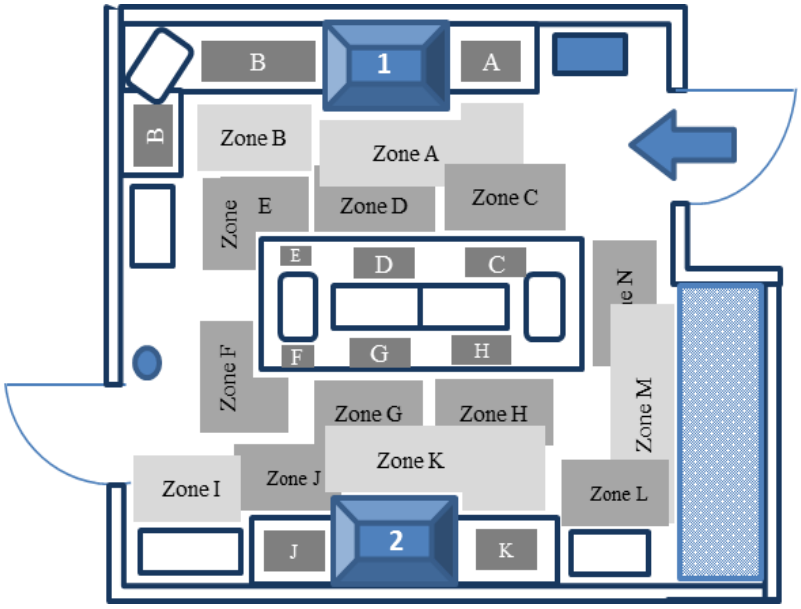


Figure 3.4b Layout of wet chemistry laboratory mentions the dedicated working zones

- Zone A: Preparation of standards and samples for GC analyses
- Zone B: Preparation of standards and samples for HPLC analyses
- Zone C: Validation of the multi-sensors probe and calibration of dissolved Oxygen probe
- Zone D: Spectrophotometer working area for analysing Ammonia, Nitrate, Nitrite, Sulphide, Phosphate and Silicate .
- Zone E: Distillation for Ammonia.
- Zone F: Filtration for sulphide and suspended solids.
- Zone G: Measurement of pH.
- Zone H: Titration cell for Alkalinity.
- Zone I: working area for dealing with the water purification system.
- Zone J: working area for drying using the oven.
- Zone K: preparation of standards and samples.
- Zone L: working area for weighing.
- Zone M: Data analysis
- Zone N: Washing area.

1. Fume hood for working with organic solvents
2. Fume hood for working with inorganic salts and mineral acids

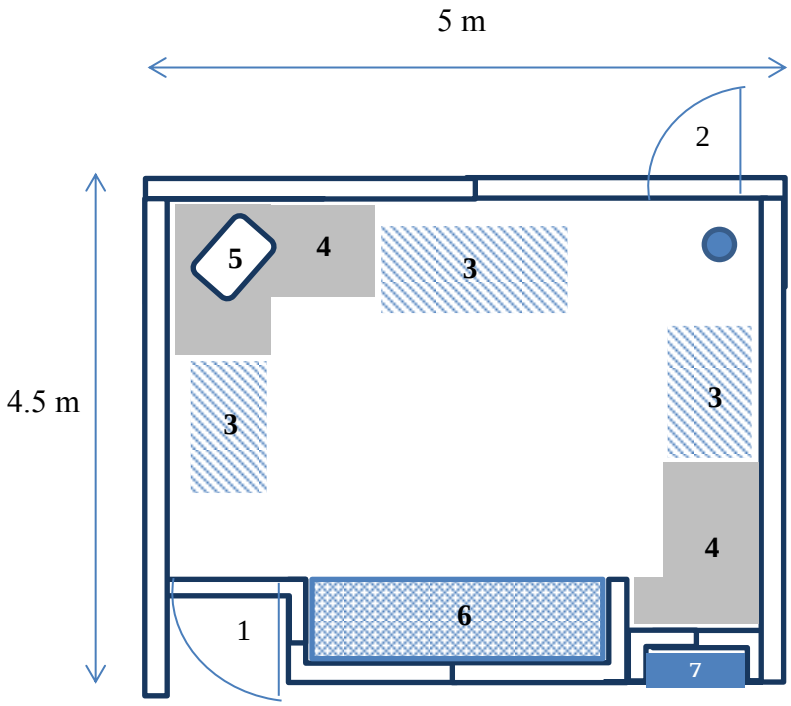
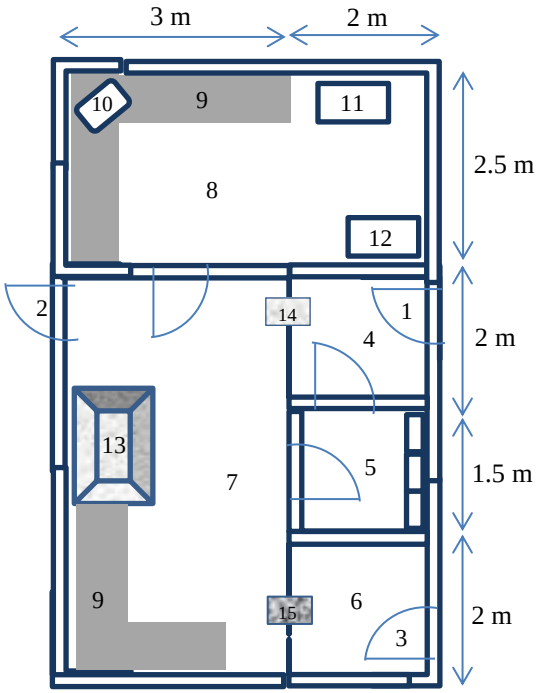


Figure 3.5 Layout of advanced analysis laboratory

1. Primary Entry/Exit
2. Emergency Exit
3. Instrument racks
4. Bench
5. Lab. sink
6. Desk
7. Electricity panel



1. Primary Entry/Exit
2. Emergency Exit
3. Entry/Exit for Washing Room
4. Anteroom
5. Clothes Changing Room
6. Washing Room
7. Working Room
8. Preparation Room
9. Wall bench
10. Lab. Sink
11. Autoclave
12. Media Refrigerator
13. Biosafety Cabinet
14. Pass-through Unit
15. Autoclave pass-through

Figure 3.6 Layout of microbiology laboratory

3.2.2.2 Heating, ventilating and air conditioning

Laboratory facilities for testing, including but not limited to energy sources, lighting and environmental conditions, shall be such as to facilitate correct performance of the tests according to ISO/IEC 17025:2005. Well-designed and well-operated heating, ventilating, and air-conditioning (HVAC) systems are essential for laboratory health and safety protection, as well as for an environment that promotes comfort, productivity, and good work practices (DiBerardinis et al, 2013). During the design, some considerations should be given to these critical aspects:

- Temperature control: Heating and air-conditioning systems must provide the uniform temperature that is required for the efficient operation of many analytical devices. Some instruments produce heat such as GC, AAS and heating mantels, thus each of the analytical instruments and auxiliary equipment should be evaluated when estimating heating and air-conditioning requirements.
- Laboratory Ventilation System: A replacement system is enough for the laboratory; the air conditioning system replaces the volume of air discharged to the atmosphere through the health and safety exhaust ventilation system. However, special consideration should be given to providing good coverage for the advanced analysis laboratory and media preparation room using local exhaust systems to control odours some toxic fumes at the source. Moreover, digestion procedures and such operations must be performed in the fume hood.

3.2.2.3 Loss prevention, industrial hygiene and personnel safety

Many laboratories have good designs but at the same time they suffer much from unforeseen events. Therefore, the best design which contains all the possible emergencies and safety services.

- Emergencies: Many emergencies should be covered by the laboratory design such as emergency fuel gas shutoff, ground fault current interrupters and master disconnect switch, emergency shower and eye wash. In addition to emergency cabinet which includes fire blanket, fire extinguisher, first aid kit, protective clothing, flash light and chemical/biological spill kit.
- Safety: Safety considerations should include alarm system, equipment safety and personnel safety.

- Chemical and biological disposal: Discarded hazardous materials, including flammable liquids, biological waste and highly toxic chemicals should be segregated in specific areas within the laboratory for disposal.

3.3 Equipment and Consumables

3.3.1 Selection of equipment

Performance of equipment varies from manufacturer to manufacturer to fulfil the market's needs in different prices. However, equipment and its software used for testing and sampling shall be capable of achieving the accuracy required and shall comply with specifications relevant to the tests concerned as stated in ISO/IEC 17025:2005. Performance and cost are crucial issue for equipment's purchasing but with other considerations:

- The work load volume
- The laboratory space
- Ruggedness needed not only to work in or with highly salty samples but also to locate the equipment in environment influenced by the sea.
- The manufacturer should have technical support agent in Portugal to guarantee the prompt availability for repairs.
- Some analytical equipment should be evaluated before purchasing by furnishing the equipment in temporary basis in the laboratory or demonstration in another laboratory.

3.3.2 Requirements of the standard methods

ISO/IEC 17025:2005 states that the laboratory shall be furnished with all items of sampling, measurement and test equipment required for the correct performance of the tests (including sampling, samples preparation, processing and analysis of test data). All the requirements of the standard methods were collected and listed under four main disciplines as follows:

- Sampling requirements
- Requirements for wet chemistry analyses
- Requirements for advanced analyses
- Microbiology requirements

Each discipline has the related parameter (s) to be analysed, the requirements listed for each parameter separately. Using this way is to avoid missing any equipment or consumable but another list will prepare for purchasing to avoid repetition of any requirement as will mention in *Chapter 4*.

3.3.2.1 Sampling requirements

These requirements have been collected from ISO 5667-3:2012 for chemical parameters and ISO 19458:2006 for microbiological parameter. Then, the non ISO standards were checked for additional requirements. Table 3.1 shows the equipment and consumables required for sampling for each parameter.

Table 3.1 The equipment and consumables for sampling

#	Parameter	Method	Type of container	Preservation	Storage time
1	Temperature	SEACAT SBE19	Plastics or glass	on site	…….
2	Salinity			Preferably on site	1 day
3	pH	ISO 10523:2008	Plastics or glass	Preferably on site	1 day
4	Alkalinity	ISO 22719:2008	Plastics or glass	Preferably on site	14 days
5	Dissolved Sulphide	ISO 10530:1992	Plastics	- Add 2 ml zinc acetate sol. - Add NaOH if the pH is not between 8,5and 9,0	7 days
6	Dissolved Oxygen	ISO 5814:2012	Plastics or glass	On site	--------
7	Ammonia	ISO 7150-1:1984	Plastics or glass	Waters shall be filtered on site	1 day
8	Nitrate	SMEWW 4500-$NO3^-$-B	Plastics or glass	--------	1 day
9	Nitrite	ISO 6777:1984	Plastics or glass	Preferably on site	1 day
10	Phosphate	ISO 6878:2004	Preferably glass, otherwise PE, PVC	Acidify to pH 1 to 2 with H_2SO_4 or HNO_3	1 Month
11	Silicate	SMEWW, 4500C-SiO_2	Plastics	--------	1 Month
12	Suspended Solids	ISO 11923:1997	Plastics or glass	--------	2 days
13	Chlorophyll & pigments	EPA, 446 EPA, 447	Plastics or glass	Filter preferably on site. Use dark-coloured bottles	1 day
14	Coloration	ISO 7887:2011	Plastics or glass	use dark-coloured bottles	5 days
15	Fecal coliforms	ISO 4831:2006	Sterile glass bottle		--------
16	Organohalogenated substances	EPA, 8021	Glass bottle with PTFE cap.	Acidify to pH 1 to 2 with HNO_3 or H_2SO_4	7 days
17	Mercury Hg	ISO 12846:2012	Plastics or borosilicate glass	Add HCl 1 ml/100 ml	2 days
18	Silver Ag	ISO 15586:2003	PE, PP, FEP	Acidify to pH 1 to pH 2 with HNO_3	6 Months
19	Arsenic As				
20	Cadmium Cd				
21	Chromium Cr				
22	Copper Cu				
23	Nickel Ni				
24	Lead Pb				
25	Zinc Zn				

Moreover, other requirements should be considered for subsurface or deep sampling. For chemical analysis, oceanographic bottles (Go-Flow, Nasen or Van Doom) are always used. Whilst, the J-Z system, consists of a sterile glass bottle, under vacuum, fitted with a rubber stopper and glass tube, is appropriate for microbiological sampling. Nevertheless, the sampling standards (ISO 5667-1:2006, ISO 5667-3:2012, ISO 5667-9:1992 and ISO 19458:2006) should be checked for special types of samples such as horizontal, vertical scan and composite samples.

3.3.2.2 Requirements for wet chemistry analyses

Regarding to the selected methods mentioned in *Chapter 2 Context*, the required equipment and consumables are listed in Table 3.2.

Table 3.2 The equipment and consumables for wet chemistry laboratory

#	Parameter	Equipment	Consumables
	Water for laboratory uses	• Purification system to produce Water Grade 1, reverse osmosis or deionization followed by filtration through a membrane filter of pore size 0.2 µm to remove particulate matter or re-distillation from a fused silica apparatus).	
1	pH	• Sample bottle of borosilicate or plastic. • Thermometer, with a 0.5 “C scale. • pH-meter, capable of being read to a discrimination of a pH of 0.01 or better. • Glass electrode and reference electrode.	• Water which is free from carbon dioxide (boiling the deionized water). • Standard buffer solutions, Potassium hydrogen tartrate (pH= 3.557 at 25^{o}C), Potassium hydrogen phthalate (pH= 4.005 at 25^{o}C), phosphate (pH= 6.865 at 25^{o}C), borax (pH= 9.180 at 25^{o}C), sodium carbonate / sodium hydrogen carbonate (pH= 10.012 at 25^{o}C),
2	Alkalinity	• Calibrated balance, capable of weighing 200 g to within ± 0.01 g. • Plastic screw-cap bottle, of capacity 125 ml, with cap. • A volumetric dispensing system, containing a constant volumetric pipette made of glass with valves at each end. • Jacketed beaker, of capacity 200 ml. A glass beaker enclosed by a water jacket of internal diameter 57 mm. • Calibrated thermometer, 0.01 °C. • Water bath, capable of being maintained at a constant	• Hydrochloric acid, 0.1 mol/kg • Sodium chloride, 0.6 mol/kg. • Deionised ultrapure water, of resistivity about 18 MΩ cm.

		temperature to within ± 0.05 °C. • Magnetic stirrer, of dimensions 38 mm x 8 mm. • Holder for burette tip, electrode, and thermometer. • EMF-measuring assembly, digital voltmeter or pH meter with ± 0.1 mv. • A very rapid response pH glass electrode, the 90 % response time during a pH change of 0.1 should be less than 10 s. • Automatic burette, of capacity 5 ml ± 0.002 ml, equipped with an anti-diffusion tip. • Transfer device for samples by mass, designed to allow dispensation from a bottle with a greased ground-glass joint in a manner that ensures that grease is not transferred to the weighing bottle. • Basin for waste. • Wash bottle	
3	Dissolved sulfide	• Filtration device with membrane filter (Pore size 0.45 µm) • Stripping apparatus for the Separation of sulphide. It consists of a reaction flask, of capacity 250 ml, with a lateral ground-glass joint attachment for the drop funnel, of capacity 100 ml, provided with a gas inlet tube ending at the bottom of the flask, vertically mounted condenser or riser tube. and an absorption vessel. • Measuring cylinder, 25 ml. • Measuring flasks, of capacity 50 rnl, 100 ml, 500 and 1 000 ml. • Measuring pipettes, of capacity 1 ml and I0 ml. • One-mark pipette, of capacity 1 ml, 2 ml, 5 ml, I0 ml, 20 ml, 50 ml and 100 ml. • Dispensers. • Microlitre syringes. • Gas supply with nitrogen, of high purity [99.996 % (m/m) pure]. • Gas flow measuring device suitable for a volume flow of 40 l/h. • **pH-meter**, equipped with an appropriate electrode. • **Spectrometer,** suitable for absorbance measurements at 665 nm. • Cuvettes, of path length 1 cm.	• Reagent water • Sulfuric acid • Sodium hydroxide • Zinc acetate • Potassium hydrogen phthalate • Hydrochloric acid • Ascorbic acid • N,N-dimethyl-1,4-phenyl di-ammonium chloride • Ammonium iron(Ill) sulphate dodecahydrate • Sodium sulfide hydrate [Na,S.xH,O, (x = 7-9)] • Iodine solution • Sodium thiosulfate • Acetic acid • Starch

4	Dissolved Oxygen	• **Measuring probe**, either of the galvanic type (for example lead/silver) or the polarographic type (for example silver/gold) with, if required, a temperature-sensitive compensating device. • **Meter**, graduated to show the concentration of dissolved Oxygen directly, and/or the percentage Saturation with Oxygen, or the current in microamperes. • **Thermometer**, graduated in divisions of 0.5 ^{o}C. • **Barometer**, graduated in divisions of 10 Pa.	• Sodium sulfite, anhydrous (Na_2SO_3) or heptahydrate, ($Na_2SO_3.7H_2O$). • Cobalt (II) salt, for example cobalt (ll) chloridehexahydrate($CoCI_2.6H_2O$).
5	Nitrate	• **Spectrophotometer**, for use at 220 nm and 275 nm with matched silica cells of 1-cm or longer light path. • **Filtration device** with membrane filter (if necessary). • **Balance** (d = 0.0001) • **Oven,** to dry potassium nitrate at 105 ^{o}C. • Measuring pipette, 1 ml, 2 ml, 5ml, 10 ml, 25ml and 50 ml. • Measuring flasks, 50 ml • Erlenmeyer flask 250ml.	• Nitrate-free water • potassium nitrate • Hydrochloric acid • Chloroform
6	Nitrite	• **Spectrophotometer**, for use at 540 nm with cells of optical path between 10 and 50 mm. • **Balance** (d = 0.0001) • **Oven,** to dry sodium nitrite at 105 ^{o}C. • **Refrigerator**, to store the standards at 2 to 5 ^{o}C • Measuring pipette, 1 ml, 5ml, 10 ml and 25 ml. • Measuring flask 50 ml, 250 ml and 1000 ml • Burette 25 ml • Amber glass bottles. • Beaker 600 and 1000 ml	• Reagent water • Orthophosphoric acid • 4-aminobenzen sulfonamide • N-(1-naphthyl)-1,2-diaminoethane dihydrochloride • Hydrochloric acid • Glass fibre paper, if necessary.
7	Phosphate	• **Spectrophotometer**, capable of operating at a wavelength 880 nm with cell of optical path length between 10 mm. • **Analytical balance** (d=0.001) • **Oven,** to dry Potassium dihydrogen phosphate at 100ºC. • Stopwatch • One mark volumetric flask 50 ml and 100ml • Pipette 1 ml, 5 ml and 10 ml • Glass cuvettes 10 mm. • Bottles 100, 250 and 500 ml for reagents.	• Potassium dihydrogen phosphate (KH_2PO_4) • Concentrated sulphuric acid (H_2SO_4) • Ammonium heptamolybdate tetrahydrate $(NH_4)6\ Mo_7\ O_{24}\ .\ 4\ H_2O$) • Potassium antimony tartrate ($K(SbO)\ C_6H_4O_6$) • Ascorbic acid ($C_6H_8O_6$).

8	Silicate	• **Spectrophotometer**, capable of operating at a wavelength 410 nm with cell of optical path length between 10 mm or longer. • **Nessler tubes**, matched, 50-mL, tall form. • **Refrigerator**	• Sodium bicarbonate, NaHCO3, powder. • Sulfuric acid, H_2SO_4. • Hydrochloric acid, HCl. • Ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$. • Ammonium hydroxid, NH_4OH. • Oxalic acid, $H_2C_2O_4.H_2O$ • Silica standard of approximately the same ionic strengths. • *Potassium chromate*, K_2CrO_4 • Sodium borate decahydrate, $Na_2B_4O_7.10H_2O$ • 1-amino-2-naphthol-4-sulfonic acid • Sodium sulphite Na_2SO_3 • Sodium bisulphite $NaHSO_3$
9	Ammonium	• **Spectrophotometer**, capable of operating at a wavelength 655nm with cell of optical path length between 10 and 50 mm. • **Distillation apparatus** • Glass bottle with well fit stopper • One mark volumetric flask 50 ml and 1000 ml • Amber glass, 1000 ml • Pipette 1 ml and 50 ml • **Balance** to weigh 130 g (d=0.1) • **Analytical balance** (d=0.001) • **Oven,** to dry ammonium chloride at 105 ^oC. • Polyethylene bottle • Water bath or incubator , capable of maintained at 25 ± 1^oC • Refrigerator to store the samples between 2 and 5 ^oC • A burette, 50 ml	• Ammonium free water, by passing through a column of a strongly acidic cation exchange resin. • Sulfuric acid • Cation exchange resin (in hydrogen form) • Sodium salicylate • Trisodium citrate dihydrate • Sodium nitroso-pentacyanoferrate(III) dehydrate [sodium nitroprusside] • Sodium hydroxide • Sodium dichloroisocyanurate. • Ammoium chloride • Pottasium hydroxide • Ethanol (95%) • Glass fibre paper • Hydrochloric acid
10	Suspended solides	• Equipment for vacuum or pressure filtration. • **Drying oven**, capable of maintaining a temperature of 105 ^oC ± 2 ^oC • **Analytical balance**, capable of weighing to accuracy at least 0.1 mg. • Drying support of suitably surfaced material, to support the filters in the drying oven. • One mark volumetric flask 100 ml and 1000 ml • Measuring cylinders different sizes • Bottles for sampling	• Borosilicate glass fibre filters • Reference suspension of microcrystalline cellulose, p = 500 mg/l.

11	Salinity, depth and temperature	• Multi-sensors probe	---------
12	Chlorophyll	• Spectrophotometer, visible multi-wavelength, with a band-pass (resolution) not to exceed 2 nm. • Centrifuge, capable of 675 g and 1000 g. • Tissue grinder, Teflon pestle (50 mm X 20 mm) with grooves in the tip with 1/4" stainless steel rod long enough to chuck onto a suitable drive motor and 30 ml capacity round-bottomed, glass grinding tube. • Petri dishes, plastic, 50 X 9 mm. • Tweezers or flat-tipped forceps. • Vacuum pump or source capable of maintaining a vacuum up to 6 in. Hg (20 KPa). • Assorted Class A calibrated pipets • Graduated cylinders, 500 ml and 1000 ml. • Volumetric flasks, Class A calibrated 25 ml, 50 ml, 100 ml and 1000 ml. • Glass rods. • Glass cells for the spectrophotometer, 1, 2, 5 or 10 cm in length. If using multiple cells, they must be matched. • Filtration apparatus consisting of 1000 or 2000 ml filtration flask, 47 mm fritted glass disk base and a glass filter tower. • Centrifuge tubes, polypropylene or glass, 15 ml capacity with non-pigmented screw-caps. • Poly-ethylene squirt bottles. • Flask or bottle 1000 ml • Measuring cylinder 100 ml • Refrigerator, to store the standards at – 20 ^{o}C • Volumetric flask 25 ml	• Filters, glass fibre, 47-mm, or 25-mm, nominal pore size of 0.7 µm. • Aluminium foil. • Laboratory tissues. • Laboratory grade detergent. • Disposable Pasteur type pipets or medicine dropper. • Acetone, HPLC grade • Hydrochloric acid • Sodium hydroxide • Chl a free of chl b and chl b substantially free of Chl a • Water, Suitable water may be obtained by passing distilled water through a mixed bed of anion and cation exchange resins.
13	Coloration	• **Spectrometer**, suitable for the visible range • **pH meter** • Thermometer • Bottles 1000 ml • Membrane filter assembly • Beaker s or cylinders	• Biological filter 0.1 µm and 0.45 µm

3.3.2.3 Microbiology requirements

To detect faecal coliforms in water using MPN as stated in regulation (EC) no. 113/2006 of the European Parliament and of the Council, of 12 December 2006, we collected the requirements of the selected standard method, ISO 4831:2006. Furthermore, some special requirements from other standards, ISO/TS 11133-1:2009, ISO/TS 11133-2:2003/Amd 1:2011 and ISO 6887-5:2010 were considered. All the requirements are listed in Table 3.3. Nevertheless, ISO 7218:2007/Amd 1:2013 should be consulted for the specifications of these requirements before purchasing.

Table 3.3 The equipment and consumables for microbiology laboratory

#	Parameter	Equipment	Consumables
1	Faecal coliforms	• Oven for dry sterilization up to 170 °C. • Autoclave for wet sterilization • pH meter, accurate to ± 0.1 pH unit at 25 °C. • Incubator, capable of operating at 30 °C ± 1 °C or 37 °C ± 1 °C. • Loop, made of platinum-iridium, or nickel-chromium, approximately 3 mm in diameter. • Test tubes, of dimensions approximately 16 mm × 160 mm and 20 mm × 200 mm. • Durham tubes, of a size suitable for use in the test tubes of dimensions 16 mm x160 mm (6.4). • Total-delivery pipettes, having nominal capacities of 1 ml and 10 ml. • Thermocouple or maximum thermometer for verification of autoclave • Thermocouple or blub for verification of incubator • Refrigerator to store the samples at 3 °C ± 2 °C or 5 °C ± 3 °C. • Gas burner or safety cabinet. • Dispensers different sizes • Vortex mixer • Heating mantel, large size with stirring systems for preparation of media • Balance (d=0.01) • Water deionizer • Glassware washer	• Non-toxic, phosphorus free detergent • Diluents, peptone water and phosphate buffer • Lauryl sulfate tryptose broth • Brilliant green lactose bile broth • Sterilization indictors, biological like *bacillus stearothermophilus* spore • Standard buffer solutions • Glycerol • Ethyl alcohol or ethylene oxide

3.3.2.4 Requirements for advanced analyses

Finisterra laboratory brings added value to its services by focusing on advanced analyses in response to legislation requirements and it uses state of art equipment such as HPLC, GC, AAS and CVAAS as shown in Table 3.4.

Table 3.4 The equipment and consumables for advanced analysis laboratory

#	Parameter	Equipment	Consumables
1	Chlorophyll	• This method uses a ternary gradient thus requiring a programmable gradient pump with at least three pump inlets for the three different mobile phases required. UV/VIS detector (cell path length, 6 mm, volume 9 mL) and PC data analysis. Tubing was made of polyether ether ketone (PEEK) • Helium or other inert gas for degassing the mobile phases OR other means of degassing such as sonication under vacuum. • Sample loops of various sizes (50-200 mL). • Guard Column. • Analytical Column -- A C reversed-phase column with end capping. A J.T. Baker 4.6 mm X 250 mm, 5 mm pore size column was used to generate the data in this method. • A visible wavelength detector with a low volume flow-through cell. Detection is at 440 nm. • A recorder, integrator or computer for recording detector response as a function of time. • Although not required, an autosampler (preferably refrigerated) is highly recommended. • Centrifuge, capable of 675 g. • Tissue grinder, Teflon pestle (50 mm X 20 mm) with grooves in the tip with 1/4" stainless steel rod long enough to chuck onto a suitable drive motor and 30 ml capacity round-bottomed, glass grinding tube. • Petri dishes, plastic, 50 X 9 mm. • Tweezers or flat-tipped forceps. • Vacuum pump or source capable of maintaining a vacuum up to 6 in.	• Filters, glass fibre, 47-mm, or 25-mm, nominal pore size of 0.7 µm. • Aluminium foil. • Laboratory tissues. • Laboratory grade detergent. • Disposable Pasteur type pipets or medicine dropper. • Acetone, HPLC grade • Methanol, HPLC grade • Ammonium acetate. • Acetonitrile, HPLC grade, • Ethyl acetate, HPLC grade, • Chl a free of chl b and chl b substantially free of chl a. • Water, Suitable water may be obtained by passing distilled water through a mixed bed of anion and cation exchange resins.

		Hg (20 KPa). • Assorted Class A calibrated pipets • Graduated cylinders, 500 ml and 1000 ml. • Volumetric flasks, Class A calibrated 25 ml, 50 ml, 100 ml and 1000 ml. • Glass rods. • Filtration apparatus consisting of 1 or 2 L filtration flask, 47 mm fritted glass disk base and a glass filter tower. • Amber 2-mL HPLC autosampler vials with screw or clamp caps. • Glass syringe, 1 or 2-mL capacity. • HPLC compatible, low-volume, acetone resistant glass fibre or PTFE syringe filters. • Refrigerator, to store the standards at – 20^{o}C or -70^{o}C.	
2	Organo-halogenated substances	• **Gas Chromatograph** - capable of temperature programming; equipped with variable constant differential flow controllers, sub-ambient oven controller, photoionization and electrolytic conductivity detectors connected with a short piece of uncoated capillary tubing, 0.32-0.5 mm ID, and data system. • Primary Column - 60-m x 0.75 mm ID VOCOL wide-bore capillary column with 1.5-µm film thickness (Supelco) or equivalent. • Confirmation column - 60-m x 0.53 ID SPB-624 wide-bore capillary column with 3.0-µm film thickness (Supelco) has been suggested as one possible option. • Photoionization detector (PID) (Tracor Model 703, or equivalent). • Electrolytic conductivity detector (HECD) (Tracor Hall Model 700-A, or equivalent). • Syringes - 5 mL glass hypodermic with Luer-Lok tips. • Syringe valves - 2-way with Luer ends [polytetrafluoroethylene (PTFE) or Kel-F]. • Microsyringe - 25-µL with a 2-in. x 0.006-in. ID, 22E bevel needle (Hamilton #702N or equivalent). • Microsyringes - 10-, 100-µL. • Syringes - 0.5-, 1.0-, and 5-mL, gas-tight with shut-off valve. • Bottles - 15-mL, PTFE-lined with screw-cap or crimp top. • Analytical balance - 0.0001 g.	• Organic-free reagent water • Methanol, CH OH - Pesticide quality or equivalent. • Vinyl chloride, (99.9% pure), CH =CHCl. • Stock standards - Stock solutions may either be prepared from pure standard materials or purchased as certified solutions. • Internal standards - It is recommended that a spiking solution containing fluorobenzene and 2-bromo-1-chloropropane in methanol be prepared. • Surrogate standards, A combination of 1,4 dichlorobutane and bromochlorobenzene

		• Volumetric flasks, Class A - Appropriate sizes with ground glass stoppers.	
3	Metals	• **Atomic absorption spectrometer** equipped with graphite furnace, background correction, the necessary hollow cathode lamps and venting system over the furnace to remove any smoke or vapours that might be harmful. • Digestion vessel, made of borosilicate glass, and having a nominal volume of 100 ml. • Reflux condenser, a straight-through type, with conical ground-glass joints and made of borosilicate glass. • Water-cooled condensers with a minimum effective length of at least 200 mm have been found suitable. • Roughened glass beads, having a diameter of 2 mm to 3 mm and acid-washed [for instance with warm • Temperature-controlled heating apparatus, capable of heating the contents of the digestion vessel to reflux temperature. • Volumetric flask, made of borosilicate glass, and having a nominal volume of 100 ml. • Graduated pipettes or dispensers. • Poly ethylene bottles for sampling • Filtrating equipment, glass or plastic • Filters paper, either membrane filter (0.45 µm) or capillary filter (0.40 µm). • Pipettes capacity varying 100 µl to 1000 µm. • Graphite tubes with platform.	• Water grade 1 according to ISO 3696 • Nitric acid, c(HNO3) = 15,8 mol/l, ρ = 1,4 kg/l. • Antifoaming agent, for instance n-dodecane ($C_{12}H_{26}$). • Ammonia solution, approximately 25 % by mass. • Hydrochloric acid, c (HCl) = 12,1 mol/l, ρ = 1,19 kg/l. (37%) • Standard stock solutions, 1000 mg/l • Palladium nitrate, 10 g/l (modifier) • Magnesium nitrate (modifier). • Ammonium dihydrogen phosphate modifier. • Nickel modifier • Argon gas
4	Mercury	• **Atomic absorption spectrometer equipped with a suitable cold-vapour generation system**, based on an automated flow injection or continuous flow or a manual or semi-automatic batch system • Radiation source like hollow cathode lamp or electrodeless discharge lamp. • Quartz tube with suitable heating and an adsorbent which enables the enrichment with amalgamation. • Data station control.	• Water grade 1 according to ISO 3696 • Potassium bromate • Potassium bromide • hydroxylammonium chloride • Tin (II) chloride • Hg-free N_2 • L-ascorbic acid • Nitric acid • Hydrochloric acid • mercury(II) chloride or mercury standard

3.3.3 Calibration and Verification plan

All equipment used in the laboratory should be kept in a state of maintenance and calibration consistent with its use. Equipment of the laboratory can be categorised as:

- General service equipment not used for making measurements or with minimal influence on measurements (e.g. hotplates and heating mantels, stirrers and vortex, non-volumetric glassware and laboratory heating or ventilation systems). This category will only be maintained by cleaning and safety checks as necessary. However, calibration and performance checks will be necessary for some equipment that affects the results significantly, namely, oven for suspended solids, water bath for alkalinity, refrigerator for samples' storage and incubator for faecal coliforms.
- Volumetric equipment (e.g. measuring flasks, pipettes and burettes) and measuring instruments (e.g. AAS, HPLC, GC, spectrophotometer, electrochemical meters, balances). Correct use, maintenance and calibration are necessary for this category. However, the laboratory has a plan to use CRM regularly to guarantee the traceability of the result and the performance of the analytical method. Thus, our calibration plan will not include the analytical instruments. The correct use, maintenance and using of CRM will not necessary assure an instrument is performing adequately. Therefore, periodic performance checks will be carried out (e.g. to check the response, stability and linearity of sources, sensors and detectors, the separating efficiency of chromatographic systems, the resolution, alignment and wavelength accuracy of spectrometers).
- Physical measurement standards, standard weights, reference thermometers, will be calibrated in regular manner.

To comply with ISO/IEC 17025 and to assure the performance of the laboratory equipment, we have designed a calibration and verification plan (Table 3.5). In this plan, the calibration periodicity is based on a guide of Portuguese Quality Institute, IPQ 1999. Whereas the acceptance criteria used are based on Maximum Permissible Error (MPE) stated by the standard methods. Nevertheless, MPE of some equipment depends on their characteristics as mentioned in our plan.

On the other hand, the verification periodicity and its acceptance criteria always depend on the laboratory work. To establish our plan, some recommendations published by Garfield in 2000 and CITAC/EURACHEM in 2002 have been considered.

Table 3.5 laboratory calibration and verification plan

#	Equipment	Calibration		Verification		
		periodicity	Maximum permissible error	parameter to be checked	Periodicity	Acceptance criteria
1	pH			Accuracy	Daily	(± 0.03)
2	Technical balance	Year	At 200 g (± 0.01 g)	Accuracy	Quarterly	At 200 g (± 0.01 g)
3	Water bath	Year	± 0.05 °C.	Temperature	Each use	± 0.05 °C.
4	Automatic burette	3 years	At 5 ml (± 0.002 ml)	Volume & Response	Daily in use	At 5 ml (± 0.002 ml)
5	Spectrophotometer			Wavelength accuracy Photometry accuracy	Biweekly	Depend on the material used
6	Oven	Year	At 105 ^{o}C (± 2 ^{o}C)	Temperature	Each use	At 105 ^{o}C (± 2 ^{o}C)
7	Analytical balance	Year	At 0.5 g (± 0.0002 g)	Accuracy	Each use	At 0.5 g (± 0.0002 g)
8	Refrigerator	Year	At 3 °C (± 2 °C)	Temperature	Daily	At 3 °C (± 2 °C)
9	Dissolved oxygen			Precision	Each use	Depend on the laboratory work
10	Incubator	Year	At 37 °C (± 1 °C)	Temperature	Daily	At 37 °C (± 1 °C)
11	Laminar flow			Surface swab	Each use	Not detected
12	HPLC			System Suitability	Quarterly	Depend on the laboratory work
13	GC			System Suitability	Quarterly	
14	GFAAS			Sensitivity check	Each use	

#	Equipment	Calibration		Verification		
		periodicity	Maximum permissible error	parameter to be checked	Periodicity	Acceptance criteria
15	CVAAS			Sensitivity check	Each use	Depend on the laboratory work
16	Measuring flask 25 ml	3 years	depends on their characteristics	The volume	Depends on use	depends on their characteristics
17	Pipette 1 ml	3 years				
18	Automatic pipette	3 years			Daily in use	
19	Micro syringe 10µL.	3 years			Daily in use	
20	Micro syringe 100µL	3 years			Daily in use	
21	Standard weights	A year		Cleaning	Daily in use	Well performance
22	Thermometer	A year			Daily in use	
23	Thermocouples	A year			Daily in use	

3.4 Human Resources

One of the key components of the quality system is to manage people as valuable resources rather than labour costs, thus designing human resources (HR) plan depending on this concept will guarantee the continuous improvement of the quality system.

The laboratory should have diverse and challenging goals regarding to its staff:

- Recruit and retain qualified employees
- Ensure that all employees have the knowledge, skills, feedback and understanding to perform their jobs
- Provide a work environment that enables people to give their best to the company.
- Comply with relevant labour law.

The laboratory management has an important role to attain these goals and to motivate and raise the morale of the staff which reflects in the productivity and job satisfaction. It's also important that laboratory managers don't just concentrate on technical issues, but that they also consider the advantages to be gained in actively and creatively managing the people issues of the laboratory (Garfield et al., 2000). Moreover, the management must then make available the resources that will be necessary to put the quality standard into practice, including appointing an appropriate person to be the laboratory's quality manager (Prichard et al., 1995).

3.4.1 Job analysis

A job analysis is the foundation of HR management. A valid job analysis provides data that should be used to develop effective recruitment, selection, performance management, and career development methodologies and it is performed to identify the basic duties, responsibilities, and specifications of this job (Mahapatro, 2010). Furthermore, ISO/IEC 17025:2005 states that "the laboratory shall maintain current job descriptions for managerial, technical and key support personnel involved in tests".

To build job descriptions for the laboratory's staff, depending on the laboratory's mission, vision and objectives which give us the data required about the laboratory's work load, the process approach concept was applied to analyse each process of the laboratory work and then to concrete the responsibilities of the laboratory's staff as shown in Figure 3.7. After reviewing of the tasks, duties and responsibilities, the minimum qualifications required for these responsibilities were collected to complete the job descriptions of the laboratory's staff as mentioned in Table 3.6.

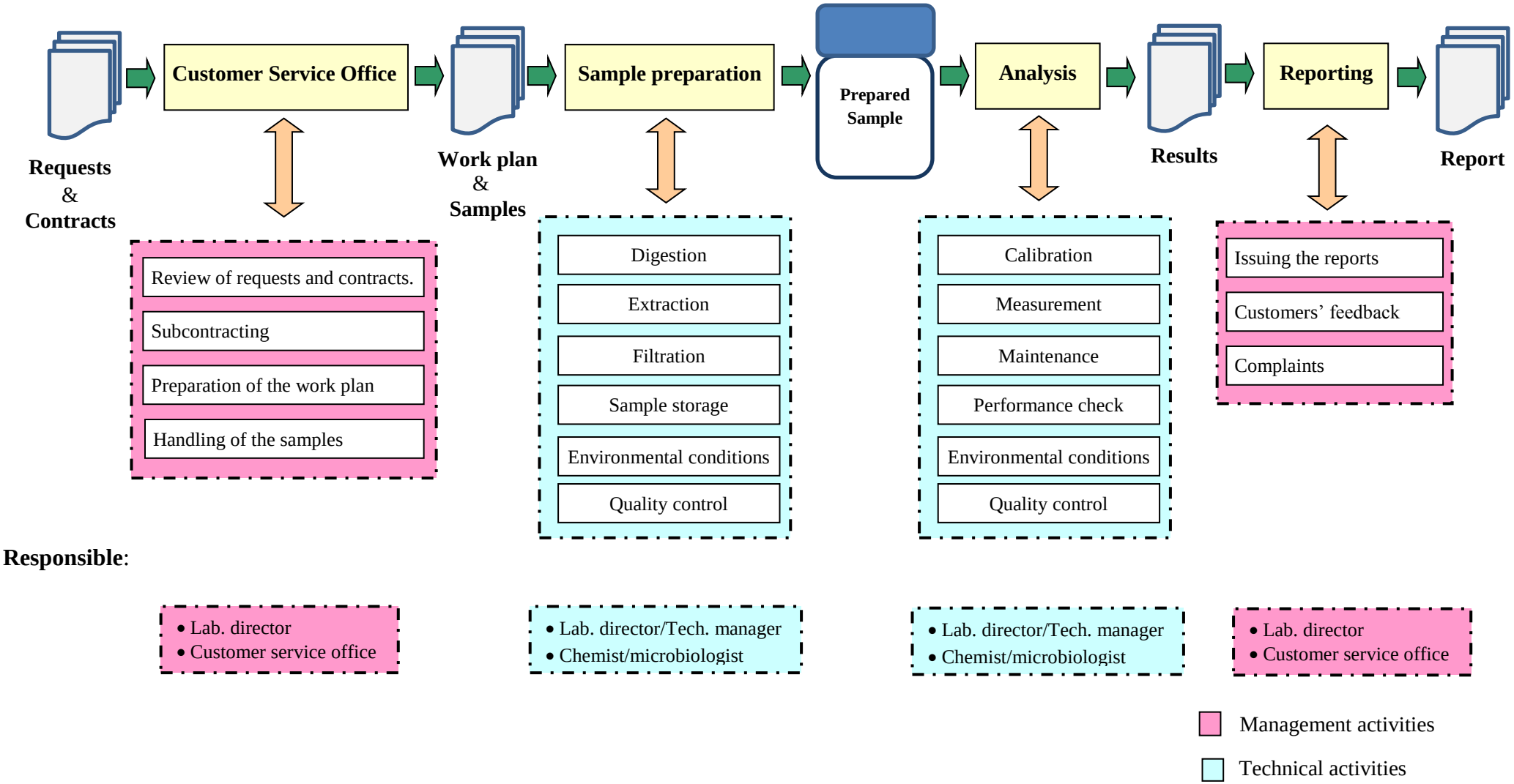


Figure 3.7 The laboratory work flow and the staff responsibilities according to the process approach

3.4.2 Human resources planning

To attain the strategic goals of an organization, the management should strive to have the right number and the right kinds of people, at the right place, at the right time, doing things, which result in both laboratory and the individual receiving maximum long-run benefits. Furthermore, the management should anticipate and plan for changes in the workforce and to be aware of upcoming needs for additional personnel such as people to staff a new function; part-time labour to help with a project; seasonal workers to fill in when employees are on vacation; replacements for those who retire (Mahapatro, 2010). By anticipating needs, the management can take action in plenty of time to make sure that the organization has the employees it needs when it needs them.

We anticipated the HR needs for the laboratory, as mentioned in Table 3.6, regarding to the work load and ISO/IEC 17025:2005 requirements, methods and equipment used in the laboratory, work area and total cost. The total number of the staff is 12 persons for optimum operation whereas 8 persons at low work load or working partially (i.e, accumulating other functions in the company).

Table 3.6 The Laboratory's needs of HR

#	Job title	No. of employees	Department	Minimum Qualifications	Responsibilities/Functions
1	Laboratory director	1	Top management	• BSc. in Chemistry or Microbiology. • Experience in Aquaculture.	Responsibility for the overall operation and administration of the laboratory, including the employment of competent and qualified personnel.
2	Quality manager	1	Top management	• BSc. in Chemistry or Microbiology. • Training courses in quality management.	Establishment and implementation of the quality system according to the legislations and standards.
3	Technical manager	1	Top management	• BSc. in Chemistry. • Training courses in Microbiology. • Experience in relevant field.	Complete responsibility of all the technical activities of the laboratory.

#	Job title	No. of employees	Department	Minimum Qualifications	Responsibilities / Functions
4	Analytical chemist	1-3	Advanced & Wet-chemistry	• BSc. in Chemistry. • Training course in microbiology.	Responsibility of the analysis of the samples and all the related activities e.g. sampling, sample preparation, quality control, monitoring of the environmental conditions, calibration and maintenance of the equipment, etc.
5	Microbiologist	1	Microbiology	• BSc. in Microbiology.	
6	Administrative	1	Customer services	• Minimum 12 years' education. • Training course in information management.	Responsible to communicate to the customers, to prepare the work plan, to handle the samples and to prepare the reports.
7	Technician	1-3	Advanced, Wet-chemistry & Microbiology	• Minimum 12 years' education. • Awareness training course for working in chemical/microbiological lab.	To work with the analytical chemist/microbiologist to analysis the samples and to achieve all the related activities. Responsibility of the washing of the glassware.
8	Labour (cleaning)	1	The laboratory	• Minimum 12 years' education.	Responsibility of the cleaning of the laboratory.

3.4.3 Orientation and Training

It is essential that new employees receive some form of orientation. Orientation programs vary, ranging from brief informal introductions to lengthy planned discussions and information sessions about the laboratory and the laboratory organization. The information impaired usually involves matters of immediate concern, such as the quality policy, goals and objectives, safety requirements, work hours, work conditions, pay periods, personal policy, benefits, organizational structure, introduction to co-workers, and other items influenced work and welfare (Garfield et al., 2000).

Training is the act of increasing the knowledge and skills of an employee for doing a particular job. Its purpose to achieve a change in the behaviour of those trained and to enable them to do their jobs better (Mahapatro, 2010). ISO/IEC 17025:2005 requires that laboratories formulate the goals with respect to the education, training and skills of the laboratory personnel. Training is equally necessary for all the staff from the new appointed up to senior (Garfield et al., 2000). Research has shown specific benefits that a small business receives from training and developing its workers, including (Mahapatro, 2010):

- Increased productivity.
- Reduced employee turnover.
- Increased efficiency resulting in financial gains.
- Decreased need for supervision.

Therefore ISO/IEC 17025:2005 states that the laboratory shall have a policy and procedures for identifying training needs and providing training of personnel. One of the most generally used training models is known as the "ADDIE" training system which consist of the following five elements making up the ADDIE model (Mahapatro, 2010):

- Assess
- Design
- Develop
- Implement
- Evaluate

This model is a powerful tool for the laboratory management to comply with the ISO/IEC 17025:2005 requirements from planning till evaluation of the training.

The laboratory can use this model regardless the training method. There are two broad types of training methods (Mahapatro, 2010):

- *On-the-job training* is delivered to employees while they perform their regular jobs. In this way, they do not lose time while they are learning. After a plan is developed for what should be taught, employees should be informed of the details. A time-table should be established with periodic evaluations to inform employees about their progress. On-the-job techniques include orientations, job instruction training, apprenticeships, internships and assistantships, job rotation and coaching.
- *Off-the-job techniques* include lectures, special study, films, television conferences or discussions, case studies, role playing, simulation, programmed instruction and laboratory training.

The effectiveness of the training should be evaluated during and/or after the process as stated in ISO 17025. Evaluation is traditionally represented as the final stage in a systematic approach with the purpose being to improve interventions (formative evaluation) or make a judgment about worth and effectiveness (summative evaluation). There are many models used for training evaluation but the most influential models, under system based approach, are Context, Input, Process, Product(CIPP) Model, Training Validation System (TVS) and Input, Process, Output, Outcome (IPO) Model (Mahapatro, 2010).

3.4.4 Performance appraisal

The on-going appraisal of the employee performance is an essential element to a functional quality system. Sometimes both supervisors and subordinates approach the appraisal session with dread and trepidation, but if they both understand the purpose of appraisal and the supervisor is objective and gives serious though to the process, most of the fears, on both sides, will be allayed (Garfield et al., 2000). The formal appraisals take place annually to review the achievements of the employee and to assess the training needs (Mahapatro, 2010). If the formal appraisal is constructive, the employee will recognize that something worthwhile has been accomplished. There should be a positive impact on the analyst's performance as well as positive impact on the employee's career through encouraging a healthier and more positive attitude about the job and chances for advancement (Garfield et al., 2000).

3.4 Quality control

Quality control (QC) is one of the most important elements of the quality system for the laboratory to produce reliable results. Moreover, it is one of the ISO/IEC 17025:2005 requirements. There are two types of quality control, internal quality control (IQC) and external quality control (EQC). Internal quality control provides confidence to the laboratory management while external quality assessment provides confidence to the customer (Prichard and Barwick, 2007).

3.4.1 Internal quality control

According to IUPAC, IQC is the set of procedures undertaken by laboratory staff for the continuous monitoring of operation and the results of measurements in order to decide whether results are reliable enough to be released (Hibbert, 2007). What we actually carry out as quality control depends on the analytical problem our work has to solve. The choices of actions are as follows:

- Measure blanks
- Measure quality control samples
- Measure repeat samples
- Measure laboratory standards and spikes

Our IQC plan (Table 3.7) was designed to fulfil the requirements of the methods that the laboratory has and to comply with ISO/IEC 17025:2005. The results of the IQC activities should be reviewed and checked against pre-defined acceptance criteria. Those acceptance criteria are derived as (ISO 5725-1:1994):

a) A theoretical or established value, based on scientific principles;
b) An assigned or certified value, based on experimental work of some national or international organization;
c) A consensus or certified value, based on collaborative experimental work under the auspices of a scientific or engineering group;
d) When a), b) and c) are not available, the expectation of the (measurable) quantity, i.e. the mean of a specified population of measurements

Furthermore, the results of IQC activities should be presented in such manner that trends are detectable as stated in ISO/IEC 17025:2005. Hence, trueness and range control charts are constructed for this purpose.

Table 3.7 Internal quality control plan

Parameter	Calibration Curve	Calibration Verification	Reagent Blank	Laboratory Control Sample	Spike	Sample Duplicate	Quality Control Sample	Other
pH	O	W				O	A	
Alkalinity			O	O		S	A	Standardization
Dissolved Sulphide	N	W	O	O	O	O	A	
Dissolved Oxygen	O					O		Zero check
Nitrate	O	W	O	O	O	O	A	
Nitrite	N	W	O	O	O	O	A	
Phosphate	N	W	O	O	O	O	A	
Silicate	O	W	O	O	O	O	A	
Ammonia	N	W	O	O	O	O	A	
Suspended Solids				O		O		
Salinity, Depth and Temperature	O	W				O	A	
Chlorophyll-Spectrophotometer	N	W	O	O	O	O	A	
Coloration	N	W				O		
Fecal Coliforms			O		O	O	A	Air blank
Chlorophyll - HPLC	N	W	O	O	O	O	A	
Organohalogenated Substances	N	W	O	O	O	O	A	
Metals - GFAA	N	W	O	O	O	O	A	
Mercury	N	W	O	O	O	O	A	

O: Once/run, N: When needed, W: Within run (minimum Once per 20 samples), S: Each sample, A: Annually

3.4.2 External quality control

In addition to the internal quality control, it is extremely useful for laboratories to obtain an independent check of their performance and to be able to compare their performance with that of other laboratories carrying out similar types of analyses (Prichard and Barwick, 2007). Failure to utilize proficiency testing can leave a crucial void in the laboratory quality system (Garfield et al., 2000). Thus, one of laboratory accreditation requirements is participation in regular proficiency testing schemes as stated in ISO/IEC 17025:2005. A key feature of proficiency testing schemes is that the assessment of laboratory performance is expressed in terms of a score that can be readily interpreted in terms of statistics (Prichard and Barwick, 2007).

To make our plan, we used EPTIS data base which is recommended by the Portuguese accreditation body IPAC to check the availability of the PT schemes depending on the quality of the PT provider; the priority was for those who is accredited with ISO/IEC 17043 and the schemes which have seawater matrix. We found that some of the laboratory analytes, pH, nutrients, metals, chlorophyll, organohalegonated substances and faecal coliforms are covered by many PT schemes as shown in Table 3.8.

Table 3.8. The available PT schemes for 2014 using EPTIS database.

#	PT Scheme	Analytes
1	AQ 1, 2, 3, 4 and AQ 11 Quasimeme Laboratory Performance Studies	Nutrients, metals, mercury, chlorophyll and halogenated organics
2	4th Campaign - Coastal Environment Quality Consult Promoting environmental quality	Metals
3	Aquacheck_ 463, QWAS_422 GCL standards	Metals, microbiology
4	PT GSCAS/2014: SEAWATER Gabinete de Servicios para la Calidad, S.A.L.	pH, conductivity, nutrients, metals and mercury
5	AM001 Quality Reliable Laboratory Services	Metals and mercury
6	SEAWATER I000001 ielab.	Nitrate, ammonia, pH microbiology, metals and mercury
7	Programme 6, AGLAE association	Salinity, pH, nutrients and suspended solids

Among these schemes, we selected the most suitable to our laboratory regarding to the cost and the program time to avoid work overloading as shown in Table 3.9.

On the other hand there are no PT schemes in seawater for the other analytes, alkalinity, dissolved oxygen, dissolved sulphide and coloration. We recommend RELACRE, the Portuguese association for accredited laboratories, which has a scheme to test these analytes in drinking water.

The external quality control plan was built based on the data available for year 2014 and the laboratory should update this plan annually.

Table 3.9 External quality control plan 2014

Parameter	January	February	March	April	May	June	July	August	September	October	November	December
pH										GSC		
Alkalinity												
Dissolved Sulphide												
Dissolved Oxygen												
Nitrate										GSC		
Nitrite										GSC		
Phosphate										GSC		
Silicate										GSC		
Ammonia										GSC		
Suspended Solids												
Conductivity, Depth and Temperature										GSC		
Chlorophyll- Spectrophotometer				AQ11								
Coloration												
Fecal Coliforms		QWAS										
Chlorophyll - HPLC										AQ11		
Organohalogenated Substances				AQ05								
Metals - GFAA										GSC		
Mercury												

Quasimeme_NL QWAS_LGC_UK GSC_Spain

4. Cost Analysis

4.1 Introduction

Cost considerations are certainly important in laboratory management and quality system. Adding a quality program will increase the cost of operation and the increased cost must be fairly judged against the benefits derived. Costs are tangible and not too difficult to assess, but some of the benefits are intangible, which makes evaluation their importance based on subjectively. The public image of the organization, the need for product improvement, the impact of government laws and regulations and complaints from the clients are examples of items which cannot be ignored (Prichard et al., 1995). Other important benefits of a good quality system that cannot be overlooked are improved laboratory credibility and staff morale. The saving from not having to reanalyse, correct, or even discard unreliable data or misjudged product samples, will often justify a significant part of the increased cost of a good quality system (Garfield et al., 2000).

Figure 4.1 is a conceptual graph that plots the cost of quality systems against the cost of failures. The cost of quality, after a setup cost, is a linear function of the activity whereas failures decrease dramatically with the most rudimentary quality system, and after a while the system is close to optimum performance. The combination of the costs and savings gives a point at which an optimum amount of money is being spent (Hibbert, 2007).

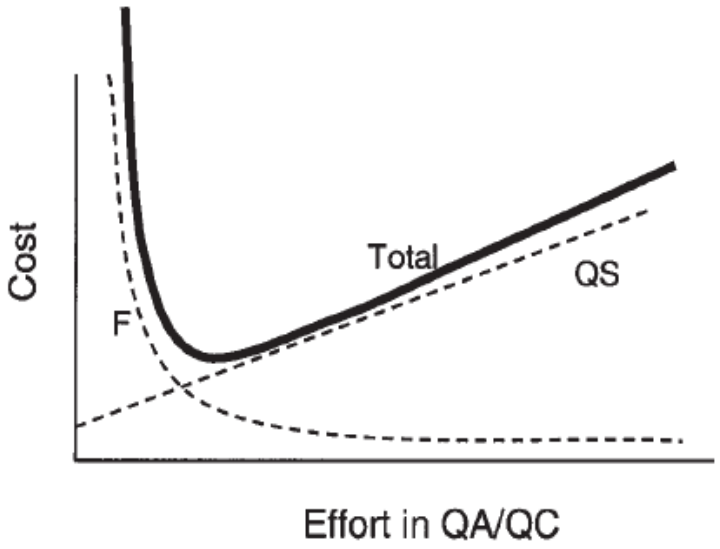


Figure 4.1. The cost of quality. F = cost of failure, QS = cost of the quality system. The minimum in the combined graph is the optimum overall cost (Hibbert, 2007).

The management may wish to develop a detailed cost versus benefits analysis in order to achieve an optimum and measurable balance. The first step in this analysis is to identify the essential quality related activities and associate them with major cost areas. The principal cost areas can be dealt with as prevention costs, appraisal costs and correction costs (Garfield et al., 2000).

- Prevention costs are those required to keep unacceptable data from being generated in the first place. They include the costs associated with performing proper laboratory planning and documentation; preparing sound procurement specifications and criteria for acceptance of new equipment, materials and services; providing sufficient and suitable training for laboratory personnel; following a rigorous schedule of equipment preventive maintenance; and performing the necessary system calibration to improve and maintain accuracy of the data produced.
- Appraisal costs are those required to continuously evaluate the system and detect any eventual error. Activities in this area include quality control measures that evaluate the performance of analysis equipment and procedures; independent audits of these quality control measures; inter- and intra-laboratory proficiency testing; and quality assurance assessment, including reporting of quality assurance activities and findings.
- Correction costs are those required to correct conditions that have been found to be out of control or less than satisfactory. These include the costs of problem investigation for determining the cause of poor data quality; the implementation of corrective and new preventative actions; and the reanalysis of samples, including in some cases resampling where invalid samples were originally taken.

Obviously, costs are not uniformly distributed among the three categories. What is often true in medicine is true here as well, namely, a small investment in prevention can produce a large dividend, in this case in saved appraisal and correction costs (Garfield et al., 2000).

4.2 Estimation of the quality system (QS) costs at Finisterra

Estimation of the total costs of the QS varies widely and is not easy to quantify. Thus, we divided the total costs into its main components, initial and running costs. The initial costs will of course depend on how much work the laboratory needs to do to bring itself up to ISO/IEC 17025:2005. In fact, quantifying the initial costs of the QS in our case, at the beginning of the laboratory life, makes it much easy, particularly to compare with the total laboratory costs using the same market prices. Both of the initial laboratory and the QS costs were studied in details and are listed in Annex I, Table 1. The main sources of the costs were sampling requirements, standard methods, equipment and accreditation including the application and initial assessment fees. Each source was studied individually as shown in Figure 4.2 which mentions the most significant sources of the QS initial costs are the standard methods costs and the accreditation fees. However, the total initial costs of the QS were studied in comparison with the total initial laboratory costs (Figure 4.3) and we found that the total initial costs of the QS are insignificant.

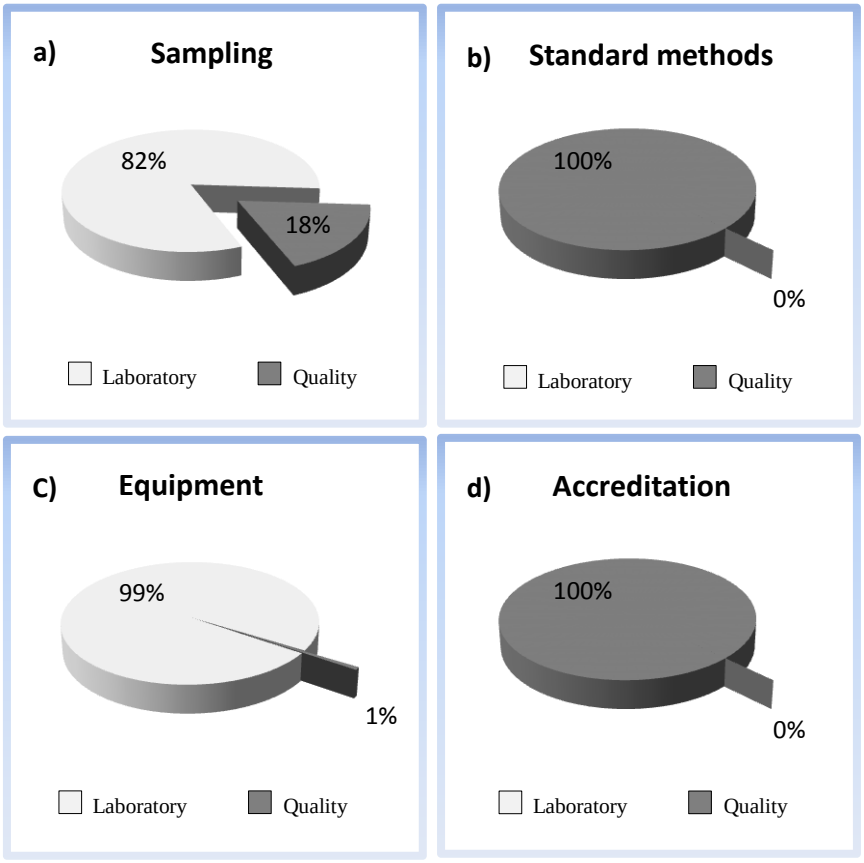


Figure 4.2 Comparison of the initial cost components of the laboratory and the QS requirements, (a) cost of sampling requirements, (b) cost of the standard methods, (c) cost of the equipment required, (d) the accreditation fees.

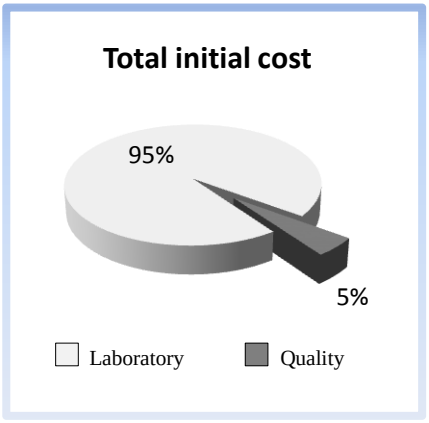


Figure 4.3 Comparison of the total initial costs of the laboratory and QS.

The next step is to quantify the running costs of the QS. The main sources of the running costs are consumables, quality control, accreditation fees, human resources and calibration, the details of these cost sources are mentioned in Annex I Table 3. We found that the QS running costs are significant in all the components in comparison with the laboratory running cost as shown in Figure 4.4. The total QS running cost was studied in comparison with the total running cost of the laboratory and our findings were the total QS running cost is 20 % of the total running costs (Figure 4.5) which is in agreement with work published by Garfield et al. in (2000). Furthermore, Prichard et al. in 1995 concluded that the operational cost of the QS is the dominant source among the QS costs and it is about 10 % taking in our considerations the operational savings regarding to existence of the QS.

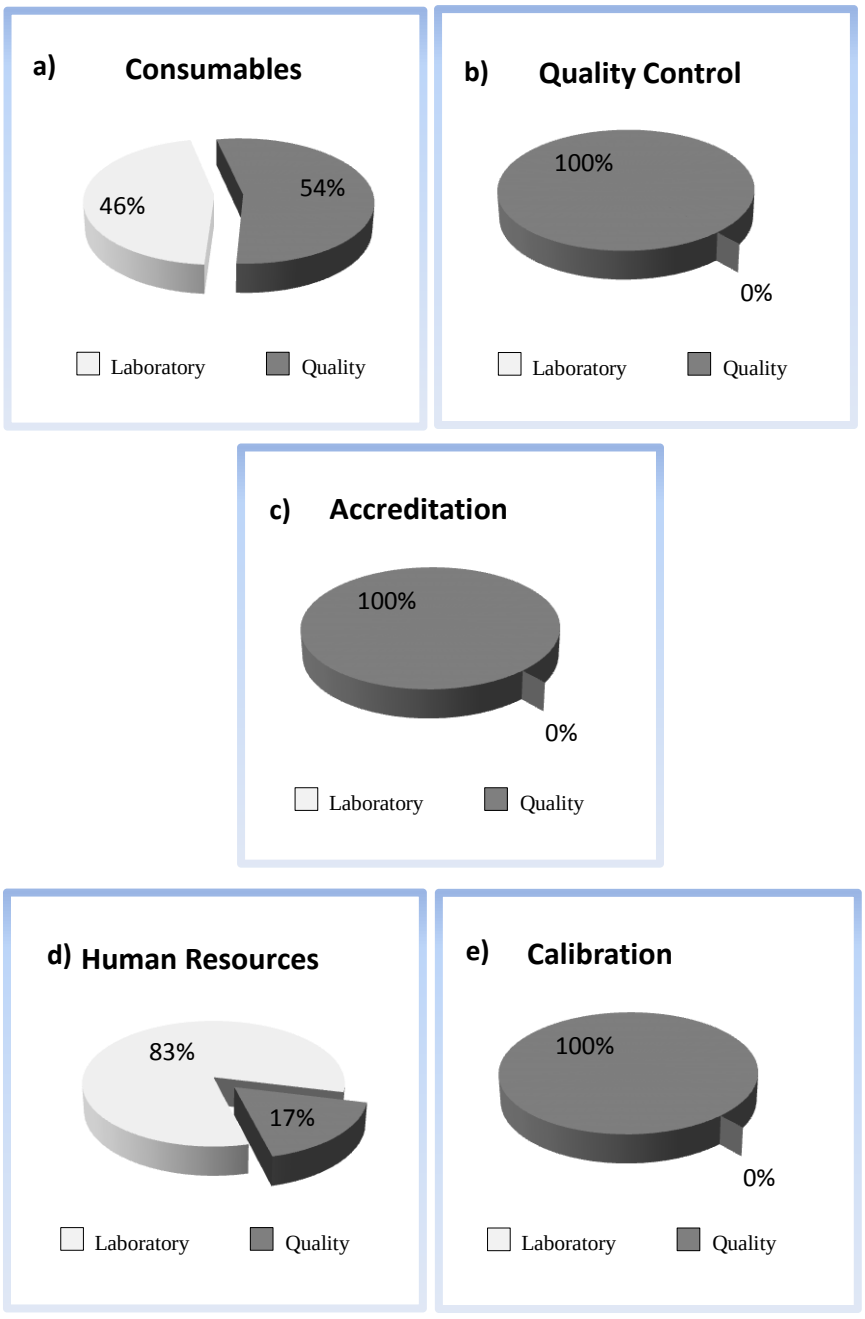


Figure 4.4 Comparison of the running cost components of the laboratory and the QS requirements, (a) cost of consumables, (b) cost of quality control, (c) the accreditation fees, (d) cost of the human resources, (d) cost of calibration.

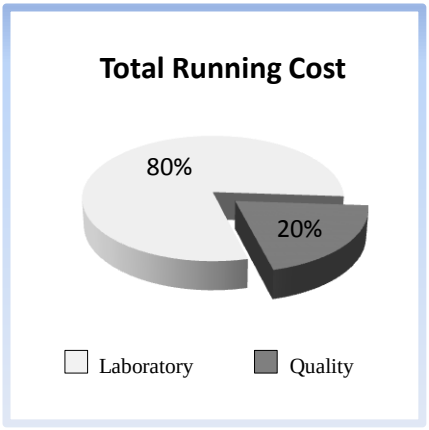


Figure 4.5 Comparison of the total running costs of the laboratory and QS.

Finally, we roughly estimated the most significant cost component regardless to the cost type, initial or running. We found that the cost of human resource is by far the largest one: one year of human resources is approximately equal to all the other costs, initial or running, as shown in Figure 4.6. It is expected that at the start of the laboratory will have a low volume of work and that some of the staff functions such as administrative and labour will not be exclusive of the laboratory but also develop work in other sections of the company. Also, the laboratory director and probably the analysts and technicians may accumulate other functions in the company, so the human resources cost is not exclusive of the laboratory. Still, this cost must be planned as, in the long run, the volume of the work will require completely dedicated staff.

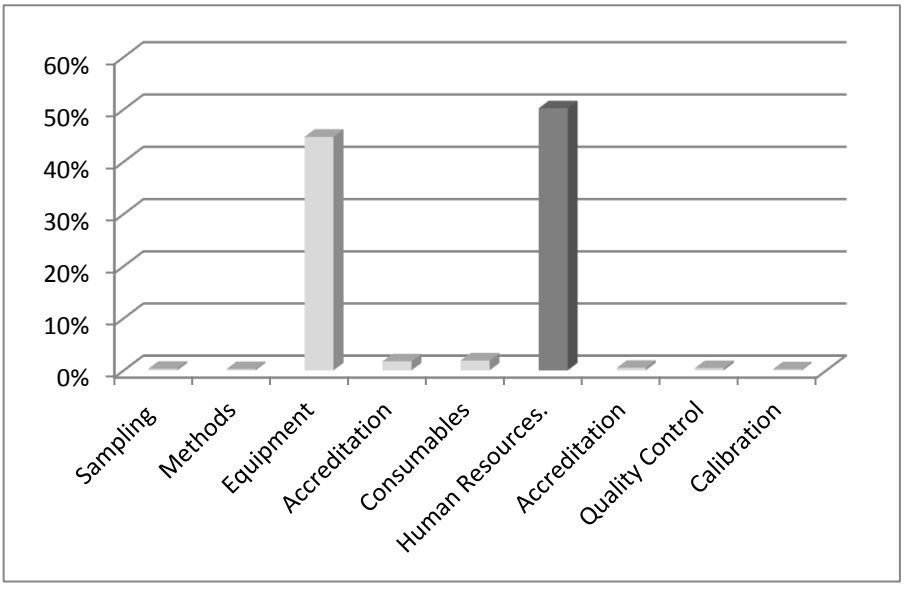


Figure 4.6 Share of each cost component in the total cost.

5. Conclusions and Recommendations

A laboratory for the environmental quality control in the aquaculture of mussels will play an important and vital role for the Finisterra Company. The determination of nutrients and chlorophyll concentrations gives the aquaculture management sufficient information about food availability for about mussel production, whilst monitoring of different pollutants, faecal coliforms, metals and organohalogenated substances gives an important indication about safety and quality of the produced mussel. Such analysis must be done locally in order to allow for a fast response of the company to changing environment conditions. Furthermore, such laboratory will provide invaluable conditions for research on the quality of the final product, providing Finisterra with an advantage over its competitors.

The present work describes a laboratory for the control of the environmental conditions on the production of mussels. The project has been designed so that the laboratory may expand in stages: the first stage providing wet chemistry analysis, involving less expensive equipment and which will be the base for the following stages. The microbiological analysis and trace metal analysis stages require separate installations and investment in new equipment which can be done at a later time.

This work has focused more on the whole organization of the quality system itself, rather than the sole design of the laboratory installations. Such organization will allow the laboratory to be accredited according to ISO 17025.

Accreditation is the recognition of competence by an independent party - in this case the Portuguese Institute for Accreditation, IPAC. It has an immediate positive impact on the company's image and assures the trust of clients, governmental institutions and researchers on Finisterra's results.

Furthermore, the organization required by the Quality System will assure a more effective and efficient work, with lower costs in the long run.

The costs of implementation and running Finisterra's laboratory have been estimated. Even though the existence of a quality system increases the material running costs significantly, these are relatively low if the total costs are taken into account. Considering the large intangible benefits of a quality system described above, we are convinced these will easily surpass its costs.

Finally, some recommendations should be considered to implement the Quality System effectively and to minimize its running cost:

- Involving of the staff in establishing of the quality system raises the awareness and prevents many mistakes during the implementation.
- Understanding the documentation system decreases the training and supervision needs.
- Following the standard methods without any modification; avoid additional work to validate the methods.
- In general, the dominant source of the cost over all the cost components is the staff cost. The laboratory can manage the staff effectively without pressure or conflict of interest. For instance, the responsibilities of the administrative person can be moved to the laboratory director, hiring of an experienced chemist to do the laboratory work can save the cost of the technical manager, the cleaning labour can serve in the laboratory as part of his/her work in another department of Finisterra.
- Regular intermediate performance checks for the equipment can extend the calibration periods.
- The laboratory can calibrate the equipment internally as mentioned in IPAC guide (OGC001, guia para a aplicação da NP EN ISO/IEC 17025).
- Periodically internal audit is a powerfully preventive action that saves the cost of non-compliance work.

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Annex I

Table 1 Estimation of the initial costs of the quality system in comparison with the total laboratory costs. The unit costs were estimated based on the current market prices and the sources indicated. These costs do not include the taxes, which must be added as 23% VAT.

#	Group	Laboratory Requirements					Quality Requirements			
		Item	Q	Estimated Cost (€)		Source	Item	Q	Total Estimated Cost (€)	Source
				Unit	Total					
1	Sampling	· Polyethylene bottles, 500 ml	12	0.55	6.6	Labbox	· Sampling Standards as shown in table 2	3	269.42	ISO store
		· Polyethylene bottles, 1 L	12	0.78	9.36	Labbox				
		· Fluorinated ethylene propylene (FEP), 1L	12	10.5	126	LAQ				
		· Amber borosilicate bottles 250 ml	12	7.47	89.64	Labbox				
		· Amber borosilicate bottles 500 ml	12	9.71	116.52	Labbox				
		· Amber borosilicate bottles 1000 ml	12	12.35	148.2	Labbox				
		· Narrow-mouthed glass bottles fitted with glass stopper, 250 ml	12	2.31	27.72	Labbox				
		· Narrow-mouthed glass bottles fitted with glass stopper, 500 ml	12	3.07	36.84	Labbox				
		· Narrow-mouthed glass bottles fitted with glass stopper, 100 ml	12	4.69	56.28	Labbox				
		· Autoclavable glass or plastic, non-toxic, bottles. 100 ml	12	2.07	24.84	Labbox				
		· Autoclavable glass or plastic, non-toxic, bottles. 250 ml	12	2.43	29.16	Labbox				
		· Autoclavable glass or plastic, non-toxic, bottles. 500 ml	12	2.99	35.88	Labbox				
		· Autoclavable glass or plastic, non-toxic, bottles. 1000 ml	12	4.01	48.12	Labbox				
		· Dark glass bottle with PTFE stopper.	12	15	180	LAQ				
		· Ice box, 30 L	2	150	300	LAQ				
Total cost					1235.2		Total cost		269.42	
2	Methods	• Published or non-official	23	-------			• Standards as shown in table 2	23	1145.85	ISO store
Total cost					0.00		Total cost		1145.85	

3	Equipment	· Water purification system to produce Water Grade 1: arium® pro DI-T	1	4990	4990	VWR	· Holmium oxide solution	3	263	VWR
		· Water purification system to produce Water Grade 3:Elix® (5 l/hora)	1	8030	8030	VWR	· Spore of bacillus stearothermophilus	5	93.9	VWR
		· High Performance Liquid Chromatography HPLC	1	45000	45000	LAQ	· Potassium dichromate	8	178	VWR
		· Gas Chromatography GC	1	40000	40000	LAQ	· Barometer, graduated	1	159	VWR
		· Graphite Furnace Atomic Absorption GFAA	1	40000	40000	LAQ	· Thermometer, with a 0.5 oC scale.	1	1.6	Labbox
		· Cold-vapour generation system, based on an automated flow injection or continuous flow equipped with quartz tube with suitable and an adsorbent which enables the enrichment with amalgamation.	1	30000	30000	LAQ	· Standard weights, M class	1	500	Labbox
		· Spectrophotometer:Spectroquant® Pharo 300	1	6430	6430	VWR	· Precision Digital Thermometers	1	88	Labbox
		· Dissolved Oxygen meter equipped with a measuring probe.	1	729.75	729.75	Labbox	· Digital auto-range multimeter.	1	38.8	VWR
		· pH-meter with ± 0.1 mv, equipped with a very rapid response pH glass electrode, the 90 % response time <10 s. during a pH change of 0.1	2	790	1580	VWR	· Maximum thermometer	1	40	LAQ
		· Condutivity-meter	1	993	993	VWR	· Thermocouple S	1	100	LAQ
		· Balance (d = 0.0001)	1	920	920	Labbox				
		· Balance (d=0.01)	2	515.2	1030.4	Labbox				
		· Oven VENTI-Line® 53L	3	1060	3180	VWR				
		· Autoclave APOUR-Line Eco	1	3310	3310	VWR				
		· Autoclavable, simple, transparent bags 40L	350	299	299	VWR				
		· Incubator, Generation 2012, 30L	1	1570	1570	VWR				
		· Refrigerator, Combined laboratory fridge/freezer, Mediline	2	1820	3640	VWR				
		· Water bath, Heating bath with immersion thermostat	1	313.95	313.95	Labbox				
		· Heating mantel	2	90.82	181.64	Labbox				
		· Glassware washer	1	1500	1500	LAQ				
		· Centrifuge, Heraeus® Labofuge® 200	1	1355	1355	VWR				
		· Tissue grinder type Potter-Elvehjem Glass, 15mL	1	23.9	23.9	VWR				
		· Vacuum pump, VACU-F1B-001 10l/min 8mbar	1	1271.1	1271.1	Labbox				
		· Rubber vacuum tubing	1	3.32	3.32	Labbox				
		· Vacuum pump acessories	1	145.33	145.33	Labbox				
		· Filtration apparatus include 1000ml filtration flask, 47 mm fritted base	1	149.02	149.02	Labbox				
		· Filtration device for sulphide	1	100	100	LAQ				
		· Filtration device for suspended solids :	1	100	100	LAQ				

3	Equipment	· Vacuum type desiccator	1	223.4	223.4	Labbox					
		· Distillation apparatus for Ammonia fully automatic, run time 2-3 min	1	9050	9050	VWR					
		· Stripping apparatus for the Separation of sulphide.	1	250	250	LAQ					
		· Digestion vessel, made of borosilicate glass, 100 ml.	1	50	50	LAQ					
		· Reflux equipment made of borosilicate glass	1	100	100	Videq					
		· Glass beads 2mm, 1Kg	1	13.2	13.2	Labbox					
		· A volumetric dispensing system, containing a constant volumetric pipette made of glass with valves at each end.	1	150	150	LAQ					
		· Jacketed beaker, of capacity 250 ml. A glass beaker enclosed by a water jacket of internal diameter 57 mm.	1	109.1	109.1	Sigma-aldrich					
		· Magnetic stirrer with heating and ceramic coated plate	1	325.38	325.38	Labbox					
		· Holder for burette tip, electrode, and thermometer:	1	13.71	13.71	Labbox					
		· Transfer device for samples by mass, designed to allow dispensation from a bottle with a greased ground-glass joint in a manner that ensure that grease is not transferred to the weighing bottle.	1	160	150	LAQ					
		· Measuring cylinder, 25 ml	6	4.18	25.08	Labbox					
		· Measuring cylinder, 50 ml	6	4.47	26.82	Labbox					
		· Measuring cylinder, 100 ml	6	5.52	33.12	Labbox					
		· Measuring cylinder, 500 ml	6	14.91	89.46	Labbox					
		· Measuring cylinder, 1000 ml	6	22	132	Labbox					
		· Erlenmeyer flask 250ml.	12	1.35	16.2	Labbox					
		· Beaker 600 mL	6	0.45	2.7	Labbox					
		· Beaker 1000 ml	6	1.2	7.2	Labbox					
		· Beaker 100 ml	6	0.35	2.1	Labbox					
		· Measuring flasks, of 50mL	12	2.36	28.32	Labbox					
		· Measuring flasks, of 100mL	12	2.66	31.92	Labbox					
		· Measuring flasks, of 500mL	6	5.19	31.14	Labbox					
		· Measuring flasks, of 1000mL	6	7.67	46.02	Labbox					
		· graduated pipettes, of capacity 5 ml	5	5.7	28.5	Labbox					
		· graduated pipettes, of capacity I0 ml.	5	6.48	32.4	Labbox					
		· One-mark pipette, of capacity 1 ml	5	11.61	58.05	Labbox					
		· One-mark pipette, of capacity 2 ml	5	11.61	58.05	Labbox					
		· One-mark pipette, of capacity 5 ml	5	11.61	58.05	Labbox					

3	Equipment	· One-mark pipette, of capacity 10 ml	5	11.82	59.1	Labbox					
		· One-mark pipette, of capacity 20 ml	5	14.92	74.6	Labbox					
		· One-mark pipette, of capacity 25 ml	5	14.92	74.6	Labbox					
		· One-mark pipette, of capacity 50 ml	5	22.61	113.05	Labbox					
		· One-mark pipette, of capacity 100 ml	5	41	205	Labbox					
		· Micro litre syringes.	3	50	150	LAQ					
		· Bottle top dispenser 2.5mL-25mL	1	198.63	198.63	Labbox					
		· Bottle top dispenser 5.0mL-50mL	1	203.1	203.1	Labbox					
		· Total-delivery pipettes:	1	119	119	Labbox					
		· Micropipette tips, polypropylene, 1-5 ml Standard Line	1000	11.78	11.78	Labbox					
		· Poly-ethylene squirt bottles: 250, 500 ml	4	15	60	Labbox					
		· Centrifuge tubes, polypropylene, 15 ml with non-pigmented screw-caps.	500	75.2	75.2	VWR					
		· Nessler tubes, matched, 50-mL, tall form.	6	18	108	VWR					
		· Test tubes 16mm	500	14.62	14.62	Labbox					
		· Durham tubes 25x61	250	44.2	44.2	VWR					
		· Caps, kIM-KAP, disposable	100	16	16	VWR					
		· Quartz Cuvette, of path length 1 cm.	2	52.62	105.24	Labbox					
		· Glass Cuvette for the spectrophotometer, 1 cm in length.	8	12.53	100.24	Labbox					
		· Glass Cuvette for the spectrophotometer,2 cm in length.	2	14.41	28.82	Labbox					
		· Glass Cuvette for the spectrophotometer,5 cm in length.	2	18.79	37.58	Labbox					
		· Glass rods 20 cm	20	0.51	10.2	Labbox					
		· Petri dishes, plastic, 50 x 9 mm.	500	43.2	43.2	Labbox					
		· Gas supply with nitrogen, of high purity [99.996 % (m/m) pure].	1	4000	4000	LAQ					
		· Gas flow meter	1	50	50	LAQ					
		· Tweezers or flat-tipped forceps 115mm	6	3.11	18.66	Labbox					
		· Drying support for filter papers in the drying oven:	24	0.6	14.4	Labbox					
		· Loop, made of platinum-iridium, or nickel-chromium, 3 mm in diameter:	1	50	50	LAQ					
		· Kolle handle 200 mm lenght + 2 Ni-Cr inoculation loops	6	4.17	25.02	Labbox					
		· Gas burner:	1	88.5	88.5	Labbox					
		· Vortex mixer:	1	141.78	141.78	Labbox					

		· Test tube rack 50tubes	4	24.6	98.4	VWR					
		· Standard holder for < Ø 30 mm tubes	4	6.63	26.52	Labbox					
		· Double Spatula, w / spoon and flat Stainless Steel 150mm	6	1.15	6.9	Labbox					
		· Claw coated cork, 210mm, opening 0-80mm	4	7.79	31.16	Labbox					
		· Micro double spatula, c / spoon and flat, stainless 150mm	6	1.55	9.3	Labbox					
		· Nuts for double frames, angle 90 °, 17mm grip	10	3.75	37.5	Labbox					
		· Glasses w / side shields, anti-UV, ultra lightweight, transparent	6	2.4	14.4	Labbox					
		· Pipette or security, rubber, universal, 3-valve, red	10	3.57	35.7	Labbox					
		· Support in stainless steel, for 16mm tubes	3	9.8	29.4	Labbox					
		· Supported in pp to 16mm tubes	3	3.82	11.46	Labbox					
		· Test tubes, PS, round bottom, 16mmX100mm, Cx 100	500	14.62	14.62	Labbox					
		· Caps for test tubes, PS, round bottom, 16mmX100mm, CX100	1000	9.56	9.56	Labbox					
Total cost					214553		Total cost			1553.86	
4	Establishing the QS	----------					Setting up the documentation system (writing and issuing)	1	700	RELACRE	
							Training,e.g awareness with ISO17025 requirements, uncertainty estimation, QC and internal audit.	3	2100		
							Accreditation fees (Application and initial assessment)	1	5549.16	IPAC	
Total cost					0.00		Total cost			8349.16	

* VWR: www. pt.vwr.com
* Labbox: www.labbox.com
*LAC: Laboratório de Análises Químicas, Algarve Universidade.
*IPAC: instituto português de acreditação .
*RELACRE: www.relacre.pt/pt

Table 2 The prices of the standard methods according to ISO store in November 2013.

Group	#	Parameter	Standard method	Price	
				CHF	€
Sampling	1	Planning	ISO 5667-1:2006	128.00	
	2	Preservation	ISO 5667-3:2012	146.00	
	3	Sampling	ISO 5667-9:1992	58.00	
Price of sampling standards				**332.0**	**269.4**
Wet-chemistry	4	Water for analytical laboratory use	ISO 3696:1987	50.00	
	5	pH	ISO 10523:2008	80.00	
	6	Alkalinity	ISO 22719:2008	86.00	
	7	Dissolved Sulphide	ISO 10530:1992	66.00	
	8	Dissolved Oxygen	ISO 5814:2012	80.00	
	9	Nitrate	SMEWW 4500-NO_3^- - B	---------	
	10	Nitrite	ISO 6777:1984	50.00	
	11	Phosphate	ISO 6878:2004	108.00	
	12	Silicate	SMEWW 4500-SiO_2- C	---------	
	13	Ammonia	ISO 7150-1:1984	58.00	
	14	Suspended solids	ISO 11923:1997	50.00	
	15	Conductivity. Depth and Temperature	SEACAT SBE19	---------	
	16	Chlorophyll	EPA, 446	---------	
	17	Coloration	ISO 7887:2011	86.00	
	18	Petroleum Hydrocarbons	Visual examination	---------	
	19	Substances affecting the taste of the shellfish	By tasting	---------	
Price of wet chemistry standards				**788.0**	**639.5**
Micro-biology	20	Fecal coliforms	ISO 4831:2006	74.00	
	21	Sampling for microbiology	ISO 19458:2006	92.00	
	22	General requirements for microbiology	ISO 7218:2007	172.00	
Price of microbiology standards				**338.0**	**274.3**
Advanced analysis	23	Chlorophyll	EPA, 447	---------	
	24	Organohalogenated substances	EPA, 8021	---------	
	25	Metals	ISO 15587-2	92.00	
			ISO 15586:2003	108.00	
			ISO 12846:2012	86.00	
Price of advanced analysis standards				**286.0**	**232.1**
Total price				**1744.0**	**1415.3**

- The currency was converted by www.oanda.com in November 2013.

Table 3 Estimation of the annual running costs of the quality system in comparison with the total laboratory costs. The unit costs were estimated based on the current market prices and the sources indicated. These costs do not include the taxes, which must be added as 23% VAT.

#	Group	Laboratory Requirements				Quality Requirements			
		Item	Q	Estimated Cost (€)	Source	Item	Q	Estimated Cost (€)	Source
1	Consumables	· 1-amino-2-naphthol-4-sulfonic acid	1	106	sigmaaldrich	· Chl a free of chl b	1	128.5	Sigma-aldrich
		· 4-aminobenzen sulfonamide	1	100	LAQ	· Chl b substantially free of Chl a	1	138	Sigma-aldrich
		· Acetic acid 99% 1L	1	22.9	VWR	· Sterilization indictor, spore of bacillus stearothermophilus	15	31.7	VWR
		· Acetone, HPLC grade 1L	1	42.1	VWR				
		· Acetonitrile, HPLC grade,1L	1	59.9	VWR	· CRM for metals in seawater: MRC As, Pb, Cd, Cr, Cu, Hg, Ni, Zn, K, N (total), P (total)	1	1340	VWR
		· Aluminium foil.	1	84.7	VWR				
		· Ammoium chloride, 1Kg	1	49.7	VWR	· E. coli strain	1	73.2	Sigma-aldrich
		· Ammonia solution, approximately 28 % by mass,1L	1	23.3	VWR	· Standard buffer solutions	3	150	LAQ
		· Ammonium acetate, 1Kg	1	70	VWR	· Conductiivity standard solution	1	50	LAQ
		· Ammonium dihydrogen phosphate modifier, 500g	1	17.8	VWR	· CRM for nutrients in seawater: MRC NH4, 2 µM; NO2 1, µM; NO2+NO3,10µM; N (total) 15 µM	12	1900	VWR
		· Ammonium hydroxid, NH4OH, 5.0M, 2L	1	29.9	sigmaaldrich				
		· Ammonium iron(Ill) sulphate dodecahydrate, 500g	1	35.4	VWR	MRC PO4 2 µM; P (total) 2 µM; SiO4 20 µM	12	1160	VWR
		· Ammonium molybdate, (NH4)6Mo7O24 .4H2O, 250g	1	120	VWR				
		· Antifoaming agent, for instance n-dodecane (C12H26).	1	60	LAQ				
		· Argon gas 10,7m3	1	160	LAQ				
		· Ascorbic acid, 100g	1	39.9	VWR				
		· Biological filter 0.1 µm and 0.45 µm,Cx 200	1	168.8	VWR				
		· Borosilicate glass fibre filters	1	100	LAQ				
		· Brilliant green lactose bile broth,500g	1	90.6	VWR				
		· Cation exchange resin (in hydrogen form)	1	200	LAQ				
		· Chloroform, 1L	1	80	VWR				
		· Cobalt (II) chloridehexahydrate(CoCI2.6H2O),100g	1	155	VWR				
		· Disposable Pasteur type pipets or medicine dropper.	1000	32	Labbox				

		· Ethanol (96%), 2.5L	1	26.68	Labbox				
		· Ethyl acetate, HPLC grade,1L	1	53.6	VWR				
		· Ethyl alcohol or ethylene oxide	1	40	LAQ				
		· Filters, glass fibre, 47-mm, or 25-mm,	1	26.4	VWR				
		· Glycerol,1L	1	27.5	VWR				
		· Hg-free N2 gas	1	160	LAQ				
		· Hydrochloric acid 37% ,2.5L	1	37.5	VWR				
		· Hydroxylammonium chloride, 250g	1	74.3	VWR				
		· Iodine solution 99.8%,100g	1	56.4	sigmaaldrich				
		· Laboratory grade detergent, 2.5L	1	35.6	VWR				
		· Hand towels, SCOTT®, Performance	6	127	VWR				
		· L-ascorbic acid	1	39.9	VWR				
		· Lauryl sulfate tryptose broth	1	85	LAQ				
		· Magnesium nitrate (modifier), 500g	1	32.5	VWR				
		· Methanol, HPLC grade, 2.5L	1	23	VWR				
		· N-(1-naphthyl)-1,2-diaminoethane dihydrochloride	1	55	LAQ				
		· N,N-dimethyl-1,4-phenyl di-ammonium chloride	1	60	LAQ				
		· Nickel modifier 100 ml	1	97.5	VWR				
		· Non-toxic, phosphorus free detergent, 2.5L	1	35.6	VWR				
		· Orthophosphoric acid 85%, 2.5L	1	46.7	VWR				
		· Oxalic acid, H2C2O4.H2O, 500g	1	9.97	LABBOX				
		· Palladium nitrate, 10 g/l (modifier), 50mL	1	134	sigmaaldrich				
		· Peptone water and phosphate buffer, 500g	1	69.8	sigmaaldrich				
		· Potassium bromate,250g	1	34.3	VWR				
		· Potassium bromide,500g	1	12.45	LABBOX				
		· Potassium chromate, K2CrO4 99.0%,100g	1	23.6	sigmaaldrich				
		· Potassium hydrogen phthalate,250g	1	31.2	VWR				
		· potassium nitrate,500g	1	11.67	LABBOX				
		· Pottasium hydroxide,1Kg	1	34.5	VWR				
		· Silica standard of approximately (Sea water Ionic strength)	1	17.7	VWR				

		· Sodium bicarbonate, NaHCO3, powder,99.7-100.3%, 500g	1	35	sigmaaldrich					
		· Sodium bisulphite NaHSO3,100g	1	31.9	sigmaaldrich					
		· Sodium borate decahydrate, Na2B4O7. 10H2O,500g	1	15.6	VWR					
		· Sodium chloride,500g	1	4.24	LABBOX					
		· Sodium dichloroisocyanurate 96%,25g	1	29.4	sigmaaldrich					
		· Sodium hydroxide,1Kg	1	15.9	VWR					
		· Sodium nitroso-pentacyanoferrate(III) dehydrate 100g	1	66.6	VWR					
		· Sodium salicylate,250g	1	25.2	VWR					
		· Sodium sulfide hydrate [Na,S.xH,O, (x = 7-9)] ≥60%,1Kg	1	23.2	sigmaaldrich					
		· Sodium sulfite, anhydrous (Na2SO3) 1Kg	1	22.7	VWR					
		· Sodium sulphite Na2SO3, 1Kg	1	22.4	VWR					
		· Sodium thiosulfate,99.5%, 500g	1	28.5	sigmaaldrich					
		· Standard stock solutions 1000 mg/l of Ag, 500mL	1	27.2	VWR					
		· Standard stock solutions 1000 mg/l ofAs, 500mL	1	26.5	VWR					
		· Standard stock solutions 1000 mg/l of Cd 500mL	1	28.2	VWR					
		· Standard stock solutions 1000 mg/l ofCr, 500mL	1	27	VWR					
		· Standard stock solutions 1000 mg/l of Cu, 500mL	1	29.2	VWR					
		· Standard stock solutions 1000 mg/l of Hg, 500mL	1	30	VWR					
		· Standard stock solutions 1000 mg/l ofNi, 500mL	1	30	VWR					
		· Standard stock solutions 1000 mg/l of Pb, 500mL	1	26.7	VWR					
		· Standard stock solutions 1000 mg/l of Zn, 500mL	1	30	VWR					
		· Starch, 100g	1	29.6	VWR					
		· Sulfuric acid 98%, 1L	1	14.2	VWR					
		· Tin (II) chloride, 250g	1	21.2	VWR					
		· Trisodium citrate dihydrate, 500g	1	18.1	VWR					
		· Zinc acetate, 250g	1	12.9	VWR					
		· Indicator tape for sterilization	1	4.36	Labbox					
		· LABKEM silica gel for desiccators 1Kg	1	14.5	Labbox					
		· Inoculation loops, calibrated 10µL	1000	41.52	Labbox					
		· Nitrile Gloves CX100 M	1	4.19	VWR					

		· Nitrile Gloves CX100 L	1	4.19	VWR				
		· Weighing, disposable, 80X50X14mm, 25ml container	1	41	Labbox				
		· Parafilm M sealing film	1	24	Labbox				
Total cost				4172.57		Total cost		4971.40	
2	Human resources	Laboratory director	1	3000	Portaria nº 1553-C/2008 published in DRI nº 252, sup. 4, 31/12/2008	Quality manager	1	2000	Portaria nº 1553-C/2008 published in DRI nº 252, sup. 4, 31/12/2008
		Technical manager	1	2000		Working time of the chemists / microbiologist for quality activities, e.g internal audit, internal quality control, checking the equipment and filling the documentation system	25 %	1500	
		Analytical chemist	3	4500					
		Microbiologist	1	1500					
		Technician	3	3600					
		Administrative (customer services responsible)	1	1200					
		Labour (for cleaning)	1	900					
Cost / month				16700		Cost / month		3500	
Total cost / year				200400		Total cost / year		42000	
3	Accreditation	----------				Surveillance fees	1	2334.48	IPAC
Total cost				0.00		Total cost		2334.48	
4	Quality Control	----------				PT for metals in seawater	1	195	GSC
						PT for nutrients, pH and conductivity	1		
						PT for chlorophyll	2	700	QLPS
						PT for halogenated organic substances in seawater	1	525	QLPS
						PT for microbiology	1	600	LGC
				0.00				2020	

5	Calibration	----------				Technical balance	1	100	Luff
						Water bath	1	70	
						Automatic burette	1	50	
						Oven	1	70	
						Analytical balance	1	100	
						Refrigerator	2	70	
						Incubator	1	70	
						Automatic pipette	2	80	
						Micro syringe 10µL.	1	40	
						Micro syringe 100µL	1	40	
						Thermometer	2	60	
						Standard weights	1	60	
				0.00				810	