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Manual on Laboratory Quality Assurance

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Manual on Laboratory Quality Assurance

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GLOSSARY OF TERMS

In the context of this document, the following terms are defined:

Quality Assurance	All the planned and systematic activities implemented within the quality system that can be demonstrated to provide confidence that a product or service will fulfil requirements for quality.
Accreditation	Procedure by which an authoritative body gives formal recognition that a body or person is competent to carry out specific tasks' (ISO Guide 21996). In the context of a laboratory making measurements, accreditation is a formal recognition that a laboratory is competent to carry out specific tests or specific types of tests.
Calibration	Operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication. A calibration may be expressed by a statement, calibration function, calibration diagram, calibration curve, or calibration table. In some cases, it may consist of an additive or multiplicative correction of the indication with associated measurement uncertainty.
Certified Reference material (CRM)	Reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure, which establishes its traceability to an accurate realisation of the units in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence' (ISO Guide 30, Terms and definitions used in connection with reference materials)
Laboratory sample	Primary material delivered to the laboratory
MicroVal	European certification organisation for the validation and approval of alternative methods for the microbiological analysis of food and beverages
NordVal	An independent third-party, reviewing alternative methods
Proficiency Testing	Evaluation of participant performance against pre-established criteria by means of inter-laboratory comparisons
Quality Control	The operational techniques and activities used to fulfil requirements for quality. Often, however, "quality assurance" and "quality control" are used interchangeably, referring to the actions performed to ensure the quality of a product, service or process.
Reference Material (RM)	Material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.' (ISO Guide 30)

Reference strains	Micro-organisms defined at least to the genus and species level, catalogued and described according to its characteristics and preferably stating its origin. (ISO 11133[1]). Normally obtained from a recognised national or international collection.
Sample	A portion of material selected to represent a larger body of material.
Sample handling	This refers to the manipulation to which samples are exposed during the sampling process, from the selection from the original material through to the disposal of all samples and test portions.
Standard Operation Procedure (SOP)	Established or prescribed method to be followed routinely for the performance of designated operations, processes. A detailed set of instructions, which describes how to carry out a task
Test portion	This refers to the actual material weighed or measured for the analysis
Test sample	The sample prepared from the laboratory sample
Traceability	'Property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties. (VIM 1993).
Uncertainty of Measurement	Parameter, associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measure. The parameter may be, for example, a standard deviation (or a given multiple of it).
Validation	The confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled. (ISO/IEC 17025).
Verification	Provision of objective evidence that a given item fulfils specified requirements. (VIM 3).

[1] ISO 11133:2014 Microbiology of food, animal feed and water -- Preparation, production, storage and performance testing of culture media

ABBREVIATIONS

AAS	Atomic absorption spectrometer
AFNOR	Association Française de Normalisation
AOAC	Association of analytical communities
ATCC	American Type Culture Collection)
CRM	Certified Reference Material
CROSQ	CARICOM Regional Organisation for Standards and Quality
EA	European Accreditation
EPTIS	PT database operated by BAM (German Institute for material research and testing)
EU	European Union
FDA	Food and Drug Administration
GLC	gas liquid chromatograph
HEPA	High-efficiency particulate arrestance
HPLC	High performance liquid chromatograph
IAAC	Inter-American Accreditation Cooperation
IEC	International Electrotechnical Commission
ILAC	International Laboratory Accreditation Cooperation
ISO	International Organization for Standardization
JANAAC	Jamaica National Agency for Accreditation
MRL	Maximum Residue Level
MU	Measurement Uncertainty
NIST	National Institute of Standards and Technology
NSP	Neurotoxic shellfish poisoning
PCR	Polymerase Chain Reaction
pH	the negative of the logarithm to base 10 of the molar concentration
PPE	Personal Protective Equipment
PT	Proficiency Testing
QA	Quality Assurance
QC	Quality Control
RM	Reference Material
SFP	Strengthening Fishery Products
SOP	Standard Operating Procedure
SI	International System of Units
TTLABS	Trinidad and Tobago Laboratory Accreditation Service
VIM	International Vocabulary of Metrology; VIM 3 – International Vocabulary of Metrology - Basic and General Concepts and Associated Terms

FOREWORD

The fishery sector is of great importance for CARIFORUM States, as it provides employment for an estimated 121,000 persons, and contributes significantly to food security and export earnings. The marine capture sector is mostly characterized by a small-scale multi-gear fishery, but several countries have also developed distant water fleets of industrial vessels. Aquaculture is also becoming more important, with some large-scale investments in shrimp and tilapia production as well as numerous experimental and small-scale operations. The fishery sector of CARICOM countries also engages in significant international trade with combined exports worth US$390 million in 2015, with imports over US$180 million (which supply not only domestic markets, but also help to sustain our tourism sector). All this business, and the resulting benefits to the people of our region, depend wholly on the fishery products we produce and market being safe for human consumption. However, ensuring such safety against the background of a diversified and globally integrated fishery sector presents significant challenges, requiring not only considerable resources, but also a high level of expertise and knowledge.

The Caribbean Regional Fisheries Mechanism was formed in 2002 with the objective to promote and facilitate the responsible utilization of the Region's fisheries and other aquatic resources for the economic and social benefits of the current and future population of the region. In line with this aim, we are therefore pleased to present this Manual, which is one of a series, which provides valuable, up-to-date, regionally relevant and practical advice on ensuring the food safety of Caribbean fishery products. The Manuals are intended for use by both fishery sector operators, as well as those involved in protecting our consumers, through the implementation and enforcement of sanitary regulations. We are sure that these documents will help to provide a solid technical basis for the ensuring the continued and sustainable growth of our seafood sector.

1 INTRODUCTION

1.1 Background

This manual was developed within the framework of the EU funded 10th EDF Sanitary and Phytosanitary (SPS) Project, under the terms of a contract "Capacity Building of regulatory and industry stakeholders in Aquaculture and Fisheries Health and Food Safety to meet the SPS requirements of international trade", implemented by Megapesca Lda, Portugal.

The primary objective of the project is to:

> *Build capacities of CARIFORUM States in health and food safety requirements of fisheries and aquaculture (inland, marine) products, and as such ensure safe food standards for fisheries products in the region, while meeting the requirements of the region's trading partners worldwide.*

The expected result is that capacities will be built at the national and regional levels for health and food safety requirements of fisheries and aquaculture (inland, marine) products. This will also ensure safe food standards for fisheries products in the region, while meeting the requirements of the region's trading partners worldwide.

This operational manual is one of eight manuals aimed at providing a structured approach to training in field, laboratory, market, and trade (import and export) activities, related to the safety of fish and fish products for human consumption. The strengthening of sanitary conditions throughout the region is expected to lead to improved health and well-being of national populations, and increased international trade in fishery products.

1.2 About this manual

This manual provides guidance on best practice for laboratories carrying out the official sanitary control of fishery products. It provides appropriate information on how to fulfil the requirements of ISO/IEC 17025[2], giving detailed guidance on requirements for undertaking chemical and microbiological testing.

ISO 17025 accreditation for official control laboratories is required by the EU, and for the international acceptance of test data. For those working towards accreditation, certification, or other compliance with particular quality requirements, the guide will help those seeking to implement these requirements in a food safety testing laboratory. This guide has been prepared to reflect the requirements for such accreditation. It details and reviews the relevant issues, including accommodation, utilities, equipment and staffing, also technical aspects of validation, calibration, use of reference materials and other quality assurance measures such that managers can make informed decisions on the practicality of the investment, the scale of the operation.

Only sound management of quality in a testing laboratory will enable it to produce test results that the international community will trust. These results often provide the basis for legal action, seizure, destruction, or rejection of consignments and have significant financial and economic consequences. Introducing and maintaining a good Quality Assurance (QA) practice, including its formal recognition by accreditation, certification etc., helps to ensure that results are valid and fit for purpose. Appropriate QA can help a laboratory to demonstrate to external parties that it has adequate facilities and equipment in place for carrying out chemical and microbiological analyses, and that the work is carried out by competent staff in a controlled manner, following a documented and validated method.

[2] ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories

This document is based on previous work undertaken in 2005 and 2010 by the EDF funded project Strengthening Fishery Product Health Conditions in ACP and OCT Countries[3] and reflects the latest updates to ISO 17025.

1.3 How to use the document

Achieving, maintaining and improving accuracy, timeliness and reliability are major challenges for laboratories conducting tests on food, including fisheries products. This manual should be used as a reference for those wishing to install effective quality assurance mechanisms in the test laboratory. It sets out the principles of laboratory work and describes how to set up and organise effective quality assurance mechanisms for a laboratory, for the official sanitary control of fishery products.

The guide is organised in chapters addressing key requirements to be considered and implemented to achieve accurate quality results, and to avoid diversion of energies into less important issues. References for further reading are provided in Annex 1.

2 ACCOMODATION AND ENVIRONMENTAL CONDITIONS

The laboratory work space and facilities must be such that the workload can be performed without compromising the quality of work, and the safety of the laboratory staff. ISO/IEC 17025, paragraph 5.3 refers to this area in more detail; see also ISO 7218[4].

2.1 Laboratory layout

The accommodation layout must facilitate all elements of the testing operation from sample receipt to issue of the final report. It must also consider steps in the testing process that must be separated from other activities. The requirements are different for a microbiology laboratory, and for different types of chemistry laboratories, although there are some similarities as discussed later.

In laboratories, there should be effective separation between neighbouring areas in which there are incompatible activities. Measures should be taken to prevent cross-contamination. The laboratory should be arranged so as to minimise risks of cross-contamination, where these are significant to the type of test being performed. The ways to achieve these objectives are, for example to:

(a) Construct the laboratory so as to ensure a direct flow of samples through the testing steps
(b) Carry out procedures in a sequential manner, using appropriate precautions to ensure test and sample integrity (e.g. use of sealed containers)
(c) Segregate activities by time or space.

[3] Manual/Handbook for the Execution of Sanitary Inspection of Fish as Raw Material and Fish-Products as Food for Human Consumption, Mission Ref: CA073GEN, May 2010, published by Strengthening Fishery Products Health Conditions in ACP/OCT Countries (Project No. 8ACPTPS137)

[4] Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations

Good practice is to have separate locations, or clearly designated areas. For the most efficient design, all related services should be located in close proximity. For optimal organization of the laboratory, consider:

- Delineation of laboratory activities. Care should be taken either to group related activities in a single room, or to clearly delineate bench space for specific activities. Measures must be taken to prevent cross-contamination of samples.
- Location of service rooms. Service rooms to accommodate autoclaves, sinks for cleaning glassware, preparation and sterilization of culture media, should be located in a central area to minimize distances and facilitate circulation paths of materials, samples and goods.

A responsible staff member should be designated to oversee cleaning and maintenance of the service rooms.

2.2 Access to laboratories and security

Irrespective of size, laboratories must maintain the conditions of security and restricted access required by clients, and any accreditation body, to minimize the risk, however small, that anyone could tamper with a sample. Control of access to, and use of, areas affecting the quality of the tests should relate to the laboratories' particular circumstances. All access points must be locked or manned to ensure that only authorized personnel are granted entry, and that visitors are registered on entry, escorted at all times and registered on leaving. There should be a clear barrier between public areas of the organisation and the laboratory and, ideally, a physical barrier such as a door with a digital lock.

2.3 Laboratory design

The typical laboratory is comprised of the testing facilities/areas, where specific testing and associated activities are carried out, and additional areas, such as administration blocks, storage rooms, archives, corridors, entrances, cloakrooms and toilets. There are specific environmental requirements for the testing facilities.

Laboratory design and layout reflects the different operations involved in the testing programme. All types of laboratory, however, have some common activities requiring additional or specialized rooms, depending on the nature of the testing operation. Common activities include:

- Sample reception
- Sample storage
- Washing up or decontamination
- Weighing activities
- Sample preparation
- Sample processing
- Test areas
- Office and data storage areas.

The following explains more about the activity areas and services/facilities required.

2.3.1 Sample reception

For any testing activity, samples are delivered to the laboratory. A key provision for accreditation is ensuring sample and/or testing data security and confidentiality. For that the laboratory usually

has a sample receipt area with restricted access which is a secure area for collecting and registering sample details, with limited access to authorized staff only. The responsible person in the sample receipt office receives the samples, completes the sample registration procedure, and notifies the relevant laboratory for storage/testing of the sample.

2.3.2 Sample storage area

Depending on the tests to be conducted, sample storage for fish, water, and other fishery products requires a varying degree of access to refrigerator and freezer space. Note, however, that freezer storage is generally for samples for chemical testing (pre- and post-analysis). Samples for microbiological testing cannot be retained in this way without damaging the integrity of the sample.

Refrigerators and freezers should ideally be in a room separate from the laboratory area but, if space is limited, they can be placed in the sample preparation room as long as there is sufficient space, and other activities are not impeded. Refrigerators and freezers can also be kept in corridors adjacent to the sample processing room, as long as they do not restrict access or compromise fire regulations. All cabinets (refrigerators or freezers) containing samples must be in an area where there can be no unauthorized access, and be fitted with a lock.

2.3.3 Washing-up room/decontamination area

To have a separate washing-up room for the cleaning/decontamination of glassware is desirable. However, in a small chemistry laboratory, this activity could be confined to a sink area in one of the operational areas. A chemical laboratory with a number of testing activities often has a common washing-up room. It is good practice to wash glassware in batches from the different sources, to minimize any possible cross-contamination. The use of an automatic washing machine is recommended as the most effective and consistent form of glassware cleaning.

For a microbiological laboratory, a separate "dirty room" must be maintained, and the above conditions apply. In addition, there must be an autoclave for the decontamination of used materials.

2.3.4 Balance room

Weighing activities can generally be divided by virtue of the type of balance and weighing range.

Analytical balances weighing a few milligrams to several grams should be maintained within a purpose-built balance room, partitioned off from other activities, and with vibration-resistant benching. A suggested balance room should be 3m × 2 m with a single vibration resistant bench along one side. A second bench would be useful for the placement of glassware or materials to be weighed. In a warm environment, the room should be air-conditioned but without a direct draught on to the balances.

A small laboratory could use a discrete area of the main laboratory for this purpose, but this is not advisable unless strict controls are put in place, because there can be a risk of laboratory contamination distorting the true analytical result. Where analytical balances are located in the main laboratory area, vibration resistant benching is essential.

2.3.5 Sample preparation room

The sample preparation room is where received samples are defrosted (if frozen), macerated and homogenized and sub-samples taken for analysis. Extraction can also take place in this area if space permits. Depending on the type of sample, this room can become wet and dirty, necessitating a design that is easy to clean. Floor drainage is ideal as it permits spillage to be washed away.

The room should be a minimum of 3 m × 3 m with a sink with taps for hot and cold running water. The benches should be sealed against the wall to permit effective cleaning, and the floor should be of good quality linoleum or similar, again sealed around the edges.

The room should be air-conditioned, providing for an optimum temperature range of 20°C – 22°C, and designed in such a way that the air flow from the unit is not directly on to the area where the samples are prepared, or on to any sensitive equipment (e.g. balances if present).

For microbiological testing, it is essential that the sampling is carried out under aseptic conditions, and sampling for microbiological tests should be kept separate from sampling for other purposes to maintain the integrity of the sample. Samples for microbiological test purposes generally require less manipulation than samples for chemical testing, where the received sample may be significantly larger, and can be prepared directly in the sample processing room.

2.3.6 Sample processing room(s)

The samples are prepared for analysis, whether chemical or microbiological, in these rooms. The space of the rooms(s) depends on the testing programme, but they will also contain much of the day-to-day apparatus (equipment and glassware) and chemicals, reagents and reference standards used in the determinations conducted by the laboratory. Such a room will also be used for sample testing, where more general procedures are used (in chemistry, for example, the physico-chemical tests for water turbidity, conductivity, nitrite content, etc.), which do not involve the use of more sophisticated equipment.

The minimum space requirements for such a room are 10m × 5 m, with benching installed to maximize the working space available, whilst retaining room to move and the requirements for free-standing equipment (e.g. refrigerators, freezers, etc.). A room of this size requires four sinks.

The provision of gas outlets for gases appropriate to the tests undertaken (e.g. compressed air, nitrogen, natural gas or a similar combustible gas), should be external to the laboratory and piped into the room. Where this is not possible, free-standing cylinders, appropriately strapped to a support to ensure stability, can be used.

Laboratories in larger institutes with up-to-date mechanical and engineering systems in place, may also provide piped vacuum to taps within the laboratory, although in most cases, portable electric vacuum pumps will be adequate.

The room should also be equipped with fume extraction facilities for use when working with hazardous reagents or volatile organic solvents.

2.3.7 Test rooms

Test rooms include all those where the determinative step of an analysis is conducted, from chemical testing to the examination of plates from the culture of microbiological test samples, or the testing for fish toxins. These are treated separately because of the differences in the procedures.

Chemistry instrument rooms and equipment

For chemical analysis, the cross contamination between samples and possible environmental contamination of samples is most relevant and to be avoided. Another concern is that chemical testing requires standards, often comprised of pure samples, or concentrated solutions of materials which are being tested for at trace levels.

Good practice in general chemical testing work should generally observe:

a. Segregated areas with their own glassware for the storage of standards and the preparation of concentrated solutions. Operating rules to ensure that only very diluted solutions of standards necessary for calibration of equipment are ever introduced into areas where samples are being handled and processed. Precautions to avoid spillage of standards, for example by carrying them inside double containers.
b. Where samples containing high levels and low levels of the same targets are being handled, for example pesticide formulations and samples for residues analysis, carry out the sample preparation work and, where possible, the instrumental analysis, in well separated rooms with their own glassware.
c. Where possible, provide separate washing up facilities for glassware with separate uses. If this is not possible, then ensure a management regime such that glassware is not interchanged, for example use clearly labelled baskets to deliver it to, and collect it from, the washroom.
d. Enforce good housekeeping and tidiness by general management pressure; have a designated time each week for cleaning and tidying the laboratory.
e. Have a system for reporting and recording all spillages. Where foreseeable, have a documented procedure for dealing with specific types of spillage.

Separate instrument rooms are normally used to house equipment such as the gas liquid chromatograph (GLC), high performance liquid chromatograph (HPLC) or atomic absorption spectrometer (AAS), which require special facilities or are best sited away from other operations.

Microbiology and equipment

The microbiology laboratory should be designed to prevent or reduce risks of cross-contamination. Separate rooms and/or separate areas and/or specific enclosures should be provided for the following activities of a microbiology laboratory:

- Sample preparation (a segregated location should be used for the preparation of powdery products likely to be highly contaminated)
- Manipulation of pathogens, e.g. Salmonella
- Media and equipment preparation, including sterilisation
- Cleaning of glassware and other equipment as well as decontamination of equipment and contaminated culture media
- Sterility assessment of foodstuffs.
- Separation of the following areas should also be considered:
    - Areas used for the preparation of culture media and the room used for its sterilization and of equipment
    - The decontamination areas and washing area.

The sample processing area should be separated from, but near to, the testing areas. If possible, circulation pathways of clean and dirty laboratory materials should never cross, and circulation pathways of contaminated waste should be isolated. All operations in the laboratory should be linked together smoothly without samples crossing, and the scope for contamination minimized. The suite should contain toilet and changing room facilities immediately upon entrance, and separated from the main laboratory and operational areas. Laboratory coats worn within the microbiological suite must not leave that suite to minimize the risk of contamination. For post-examination pathways, after the analysis of the samples, results must be accurately recorded, properly filed, and delivered on time to the right person. Communication systems appropriate to the size and complexity of the laboratory, including the efficient and reliable transferring of messages, should be part of the laboratory design.

Special requirements for fish toxin testing

Fish toxin testing uses either procedures based on HPLC, or a mouse bio-assay (MBA). The MBA necessitates a separate air-conditioned room where test mice are housed prior to use, and for an observation period after use. The size of the room depends upon the scale of operations, but in most cases 3m × 3 m will be perfectly adequate. The room should have a minimum of four power outlets and a sink, 40 cm × 30 cm or similar, with hot and cold running water.

2.3.8 Physical aspects of premises and rooms-

The testing premises should be fitted out in the following ways in order to reduce the risks of contamination:

- Smooth surfaces on walls, ceilings, floors and benches (the smoothness of a surface is judged on how easily it may be cleaned). Tiles are not recommended as bench covering material; Laboratory work benches should be constructed of materials that are durable and easy to disinfect.
- Concave joints between the floor, walls and ceiling
- Minimal opening of windows and doors while tests are being carried out
- Sun shades placed on the outside
- Easy access for cleaning of internal sun shades, if it is impossible to fit them outside
- Fluid conveying pipes not passing above work surfaces, unless placed in hermetically sealed casings
- A dust-filtered air inlet for the ventilation system
- Separate hand-washing arrangements, preferably non-manually controlled;
- Cupboards up to the ceiling
- No rough and bare wood
- Wooden surfaces of fixtures and fittings adequately sealed
- Stored items and equipment arranged to facilitate easy cleaning
- No furniture, documents or other items other than those strictly necessary for testing activities.

This list is not exhaustive (for more information see Annex 1), and not all examples will apply in every situation. Ceilings, ideally, should have a smooth surface with flush lighting. When this is not possible (as with suspended ceilings and hanging lights), the laboratory should have documented evidence that they control any resulting risks to hygiene, and have effective means of overcoming them, e.g. a surface-cleaning and inspection programme.

Decontamination procedures may be appropriate where environment or equipment is subject to change of use or where accidental contamination has occurred.

Samples should be separately secured, ideally in locked storage, and data should be tidied away into drawers or cupboards.

Samples, reagents, measurement standards, and reference materials must be stored so as to ensure their integrity. In particular, samples must be stored in such a way that cross contamination is not possible. The laboratory should guard against their deterioration, contamination and loss of identity.

The technical requirements for accommodation and environmental conditions that can affect the results of tests should be documented.

2.4 Laboratory services and maintenance and inspection

Laboratory maintenance covers the basic operations of cleaning, the key services of power, water and drainage, fume cupboard and air circulation/air-conditioning systems, damage to the working environment (e.g. benches, floors, etc.) and pest control where appropriate. Procedures must be put in place to deal with each of these operations, with a responsible officer designated to ensure full compliance.

2.4.1 Power, water and drainage

There is need for a stable power supply for sensitive equipment, and a backup power supply or emergency generator for times when the laboratory's primary power source is down. A fluctuation of electric voltage in the laboratory is one of the most important reasons, reducing the longevity of the equipment and sometimes damaging them. Therefore, all the voltage-sensitive equipment should be provided with voltage protection devices like stabilizers, servo stabilizers, or constant voltage transformers, as recommended by the manufacturers of the equipment.

2.4.2 Fume cupboards

Fixed fume cupboards and portable fume extraction units and chambers require regular maintenance to ensure that they function effectively. They should be periodically tested on the efficiency of air flow (the face velocity) for each unit.

Where filters are fitted to the systems, they should be checked at a frequency as defined by the manufacturer/supplier. Contaminated filters should be disposed of in the appropriate manner.

2.4.3 Air circulation and air-conditioning systems

Workrooms should be appropriately ventilated and at a suitable temperature. This may be done by natural or forced ventilation, or by the use of an air conditioner. Where air conditioners are used, filters should be appropriate, inspected, maintained and replaced according to the type of work being carried out.

For laboratory work, room temperature is taken to be about 20-25°C with an average of 23°C (about 73.4 degrees Fahrenheit (°F)). The typical laboratory can have both "hot spots" and "cold spots," depending on air vent supply-return locations and air flow patterns. This in turn affects both the assumed and measured room temperature. Temperature mapping of the laboratory work areas can pinpoint areas of temperature instability.

In the microbiological suites, samples/reference materials should be handled only in laminar flow cabinets under a filtered, clean air supply. Natural ventilation is not recommended in clean rooms or workrooms where pathogens are handled.

2.4.4 Work environment

The floors, walls, ceilings, laboratory bench tops and furniture should be subjected to regular maintenance and repair, to prevent cracks where dirt might accumulate and thus become a source of contamination.

2.4.5 Hygiene and cleaning

The routine cleaning of general laboratory areas, as distinct from the specific cleaning up of microbial or chemically contaminated areas or used glassware and apparatus, is important to

minimize the build-up of dirt, spilled materials and, on occasion, insect populations. Regular cleaning and disinfection should be carried out in order to keep the premises in a condition suitable for conducting tests. It is important that all areas of the laboratory are cleaned and maintained on a regular basis.

A periodic pest survey should be undertaken and control actions taken if required.

2.4.6 Waste disposal

Procedures for waste disposal are important to ensure that laboratories are kept free from unwanted and used materials, to minimize the risk of contamination, and to ensure that quantities of hazardous materials (chemical and biological) are kept to a minimum, and disposed of with due regard to public and environmental health.

Laboratory waste management is a critical issue. All potentially harmful and dangerous materials (including liquids and radioactive materials), must be treated in a specific way before disposal. Separate waste containers should be used depending on the nature of the waste, and must be clearly identified by a colour code. Specific attention should be given to the management of potentially harmful contaminated waste such as sharps, needles or broken glassware. Containers for sharps must be available on work benches so they are conveniently accessible to staff.

Each working day, all waste bins should be emptied in a manner following the defined procedures of the organization for the level of hazard associated with the waste. Items that have come in contact with microbial samples (e.g. pipettes and pipette tips) are usually discarded in jars containing disinfectant. Periodically, the containers must be emptied and the contents decontaminated in an autoclave.

All containers containing waste (e.g. contaminated materials, waste solvent, etc.) must be adequately labelled, and reactive substances kept separate. All waste must be kept in a locked, ventilated store whilst awaiting disposal. With some biological waste (e.g. fish tissue, dead mice from fish toxin testing), there may be a need for refrigerated or freezer storage whilst the materials await disposal.

When placing equipment in the laboratory, be sure to consider how liquid wastes will be handled. It is important to be aware of, and comply with, local and national requirements for liquid waste disposal. Under no circumstances should waste solvent be disposed of by burial or tipping on to soil where it may penetrate the ground and enter underground water, or contaminate the soil. No materials that could leach into the underground water supply should be disposed of in this way. Waste solvent should be incinerated or in worst case scenarios, volatilised by exposure to the sun on a windy day.

2.4.7 Environmental monitoring

The laboratory should monitor, control and record environmental conditions as required by the relevant specifications for methods and procedures, or where they influence the quality of the results. Due attention should be paid, for example, to biological sterility, dust, electromagnetic disturbances, radiation, humidity, electrical supply, temperature, and sound and vibration levels, as appropriate to the technical activities concerned. Tests and calibrations should be stopped when the environmental conditions jeopardize the results of the tests and/or calibrations.

2.5 Hygiene and safety

Measures should be taken to ensure good housekeeping in the laboratory. Special procedures should be prepared where necessary (see above).

As a general rule, diagnostic laboratories working with pathogens in food safety should be designed and organized for biosafety level 2.

There should be a documented cleaning programme for laboratory fixtures, equipment and surfaces. It should take into account the results of environmental monitoring, and the possibility of cross-contamination. There should be a procedure for dealing with spillages.

Protective clothing appropriate to the type of testing being performed (including protection for hair, beard, hands, shoes, etc., if necessary) should be worn in the microbiological laboratory and removed before leaving the area. This is particularly important in the molecular biology laboratory, where e.g. movement from an area of high deoxyribonucleic acid (DNA) load to an area of low DNA load may accidentally introduce cross-contamination. A change of the laboratory coat may suffice when moving between areas.

Adequate hand washing facilities should be available, and a policy regarding glove use should be in place. For reduction of contamination it is advised to provide separate hand-washing arrangements, preferably non-manually controlled.

In the field of personal hygiene, the following precautions should be taken to avoid contamination of the samples and culture media, and to avoid risk of infection for personnel:

- Wear laboratory clothing, clean and in good conditions, texture inflammable; do not wear this clothing outside the work areas and possibly cloakrooms
- Wear protection of hair and beard
- Wash hands thoroughly
- Avoid speaking etc. when inoculating
- Take precautions that any persons having infections do not invalidate results
- Do not put food for personal consumption in the laboratory refrigerators.

As a quality manager, it is necessary to develop a complete and thorough description of basic safety rules and organization, and ensure that personnel are trained in their specific duties when new activities or techniques are introduced into the laboratory.

Each member of the laboratory staff must be familiar with all potential hazards, and the materials safety data sheet supplied with each chemical should be available for immediate reference. Procedures should be put into place to deal with all potential hazards, and to minimize any risks associated with their use.

3 PERSONNEL

Personnel are the most important laboratory resource. The provision of effective laboratory services requires a combination of good management, effective staff supervision and well-trained staff. Recruiting and retaining qualified staff is essential to laboratory quality. ISO/IEC 17025, paragraph 5.2 refers specifically to this issue.

Management of personnel is critical to the success of a quality management programme. Several elements are important in this management process. Job descriptions should reflect all skills needed and accurately describe tasks, roles, and authorities. The competency of personnel will need to be evaluated at the time of hiring and on a regular basis. Continuing education is vital to personnel competency, but does not need to be expensive. New testing methodologies and instruments are constantly introduced to the marketplace, and employees need to update their knowledge and skills.

As Head of Laboratory it is important to hire an appropriate number of staff to cover the workload, and to train all employees in their specific duties, to provide orientation for new

employees, and to provide opportunities for continuing education. New techniques or updates for existing methods can be introduced using continuing education courses. Annual employee performance appraisals should be conducted.

3.1 Staff requirement

The laboratory management should define the minimum levels of qualification and experience necessary for the key posts within the laboratory. It is important for the head of laboratory to hire an appropriate number of staff to cover workload. These should include:

- Technical manager/laboratory manager
- Chemists
- Microbiologists
- Laboratory technicians
- Support staff (secretarial, cleaners, driver, building maintenance etc.).

Staff numbers will obviously reflect the volume of work that the laboratory has to undertake. In most cases, additional support will be at the technician and support grade level. In exceptional cases, an additional chemist or microbiologist may be required.

3.2 Staff qualifications

Personnel performing specific tasks should be qualified on the basis of appropriate education, training, experience and/or demonstrated skills, as required.

The laboratory management should ensure the competence of all who operate specific equipment, perform tests, evaluate results, and sign test reports. Personnel performing specific tasks should be qualified on the basis of appropriate education, training, and experience.

At the technical level, there should be a competent technical manager or laboratory manager, responsible for overseeing all analyses performed, to provide training as required, and to certify the competence of the staff conducting the tests.

Microbiological testing should be either performed or supervised by an experienced person, qualified to degree level in microbiology or equivalent. Alternative qualifications may meet requirements, where a member of staff has extensive relevant experience relating to the laboratory's scope of accreditation. Staff should have relevant practical work experience before being allowed to perform work within the scope of accreditation without supervision, or before being considered as experienced for supervision of accredited work. Specific national regulations may override this.

The technicians could be graduates, but this is not critical provided they have some basic chemistry/microbiology qualifications (A level, diploma or equivalent) and receive appropriate on-the-job training.
The personnel in charge of performing tests should have a good knowledge of the microorganism sought, and sufficient practical experience with microbiological techniques. They should be able to interpret the accuracy and precision required to yield acceptable results. For this they could take part in PTs, use reference materials or achieve self-assessment tests for enumeration of microorganism.

Chemical analysis must be carried out by, or under the supervision of a qualified, experienced and competent analyst. In the chemistry section, the technical/laboratory manager and chemists should

be at graduate level with experience of analytical chemistry. Other senior laboratory staff will normally possess similar competencies. Lower formal qualifications may be acceptable when staff have extensive relevant experience and/or the scope of activities is limited. Staff qualified to degree level will normally have at least two years' relevant work experience before being considered experienced analysts. Staff undergoing training should be adequately supervised. In certain circumstances, the minimum requirements for qualifications and experience for staff carrying out particular types of analysis may be specified in regulations.

If the laboratory provides opinions and interpretations of test results in reports, this should be done by authorised personnel with suitable experience and relevant knowledge of the specific application, as well as legislative and technological requirements and acceptability criteria. The management shall authorize specific personnel to perform particular types of sampling, tests, to issue test reports, to give opinions and interpretations and to operate particular types of equipment.

3.3 Staff training

The laboratory management should formulate the goals according to the education, training and skills of the laboratory personnel. The laboratory should have a policy and procedures for identifying training needs, and providing training of personnel. The training programme should be relevant to the present and anticipated tasks of the laboratory, and its effectiveness evaluated.

The laboratory management should ensure that all personnel have received adequate training for the competent performance of tests and the operation of equipment. Each member of staff must be trained in all aspects of their duties, whether it is in the use of specific items of equipment, or full analytical procedures.

For a microbiologist this should include training in basic techniques, e.g. plate pouring, counting of colonies, aseptic technique, etc., using objective criteria to determine acceptability. Personnel may only perform tests on samples if they are either recognised as competent to do so, or if they do so under adequate supervision. Ongoing competence should be monitored, with provision for retraining where necessary. Where a method or technique is not in regular use, verification of personnel performance before testing is undertaken may be necessary. The critical interval between performances of tests should be established and documented. The interpretation of test results for identification and verification of micro-organisms is strongly connected to the experience of the performing analyst and should be monitored for each analyst on a regular basis.

Where appropriate, this will include training in the principles and theory behind particular techniques. In some cases, it may be more appropriate to relate competence to a particular technique or instrument rather than to methods. For example, in contaminates testing, analytical chemists use a diverse range of methods to investigate the chemical nature of substances. The aim is to identify and understand the substance and how it behaves in different conditions. Analytical chemists analyse samples using a range of techniques such as AAS, high performance liquid chromatography, and spectroscopy and they can specialise in areas such as quality control.

Procedures should exist for periodic review of performance and re-training if necessary. Participation in inter-laboratory and Proficiency Testing (PT) schemes is an important tool for monitoring laboratory performance and its staff (see section 11). The competence of personnel to perform tests should be documented in relation to the results of internal and external quality control. The effectiveness of the training programme, as well as the identification of further training needs, should also be evaluated based on these results.

All personnel should receive relevant updated information as necessary in hygiene and laboratory safety matters.

The laboratory should maintain an up-to-date record of the training that each member of staff has received, showing that individual members of staff have been adequately trained, and that their competence to carry out particular tests has been assessed. In some cases, it may be pertinent to state any limitations in evidence about competence.

The records should typically include:

a) Academic qualifications
b) External and internal courses attended
c) Relevant on-the-job training (and retraining as necessary)
d) Possibly also: participation in Quality Control and/or PT schemes, with associated data
e) Technical papers published and presentations given at conferences.

4 EQUIPMENT AND MAINTENANCE

ISO/IEC 17025, paragraph 5.5; ISO 7218 (see Annex 1) and ILAC P10[5]

This section provides an overview of the arrangements required for monitoring the chemical and microbiological safety of fishery products

Proper management of the equipment in the laboratory is necessary to ensure accurate, reliable, and timely testing. A good equipment management programme helps to maintain a high level of laboratory performance, reduces variation in test results, and improves the technologist's confidence in the accuracy of testing results. It lowers repair costs, and reduces interruption of services due to breakdowns and failures;

When putting an equipment management programme in place, the following elements should be considered.

- Selection and purchasing: When obtaining new equipment, what criteria should be used to select equipment?
- Installation: For new equipment, what are the installation requirements and who will install the new instruments?
- Calibration and performance evaluation: What is needed to calibrate the equipment and validate that it is operating correctly? How will these important procedures be conducted for both old and new instruments?
- Maintenance: What maintenance schedule is recommended by the manufacturer? Will the laboratory need additional preventive maintenance procedures? Are current maintenance procedures being conducted properly?
- Troubleshooting: Is there a clear procedure for troubleshooting for each instrument?
- Service and repair: What is the cost? Can the laboratory obtain the necessary service and repair in its geographical area?
- Retiring and disposing of equipment: What must be done to dispose of old equipment when it needs to be replaced?

It is the responsibility of the head of laboratory or the Technical Manager to oversee all the equipment management systems in the laboratory, and to ensure that all persons who will be using the instruments have been appropriately trained to both properly operate the instrument and perform all necessary routine maintenance procedures.

[5] ILAC P10:01/2013: ILAC Policy on the Traceability of Measurement Results (find ILAC publications at http://ilac.org/publications-and-resources/publications-list/)

Equipment management responsibility may be specifically assigned to a technologist in the laboratory. In many laboratories, there is a person who has good skills with equipment maintenance and troubleshooting. Giving this person the role of oversight of all equipment is recommended.

Oversight of an equipment management programme includes:

a) Assigning responsibilities for all activities
b) Ensuring that all personnel are trained in operation and maintenance
c) Monitoring the equipment management activities, including - reviewing all equipment records routinely
d) Updating maintenance procedures as necessary
e) Ensuring that all procedures are followed.

Note: day-to-day maintenance should be the responsibility of the technical operator. Everyone who uses the equipment should be trained in calibration and daily maintenance.

4.1 Equipment requirements

All equipment used in laboratories should be of a specification sufficient for the intended purpose, and kept in a state of maintenance and calibration consistent with its use.

Equipment normally found in chemical and microbiological laboratories can be categorised as:

i. General service equipment not used for making measurements or with minimal influence on measurements (e.g. hotplates, stirrers, non-volumetric glassware and glassware used for rough volume measurements such as measuring cylinders) and laboratory heating or ventilation systems;
ii. Volumetric equipment (e.g. flasks, pipettes, pyknometers, burettes etc.) and measuring instruments (e.g. hydrometers, U-tube viscometers, thermometers, timers, spectrometers, chromatographs, electrochemical meters, balances etc.).
iii. Physical measurement standards (weights, reference thermometers);
iv. Computers and data processors.

A good overview, and guidance as to the general requirements for equipping basic chemistry and microbiological laboratories in the fisheries sector (including common items such as those for sample preparation) is provided by the Strengthening Fishery Products (SFP) guide[6]. The guide also provides specifications for individual items of equipment, and indicates quantities of chemicals and reagents as "start-up" quantities for a general purpose laboratory. For equipment specifications in microbiology laboratories see also ISO 7218[7].

4.2 Equipment maintenance and inspection

Routine or preventive maintenance is the procedure by which the laboratory tries to minimize the likelihood of instrument malfunction, which can range from inconsistencies in the results obtained to a complete breakdown. Such maintenance operates at two levels, maintenance that

[6] Guide to the development and maintenance of fishery product testing laboratories LTI040GEN, 2010; SFP programme, EU
[7] Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations

can be conducted by laboratory staff, and maintenance that necessitates the visit of an external engineer.

When purchasing new equipment, and particularly with sophisticated analytical equipment, it is important to ensure that the engineer doing the installation delivers a course in routine maintenance to laboratory personnel, covering issues that the laboratory itself can undertake. This normally only covers the replacement or cleaning of certain easy to access parts, but frequently attention to such parts can reduce the risk of instrument malfunction.

With additional training and experience, however, more complex tasks can be attempted including, for example, replacing detector units in a GLC or cleaning the source in a mass selective detector. The operators should be clear, however, that they should only try to resolve issues for which they have been trained, to avoid the risk of causing further damage or affecting the calibration of the instrument.

Operations that the laboratory personnel are required to undertake should be listed in a laboratory procedure, together with details of the frequency of such operations, and the way in which such operations should be conducted.

Maintenance of any item of equipment is essential to maximize its operational life, to ensure that it functions to an acceptable standard, and to minimize the risk of its malfunctioning and causing delays to the laboratory and to the testing of samples that have been submitted.

For microbiological laboratories, attention should be paid to the avoidance of cross-contamination arising from equipment. For example, disposable equipment should be clean and sterile when new, and re-used glassware should be properly cleaned and sterilised. Ideally, laboratories should have a separate autoclave for decontamination. If precautions are taken to separate decontamination and sterilisation loads, one autoclave is acceptable, provided that an adequate and documented cleaning programme is in place to address both the internal and external environment of the autoclave.
Typically, the following items of equipment will be maintained by cleaning and servicing, inspecting for damage, by general verification of suitability and, where relevant, sterilising:

1. **General service equipment** not used for making measurements or with minimal influence on measurements (e.g. hotplates, stirrers, non-volumetric glassware and glassware for rough volume measurements, e.g. measuring cylinders) and laboratory heating or ventilation systems. General service equipment will typically be maintained by cleaning and safety checks as necessary. Calibrations or performance checks will be necessary where the setting can significantly affect the test or analytical result (e.g. the temperature of a muffle furnace or constant temperature bath). Such checks need to be documented.

2. **Volumetric equipment** (e.g. flasks, pipettes, pyknometers, burettes etc.) and measuring instruments (e.g. hydrometers, U-tube viscometers, thermometers, timers, spectrometers, chromatographs, electrochemical meters, balances etc.). The correct use of this equipment is critical to analytical measurements and therefore it must be correctly used, maintained and calibrated in line with environmental considerations. The performance of some volumetric and related glassware is dependent on particular factors, which may be affected by cleaning methods. As well as requiring strict procedures for maintenance, such apparatus may therefore need more regular calibration, depending on use. For example, the performance of pyknometers, U-tube viscometers, pipettes, and burettes is dependent on "wetting" and surface tension characteristics. Cleaning procedures must be chosen so as not to compromise these properties.

3. **Physical measurement standards** (weights, reference thermometers). Wherever physical parameters are critical to the correct performance of a particular test, the laboratory shall have, or have access to, the relevant measurement standard as a means of calibration. In some cases, a test and its performance is actually defined in terms of a particular piece of equipment and checks are necessary to confirm that the equipment conforms to the relevant specification. For example, flashpoint values for a particular flammable sample are dependent on the dimensions and geometry of the apparatus used in the testing.

4. **Computers and data processors.** The chemical testing environment creates particular hazards for the operation of computers and storage of computer media. Particular care should be taken to avoid damage due to chemical, microbiological, or dust contamination, heat, damp, and magnetic fields. Initial validation should verify as many aspects of a computer's operation as possible. Similar checks should be carried out if the computer's use is changed, or after maintenance, or revision of software.

5. **Computer controlled automated systems** operated either simultaneously or in controlled time sequence, will normally be validated by checking for satisfactory operation (including performance under extreme circumstances) and establishing the reliability of the system, before it is allowed to run unattended. There should be validation of individual components, plus an overall check on the dialogue between individual components and the controlling computer. Electronic transfer of data should be checked to ensure that no corruption has occurred during transmission. This can be achieved on the computer by the use of 'verification files' but, wherever practical, the transmission should be backed-up by a hard copy of the data.

Maintenance of essential equipment should be carried out at specified intervals as determined by factors such as the frequency of use. Detailed records should be kept. Examples of maintenance of equipment and intervals for a microbiological laboratory are given in Table 1. The information is provided for guidance purposes only and the frequency will be based on the need, type and previous performance of the equipment. Guidance on Equipment Validation and Verification of Performance id given in Table 2.

TABLE 1: GUIDANCE ON MAINTENANCE EQUIPMENT

Type of equipment	Requirement	Suggested frequency
(a) Incubators (b) Fridges (c) Freezers, ovens	Clean and disinfect internal surfaces	a) Monthly b) When required (e.g. every 3 months) c) When required (e.g. annually)
Water baths	Empty, clean, disinfect and refill	Monthly, or every 6 months if biocide used
Centrifuges	a) Service b) Clean and disinfect	a) Annually b) Each use
Autoclaves	a) Make visual checks of gasket, clean/drain chamber b) Full service c) Safety check of pressure vessel	a) Regularly, as recommended by manufacturer c) Annually or as recommended by manufacturer d) Annually
Safety cabinets Laminar flow cabinets	Full service and mechanical check	Annually or as recommended by manufacturer
Microscopes	Full maintenance service	Annually
pH meters	Clean electrode	Each use
Balances, gravimetric diluters	a) Clean b) Service	a) Each use b) Annually
Stills	Clean and de-scale	As required (e.g. every 3 months)
De-ionisers, reverse osmosis units	Replace cartridge/membrane	As recommended by manufacturer
Anaerobic jars	Clean/disinfect	After each use

Source: Eurachem Guide ALM 2013

TABLE 2: GUIDANCE ON EQUIPMENT VALIDATION AND VERIFICATION OF PERFORMANCE

Type of equipment	Requirement	Suggested frequency
Temperature controlled equipment (incubators, baths, fridges, freezers)	a) Establish stability and uniformity of temperature b) Monitor temperature	a) Initially, periodically, at documented frequency, and after repair/ modification b) Daily. each use
Sterilising ovens	a) Establish stability and uniformity of temperature b) Monitor temperature	a) Initially, periodically, at documented frequency, and after repair/ modification b) Daily/each use
Autoclaves	a) Establish characteristics for loads/cycles b) Monitor temperature/time	a) Initially, periodically, at documented frequency, and after repair/ modification b) Daily/each use
Safety cabinets	a) Establish performance b) Microbiological monitoring c) Air flow monitoring	a) Initially, every year and after repair/ modification b) Weekly c) Daily/each use
Laminar air flow cabinets	a) Establish performance b) Check with sterility plates	a) Initially, and after repair/modification b) Weekly
Timers	Check against national time signal	Annually
Microscopes	Check alignment	Daily/each use
pH meters	Adjust using at least two buffers of suitable quality	Daily/each use
Balances	Check zero, and reading against check weight	Daily/each use
De-ionisers and reverse osmosis units	a) Check conductivity b) Check for microbial contamination	a) Weekly b) Monthly
Gravimetric diluters	a) Check weight of volume dispensed b) Check dilution ratio	a) Daily/each use b) Daily/each use
Media dispensers	Check volume dispensed	Each adjustment or replacement

Pipettors/pipettes	Check accuracy and precision of volume dispensed by gravimetric method	Regularly (to be defined by taking account of the frequency and nature of use)
Spiral platers	a) Establish performance against conventional method b) Check stylus condition and the start and end points c) Check volume dispensed	a) Initially and annually b) Daily/each use c) Monthly
Type of equipment	**Requirement**	**Suggested frequency**
Colony counters	Check against number counted manually	Annually
Centrifuges	Check speed against a calibrated and independent tachometer	Annually
Anaerobic jars/incubators	Check with anaerobic indicator	Daily/each use
Laboratory environment	Monitor for airborne and surface microbial contamination using, e.g. air samplers, settle plates, contact plates or swabs	Weekly for total count and moulds: Biannually for pathogens or as otherwise decided by the laboratory based on activities and historical trends and results

This information is provided for guidance purposes and the frequency will be based on the need, type and previous performance of the equipment.

Source: Eurachem Guide: Accreditation for Microbiological Laboratories, second edition (2013)

For verification and calibration see 8.3

4.3 Preventive maintenance requiring a service engineer

Preventive maintenance includes measures such as systematic and routine cleaning, adjustment and replacement of equipment parts at scheduled intervals. Manufacturers generally recommend a set of equipment maintenance tasks that should be performed at regular intervals: daily, weekly, monthly or yearly. Following these recommendations will ensure that the equipment performs at maximum efficiency and will increase the lifespan of the equipment. This will also help to prevent inaccurate test results due to equipment failure, delays in reporting results, low productivity and large repair costs.

Service engineers are trained to be able to replace most parts of an instrument and to check that critical components are functioning according to their specification. They will dismantle elements of the instrument and check a wide range of functions, including essential components for calibration (e.g. gas flows, spectral wavelengths, temperature functions, etc.), and replace

components that do not function to their specification. Such routine maintenance by an external trained engineer of instruments used in the testing and measurement of sample parameters is required for ISO 17025.

Preventive maintenance is generally undertaken at intervals of 6 months. A maintenance plan will include preventive maintenance procedures as well as provision for inventory, troubleshooting and repair of equipment.

It is recommended that a label be attached to the instrument indicating when the next maintenance or service should be performed. The laboratory should keep an inventory log of all equipment in the laboratory. The log should be updated with information on new equipment and include documentation of when old equipment is retired.

5 REAGENTS AND CULTURE MEDIA

ISO/IEC 17025, paragraphs 4.6, 5.5; ISO/TS 11133-1[8]; ISO/TS 11133-2[9]

5.1 Reagents

The quality of reagents and other consumable materials must be appropriate for their intended use. Consideration needs to be given to the selection, purchase, reception and storage of reagents.

Laboratories should ensure that the quality of reagents used is appropriate for the test concerned. They should verify the suitability of each batch of reagents critical for the test, initially and during its shelf-life, using positive and negative control organisms traceable to recognised national or international culture collections

The grade of any critical reagent used (including water) should be stated in the method, together with guidance on any particular precautions which should be observed in its preparation, storage and use. These precautions include toxicity, flammability, and stability to heat, air and light, reactivity to other chemicals, reactivity to particular containers, and other hazards. Reagents and reference materials prepared in the laboratory should be labelled to identify substance, strength, solvent (when not water), any special precautions or hazards, restrictions of use, and date of preparation and/or expiry. The person responsible for the preparation shall be identifiable either from the label or from records.

The correct disposal of reagents does not directly affect the quality of sample analysis, but is a matter of good laboratory practice, and should comply with national environmental or health and safety regulations.

Where the quality of a reagent is critical to a test, the quality of a new batch should be verified against the outgoing batch before use, provided that the outgoing batch is known to be still serviceable.

[8] Microbiology of food and animal feeding stuffs -- Guidelines on preparation and production of culture media -- Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory

[9] Microbiology of food and animal feeding stuffs -- Guidelines on preparation and production of culture media -- Part 2: Practical guidelines on performance testing of culture media.

5.2 In-house prepared media and reagents

Culture medium is defined as a formulation of substances, in liquid, semi-solid or solid form, which contain natural and/or synthetic constituents intended to support the multiplication (with or without inhibition of certain microorganisms), identification or preservation of viability of microorganisms.

The suitable performance of culture media, diluents and other suspension fluids prepared in-house should be checked, where relevant, with regard to:

- Recovery or survival maintenance of target organisms
- Inhibition or suppression of non-target organisms
- Biochemical (differential and diagnostic) properties
- Physical properties (e.g. pH, volume and sterility).

Raw materials (both commercial dehydrated formulations and individual constituents) should be stored under appropriate conditions, e.g. cool, dry and dark. All containers, especially those for dehydrated media, should be sealed tightly. Dehydrated media that are caked or cracked, or show a colour change, should not be used. Distilled deionised, or reverse osmosis produced water, free from bactericidal, inhibitory or interfering substances, should be used for preparation unless the test method specifies otherwise.

Shelf-life of prepared media under defined storage conditions should be determined and verified.

The accurate preparation of culture media is one of the fundamental steps in microbiological analysis. The water quality used is important. It should be distilled water or water of equivalency, i.e. free from substance likely to inhibit or influence the growth of microorganism under the test conditions. If chlorinated water is used to prepare distilled water, the chlorine needs to be neutralised prior to distillation. For more information, see ISO 7218 (see Annex 1). Distilled water must be stored in containers made from inert material. A good quality distilled water should exhibit a resistivity of at least 300 000 Ohm-cm.

The media can be prepared either from dehydrated basic ingredients, or from dehydrated complex media. Bottles containing dehydrated media or ingredient must be kept in a dry place, away from light and at a temperature as stated by the manufacturer. They should not be used beyond shelf-life. The bottles must be quickly and carefully closed after sampling. A dehydrated medium that shows signs of caking or solidifying when water is introduced should not be used.

Culture media dispensed in tubes or bottles, and reagents that are not used immediately, must be protected against light and desiccations. Thy should be refrigerated for a maximum period of 3 months, or between 18 and 23 °C for a maximum of 1 month, under conditions that prevent their composition being modified, unless otherwise specified in International Standards.

Media that has become dehydrated should never be used. Prior to use, it is desirable that the culture media be in equilibrium with the conditions of the laboratory.

Quantitative procedures for evaluation of recovery or survival should be performed according to EN ISO 11133 (see above). This is a mandatory standard for all accredited laboratories that perform microbiological food and water testing using culture media. It defines the preparation and quality control of all types of culture media, ranging from dehydrated to ready-to-use media, for classical or alternative microbiological testing methods. It covers requirements for the preparation, production, storage, and performance testing of culture media.

5.3 Ready-to-use-media

All media, including diluents and other suspension fluids, procured ready-to-use or partially complete, also require performance evaluation (as in section 5.2) before use. Evaluation of

performance for recovery or survival of target organisms, and the inhibition or suppression of non-target organisms, should be fully quantitative. Attributes (e.g. physical and biochemical properties) should be evaluated using objective criteria.

Where the manufacturer of ready-to-use or partially complete media is covered by a recognised quality system (i.e. ISO 9000 series), and the media are quality controlled according to ISO 11133[10], relevant information (certificates) needs to be reviewed for acceptability, but quality control does not need to be repeated. Suppliers must conduct rigorous qualitative and/or quantitative testing on all ISO 11133 compliant culture media that they provide to laboratories. Laboratories that source their culture media from a supplier that applies the standard can ensure that the media is manufactured and certified according to the latest international standard, EN ISO 11133:2014, by procuring the quality control certificate as a supporting document. Ultimately, this standard should reduce the workload for the qualification of new culture media batches procured from suppliers. In the supporting document, suppliers should provide quantitative information about the growth of both "wanted" microorganisms (bacteria that should grow on a specific medium) and "unwanted" microorganisms (bacteria that should not grow on a specific medium). The highest quality media will support only the growth of "wanted" microorganisms.

As part of this performance evaluation, the user laboratory needs to have adequate knowledge of the manufacturer's quality system and the product specifications, which include at least the following:

- Name of the media and list of components, including any supplements
- Shelf-life and the acceptability criteria applied
- It is necessary to comply with the manufactures instructions: expiry date, storage temperature and conditions, conditions for use (pH etc.) and efficiency control.

5.4 Labelling

Laboratories should ensure that all reagents (including stock solutions), media, diluents, and other suspending fluids are adequately labelled to indicate, as appropriate, identity, concentration, storage conditions, date of opening, preparation date, validated expiry date and/or recommended storage periods. The person responsible for preparation should be identifiable from records.

6 SAMPLING

17025 ISO/IEC, paragraphs 5.7 and 5.8; ISO 7218 (see Annex 1), ISO 6887[11] and ISO 19458[12]

6.1 Sample taking and transport

In many cases, testing laboratories are not responsible for primary sampling to obtain test items. Where they are responsible, it is strongly recommended that this sampling be covered by quality assurance and ideally by accreditation.

[10] Microbiology of food and animal feeding stuffs -- Guidelines on preparation and production of culture media -- Part 2: Practical guidelines on performance testing of culture media.
[11] Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions
[12] Water quality - Sampling for microbiological analysis (ISO 19458:2006); German version EN ISO 19458:2006

The way samples are taken will depend on the reason for the analysis. Sampling plans may be random, systematic or sequential, and they may be undertaken to obtain quantitative or qualitative information, or to determine conformance or non-conformance with a specification.

All interested parties should agree upon the sampling plan to be used. In the case of bulk products, locations for sub-sampling (and the sampling techniques) should be included in the sampling plan.

Before starting sampling, the minimum quantity required for analysis and any instructions on pooling sub-samples on site shall be agreed with the client. Other details should also be agreed with the customer, to ensure correct interpretation of the results of analysis. For example, it is important to decide:

i. What kind of product and which batches are to be sampled
ii. Sampling techniques for microbiological analysis
iii. The purpose of the analysis of the product (survey or analysis of a batch) is to know the
microbial quality of the product itself or to know the quality of the product as given to the consumer)
iv. Whether sterile or non- sterile tools will be used.

In many areas of food and water testing, the problems associated with sampling have been addressed and methods have been validated and published, e.g. sampling for heavy metals[13]. Laboratories are prepared to receive samples by the competent authority, based on their sampling strategy, and sampling procedures for objective sampling, selective sampling and suspect sampling. For EU sampling and analysis, methods used in the context of official controls should comply with relevant Community rules or if no such rules exist, with internationally recognized rules or protocols (e.g. CEN, the European Committee for Standardisation or other standards, e.g. ISO or Guidelines of Codex Alimentarius[14]).

In general sampling should only be performed by trained personnel. Whenever the laboratory is responsible for sampling, the personnel to be involved should also be authorised for sampling. Microbiological sampling should be carried out aseptically using sterile equipment. Environmental conditions, for instance air contamination and temperature, should be monitored and recorded at the sampling site. Time of sampling should be recorded.

It is important to cause minimum disruption at the sampling site and to follow security instructions. The properties of the analyte(s) of interest should be considered. Volatility, sensitivity to light, thermal lability, and chemical reactivity, may be important considerations in designing the sampling strategy and choosing equipment, packaging and storage conditions.

Equipment used for sampling, subsampling, sample handling, sample preparation and sample extraction, should be selected to avoid unintended changes to the nature of the sample which may influence the results. The significance of gravimetric or volumetric errors during sampling should be considered, and any critical equipment calibrated. It may be appropriate to add chemicals such as acids, or antioxidants to the sample to stabilise it. This is of importance in trace analysis, where there is a danger of adsorption of the analyte onto the storage vessel.

[13] European Commission Regulation 333/2007 laying down the sampling methods and the methods of analysis for the official control of the lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs

[14] Codex Alimentarius: General guidelines on sampling, CAC/GL 50-2004 ISO 7218: Microbiology of food and animal feeding stuffs; ISO 18593: Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs NMKL (Nordic Committee on Food Analysis) Procedure No. 12: Guide on Sampling for Analysis of Foods

Whatever strategy is used for the sampling; it is of vital importance that the sampler keeps a clear record of the procedures followed in order that the sampling process may be repeated exactly. It is important when documenting a sampling procedure to ensure that all the terms used are clearly defined, so that the procedure will be clear to other users (e.g. by standards).

Basically, the sampling procedure reduces the original consignment through lots or batches, increments, primary or gross samples, composite or aggregate samples, subsamples or secondary samples, to a laboratory sample. The laboratory sample, if heterogeneous, may be further prepared to produce the test sample. The laboratory sample, or the test sample, is deemed to be the end of the sampling procedure. Operations within this procedure are likely to be subject to sampling uncertainties. The measurement uncertainty associated with sub-sampling etc. should always be included in the test result measurement uncertainty, but the measurement uncertainty associated with the basic sampling process is commonly treated separately (see also 7.4).

The laboratory should have procedures for recording relevant data and operations relating to sampling that form part of the testing to be undertaken. These records should include the sampling procedure used, identification of the sampler, environmental conditions (if relevant), and diagrams or other equivalent means to identify the sampling location as necessary and, if appropriate, the statistics on which the sampling procedures are based.

Samples must be handled and labelled in such a way as to guarantee their legal and analytical validity. Sample handling procedures, including transport, should not affect the microbiological quality of samples in any way. In all cases it is important to retain the microbiological quality of the product, so the sampling technique should not modify the sample (e.g. chilled or frozen where appropriate). The conditions should be monitored and records kept. Where appropriate, responsibility for transport and storage, between sampling and arrival at the testing laboratory, should be clearly documented. Testing of the samples should be performed as soon as possible after sampling and should conform to relevant standards and/or national/international regulations.

In transportation of the sample to the laboratory, it should be ensured that they are kept under conditions that prevent any alteration in the number of microorganism present. Preference should be given to those means of transport that are the fastest.

Unique identification of samples and labelling requirements should be defined. Sufficient information should be recorded in the sampling report, to allow traceability of the samples, and allow interpretation of the results of analysis.

6.2 Sample registration

The laboratory should have procedures that cover the delivery of samples and sample identification. Upon receipt of the test item, abnormalities should be recorded. If there is insufficient sample, or the sample is in poor condition due to physical deterioration, incorrect temperature, damaged packaging or deficient labelling, the laboratory should consult with the customer before deciding whether to test or refuse the sample. In any case, records should be maintained, and the condition of the sample indicated on the test report.

If the sample is accepted, the procedure for sample registration include:

- Issue of a receipt to the person bringing the samples
- A record detailing the nature and numbers of samples received
- Note of condition on receipt (e.g. frozen, partially defrosted, signs of decomposition) when necessary, temperature
- Characteristics of the sampling operation (sampling date, sampling conditions, etc.)
- Details of the tests required
- Name and contact details for the sample originator

- Provision to give the sample a unique registration number/code to ensure that samples cannot be confused or mixed up. This is vital; samples entering the testing area should be anonymous with the identity of the supplier known only to the sample registration officer, and the officer designated to prepare the report containing the test results.
- Date and time of receipt.

6.3 Sample handling and identification/handling of test item

Microbial flora may be sensitive to factors such as temperature or duration of storage and transport, so it is important to check and record the condition of the sample on receipt by the laboratory.

Attention should be paid to the storage temperature and to the examination deadlines for the following products:

- Stable products; as early as possible and before the storage limit date
- Fresh and refrigerated products: within 24 h after receipt. If a longer storage period cannot be avoided, freeze the sample asap at a temperature below -18°C and mention this in the test report since in certain products freezing modifies the composition of the flora
- Pasteurized or similar products; as early as possible and before the storage limit date
- Spoiled stable units: as soon as possible and in less than 48 h.

Samples awaiting test should be stored under suitable conditions to minimise changes to any microbial population present. Storage conditions should be defined and recorded.

The packaging and labels from samples may be highly contaminated and should be handled and stored with care so as to avoid any spread of contamination.

The laboratory should have a system for identifying test items. The identification should be retained throughout the life of the item in the laboratory. The system should be designed and operated so as to ensure that items cannot be confused physically, or when referred to in records or other documents. The system should, if appropriate, accommodate a sub-division of groups of items, and the transfer of items within and from the laboratory.

Some samples, those involved in litigation for example, may have special labelling and documentation requirements. Labels may be required to identify all those who have been involved with the sample, including the person taking the sample and the analysts involved in the testing. This may be supported by receipts, to testify that one signatory (as identified on the label) has handed the sample to the next signatory, thus proving that sample continuity has been maintained. This is commonly known as "chain of custody".

The laboratory should have procedures and appropriate facilities for avoiding deterioration, loss, or damage to the test or calibration item during storage, handling and preparation. Handling instructions provided with the item should be followed. When items have to be stored or conditioned under specified environmental conditions, these conditions should be maintained, monitored and recorded. Where a test or calibration item or a portion of an item is to be held secure, the laboratory should have arrangements for storage and security that protect the condition and integrity of the secured items or portions concerned.

Samples must be stored at an appropriate temperature and in such a manner that there is no hazard to laboratory staff, and the integrity of the samples is preserved. Storage areas should be kept clean and extremes of environmental conditions (e.g. temperature, humidity) avoided. If necessary, environmental monitoring should be used. An appropriate level of security should be exercised to restrict unauthorised access to the samples.

All staff concerned with administration of the sample handling system should be properly trained. The laboratory should have a documented policy for the retention and disposal of samples. The disposal procedure should take into account the guidelines set out above.

Sub-sampling by the laboratory immediately prior to testing is considered part of the test method. It should be performed according to national or international standards, where they exist, or by validated in-house methods. Sub-sampling procedures should be designed to take account of uneven distribution of micro-organisms (general guidance given in ISO 6887 and ISO 7218, see above).

Where the laboratory has not been responsible for the sampling stage, it should state in the report that the samples were analysed as received. If the laboratory has conducted or directed the sampling stage, it should report on the procedures used and comment on any consequent limitations imposed on the results.

6.4 Sample preparation

Once received into the laboratory, the laboratory sample(s) may require further treatment such as subdivision prior to analysis.

The analytical operations begin with the measuring out of a test portion from the laboratory sample, or the test sample, and proceeds through various operations to the final measurement. For microbiological testing, in order to avoid contamination of the environment and of the test portioned, it is recommended to use special premises or a safety cabinet, otherwise in clean and disinfected areas.

Care should be taken to secure sample homogeneity. Where a material is clearly in two or more physical phases, it may be appropriate to separate the phases and treat them as separate samples. Similarly, it may be appropriate to combine and homogenise the phases to form a single sample.

Sample preparation describes the procedures followed to select the test portion from the sample (or subsample), and includes in-laboratory processing, mixing, reducing, coning and quartering, riffling, milling and grinding. Test portion refers to the actual material weighed or measured for the analysis.

Unless otherwise specified, the test portion taken for analysis must be representative of the laboratory sample. To ensure that the test portion is homogeneous, it may be necessary to reduce the particle size by grinding or milling. If the laboratory sample is large, it may be necessary to subdivide it prior to grinding or milling. Care should be taken to ensure that segregation does not occur during subdivision. In some cases, it will be necessary to crush or coarsely grind the sample prior to subdivision into test samples.

The sample label is an important aspect of documentation, and should unambiguously identify the sample to related plans or notes. Labelling is particularly important further into the analytical process, when the sample may have been divided, sub-sampled, or modified in some way. In such circumstances, additional information may be appropriate, such as references to the main sample, and to any processes used to extract or subsample the sample.

Depending on the instrument technique applied in chemical analysis, various methods in sample preparation involve extraction, concentration, clean-up, derivatisation and use of matrix modifier etc. For heavy metals analysis in fish, the use of a microwave digester is recommended to achieve a better extraction.

Many standard methods of analysis contain a section that details the preparation of the laboratory sample prior to the withdrawal of the test portion for analysis, and there are also standard methods or legal requirements related to sample preparation, e.g. by the EU or other

international organisation, e.g. the Food and Drug Administration's Bacteriological Analytical Manual (FDA BAM)[15].

7 TEST METHODS AND VALIDATION

ISO/IEC 17025 paragraphs 5.4.2, 5.4.3, 5.4.4; ISO 7218 (see Annex 1), ISO TR 13843[16], ISO 16140[17]; Eurachem Guide on method validation[18]

7.1 Selection of test methods

The laboratory should use appropriate test methods to meet the specific needs in each case. It is the laboratory's responsibility to use methods that are appropriate for the required application. The laboratory may use its own judgement, it may select a method in consultation with the customer, or the method may be specified in regulation or by the customer.

For a laboratory, it is always preferable to use standard methods, such as methods published as national, regional or national standards, or in scientific textbooks or by widely recognised and reputable organisations.

Test methods being used by laboratories are normally from standard bodies or other professionally established international bodies like the Association of Official Analytical Chemist (AOAC International). These methods have normally been validated. Quality standards often favour the use of standard or collaboratively tested methods, and they are desirable in situations where a method is to be widely used, or defined in regulation.

Laboratories can also use other methods, provided that the method is suitable for the purpose intended, adequately validated and documented, and that the results provided are traceable to stated references at an appropriate level of uncertainty. Such non-standard methods (not covered by standard methods) should be agreed with the customer. In practice, methods used by laboratories fall into one of three categories:

i. Standard methods published as standard specifications, for example ISO standards, national standards, or such that are published in the scientific literature
ii. Documented in-house methods which are the laboratory's own methods
iii. Documented in-house methods based on standard specifications.

The laboratory should validate non-standard methods, laboratory-designed/developed methods, standard methods used outside their intended scope, and amplifications and modifications of standard methods to confirm that the methods are fit for the intended use.

All methods developed in-house must be adequately validated, documented and authorised before use. Where they are available, matrix matched reference materials should be used to determine any bias or, where this is not possible, results should be compared with other technique(s), preferably based on different principles of measurement. Measurement of the recovery of gravimetrically added spike analyte, measurement of blanks and the study of interferences and matrix effects can also be used to check for bias or imperfect recovery. Estimation of uncertainty

[15] www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm
[16] Water quality - Guidance on validation of microbiological methods
[17] Microbiology of the food chain -- Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method
[18] B. Magnusson and U. Örnemark (eds.) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, (2nd ed. 2014). ISBN 978-91-87461-59-0. Available from www.eurachem.org

(see 7.4) must form part of this validation process and in addition to covering the above factors, should address issues such as sample homogeneity and sample stability. Advice on method validation is given in Section 7.2.

The detailed procedure to be followed in each case varies with the nature of the method, i.e. qualitative, semi-quantitative and quantitative. The method/technique used will dictate the aspects of validation to be considered. The validation should be as extensive as needed to meet the needs of the given application of field of application.

Result obtained should be recorded together with the procedure used for the validation, and a statement as to whether the method is fit for the intended use. In some cases, validation may include procedures for sampling, handling and transportation.

The documentation of methods should include validation data, limitations of applicability, and procedures for quality control, calibration and document control. A laboratory may find it convenient to adopt a common format for documenting methods (e.g. as used in ISO standards). An example for a test method Standard Operating Procedure (SOP) is provided in Annex 2.

7.2 Documentation of methods

It is recommended to write Standard Operating Procedures (SOPs) for all tests performed by the laboratory. SOPs for the different tests can best be written by the staff members who most often perform the test. These staff members know best all the small details that make the tests perform optimally, and can describe these in the SOPs. Several portals exist that provide generic SOP templates for many tests performed in fisheries laboratories. However, it is extremely important to keep in mind that these generic SOPs need to be checked and adapted to the situation in your laboratory. To achieve standardization and assure quality you have to *write what you do and do what you write*. If the generic SOPs are not checked and adapted to the situation in the laboratory, the procedures performed in that laboratory are still not standardized and quality is not assured.

In addition, advice on documentation of methods is available from other sources such as national standardisation bodies and accreditation bodies. Each copy of the method should show issue number/date, issuing authority, and copy number.

Irrespective of whether the method is in-house or standard, the staff must have documentation to enable it to be applied properly and consistently. In the case of standard methods, this may be covered by providing staff with access to the standard specification. It will, however, normally be necessary to supplement this with instructions on the use of particular models of instrument and also with information on local quality control regimes, and the quality control data to be collected.

In-house methods will need complete documentation. Annex 2 contains a suggested format. This can also be used as a checklist for determining whether published documentation is adequate. If it does not cover all of the points noted in Annex 2, then any omissions will need to be detailed for in in-house-generated documentation. Where the method is an in-house method based on a standard specification, there will need to be documentation specifying the variations from the standard, and cross-referring to the specification.

All the documentation of methods must be issued as controlled documents. This is typically done by compiling a methods manual consisting of in-house methods documentation, any supplementary documentation for standard methods and a list of standard methods used by the laboratory. The methods manual should also contain information on where the standard specifications can be found in the laboratory, and should refer to the appropriate instrument manuals and instructions.

It must be possible to determine from records which is the most up-to-date version of each method authorised for use. The laboratory should always use the current version of a standard/method, which may state that it conforms to the policy of using standard methods wherever possible. Obsolete methods should be withdrawn, but must be retained for archive

purposes, and clearly labelled as obsolete. The difference in performance between revised and obsolete methods should be established so that it is possible to compare new and old data.

Any report must, of course, specify exactly which method was used and note any deviations from the standard procedure.

7.3 Validation and performance criteria

Checks need to be carried out to ensure that the performance characteristics of a method are understood, and to demonstrate that the method is scientifically sound under the conditions in which it is to be applied. These checks are collectively known as validation.

Method validation is defined as the conformation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled. The aim is to establish the operational limits and performance characteristics to a new, modified or inadequately characterised test method.

Method validation is needed to provide evidence that the results are accurate and reliable, and to demonstrate that the test method is fit for purpose. Validation of a method establishes, by systematic laboratory studies, that the method is fit-for-purpose, i.e. its performance characteristics are capable of producing results in line with the needs of the analytical problem.

The important performance characteristics include:

- Selectivity & specificity: Description of the measure and ability to accurately measure the analyte in the presence of interferences
- Linearity: Ability of the analytical method to produce test results which are proportional to the concentration of analyte in samples within a given concentration range
- Accuracy: Closeness of the measured value to the true value for the sample
- Range: The concentration interval over which acceptable accuracy, linearity and precision are obtained
- Limit of detection: Lowest concentration of the analyte that can be confidentially detected by the method
- Limit of quantitation: The lowest concentration that can be determined with an acceptable level of repeatability, precision and trueness
- Precision: Repeatability, reproducibility: Measure of the degree or repeatability: the amount of scatter in the results obtained from multiple analysis of a homogenous sample
- Robustness: Ability of the test method to remain unaffected by small and deliberate changes, e.g. temperature
- Recovery: Assesses the efficiency of the method in detecting all the analytes present.

The above characteristics are interrelated, many of them contributing to the overall measurement uncertainty, and the data generated may be used to evaluate the measurement uncertainty (see 7.4) during validation. For more information, reference is provided to the Eurachem guide on method validation[19].

[19] B. Magnusson and U. Örnemark (eds.) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, (2nd ed. 2014). ISBN 978-91-87461-59-0. Available from www.eurachem.org

The key to determining how much validation is needed for a method is to be found in the 'fit for purpose' requirement. The laboratory must show that the method as applied by it is suitable for the purpose claimed or demanded by customers.

The validation of a standard or collaboratively tested methods should not be taken for granted. If the method is a standard published method, however, most of these factors will already have been investigated and specified as part of the method documentation. However, whatever the origin of the method, some validation will be required to establish that the performance of the method in that particular laboratory is satisfactory. Even if typical accuracy and precision data are published with the method, and the method is followed precisely as written down in the literature, a laboratory cannot automatically assume that it will reproduce these figures. There is no guarantee that the laboratory's skills, or the performance of its instruments, are of the same standard as those used to generate the standard validation data. The laboratory must always test its own capability directly. Even if the validation is complete, the user will still need to verify that the documented performance characteristics (e.g. trueness and precision) can be met in their own laboratory.

The laboratory should satisfy itself that the degree of validation of a particular method is adequate for the required purpose, and that the laboratory is itself able to verify any stated performance criteria.

However, when laboratories use test methods that are not from standard source, full validation will be required A few laboratories take this approach and the norm is rather to adopt, and perhaps slightly modify, standard methods.

In the case of method development from scratch in-house, a much more comprehensive approach, covering the other parameters described above, will be required. If the laboratory has devised the method itself, then adequate validation might well be a very complex and involved process, requiring a demonstration of the scope of applicability of the method in terms of samples and numerical range, selectivity, robustness in use, accuracy, precision, bias, linearity, detection limit and any other relevant characteristics.

Good practice in method validation is described in a Eurachem Guide (footnote 21). When stating validation data, it is advisable to state any conventions followed. The following standards can assist laboratories in obtaining method validation data: ISO/TR 13843 and ISO 17994[20]. As to terminology used in microbial validation, see ISO 16140-1:2016[21].

The extent of validation must be clearly stated in the documented method so that the user can assess the suitability of the method for their particular needs.

Validation of microbiological test methods should reflect actual test conditions. This may be achieved by using naturally contaminated products, or products spiked with a predetermined level of contaminating organisms. The analyst should be aware that the addition of contaminating organisms to a matrix only mimics in a superficial way the presence of the naturally occurring contaminants. However, it is often the best and only solution available.

Qualitative microbiological test methods, such as where the result is expressed in terms of detected/not detected, and confirmation and identification procedures, should be validated by determining, if appropriate, the specificity, sensitivity, relative trueness, positive deviation, negative deviation, limit of detection, matrix effect, repeatability and reproducibility. For quantitative microbiological test methods, the specificity, sensitivity, relative trueness, positive deviation, negative deviation, repeatability, reproducibility and the limit of quantification within a defined variability should be considered and, if necessary, quantitatively determined. The differences due

[20] Water quality -- Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods
[21] Microbiology of the food chain – Method validation – Part 1: Vocabulary

to the matrices must be taken into account when testing different types of samples. The results should be evaluated with appropriate statistical methods.

Laboratories should retain validation data on commercial test systems (such as test kits) used in the laboratory. This validation data may be obtained through collaborative testing and from validation data submitted by the manufacturers and subjected to third party evaluation (e.g. A FNOR, NordVal, Microval, and AOAC). If the validation data are not available, or not wholly applicable, the laboratory is responsible for completing the validation of the method.

ISO 16140-2:2016[22] provides new insights on the validation of proprietary microbiological methods for food testing laboratories. It provides reliable common protocol for the validation of alternative methods. With this new protocol, the data generated will also provide potential laboratories with performance data for a given method, thus enabling them to make an informed choice on the adoption of a particular (alternative) method.

Test methods should be validated when a new test method is being developed, or an established test method is modified, and when quality control indicates that an established test method is changing with time, by demonstrating the equivalence between two methods.

Validation of new equipment and associated techniques

Instrument validation is a series of processes through which the system is tested to verify or validate the performance specifications published by the manufacturer of the instrument. Prior to testing, it is important to evaluate the performance of new equipment, to ensure it is working correctly with respect to accuracy and precision.

If the equipment and associated techniques are new, validation processes will be important. Validation can be carried out by running samples in parallel using both old and new equipment and methods for a period of time, to determine that the expected results can be obtained. These validation procedures should be recorded in detail. In order to verify that equipment is working according to the manufacturer's specifications, it is necessary to monitor instrument parameters by performing periodic function checks. This should be done before using the instrument initially, then with the frequency recommended by the manufacturer. These function checks should also be done following any instrument repairs. Some examples of function checks are daily monitoring of temperatures and checking the accuracy of wavelength calibration.

7.4 Verification

In the preferable case of standard and validated methods being used, the laboratory is still required to prove that it can implement them in a reliable way. This is called verification (see further "Eurachem Terminology in Analytical Measurement"[23]).

Verification is necessary to provide objective evidence that the laboratory has the ability to achieve acceptable results for a given test method, and to prove that an externally validated test method is acceptable for its intended use.

The method verification is a simplified validation process employed to check or verify a test method's performance characteristics. It typically includes subsets of parameters evaluated when a complete validation is performed.

[22] Microbiology of the food chain – Method validation – Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method,
[23] VJ Barwick and E Prichard (Eds.), Eurachem Guide: Terminology in Analytical Measurement – Introduction to VIM 3 (2011).

Verification is done before a new externally validated test method is employed by the laboratory to report test results. For this the laboratory should:

- Develop a clear, detailed verification procedure that defines the parameters o be evaluated
- Define and approve the acceptance criteria (e.g. manufacturers' package insert) to be used in analysing the results
- Compare experimental results to the previously established performance characteristics
- Based on the results, accept or reject the test methods
- Summarise the data collected from the verification study in a final report.

The following is the most common subset of characteristic used in verification studies:

For verification of quantitative methods, the laboratory, in most cases, determines repeatability, measurement uncertainty and limit of quantitation, and for qualitative methods the limit of detection.

In addition, test methods using kits or laboratory instruments need to be evaluated for the ability to detect (sensitivity, specificity, positive and negative predictive value), and to determine normal and reportable ranges.

Verification of manufacturers' performance claims

Manufacturers provide performance evaluations for testing methods using their kits or instruments, and include the information in the package inserts or operator's manuals. However, laboratories need to verify the manufacturer's performance claims, and demonstrate they can get the same results using the kits or equipment in their laboratory, with their personnel. Some of the steps that should be followed to verify performance include testing samples with known values, and comparing the results with the expected or certified value and, if equipment is temperature controlled, establishing the stability and uniformity of the temperature.

7.5 Relationship between method validation and quality control

Method validation can be seen as a two stage process, establishing precision and accuracy. In the first instance, the laboratory needs to establish the extent to which it can reproduce measurements, and hence show that it can deliver consistent data within known limits. This is only the first phase, however, since a laboratory which can reproduce measurements well might still have a bias in its data. It could, so to speak, be consistently wrong. In order to address this issue, the laboratory will have to look outside and test itself against agreed reference points. The ongoing reliability and comparability of data can be guaranteed only through the implementation of quality assurance, including the application of method validation according to international accepted procedures and performance criteria.

7.6 Estimation of measurement uncertainty

ISO/IEC 17025 specifies the need for laboratories to estimate the measurement uncertainty, taking into account all components that may affect the result. ISO/IEC 17025 requires laboratories to evaluate their measurement uncertainty (MU) for the analyses they conduct, and to report it when relevant. There is also a requirement to report measurement uncertainty under specific circumstances, for example, where it is relevant to the interpretation of the test result (which is often the case). Thus, statement of measurement uncertainty in test reports should become common practice.

The MU estimation gives a measure of the confidence that can be put on the analytical results, not on the laboratory competency. MU provides laboratories and customers with valuable information about the accuracy and reliability of test data. It tells how the results represent the value of the quantity being measured. It gives confidence in comparability of results which helps to reduce barrier of trade. It also shows whether the result is within the acceptable limits or outside of it.

Measurement uncertainty characterises the range of values within which the true value is asserted to lie, with a specified level of confidence. Every measurement has an uncertainty associated with it, resulting from errors arising in the various stages of sampling and analysis, and from imperfect knowledge of factors affecting the result. For measurements to be of practical value, it is necessary to have some knowledge of their reliability or uncertainty. A statement of the uncertainty associated with a result conveys to the customer the 'quality' of the result.

A statement of uncertainty is a quantitative estimate of the limits within which the value of a measure (such as an analyte concentration) is expected to lie. Uncertainty may be expressed as a standard deviation, or a calculated multiple of the standard deviation. In obtaining or estimating the uncertainty relating to a particular method and analyte, it is essential to ensure that the estimate explicitly considers all the possible sources of uncertainty, and evaluates significant components. Repeatability or reproducibility, for example, are usually not full estimates of the uncertainty, since neither takes full account of any uncertainties associated with systematic effects inherent in a method.

A wide variety of factors make any analytical measurement result liable to deviate from the true value. For example, temperature effects on volumetric equipment, reflection and stray light in spectroscopic instruments, variations in electrical supply voltages, individual analysts' interpretation of specified methods and incomplete extraction recoveries, all potentially influence the result. As far as reasonably possible, such errors must be minimised by external control, or explicitly corrected for, for example, by applying a suitable correction factor. The exact deviation of a single measurement resulting from the (unknown) true value is, however, impossible to obtain. This is because different factors vary from experiment to experiment, and because the effect of each factor on the result is never known exactly. The likely range of deviation must therefore be estimated.

The primary task in assigning a value to the uncertainty of a measurement, is the identification of the relevant sources of uncertainty, and the assignment of a value to each significant contribution. The separate contributions must then be combined in order to give an overall value.

A record should be kept of the individual sources of uncertainty identified, the value of each contribution, and the source of the value (for example, repeat measurements, literature reference, certified reference material (CRM) data etc.). In identifying relevant sources of uncertainty, consideration must be given to the complete sequence of events necessary to achieve the purpose of the analysis. Typically, uncertainty contributions for analytical results might fall into four main groups:

i. Contributions from short-term random variability, typically estimated from repeatability experiments.
ii. Contributions such as operator effects, calibration uncertainty, scale graduation errors, equipment and laboratory effects, estimates from inter-laboratory reproducibility trials, in-house inter-comparisons, proficiency test results or by professional judgement.
iii. Contributions outside the scope of inter-laboratory trials, such as reference material uncertainty.
iv. Other sources of uncertainty, such as sampling variability (inhomogeneity), matrix effects, and uncertainty about underlying assumptions (such as assumptions about completeness of derivatisation).

The uncertainty contributions for each source must all be expressed in the same way, ideally as standard deviations or relative standard deviations. MU is usually written as an expanded uncertainty and provides an interval within which the value of the measure is believed to lie with higher level of confidence. It is obtained by multiplying the combined standard uncertainty by a coverage factor k where k is based on the level of confidence desired. For a level of confidence of 95%, k is 2.

Microbiological tests generally preclude the rigorous, metrologically and statistically valid calculation of measurement uncertainty as described in the ISO Guide to the expression of uncertainty in measurement[24]. It is generally appropriate to base the estimate of measurement uncertainty on repeatability and intermediate precision (within laboratory reproducibility) data. The individual uncertainty components should be identified and demonstrated to be under control and their contribution to the variability of results evaluated. Some components (e.g. pipetting, weighing, dilution effects and incubator effects) may be readily measured and easily evaluated, to demonstrate a negligible contribution to the overall measurement uncertainty. Other components (e.g. sample stability and sample preparation) cannot be measured directly and their contribution cannot be evaluated in a statistical manner, but their importance to the variability of results should also be considered.

8 MEASUREMENT TRACEABILITY, CALIBRATION AND PERFORMANCE VERIFICATION

ISO/IEC paragraph 5.4.6; ISO 7218 (see Annex 1), and ILAC P10[25]

8.1 Meaning of traceability

Traceability is the property of the result of measurement that can be related to appropriate measurements standards, generally international standards, through an unbroken chain of comparison (traceability chain), in which all uncertainties are indicated.

The standards referred to can be a material measure, measuring instrument, reference material, or measuring system that defines, realises, conserves or reproduces a unit, of one or more values of a quantity, to serve as a reference. It should be noted that the instrument itself is not traceable, but the result produced by the instrument is. Traceability applies to both physical and chemical measurements.

Traceability by definition is the "Property of the result of a measurement, or the value of a standard whereby it can be related to stated references, usually national or international standards. For further reading a guide on the traceability of chemical measurements is available[26].

Traceability concerns the requirement to relate the results of measurements to the values of standards or references, the preferred reference points being the internationally recognised system of units, the SI. This is achieved using primary standards (or other high level standards), that are used to establish secondary standards that can be used to calibrate working level standards and related measuring systems. Traceability is established at a stated level of measurement uncertainty, where every step in the traceability chain adds further uncertainty. Traceability is

[24] JCGM 100:2008 GUM 1995 with minor corrections Evaluation of measurement data — Guide to the expression of uncertainty in measurement
[25] ILAC Policy on the Traceability of Measurement Results, ILAC P10:01/2013
[26] EURACHEM/CITAC Guide 2003: Traceability in Chemical Measurement A guide to achieving comparable results in chemical measurement Drafting Editors S L R Ellison (LGC, UK) B King (UK) M Rösslein (EMPA, Switzerland) M Salit (NIST, USA) A Williams (UK)

important because it provides the linkage that ensures that measurements made in different laboratories, or at different times, are comparable. It is a matter of choice, whether to claim traceability to local references, or to international references.

8.1.1 Traceability to international standard

The ISO 17025 requires that a laboratory has a calibration system in place which ensures that, within known limits of uncertainty of measurement, any tests which it makes are comparable with those of any other laboratory.

Calibration is a set of operations that establishes, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure, and the corresponding values realized by standards[27]. The usual way to perform calibration is to subject known amounts of the quantity (e.g. using a measurement standard or reference material) to the measurement process, and monitor the measurement response.

The key element in achieving this is to ensure that all equipment in all laboratories having an impact on the validity of tests, is calibrated so that there is an unbroken chain of comparisons leading from the equipment to a recognised international standard of measurement.

Wherever possible, this international standard is required to be the corresponding SI (International System of Units) unit of measurement. The ideal way in which the system works in practice is that a country establishes a national metrology system, where a central metrology laboratory holds the national standards for all measurements. This central laboratory establishes a link into the international measurement system by, from time to time, checking its standards against those of other countries, and participating in inter-laboratory measurement audit exercises. In the latter case, the laboratories circulate references, for example a mass or a thermometer, and all compare them with their own standards, so establishing a basis of agreement, or otherwise, between the national calibration laboratories. Laboratories and industry requiring calibrations can then go to their own national metrology laboratory to have their equipment calibrated, in the knowledge that the calibration is internationally traceable.

Such national metrology systems do not exist in all countries, and in these cases it will be necessary for laboratories seeking ISO 17025 compliance to establish traceability by having calibrations performed by agencies outside the country that are able to provide the necessary traceability. These could, for example, be performed in a national metrology laboratory in a nearby country. Some equipment can be sent to the calibration laboratory for calibration and then shipped back to the laboratory, but many systems are either too bulky for this approach or need calibration on site, for example balances, as calibration is invalidated by their being moved. This inevitably means bringing calibration personnel and references to the laboratory site. This extra-national approach to traceable calibration is both inconvenient and expensive, so it is in the interests of countries seeking to establish a network of ISO 17025 compliant testing and calibration laboratories, to seriously consider establishing a national metrology system.

Key issues on which the accreditation body will need to be satisfied with in any calibration are:

- That the references used are properly calibrated and provide international traceability
- That the calibration procedures being used are scientifically sound, of known performance characteristics, for example uncertainty of measurement, and subject to proper quality control
- That staff carrying out the procedures are properly trained and competent in the calibrations performed.

[27] International Vocabulary of Metrology – Basic and General Concepts and Associated Terms (VIM 3rd edition) JCGM 200:2012 (JCGM 200:2008 with minor corrections)
hwww.bipm.org/en/publications/guides/vim.html

It is strongly recommended that any laboratory intending to use a calibration service, even a national metrology laboratory, should enter a dialogue with the body chosen as a potential accreditor for the laboratory along the following lines:

- Determine whether the calibration service has ISO 17025 accreditation, and who its accreditation body is. Ask the proposed accreditation body for your laboratory whether they have mutual recognition for calibration with the accreditation body of the calibration service.
- If the calibration service is not accredited, ask the proposed accreditation body for your laboratory whether they have any policy on the acceptance of calibrations from the proposed calibration service.

Essentially, the rule is to establish, at as early a stage as possible, that the proposed accreditation body will be likely to accept the calibrations the laboratory is proposing to rely upon. There is no point in spending time, money and effort on setting up calibrations which are simply rejected at assessment.

8.2 Calibration

The overall programme for calibration in the laboratory should be designed to ensure that all measurements with a significant effect on test results are traceable to a measurement standard, preferably a national or international measurement standard such as a reference material. Where appropriate and where feasible, certified reference materials should be used (see section 9.1.). Where formally designated measurement standards are not available, a material with suitable properties and stability should be selected or prepared by the laboratory and used as a laboratory measurement standard. The required properties of this material should be characterised by repeat testing, preferably by more than one laboratory and using a variety of validated methods (see ISO Guide 35[28]).

Analytical tests may be sub-divided into general classes depending on the type of calibration required:

a) Some analytical tests depend critically on the measurement of physical properties, such as weight measurement in gravimetry and volume measurement in titrimetry. Since these measurements have a significant effect on the results of the test, a suitable calibration programme for these quantities is essential. In addition, the calibration of measuring devices used to establish the purity or amount concentration of the chemical standards, need to be considered.
b) New or newly acquired equipment must be checked by the laboratory before use to ensure conformity with specified design, performance and dimension requirements.
c) Instruments such as chromatographs and spectrometers, which require calibration as part of their normal operation, should be calibrated using reference materials of known composition (probably solutions of pure chemicals).
d) In some cases, calibration of the whole analytical process can be carried out by comparing the measurement output from a sample, with the output produced by a suitable reference material that has been subjected to the same full analytical process as the sample. The reference material may be either a synthetic mixture prepared in the laboratory from materials of known (and preferably certified) purity, or a purchased certified matrix reference material. However, in such cases, a close match

[28] ISO Guide 35, *Certification of reference materials — General and statistical principles*

between the test sample and the matrix reference material, in terms of the nature of the matrix, and the concentration of the analyte, has to be assured.

e) However, in many cases, calibration is only performed on the final measurement stage. For example, calibration of a gas chromatography method may be carried out using a series of measurement standards which are synthetic solutions of the analyte of interest at various concentrations.

Individual calibration programmes should be established depending on the specific requirements of the analysis. Also, it may be necessary to check instrument calibration after any shutdown, whether deliberate or otherwise, and following service or other substantial maintenance.

Chemical measurements are made by calculating the value from a measurement equation that involves the measured values of other quantities, such as mass, volume, concentration of chemical standards etc. For the measurement of interest to be traceable, all the measurements associated with the values in the measurement equation used to calculate the result must also be traceable. Other quantities not present in the measurement equation, such as pH, temperature etc. may also significantly affect the result. Where this is the case, the measurements used to control these quantities also need to be traceable to appropriate measurement standards.

Establishing the traceability of physical quantities such as mass, volume, etc., is readily achieved using transfer standards, at the level of uncertainty needed for chemical measurements. The problem areas for laboratories are usually method validation and calibration. Validation establishes that the method measures what it is intended to measure, e.g. methyl mercury in fish. Validation establishes that the measurement equation used to calculate the results is valid.

The strategies available to address method bias include:

- Use of primary or reference methods of known and small bias
- Comparisons with closely matched matrix Certified Reference Materials (CRMs)
- Measurement of gravimetrically spiked samples and blanks
- Study of losses, contamination, interferences and matrix effects.

8.3 Calibration programme

All equipment used for tests, including equipment for subsidiary measurements (e.g. for environmental conditions), having a significant effect on the accuracy or validity of the result of the test or sampling, should be calibrated before being put into service. The laboratory should have an established programme and procedure for the calibration of its equipment.[29]

Per definition, calibration is the process of comparing a measuring instrument against a measurement standard, to establish the relationship between the values indicated by the instrument and those of the standard (standard in measurement is considered to be reference base). The purpose of calibration is to eliminate or reduce bias in the laboratories measurement system relative to the reference base. The calibration procedure compares unknown or test items(s) or instruments with reference standards according to a specific algorithm.

The laboratory must establish a programme for the calibration and performance verification of equipment that has a direct influence on the test results. The level and frequency of such calibration and performance verification will be determined by documented experience based on

[29] Note: Such a programme should include a system for selecting, using, calibrating, checking, controlling and maintaining measurements standards, reference materials used as measurement standards and measuring and test equipment used to perform tests.

need, type, and previous performance of the equipment, and should be at least that recommended by the manufacturer The frequency of calibration required will also depend on the stability of the measurement system, the level of uncertainty required, and the criticality of the work.

Procedures for performing calibrations should be adequately documented, either as part of specific analytical methods or as a general calibration document.

The laboratory should also have a programme and procedure for the calibration of its reference standards. Reference standards should be calibrated by a body that can provide traceability (as described in section 8.1.1.). Such reference standards of measurement held by the laboratory should be used only for calibration. Reference standards should be calibrated before and after any adjustment.

Examples of calibration intervals and typical performance checks for various laboratory instruments are given below, and in Tables 1 and Table 2 (see section 4.2). These tables include typical calibration intervals for types of simple instruments, and indicates the parameters which may require calibration in more complex analytical instruments.

8.3.1 Temperature measurement devices

Where temperature has a direct effect on the result of an analysis, or is critical for the correct performance of equipment, temperature measuring devices (e.g. thermocouples and platinum resistance thermometers (PRTs) used in incubators and autoclaves) should be of an appropriate quality to achieve the accuracy required. It is preferable that, for health and safety reasons, mercury and toluene liquid-in-glass thermometers are not used in the laboratory. Calibration of these devices should be traceable to national or international standards for temperature.

The stability of temperature, uniformity of temperature distribution and time required to achieve equilibrium conditions in incubators, water baths, ovens and temperature-controlled rooms should be established initially, and then periodically, at a documented frequency, in particular with respect to typical usage (for example position, space between, and height of, stacks of Petri dishes). Laboratories should monitor daily, or according to usage, the operating temperature of this type of equipment and retain records.

8.3.2 Autoclaves, including media preparators

The following paragraphs outline the generally accepted approach to calibration, and the establishment and monitoring of performance. However, it is recognised that quantitative testing of materials and items processed by autoclaving may also provide equivalent assurance of quality.

- Autoclaves should be capable of meeting specified time and temperature tolerances. Pressure cookers fitted only with a pressure gauge are not acceptable. Sensors used for controlling or monitoring operating cycles require calibration, and the performance of timers should be verified.
- Initial validation should include performance studies (spatial temperature distribution surveys) for each operating cycle, and each load configuration used in practice. This process must be repeated after significant repair or modification (e.g. replacement of thermo-regulator probe or programmer, modification of loading arrangements or operating cycle), or where indicated by the results of quality control checks on media. Sufficient temperature sensors should be positioned within the load (e.g. in containers filled with liquid/medium) to enable location differences to be demonstrated. In the case of media preparations, where uniform heating cannot be demonstrated by other means, the use of two sensors, one adjacent to the control probe and one remote, would

generally be considered appropriate. Validation and re-validation should consider the suitability of come-up and come-down times as well as time at sterilisation temperature.

- Clear operating instructions should be provided based on the heating profiles determined for typical uses during validation/re-validation. Acceptance/rejection criteria should be established and records of autoclave operations, including temperature and time, maintained for every cycle.
- Monitoring may be achieved by one of the following:

    i Using a thermocouple and recorder to produce a chart or printout
    ii Direct observation and recording of maximum temperature achieved and time at that temperature.

In addition to directly monitoring the temperature of an autoclave, the effectiveness of its operation during each cycle may be checked by the use of chemical or biological indicators for sterilisation/decontamination purposes.

Autoclave tape or indicator strips should be used only to show that a load has been processed, not to demonstrate completion of an acceptable cycle.

8.3.3 Weights and balances

Weights and balances should be calibrated traceably at regular intervals (according to their intended use).

8.3.4 Volumetric equipment

Volumetric equipment such as automatic dispensers, dispenser/diluters, mechanical hand pipettes and disposable pipettes should be initially verified by the laboratory, and then regular checks should be carried out to ensure that the equipment is performing within the required specification. Verification should not be necessary for glassware which has been certified to a specific tolerance. Equipment should be checked for the accuracy of the delivered volume against the set volume (for several different settings in the case of variable volume instruments), and the precision of the repeat deliveries should be measured.

For ‘single-use’ disposable volumetric equipment, laboratories should obtain supplies from companies with a recognised and relevant quality system.

8.3.5 Other equipment

Conductivity meters, oxygen meters, pH meters and other similar instruments should be verified regularly or before each use. The buffers used for verification purposes should be stored in appropriate conditions and be marked with an expiry date. Where humidity is important to the outcome of the test, hygrometers should be calibrated, the calibration being traceable to national or international standards. Timers, including the autoclave timer, should be verified using a calibrated timer or national time signal. Where centrifuges are used in test procedures, an assessment should be made of the criticality of the centrifugal force. Where it is critical, the centrifuge will require calibration.

Weights and balances should be calibrated traceably at regular intervals (according to their intended use).

TABLE 3: GUIDANCE ON CALIBRATION AND CALIBRATION CHECKS

Type of equipment	Requirement	Suggested frequency
Reference thermometers (liquid-in-glass)	Full traceable re-calibration Single point (e.g. ice-point check)	Every 5 years Annually
Reference thermocouples	Full traceable re-calibration Check against reference thermometer	Every 3 years Annually
Working thermometers & Working thermocouples	Check against reference thermometer at ice point and/or working temperature range	Annually
Balances	Full traceable calibration	Annually in the first 3 years, followed by less frequently, based on satisfactory performance
Calibration weights	Full traceable calibration	Every 5 years
Check weight(s)	Check against calibrated weight or check on balance immediately following traceable calibration	Every two years
Volumetric glassware	Gravimetric calibration to required tolerance	Annually
Pipettors/pipettes	Full traceable calibration	Annually
Microscopes	Traceable calibration of stage micrometre (where appropriate)	Initially
Hygrometers	Traceable calibration	Annually
Centrifuges	Traceable calibration or check against an independent tachometer, as appropriate	Annually

This information is provided for guidance purposes and the frequency will be based on the need, type and previous performance of the equipment.

Source: Eurachem Guide, Accreditation for Microbiological Laboratories, 2013

9 REFERENCE MATERIALS

ISI/IEC 5.6.3, ISO/TS 11333, ISO Guide 3 refers to the use of reference materials.

9.1 Reference materials

Reference materials and certified reference materials (see definition in Glossary of Terms) provide essential traceability in measurements.

Standards for the accreditation of testing laboratories demand the using of reference materials. The use of reference materials (RM) and CRM is an important procedure to avoid mistakes in the laboratory routine. They are 'controls' or standards used to check the quality and traceability of products and provide a kind of benchmark for a measurement.

They are used for example to:

- Demonstrate the accuracy of results; For measurement verification
- Calibrate equipment
- Monitor laboratory performance
- Validate methods
- Enable comparison of methods
- Demonstrate quality of culture media
- Demonstrate consistent performance of kits
- Evaluate measurement uncertainty and for training purposes.

The use of appropriate reference materials can provide essential traceability, and enable analysts to demonstrate the accuracy of results, calibrate equipment and methods, monitor laboratory performance and validate methods, and enable comparison of methods by use as transfer (measurement) standards. Their use is strongly encouraged wherever appropriate.

A series of ISO Guides relating to reference materials are available (see Annex 1).

Reference materials may take a variety of forms, including pure substance RMs, matrix RMs and solutions or mixtures. The International Laboratory Accreditation Cooperation (ILAC) describes the following five types of reference material:[30]

1. Pure substances; essentially pure chemicals, characterised by chemical purity and/or trace impurities.
2. Standard solutions and gas mixtures, often prepared gravimetrically from pure substances.
3. Matrix reference materials, characterised by the composition of specified major, minor or trace chemical constituents. Such materials may be prepared from matrices containing the components of interest, or by preparing synthetic mixtures.
4. Physico-chemical reference materials, characterised by properties such as melting point, viscosity, or optical density.
5. Reference objects or artifacts, characterised by functional properties such as taste, odour, octane number, flash point and hardness. This type also includes microscopy

[30] "*ILAC G9:2005 - Guidelines for the Selection and Use of Reference Materials*" (PDF). 2005. Retrieved 30 May 2013.

specimens characterised for properties ranging from fibre type to microbiological specimens.

The following are all examples of reference materials:

- 95% pure sodium chloride
- An aqueous solution containing 1% (w/v) copper (II) sulphate and 2% (w/v) magnesium chloride
- A powdered polymer with a particular weight distribution range
- A crystalline solid melting in the range 150-151°C
- A dried milk powder containing a known amount of vitamin C
- Polychlorinated biphenyl (PCB) congers in fish tissue, CRM (Sigma-Aldrich; matrix material; HPLC, GC suitable).

If possible, reference materials should be used in appropriate matrices.

Many basic test methods, especially in analytical chemistry, are intrinsically traceable. There is no need to have a certified reference for most titrations, for example. Here, traceability is provided via the calibrations of the balance and volumetric apparatus. The purist may argue that certification of the purity of the reagents that are weighed or measured is necessary but, provided the origin of the compounds is known and they are of known specification, it would be a harsh interpretation of the standard to insist upon this. This is not to be taken to mean that methods which use basic measurements, such as titrations, need never be tested against certified references. In many methods, there are preparation steps prior to the end measurement which need to be challenged. For example, a nitrogen determination in food by a Kjeldahl procedure involves a digestion and distillation prior to titration. Such a method would need to be validated using a suitable certified reference soil where available.

Reference materials are particularly important for analytical chemistry. Since most analytical instrumentation is comparative, it requires a sample of known composition (reference material) for accurate calibration. These reference materials are produced under stringent manufacturing procedures and differ from laboratory reagents in their certification, and the traceability of the data provided. For many types of analysis, calibration may be carried out using reference materials prepared within the laboratory from chemicals of known purity and composition. Some chemicals may be purchased with a manufacturer's certificate stating purity. Alternatively, chemicals of a stated but uncertified purity may be purchased from reputable suppliers.

Whatever the source, it is the users' responsibility to establish that the quality of such materials is satisfactory. Sometimes additional tests will need to be carried out by the laboratory. Normally a new batch of a chemical should be checked against the previous batch. Ideally, all chemicals to be used for RM purposes should be purchased from producers with demonstrated quality assurance (QA) systems. Due regard should be paid to the manufacturers recommendations on storage and shelf life. In addition, caution is needed, as suppliers do not always provide information about all impurities.

The composition of the certified reference material should be as close as possible to that of the samples. Where matrix interferences exist, ideally a method should be validated using a matched matrix reference material certified in a reliable manner. If such a material is not available, it may be acceptable to use a sample spiked with a reference material.

Reference materials and certified reference materials should be clearly labelled so that they are unambiguously identified and referenced against accompanying certificates or other documentation. Information should be available indicating shelf life, storage conditions, applicability, and restrictions of use. Reference materials made up within the laboratory, e.g. as solutions should be treated as reagents for the purposes of labelling.

Reference materials and measurement standards should be handled in a way that safeguards against contamination or degradation. Staff training procedures should reflect these requirements.

For more information, please refer to ISO Guide 33:2015 Reference materials – Good practice in using reference materials available from www.iso.org.

9.1.1 Certified reference materials

A certified reference material (CRM) is a particular form of measurement standard. Quality management systems involving laboratory accreditation under national and international accreditation standards such as ISO/IEC 17025 require metrological traceability to Certified Reference Materials (where possible) when using reference materials for calibration.

A CRM (often referred to as a matrix CRM) is a sample for which the test results are firmly established and agreed, ideally on an international basis. They are sold by some national standards bureaux and similar organisations and usually verified by highly respected reference laboratories or by inter-laboratory calibration. Acceptable procedures for certification of reference materials are detailed in ISO Guide 35[31]. This source also contains much information that can be equally applied in the production of in-house reference materials.

Whilst CRMs are preferred where available, their availability is limited. Reference Materials that do not meet all the criteria for certified reference materials are more widely available: the principal difference is the additional evidence of metrological traceability and statement of measurement uncertainty provided on the certificate for certified reference materials.

It is important that any certified reference material used has been produced and characterised in a technically valid manner. Users of CRMs should be aware that not all materials are validated with the same degree of rigour. Details of homogeneity trials, stability trials, the methods used in certification, and the uncertainties and variations in the stated analyte values, are usually available from the producer and should be used to judge the pedigree. The material must be accompanied by a certificate that includes an estimate of uncertainty of the certified value. ISO Guide 34[32] and an ILAC Guide deal with criteria for the competence of reference material producers. These guides may provide the basis for future assessment of reference material producers. Preparation of certified reference materials is described in general in ISO Guide 34 and in more detail in ISO Guide 35[33]. General steps required in production of a certified reference material typically include:

- Collection or synthesis of material
- Sample preparation (including homogenization, stabilization, bottling etc.)
- Homogeneity testing
- Stability assessment
- Value assignment ("characterization" in ISO REMCO terms).

In addition it may be important to assess the commutability of a reference material; this is especially important for biological materials.

A laboratory that wishes to calibrate and validate its methods can check its performance by testing the CRM and so establish traceability. Laboratories that can achieve correct results with the CRM should, in theory, agree on any other test for the same parameters in the same matrix. To be effective, a CRM must be typical of the samples which the laboratories are testing on a routine basis. A method for effluents will, ideally, require a CRM which is a typical effluent, and fish testing

[31] ISO Guide 35, Certification of reference materials — General and statistical principles
[32] ISO Guide 34, General requirements for the competence of reference material producers
https://www.iso.org/obp/ui/#iso:std:iso:guide:34:ed-3:v1:en
[33] ISO Guide 35, Reference materials — General and statistical principles for certification
https://www.iso.org/obp/ui/#iso:std:iso:guide:35:ed-3:v1:en

will need CRMs that are typical of the fish the laboratory normally tests. CRMs must be stable and highly homogeneous, as well as of established composition or properties. This is readily achieved in some areas, such as in the chemical analysis of alloys, the measurement of physical properties such as mass, dimension, etc., and with some geological samples, but things are not so simple in other areas of testing.

Microbiology provides a different sort of problem since sample stability is virtually impossible. However, in microbiology there are certified reference cultures which provide a definition of particular organisms so that laboratories can verify that their test systems are adequately selective. Relatively recently, quantitative microbiology references have become readily available. These are generally based on the impregnation of cultures onto plastic supports of controlled surface porosity.

European Reference Materials or ERM are certified reference materials of high quality and reliability produced by members of the European Reference Materials consortium in the European Union .European Reference Materials are a tool for improving the confidence in, and the mutual recognition, of test results and certificates in the European market. The CRMs comply with high metrological requirements, ensuring traceability of measurements results, and are the end-point of the traceability chain, thus being primary standards in chemistry[34].

Where certified reference materials are not available, there are several alternative strategies, but the main approach is participation in inter-laboratory proficiency exercises (see Section 11.2). Such schemes give a laboratory a measure of its data relative to other similar laboratories and, if organised properly, provide a very effective addition to the use of certified references. Accreditation bodies will always expect participation in appropriate proficiency schemes but, where certified references are available, these will be expected to be used as well.

Understanding the hierarchy and validity of reference materials

A reference material is any material or substance where one or more property values are sufficiently homogeneous and well accepted that they can be used for checking methods or apparatus. Two key types of reference materials are a) single compounds or items of established purity or properties, and b) matrix references which are specific types of sample where accepted values of one or more determinants have been established.

The key to the reference material is in the acceptance. The highest level of acceptance is a CRM, but even this term is somewhat variable in meaning. Strictly speaking, the only certified reference material of impeccable pedigree is one complying with the definition of a CRM in ISO Guide 30[35], which means one produced according to ISO Guide 35[36] by an organisation complying with ISO Guide 34 and where the certificate complies with ISO Guide 31[37]. Until relatively recently, there was no accreditation system for reference materials producers, so any claims which might be made regarding a reference material and its producer were effectively self-certified.

What happened in practice was that suppliers of commercial reference materials made an evaluation, and purchasers relied on the credibility of the supplier and on the content of the certificates provided to give confidence that the values quoted for the reference material were reliable. Whether the reference material came with enough information to enable it to be classed as a CRM, or only as a RM, was a matter of interpretation. The basis of any particular supplier's interpretation of the terms can normally be found in their catalogue.

Note that in the US the terms NIST Reference Material or Standard Reference Material (SRM) are generally regarded as equivalent to CRM. In practice, a reference material obtained from a reliable

[34] http://www.erm-crm.org/
[35] ISO Guide 30, Terms and definitions used in connection with reference materials
[36] ISO Guide 35, Certification of reference materials — General and statistical principles
[37] ISO Guide 31, Reference materials — Contents of certificates and labels

organisation, such as NIST (National Institute of Standards and Technology), the European Reference Materials or ERM, or the Laboratory of the Government Chemist (LGC), would be very widely recognised and could usually be regarded as a reliable basis for checking the accuracy of methods. Now, however, the situation is changing rapidly.

Accreditation of reference materials producers against ISO Guide 34

General requirements for the competence of reference materials producers are now increasingly widespread, and many accreditation bodies are beginning to insist that only results from reference materials from accredited producers are acceptable as demonstrating traceability of measurement.

In practice, reference materials producers will also form part of the certification chain, and will need to have laboratories to monitor preparation of materials, and to participate in the setting of certified values. This means that not only must the materials producer show compliance with ISO Guide 34 as regards certification, but they must also have an ISO 17025 accredited laboratory. Guidance on the use of certified reference materials in analytical chemistry can be found in ISO Guide 32[38]. It should also be noted that reference materials, as interpreted by most ISO 17025 accreditation bodies, include materials such as standard solutions and buffers that are frequently purchased by laboratories from laboratory chemicals suppliers. Increasingly, accreditation bodies are insisting that laboratories should use only those from ISO Guide 34 accredited sources.

In summary, it is now very important to check with your accreditation body on its attitude to acceptance of materials as references before committing to buy any particular item.

9.1.2 Reference cultures

Reference culture is a microorganism preparation that is acquired from a culture type collection.

Traceable reference cultures are required for establishing acceptable performance of media (including test kits), for validating methods, and for assessing/evaluating on-going performance. To demonstrate traceability, laboratories should use reference strains of micro-organisms obtained directly from a recognised national or international collection, where these exist. When traceable reference cultures are not readily available, commercial derivatives traceable to them could alternatively be used, provided that the relevant properties for its intended use have been shown by the laboratory to be equivalent at the point of use.

Reference strains may be sub-cultured once to provide reference stocks. Purity and biochemical checks should be made in parallel as appropriate. It is recommended to store reference stocks in aliquots either deep-frozen or lyophilised. Working cultures for routine use should be primary subcultures from the reference stock . If reference stocks have been thawed, they must not be re-frozen and re-used.

Working cultures should not be sub-cultured unless it is required and defined by a standard method or laboratories can provide documentary evidence that there has been no change in any relevant property. Working stocks shall not be sub-cultured to replace reference stocks. Commercial derivatives of reference strains may only be used as working cultures.

Cultures from the ATCC (American Type Culture Collection) Bacteriology Collection e.g. are useful in a variety of applications, as e.g. as quality control organisms for commercial identification systems. They have a wide selection of extremophile strains from a variety of environmental sources, and genomic DNA from well-characterized microbial strains suitable for amplification by Polymerase Chain Reaction (PCR). From their website it is possible to download ATTC Culture Guides Strains (www.lgcstandards-atcc.org).

[38] ISO Guide 32, Calibration in analytical chemistry and use of certified reference materials

EN ISO 11133:2014[39] contains detailed instructions for the maintenance of microbial strains, and the preparation and standardization of working cultures and inoculation suspensions. The standard provides comprehensive specification tables for most culture media for both food and water microbiology. These tables include the medium's target microorganism, relevant ISO standard, each medium's function to be tested (productivity, selectivity, specificity, the appropriate control strains for each medium's function, including their World Data Centre for Microorganisms numbers, and test criteria for characteristic reactions and other practical information.

9.2 Use of spikes

Spikes are widely used for method validation and calibration in chemistry and microbiology. They provide a reasonable alternative to certified references, if the spiking material is adequately authenticated, ideally by certification of its purity.

On the face of it, a spike has the advantage that the laboratory can spike into a matrix which is typical of its normal sample stream. However, it is valid to question whether a material spiked into the sample artificially is present in the same distribution and speciation as the actual target. The strength of this argument depends on the matrix. A metal ion spiked into a water sample might well be regarded as a valid approach, but a pesticide spiked into a food sample may be questioned on the grounds that the pesticide in real samples may have been systemically absorbed by the crop used to make the food, and so may be bound into the cell structure.

A spike is generated by taking a real sample and adding a known amount of the target in question. Ideally, the base sample for the spike should have little or none of the target present before spiking. If this is not possible, the spike level should be large compared to the natural level present. Of course, the natural level must be known in this instance. The spike must be thoroughly mixed and distributed homogeneously throughout the matrix. The spike does not provide true traceability, but it can be reasonably assumed that laboratories that can demonstrate good recoveries of spikes have good accuracy.

The use of spikes is especially important where laboratories are carrying out tests in complex matrices which may affect the results. Examples are water analysis, where matrix effects are common, and microbiology, where components of the sample may well affect the viability of organisms. The spike, at the very least, demonstrates that the laboratory would detect the material or organism being sought if it were present. As more certified reference materials become available, accreditation bodies are, however, increasingly expecting method validation to involve their use and are less inclined to accept validations based on spiking.

[39] Microbiology of food, animal feed and water -- Preparation, production, storage and performance testing of culture media

10 REPORTING OF RESULTS

ISO/IEC 17025 paragraph 5.10; ISO 19036[40], ISO 8199[41], ISO 7218 (see Annex 1)

The results of each test or series of tests carried out by the laboratory should be reported accurately, clearly, unambiguously and objectively, and in accordance with any specific instructions in the test methods.

Results are usually reported in a test report and should include all the information requested by the customer, and necessary for the interpretation of the test results, and all information required by the method used.

Each test report should include at least the following information, unless the laboratory has valid reasons for not doing so:

- Title (e.g. "Test Report")
- The name and address of the laboratory, and the location where the tests were carried out, if different from the address of the laboratory
- Unique identification of the test report (such as the serial number), and on each page an identification in order to ensure that the page is recognized as a part of the test report, and a clear identification of the end of the test report
- The name and address of the customer
- Identification of the method used
- A description of, the condition of, and unambiguous identification of the item(s) tested
- The date of receipt of the test (s) where this is critical to the validity and application of the results, and the date(s) of performance of the test.

Test reports may be issued as hard copy or by electronic data transfer.
In addition to the above, test reports shall, where necessary for the interpretation of the test results (including sampling if applicable), include:

- Deviations from, additions to, or exclusions from the test method, and information on specific test conditions, such as environmental conditions
- Where relevant, a statement of compliance/non-compliance with requirements and/or specifications
- Where applicable, a statement on the estimated uncertainty of measurement. Information on uncertainty is needed in test reports when it is relevant to the validity or application of the test results, when a customer's instruction so requires, or when the uncertainty affects compliance to a specification limit
- Where appropriate and needed, opinions and interpretations.

And additional information which may be required by specific methods, customers or groups of customers.

[40] Microbiology of food and animal feeding stuffs - Guidelines for the estimation of measurement uncertainty for quantitative determinations
[41] Water quality - General guidance on the enumeration of micro-organisms by culture

If test reports contain the results of sampling the following, where necessary for the interpretation of test results, shall be included:

- The date of sampling
- Unambiguous identification of the substance, material or product sampled (including the name of the manufacturer, the model or type of designation and serial numbers as appropriate)
- The location of sampling, including any diagrams, sketches or photographs
- A reference to the sampling plan and procedures used
- Details of any environmental conditions during sampling that may affect the interpretation of the test results
- Any standard or other specification for the sampling method or procedure, and deviations, additions to or exclusions from the specification concerned.

With tests performed for internal customers, or according to a written agreement with the customer, results can be reported in a simplified way. Any information not reported to the customer should be readily available in the laboratory which carried out the tests.

Additional information on expressing test result in microbiological testing is specified in ISO 7218 (see Annex 1).

For quantitative methods in microbiology, results are expressed as the number of Colony Forming Units (CFU) per volume, or grams of sample analysed. Below 10 CFUs per plate, precision decreases significantly, and laboratories are advised to reflect this in their test reports. If the result of the enumeration is negative, it should be reported as "not detected for a defined unit" or "less than the detection limit for a defined unit". If preferred, and in order to comply with national technical and health regulations, the result may also be reported as "zero for a defined unit". Qualitative test results should be reported as "detected/not detected in a defined quantity or volume". They may also be expressed as "less than a specified number of organisms for a defined unit", where the specified number of organisms exceeds the detection limit of the method, and this has been agreed with the customer.

Where an estimate of the measurement uncertainty of the test result is expressed on the test report, any limitations have to be made clear to the customer, particularly if the estimate does not include the component contributed by the distribution of micro-organisms within the sample.

Laboratories may have to check if the standards used have their own specific requirements regarding the expression of results. International standards, such as ISO standards e.g. provide additional or concrete information on expression of test results, on performance characteristics and results and on calculation of results.

11 QUALITY ASSURANCE OF RESULTS/QUALITY CONTROL OF PERFORMANCE

ISO/IEC 17025, paragraph 5.9; Eurachem Proficiency Testing Guide[42]

Method validation is typically an exercise undertaken when a laboratory devises or adopts a method. Having established the performance characteristics of the method, it is necessary to put measures in place to ensure that the demonstrated performance is maintained in routine use, and to detect deviations from the ideal performance. These measures are generally encompassed by the term quality control. Quality control is a discipline-specific activity but, in general terms, the ideal approach to it is to have samples items available for which the expected result is known. These are passed through the test process along with normal items for test and the data generated from the controls is compared with the expected values.

11.1 Internal quality control

Internal quality control consists of all the procedures undertaken by a laboratory for the continuous evaluation of its work. The main objective is to ensure the consistency of results day-to-day and their conformity with defined criteria.

A programme of periodic checks is necessary to demonstrate that variability (i.e. between analysts and between equipment or materials etc.) is under control. All tests included in the laboratory's scope of accreditation need to be covered. The programme may involve:

- The use of spiked samples with variable contamination levels, including target and background flora
- The use of spikes/naturally contaminated samples from a range of matrices;
- The use of reference materials (including PT scheme test materials)
- Replicate testing
- Replicate evaluation of test results, i.e. counting of colonies in petri dishes by two analysts.

The internal quality control programme must be adapted to the actual frequency of tests performed by the laboratory. It is recommended that, where possible, tests should incorporate controls to monitor performance. It is also advised that data from reference materials and spiked samples be plotted, to assist in the evaluation of trends in a visual manner.

In special instances, a laboratory may be accredited for a test that it is rarely called on to do. It is recognised that in such cases an on-going internal quality control programme may be inappropriate, and that a scheme for demonstrating satisfactory performance carried out in parallel with the testing, may be more suitable. However, this does not eliminate the need to participate in PT schemes at acceptable frequency. In any case, the laboratory should be aware of the inherent risk associated with such an approach and take all appropriate measures.

Internal laboratory quality control provides evidence of reliability of analytical results. Monitoring of analytical performance on an on-going bass is an important element of quality management in the laboratory. It is during the stage of method development and validation that the analytical method applied in routine analysis is documented as fit for purpose each time. This is accomplished by analysis of RM or controls sample under the same condition.

[42] Selection, Use and Interpretation of Proficiency Testing (PT) Schemes Second Edition 2011; Editors Ian Mann (SAS, Switzerland) Brian Brookman (LGC Standards, UK)

The data obtained regularly from the quality control materials are, in general, evaluated by control charts. Control charts are extremely valuable in providing a means of monitoring the total of the performance of the analyst, the instruments, and the test procedure, and can be utilized by any laboratory. There are a number of different types of control charts, but they all illustrate change over time. It is a graphical and analytic tool for monitoring process variation. The natural variation in a process can be quantified using a set of control limits. Control limits help distinguish common-cause variation from special-cause variation. Typically, action is taken to eliminate special-cause variation and bring the process back in control. It is also important to quantify the common-cause variation in a process, as this determines process capability. Very often Shewhart charts are used. For more see EURACHEM / CITAC Guide CG 4, Quantifying Uncertainty in Analytical Measurement, Third Edition QUAM: 2012.P1 and ISO 7870:1993 Control charts - General guide and introduction.

11.2 External quality assessment (proficiency testing)

Laboratories should regularly participate in proficiency testing (PT), relevant to their scope of accreditation. Preference should be given to proficiency testing schemes that use appropriate matrices.

Participation in proficiency testing schemes is mandatory when a laboratory is ISO accredited, provided that appropriate schemes are available. If this is not the case, the laboratory should participate in inter-laboratory comparisons organised by a sufficient number of other laboratories, on the basis of a well-documented protocol.

PTs are inter-laboratory comparisons that are organized regularly to assess the performance of analytical laboratories, and the competence of the analytical personnel. Proficiency testing has been in use by laboratories for many years. It is the most commonly employed type of external quality assurance, as it is able to address many laboratory methods.

PTs are programs in which multiple samples are periodically sent to members of a group of laboratories for analysis and/or identification; whereby each laboratory's results are compared with those of other laboratories in the group and/or with an assigned value, and reported to the participating laboratories and others.

PT is available for most of the commonly performed laboratory tests, and covers a range of chemistry and microbiology testing. Provision of PT is now essentially a commercial activity, and laboratories subscribe to suitable schemes. Accreditation of PT operators against ISO 17043[43] is now becoming extensive and, although ISO 17025 does not currently insist that an accredited proficiency test scheme be used by laboratories, accreditation bodies are increasingly insisting that, where an appropriate accredited scheme exists, it should be used.

In the PT process, laboratories receive samples from a PT provider. This provider may be an organization (non-profit or for-profit) formed specifically to provide PT. Other providers of PT include central reference laboratories, government health agencies, and manufacturers of kits or instruments. In a typical PT programme, challenge samples are provided at regular intervals. An optimal frequency will be 3–4 times yearly. If the programme cannot provide challenges with this frequency, the laboratory may be able to seek additional sources. The laboratories participating in the programme analyse the samples and return their results to the central organization. Results are evaluated and analysed, and the laboratories are provided with information about their performance, and how they compared with other participants. The participating laboratories use the information regarding their performance to make appropriate changes and improvements. To be successful, PT instructions must be followed carefully, all paper work completed accurately, and results submission deadlines met.

[43] Conformity Assessment - General requirements for proficiency testing

All PT results, as well as corrective actions, should be recorded, and the records maintained for an appropriate period of time.

PT is a tool to measure laboratory performance. Therefore, there must be no difference in the treatment of PT samples. PT providers make every effort to produce samples that exactly mimic, or closely resemble, usual samples received. PT samples must be processed by normal testing method(s) and involve personnel who routinely perform the testing.

PT participation is valuable only if the information received is directed to improvement in the laboratory. It is important to remember that PT does have some limitations, and it is not appropriate to use PT as the only means for evaluating the quality of a laboratory. PT results are affected by variables not related to samples, including preparation of the sample, matrix effects, clerical functions, selection of statistical methods of evaluation, and peer group definition. PT will not detect all problems in the laboratory, particularly those that occur in the pre-examination and post-examination procedures. A single unacceptable result does not necessarily indicate that a problem exists in the laboratory

Laboratories should use external quality assessment not only to assess laboratory bias, but also to check the validity of the whole quality system.

Although accreditation bodies may specify minimum participation in PT schemes, it is the responsibility of the laboratory to demonstrate that the frequency and extent of their participation is appropriate for their scope. The document EA-4/18[44] may give useful support with the use of sub-disciplines, i.e. an area of technical competence defined by a minimum of one measurement technique, property and product, which are related. This facilitates the optimisation of the extent of participation in proficiency testing. Further to this, the Eurachem guide on selection, use and interpretation of PT schemes[45] may help in the interpretation of the results from PT participation. The main topics covered by the Guide are:

- The aims and benefits of participation in PT schemes
- How to select the most appropriate PT scheme
- Understanding the basic statistics and performance scoring used by the PT providers
- Using and interpreting the PT results in order to improve the overall performance of the laboratory.

Laboratories are encouraged to subscribe to ISO/IEC 17043[46] accredited PT schemes. Other providers should be used only where the laboratory has assessed their competency. Annex 3 to ISO 17043 is a useful reference for laboratories, as it gives guidance on the issues to be considered when choosing a PT provider.

Laboratories undertaking chemical tests and microbiological examination of official control samples of fish and fishery products are expected to take part in a PT scheme. For detailed information on schemes and dates, see the EPTIS database[47].

[44] EA-4/18 TA:2010, Guidance on the level and frequency of proficiency testing participation, European co-operation for Accreditation.

[45] Selection, Use and Interpretation of Proficiency Testing (PT) Schemes by Laboratories, I. Mann, B. Brookman (Eds), 2nd Edition, 2011, Eurachem www.eurachem.org.

[46] Conformity assessment - General requirements for proficiency testing (ISO/IEC 17043:2010)

[47] https://www.eptis.bam.de/en/index.htm

12 ISO/IEC 17025 ACCREDITATION REQUIREMENTS FOR TESTING LABORATORIES

12.1 Overview

It is a requirement of the EU that laboratories providing testing services to competent authorities for the testing of fish and fishery products be accredited to the ISO 17025 standard for the test methods involved. Obtaining this standard requires total management support and investment, appropriate accommodation, equipment, and supporting facilities and well-trained/well-supervised staff. Accredited status allows for the exchange of data and its acceptance that they are from a source that has attained, and maintains through appropriate procedures, a recognized standard of delivery.

The principle is to ensure, as far as is practicable, that the service package – the whole operational infrastructure – is adequate for the provision and maintenance of a testing service that is fully competent, demonstrates that competence through the quality assurance measures that have been put in place, and through the measures that it takes to ensure effective liaison with its client and the confidentiality of the service to that client.

The requirements are broad and necessitate diligence in ensuring compliance with the procedures developed to meet those requirements. ISO 17025 accreditation is specific to individual test methods and, for a laboratory involved in testing for a wide range of analytes, each of the test methods should be accredited to meet EU requirements.

Accreditation is, however, viewed by some laboratories to be wasteful in time and resources, with a resulting increase in the time taken to conduct an analysis. On the other hand, it allows for increased confidence in the test results and professional recognition of the quality of the services provided. The documentation prepared in support of accreditation also makes very useful training aids for new staff or those being developed to take on broader duties. The provisions and requirements for ISO 17025 accreditation are detailed in Annex 2; a simple checklist can easily be prepared from this to enable auditing or monitoring of the degree of preparedness or compliance of an organization for accreditation.

12.2 Regional accreditation bodies

A number of organisations exist worldwide that are authorized to assess the status of laboratories and confer accredited status such as the TTLABS (Trinidad and Tobago Laboratory Accreditation Service), of Trinidad and Tobago and JANAAC (Jamaica National Agency for Accreditation) of Jamaica – both IAAC (Inter-American Accreditation Cooperation) full member and ILAC affiliate member. The CARICOM Regional Organisation for Standards and Quality (CROSQ) is in the process of establishing a regional accreditation mechanism amongst its Member States, with the objective of co-ordinating for laboratory accreditation regionally in a manner that leverages capacity, and ensures quality service while being cost-effective, internationally recognized, and accepted globally. This regional approach to accreditation envisages the two National Accreditation Bodies of the region TTLABS and JANAAC working in cooperation with CROSQ and the local national accreditation focal points.

Details of other regional accreditation bodies can be obtained via the Internet using a site such as www.fasor.com/iso25/, which lists national accreditation bodies.

12.3 The accreditation procedure

The accreditation procedure starts with the engagement of a recognized accreditation body that will provide the necessary service. In some parts of the world, access to such bodies is limited. The reference given above in Section 1.5.1 will facilitate this process.

This body will liaise with the organization applying for accreditation, and a timescale for the submission of the primary documentation (the Quality Manual) will be agreed. A pre-assessment to observe the general standard of operation, and to highlight significant problems, will generally follow, then the main accreditation audit takes place during which the laboratory operation will be observed, and the generated data scrutinised. At the end of the audit, the organization will be told whether it has succeeded or failed and a written report from the accreditation body will follow. This report will detail any non-compliances observed (generally where the procedure is inadequate or ineffective or where its implementation could be improved), together with a timescale for the resolution of these. Following confirmation and evidence of the closure of these non-compliances, a date will be given for the formal award of the accreditation. The organization will then be periodically visited by the accreditation body to ensure the maintenance of standards, and to allow for the accreditation of additional test procedures. Application for accreditation of additional test procedures is generally much more straightforward than the initial application as the organizational documentation (the *Quality Manual* and associated procedures) will already be in place.

Each of these steps is discussed in more detail below.

12.3.1 The Quality Manual

The *Quality Manual* describes the organization and its operation with an overview of all of the elements appropriate to ensure the efficiency of the testing service and its relationship with clients including issues such as:

- The management structure
- Management responsibilities
- Management review
- Role of the quality manager
- Finance
- Equipment and materials procurement
- Procedures for recruitment
- Staff training
- Certification of staff competence
- Relations with the client
- Site security and client confidentiality
- Sample acceptance and reception
- Data storage
- Archiving and retention of records
- Preparation, certification and despatch of test reports
- Quality assurance including PT
- Internal audits

- Preventive action
- Complaints procedure
- Corrective actions
- Authorization, review and withdrawal of procedures.

To accompany the Quality Manual, a set of procedures should be developed, which set out in greater detail the operation and requirements of each of the issues described in the manual.

12.3.2 Laboratory procedures

At laboratory level, a further series of procedures, the SOPs, are developed to describe the use and maintenance of key items of equipment, laboratory quality assurance issues and the analytical procedures themselves.

Examples of SOPs required for analytical procedures in a fisheries testing environment may include:

- SOP for the analysis of lead, cadmium and mercury in fish tissue and water
- SOP for the determination of histamine in fish tissue
- SOP for the analysis of dissolved oxygen in sea water
- SOP for the detection and enumeration of *Escherichia coli* and coliforms in water
- SOP for the enumeration of *Clostridium perfringens* including spores in water
- SOP for the extraction and testing of ciguatoxin by mouse bioassay etc.

General laboratory procedures may include:

- SOP for the cleaning of glassware (chemistry or microbiology)
- SOP for the calibration of volumetric glassware
- SOP for the calibration of thermometers
- SOP for the calibration of balances
- SOP for the calibration of the pH meter
- SOP for the measurement and recording of operating temperatures in ovens, refrigerators, freezers and autoclaves
- SOP for the use and maintenance of each individual main item of equipment
- SOP for sample storage and disposal
- SOP for the retention of reagents and the disposal of waste/obsolete materials.

12.3.3 Forms

To assist with data recording, the development of easy to complete forms is useful, and will also help with ease of reference/comparison of data. Forms should be numbered with an appropriate code, and reference made in the procedure to the associated form.

12.3.4 Validation

The validation of test procedures is a routine operation when introducing a procedure into a laboratory. Essentially it is proving that the laboratory can develop comparable data to that generated by the authors of the procedure, and by peers who are also using the procedure. For accreditation of a test procedure, the validation data must be available for inspection at the accreditation audit.

Validation data will normally include statistically evaluated:

- Recovery data for multiple laboratory-treated samples
- Data for untreated samples and reagent blanks
- Data to demonstrate repeatability of the result for laboratory-treated samples
- Data to demonstrate reproducibility (between operators) for laboratory spiked samples.

Full laboratory quality control data (e.g. for sample storage conditions, etc.) also need to be available for assessment.

If the procedure for accreditation concerns only one matrix, such as wet fish tissue, then the validation data are confined to this matrix. If other matrices are involved (such as smoked fish or fish feed from aquaculture,) then validation data need to be produced for these. Separate accreditation requests are needed where the matrices are significantly different, even if the test analyte is the same (e.g. pesticide residues).

12.4 Implementation

The accreditation process should be led and developed by a small working group appointed by management and, generally, led by the quality manager. It is important that the group is formally trained in the requirements for ISO 17025 accreditation. This helps to focus effort, allows for a better understanding of the requirements and reason for such controls, and minimises wasted effort.

The Quality Manual and associated procedures are written by the quality manager and the team, in consultation with management. The laboratory procedures are written by the laboratory staff who are familiar with the equipment, techniques, etc., and who are best placed to develop such documents. All procedures are reviewed, following a procedure developed and agreed by the working group. Each document bears the name of the person who developed the procedure (the "owner") and the person designated to authorize the procedure for use. The procedure is given a version or revision number which will change each time the procedure is revised and re-issued.

Once a procedure has been developed and implemented, it is essential that there is full compliance and this can, initially, be difficult to achieve. Where time permits, the informal introduction of a procedure allows for staff to become familiar with the procedure and to highlight any issues necessitating amendment. Once the procedure is fully implemented, all detected non-compliances must be recorded, examined and appropriate (corrective) action taken. Evidence of this will be required by the accreditation assessment team.

Although the procedures must be comprehensive, they should not be over-elaborated; they should do the job without being too prohibitive. Also, ensure that what is written does not involve staff being tied to practices that were not intended.

The quality manager is responsible for implementing and operating the quality system on a day-to-day basis. He/she is normally responsible for administering the controlled document system, for

compiling the quality manual, and for organising the review and audit of the quality system. The quality manager must have direct access to the highest level of management in the organisation and to the laboratory technical management. An accreditation body will generally regard the quality manager as the person who provides day-to-day guardianship of the quality standard, and so represents their interests within the organisation.

12.5 Recurrent costs of the laboratory operation

The annual costs of running a laboratory are high and often underestimated, particularly by those unused to the specific requirements of a laboratory, and financial constraints can result in restrictions on the operational programme. The factors commonly considered when assessing the cost of a laboratory include:

- Rent (where appropriate)
- Staff salary costs
- The cost of power, water and drainage
- Telephone charges
- Stationery
- Vehicles (where appropriate)
- Provision for replacement equipment
- Equipment depreciation charges.

There are other costs, however, which significantly contribute to the annual cost of the establishment, and which are often either not considered or are underestimated. These other recurrent costs relate to:

- The maintenance and servicing of equipment
- The procurement of essential chemicals and reagents; the type and quantities will depend on the nature of the testing service and on the number of tests conducted
- Reagents, etc., used in the method validation (quality assurance) and additional to the quantities calculated based on the number of samples actually tested
- Reagents, materials, etc., used in staff training
- The provision of laboratory personal protective equipment (PPE)
- Provision for glassware replacement
- Participation in PT schemes
- Accreditation (the cost of the audit including fees and allowances for the audit team and certification charges).

Awareness of these other considerations allows for a better estimate of the overall costs of a laboratory and better budget estimation/budget bidding, or even acts as a factor in deciding whether or not the establishment of a laboratory is a viable proposition.

It is not possible to indicate or estimate the likely costs of all of the above, although some guidance is provided below.

- The maintenance and servicing of equipment – perhaps between 5% and 10% of the purchase price.

- The procurement of essential chemicals and reagents – impossible to estimate as it depends upon the nature and range of tests undertaken and sample numbers.
- Reagents, etc., used in the method validation – provision to cover 20% additional usage of reagents above that required based on pure sample numbers is recommended.
- Reagents, materials, etc., used in staff training – possibly 5–10% additional usage of reagents above that required based on pure sample numbers.
- The provision of laboratory PPE – perhaps 500 – 1,000 euros; depending upon staff numbers.
- Provision for glassware replacement – perhaps between 5% and 10% of the initial procurement cost.
- Participation in PT schemes – assume a cost of approximately 500 euros per disciplinary round. Need to calculate the full cost to reflect the frequency of testing (times per year) and the number of specific tests involved.
- Accreditation – assume a cost of approximately 10,000 euros for the initial accreditation and 8000 euros for each successive audit.

Efficient accounting over the first few years of operation will enable a more accurate assessment of the costs to be made.

ANNEX 1: FURTHER READING

This guide is based on a number of different sources of information. These are listed below, and may be consulted for additional information regarding the nature and characterisation of the different hazards identified.

Laboratory Manual

Guide to the development and maintenance of fishery product testing laboratories LTI040GEN, 2010; SFP programme, EU Health Conditions in ACP/OCT countries, Secretariat of the ACP Group of States
SFP-ACP/OCT Management Unit, REG/70021/000
http://www.megapesca.com/files/manual.rar

ISO 7218: Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations (ISO 7218:2007 + Amd 1:2013)

ISO/IEC 17025:2005: General requirements for the competence of testing and calibration laboratories

Eleftheriadou and K. C. Tsimillis (Eds.), Eurachem Guide: Accreditation for Microbiological Laboratories, Second edition (2013), Subject to journal requirements, ISBN: 978-91-87017-92-6. Available from www.eurachem.org

CITAC (The Cooperation on International Traceability in Analytical Chemistry) and EURACHEM (A Focus for Analytical Chemistry in Europe) (2002): Guide to Quality in Analytical Chemistry, An Aid to Accreditation

UNIDO, Vienna (2009): Complying with ISO/IEC 17025: A practical guidebook for meeting the requirements of laboratory accreditation schemes based on ISO/IEC 17025:2005 or equivalent national standard

Useful Website Addresses

AOAC - AOAC INTERNATIONAL is a globally recognized, 501(c)(3), independent, third party, not-for-profit association and voluntary consensus standards developing organization.

www.aoac.org

APLAC - New Zealand's premier accreditation body.

www.ianz.govt.nz/aplac

BIPM - Intergovernmental organization through which Member States act together on matters related to measurement science and measurement standards.

www.bipm.fr

EA - European co-operation for Accreditation.
www.european-accreditation.org

EPTIS - Help to find a PT scheme for the laboratory.
www.eptis.bam.de

EURACHEM – A network of organisations in Europe having the objective of establishing a system for the international traceability of chemical measurements and the promotion of good quality practices. www.eurachem.org

ILAC - International organisation for accreditation bodies.
www.ilac.org

ILAC -International Laboratory Accreditation Cooperation.
www.ilac.org

ISO - The International Organization for Standardization.
www.iso.ch

ANNEX 2: SUGGESTED FORMAT FOR IN-HOUSE METHODS DOCUMENTATION

Each page of the method must show the method number, the date of first issue and the date of the current version. Pages should be numbered in the format Page....of....

The method number is of critical importance since it provides an unambiguous identifier for the method.

A suitable arrangement for the page header is:

Method No. M/0001 Page 1 of 10 First issued: February 2015 (or version number). This issue: March 2016

The following sections should be included in the documentation except where the quality manager decides a section is inappropriate.

Title: The title should be brief but must include a reference to the property to be measured objective.

Scope: Clearly identify the range of items to which the test is applicable and any limitations on the range of any parameters which are measured, for example suitable for measuring lead in fishery products in the range ... to ....ppm.

Principles of the method: Give a brief description of the principles behind the measurement, e.g. a coloured complex is formed between the metal ions and dithiazone. The concentration is determined by comparison of the absorbance of the solution at 259nm with the absorbance produced by solutions of known concentration.

Sample requirements for test methods: Note the type of sample to which the test can be applied. This section also contains instructions for any special sampling techniques, sample handling and preservation, sample preparation or pre-treatment required. Alternatively, it can refer to other documents in which these procedures are described.

Materials: Any materials or consumables used by the method must be specified together with any required standards of purity or performance. Any quality checks on reagents must be described or the method for carrying out the checks must be referred to. Avoid referring to specific suppliers or products in this section, unless the source is critical to obtaining the correct quality of material, otherwise you run the risk of having a non-conformance merely because your usual supplier had no stocks and you used an alternative.

If you think that giving a supplier is useful to staff, then use a form of words such as:

High performance liquid chromatography column, reversed phase ODS silica, 10cm, 5 mm i.d. A suitable product is Chromatography Supplies Cat. No. LC/98765/ODS.

Equipment and calibration: Provide a brief description of the equipment with instructions on whether calibration is required before each use and how this calibration is to be carried out. Calibration instructions need not be included in the method, but a reference to where they can be found is then essential. Routine calibration as described in the equipment log need not be covered here; only such calibration as is part of the method need be included. Instruction to check that calibration markings and labels are up to date is a wise precaution, however.

Reference standards, including any certified reference materials, required for calibration should be specified. It is also appropriate here to specify any quality control standards used and to indicate the basis of their calibration, for example by checks against certified reference materials.

Setting up and checking Instructions for setting up the equipment must be given here, followed by instructions for any checks required to confirm that the equipment is operating properly prior to use. The criteria for passing the tests must be given and instructions included on the information to be recorded about the tests. It should be clear from this section what action is required when check criteria are not met. This need not be a detailed description of how to remedy particular problems but might refer to a manual or merely instruct the user to refer the problem to, for example the laboratory manager.

Environmental factors: Any environmental variables which should be taken into account or measured and recorded as part of the test or calibration must be noted. This would be relevant, for example, in the case of most calibrations and in materials testing where certain ambient temperature ranges may need to be adhered to for the test or, possibly, the temperature of the test may need to be recorded in the report.

Interferences: Any interferences, for example spectral, chemical, physical, etc., which might affect the results should be detailed with any precautions to be taken to minimise such effects.

Procedure: A detailed description of the procedure must be given, including any quality control measurements required, for example duplicate or reference measurements. The level of detail is difficulty to specify for any particular type of test, however the description must define the procedure adequately to enable it to be carried out in a consistent manner by different staff. The procedure goes to a trained staff.

Recording data: This section must give precise instructions on the data to be recorded from test/calibration items and for quality control. The format of any tables for results must be specified. Where worksheets are used, an example should be included with specimen data filled in.

Calculations: Full details of any calculations to be carried out must be included, with instructions of how calculations are to be checked, for example by a second person. Where calculations are done on a computer, for example by spreadsheet, there should still be a description of the calculations required and a clear identification of which sheet is to be used, for example file name.

Quality assessment: This section must specify precisely the criteria to be used to judge when results meet the necessary quality standards. This may include details of the correspondence required between duplicates or the values required to be returned for quality control. The objective, again, is to achieve consistency. There should be enough detail here to ensure that any person using the guidelines will come to the same conclusions. This normally means defined quantitative criteria or reference to rules for interpreting statistical quality control data.

Instructions on the response required to a failure in quality control must be given. This may simply be a requirement to re-run the test or calibration. Where this is not technically possible, it will normally be necessary for the laboratory manager to make a decision and, in most instances, to contact the client.

Performance characteristics (uncertainty): The known performance characteristics of the method should be given. This will generally be determined when the method was first validated but, where values are subject to review as part of the quality system, it may be necessary to refer to another document, for example records held by the laboratory manager on current performance. Either the uncertainty of measurement must be specified or instructions provided on how this is to be calculated in any particular instance.

Reports: The data to be included in the formal report which will be sent to the client must be described. This section should include details of the units to be used and any qualifiers to be added to reports, for example uncertainty estimates. This section is not necessarily relevant to the person actually carrying out the test but is necessary in order to have a complete specification of the test for audit purposes.

Safety: Any safety precautions to be taken and any hazards known to be associated with the method must be specified. An ISO 17025 assessment does not deal with safety, but the inclusion of safety information in methods is generally regarded as good practice.

Site use: Where methods are carried out on site, any special precautions needed to ensure that data is valid must be noted. This should include checks on instruments or references to confirm that they have not suffered in transit. If site checks are not possible, then the equipment should be checked before leaving the laboratory and immediately upon its return.

References: To be made to any standard specifications or published methods of relevance. Any manuals, technical documents or other relevant sources of information must be listed.

Authorisation: The signatures, with dates, of the laboratory manager and the quality manager, with dates, accepting the method for use, must appear. The laboratory manager is responsible for ensuring that the method is technically sound and that all relevant validation has been carried out and evaluated. The quality manager will normally carry out a check to ensure that all of this has been done and will, in addition, check that the level of documentation and its content complies with the requirements of the quality policy as expressed in the quality manual.

Source: COMPLYING WITH ISO/IEC 17025. A practical guidebook for meeting the requirements of laboratory accreditation schemes based on ISO/IEC 17025:2005 or equivalent national standard, 2009, UNIDO.