United Nations
Educational, Scientific and
Cultural Organization

GRÓ FTP
Fisheries Training
Programme
Under the auspices
of UNESCO

grocentre.is/ftp Final Project 2021

GUIDELINES FOR PREPARATION TO OBTAIN ACCREDITATION OF MICROBIOLOGICAL METHODS AT THE NATIONAL FISHERIES AND AQUACULTURE AUTHORITY (NAFAA) QUALITY CONTROL LABORATORY

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ABSTRACT

Fish processing establishments in Liberia are faced with the challenge of complying with ever-changing regulations requiring them to provide evidence that they are producing safe foods. Food testing laboratories aid in mitigating food safety issues by providing evidence that a manufacturer's food safety system is acceptable. To perform these activities, laboratories must adhere to certain standards such as ISO/IEC 17025 General Requirements for the Competence of Testing and Calibration Laboratories. However, implementing ISO/IEC 17025 practices in Liberia is challenging due to the lack of available guidance. The goal of NaFAA has been to prepare for ISO/IEC 17025 accreditation and provide accredited testing services to fish industries. This report presents a guideline for implementing a quality management system, including organizational structure, policies, laboratory layout, monitoring program, and standard operating procedures for the NaFAA laboratory to be fully established and prepared to obtain ISO/IEC 17025 accreditation. This report includes 3 SOPs and 18 forms. Media qualification verification procedures were developed to evaluate growth and quality parameters over the shelf life of the media packaged in the quality control department of NaFAA. This report provides the NaFAA laboratory with methods and procedures that can guide the implementation of a quality management system to become ISO/IEC 17025 accredited.

Key words: fish processing, food safety, laboratory standards, accredited testing services, quality management system, NaFAA laboratory, Liberia.

This paper should be cited as:

Noah, V. 2021. *Guidelines for preparation to obtain accreditation of microbiological methods at the National Fisheries and Aquaculture Authority (NaFAA) quality control laboratory.* GRÓ Fisheries Training Programme under the auspices of UNESCO, Iceland. Final project. https://www.grocentre.is/static/gro/publication/1748/document/Noah21prf.pdf

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1 INTRODUCTION

Fisheries play a vital role in Liberia, providing livelihoods for fishers and fish processors (Bureau of National Fisheries, 2014). Liberia's coastal areas are home to approximately 58% of the country's population (FAO, 2021). The fisheries sector provides full-time employment and livelihoods for about 11,250 people, with an estimate pointing to hundreds of thousands more employed in the sector on a part-time basis (FAO, 2021). Approximately 86% of Liberia's total capture fisheries production is from marine fisheries, while 14% comes from inland waterbodies (NaFAA, 2018). Fish is an essential source of animal protein for Liberians (Bell, 2019) and provides important several nutritional benefits to the population. The average fish and seafood product consumption across the population was measured at 4.34 kg per person in 2010 and increased to 5.88 kg in 2017 (FAO, 2020).

Fish is a perishable food that is highly susceptible to spoilage and microbial deterioration (Tavares, et al., 2021). Its consumption has become a fundamental aspect of life worldwide (FAO, 2020). However, if fish products are infected with foodborne pathogens, it can lead to sickness and possibly even death. The fish processing industries in Liberia are faced with the challenge of complying with ever-changing regulations that require them to provide evidence that the food products they produce are manufactured in an acceptable environment and are free of physical, chemical, and biological hazards, ensuring the safety of consumers. Complying with these regulations can be accomplished by submitting samples to food testing laboratories that meet specific criteria of acceptability or are accredited (FDA, 2011).

Food producers and manufacturers rely on ISO-accredited laboratories to provide trustworthy results proving their products are indeed safe to sell. ISO, in combination with the International Electrotechnical Commission (IEC), created guidelines for verifying the acceptability of food testing laboratories, which is now the basis for accreditation of food testing laboratories. This standard is known as ISO/IEC 17025 General Requirements for the Competence of Testing and Calibration Laboratories (ISO/ IEC 17025, 2005; ISO 17025, 2017). The ISO/IEC 17025 standard contains all of the requirements necessary for a food testing laboratory to become ISO accredited and provides guidelines and specifications on how to properly manage a food testing facility from the time samples arrive to when the final results go to the client, regardless of the company’s size or how many employees they have (ISO/ IEC 17025, 2005; ISO 17025, 2017). Following these guidelines also helps to ensure that no matter where you are in the world, a specific test, process, material, or procedure is performed in the same way and will give approximately the same result while also being fit for its intended purpose (ISO 17025, 2017).

The NaFAA laboratory in Liberia is relatively small (five employees), but despite these challenges, it is possible for the laboratory to successfully implement a Quality Management System (QMS), generate all required documentation, and implement support programs in order to obtain ISO/IEC 17025 accreditation. The Liberia Coca-Cola bottling company (LCCBC) was granted ISO 17025:2005 accreditation for methods within their water treatment plant, microbiology, and analytical laboratory, for the enumeration and detection of pathogenic bacteria, and other microbiological and analytical methods for water and beverages (ECCBC, 2018).

The challenges of food safety and the enhancement of health security are of growing concern in the world (Negri, 2009). About 30% of fish landed globally are lost to spoilage due to microbial activity (Rippen & Skonberg, 2012). Additionally, about 30-60% of fish harvested in Liberia are wasted due to spoilage, resulting in huge financial losses for fishers (NaFAA, 2018).

The evaluation of the quality and safety of fish in Liberia is poor. Currently, Liberia's National Standard Laboratory (NSL) is the only institution that carries out microbiological evaluation on fish and fishery products. They comply with ISO 17025:2005 international standards. However, their microbiological parameters are not accredited. Due to this, the fish products evaluated do not meet the requirement for export to the international market.

Currently, Liberia exports over 200 metric tons of fishery products annually to regional countries. However, due to this failure to meet the required standards, Liberia cannot access the international market (Africa, 2019). The EU market, the leading market for fish and fishery products globally, requires the laboratory of a third country to be accredited. Liberia could benefit greatly from exporting fish and fishery products into the international market. The Quality Control Laboratory of NaFAA needs to establish testing procedures and a quality system that will serve as a step towards acquiring ISO/IEC 17025 accreditation to ensure that the nation’s fish products meet European food safety requirement standards and pose no threat to European consumers (Scholaert, 2020).

1.1 Problem statement

The export of food products from Liberia to the international market is subject to the condition that an accredited institution has evaluated their safety. The NaFAA microbiology laboratory will be installed within the next three months and following the installment, testing procedures and laboratory's quality control system must be finalized and activated in order for the lab to be accredited to the ISO 17025 standard. However, there are no testing procedures or documentation control guidelines of microbiological testing methods currently on file at the Quality Control Laboratory of NaFAA to ensure the quality and safety of fish products meant for local and international markets.

1.2 Justification

The National Fisheries and Aquaculture Authority (NaFAA) has been considering the issue of accrediting its laboratory to comply with international standards. However, there is still a need to finalize the quality management system according to the ISO 17025. This study will serve as a step towards acquiring accreditation for the Quality Control laboratory of NaFAA. In addition, the implementation of this guide will enhance competency, reliability of services offered to the customers/clients, and compliance with national and international standards.

1.3 Goal and objectives

The project's goal is to provide a roadmap for the microbiology laboratory of NAFAA to meet all requirements determined by ISO for obtaining accreditation to ISO/IEC 17025:2005 standards. The main objective was to prepare a guideline for establishing and maintaining a fully

functioning food testing microbiology laboratory at NaFAA that will be capable of providing food testing services, guidance, and training to the food industries of Liberia to adequately prepare the laboratory to obtain and maintain ISO/IEC 17025 accreditation. The specific objectives were to carry out an analysis on the clauses of the ISO/IEC 17025 standard, to establish a quality management system (QMS) unique to the quality control laboratory that will implement the proper organizational structure, policies, programs, and detailed standard operating procedures (SOPs) to govern all aspects of the microbiology laboratory to meet ISO/IEC 17025 requirements for accreditation, to developed a road map for establishing an accredited laboratory at NaFAA, and to develop an implementation guideline for the microbiology laboratory of NaFAA.

2 GENERAL REQUIREMENTS FOR ACCREDITATION

Accreditation of a microbiological laboratory is the process by which an independent and authorized agency accredits the quality management system and competence based on certain pre-defined standards (WHO, 2007). Accreditation is done once and then maintained with regular audits from a third party (Eurachem, 2013).

For any food testing laboratory to obtain ISO/IEC 17025 accreditation, they must first meet certain requirements, have a functioning laboratory in place that complies with ISO/IEC 17025 guidelines/requirements, and submit certain pre-audit documents to the accrediting body of their choice (ISO/ IEC 17025, 2005). Accreditation involves obtaining and reviewing a copy of the ISO/IEC 17025 standard and other supporting documentation (A2LA, 2015a). Next, the laboratory must obtain the accordance checklist that details all the ISO/IEC 17025 standard requirements that must be met prior to scheduling an initial assessment. The laboratory must then provide evidence to the accrediting body that they have obtained copies of the standards and the checklist to guide them through their laboratory compliance process, which assures the accreditation body that they are implementing the appropriate standards (A2LA, 2016a).

After taking the initial steps for accreditation, the laboratory must develop a unique quality management system (QMS) that meets its establishments' needs by altering or updating all current processes, policies, and procedures to meet ISO/IEC 17025 requirements prior to submitting to an initial assessment (ISO 17025, 2005; AOAC, 2015; Sampaio, 2009). This is by far the most challenging part of becoming accredited, as it can take months or even years, depending on the available resources and current laboratory capabilities, to implement all parts of the standard into the current laboratory system. As part of this process, the laboratory must: (A2LA, 2015a; A2LA, 2016a)

1. Develop a draft scope of accreditation detailing what food analysis application(s) they intend to become accredited for
2. Implement all parts of the QMS, including management structure, employee policies, quality assurance, laboratory procedures, records, and other
3. Perform internal audits against the standard/checklist to verify that the laboratory meets all the ISO/IEC 17025 requirements and is ISO compliant
4. Conduct management review meetings to discuss issues and track/trend available laboratory data to show improvement and predict areas of concern.

Once the QMS is in place and the laboratory has completed all required pre-accreditation tasks, the laboratory may apply to the accrediting body to begin the accreditation process. During this stage of the process, the laboratory will be required to submit examples of its current documentation, standard operating procedures (SOPs), equipment lists, records, internal audit findings, corrective actions taken, a completed conformity assessment checklist, and other supporting information as proof that they are currently meeting ISO standards (PJLA, 2009).

After submission of the application, the laboratory will be assigned an assessor that will conduct the pre-assessment (desk audit of the laboratory's documentation) followed by an initial assessment (onsite assessment) to determine if the laboratory should receive ISO/IEC 17025 accreditation for its intended scope (PJLA, 2009; A2LA, 2015a; A2LA, 2016). If the laboratory passes these assessments, they will become accredited for the scope of work they submitted (ISO 17025, 2017).

2.1 Maintaining ISO 17025 Accreditation

There are several conditions that an accredited laboratory must meet to maintain ISO accreditation (ISO 17025, 2005) including:

- Provide accommodation to the accrediting body by giving access to documentation, the laboratory, or laboratories where the applicable analyses are taking place, and to all equipment, personnel, records, complaints, and past assessments
- Comply with the standards
- Maintain impartiality and integrity in all its dealings
- Retain records, both quality and technical, for required time frames and making them accessible to an auditor within a reasonable amount of time
- Only claim accreditation status for methods on its scope of accreditation
- Pay all fees associated with maintaining accreditation to the accrediting body.
- Never mislead clients or use accreditation in a misleading manner
- Inform the accrediting body of any changes to the organization, management, personnel, accredited methods on scope, or any other changes that could affect the laboratory's accreditation status.

If all these conditions, and other conditions not mentioned here, are met, the laboratory may retain its ISO/IEC 17025 accreditation and continue to receive all the benefits of being an accredited laboratory.

2.2 Benefits and Challenges of Accreditation

Implementing the ISO/IEC 17025 standard within a food testing laboratory provides a structure for the laboratory to gain ISO accredited status while affording many benefits to the laboratory. Some of these benefits include increased reliability of and trust in the data being generated; minimized errors in laboratory analysis, sample processing, and reporting of results; reduced costs; improved quality of work being performed; improved training and competency of staff;

and recognition within the food industry (Rodima, et al., 2005; ILAC, 2001). Even though there are many benefits of obtaining ISO/IEC 17025 accreditation for food testing laboratories, many microbiological laboratories do not attempt to obtain accreditation because it is challenging and requires many resources. Implementation of ISO practices involves the development of a quality management system (QMS) and the creation of documentation (Zapata-García, Llauradó, & Rauret, 2007; Grochau, Ferreira, Ferreira, & Caten, 2010), implementation of in-house control programs such as environmental monitoring, training (Honsa & McIntyre, 2003), and media qualification programs, and the verification of all in-scope methods used within the laboratory (AOAC, 2015).

The main challenges associated with implementing all the requirements to obtain ISO accreditation include the time and difficulty of developing and implementing the quality management system and developing the documents, forms, policies, and programs to meet the requirements (Vlachos, Michail, & Sotiropoulou, 2002; Zapata-García, Llauradó, & Rauret, 2007). Currently the microbiology laboratory of NaFAA is not prepared to obtain accreditation. The laboratory will be starting from the ground up. Therefore, obtaining accreditation will be a financially burdensome task that will take several months to years to accomplish.

2.3 The Quality Management System

The Quality Management System of any laboratory is based on ISO 17025 and is defined as "the organizational structure, responsibilities, procedures, processes, and resources for implementing quality management" that "control how quality policies are implemented and achieve quality objectives" (Allen, 2013). Its objective is to simplify the relationships between different levels (staff and clients), define specific goals, organize procedures, and documentation in general, fulfill the standards and permit all staff members to access information (ISO 17025, 2005). According to this standard, the organization should have a well-defined structure, and management must maintain the system. The quality manager should have access to the highest level of control and defined responsibility and authority for ensuring that the quality system is implemented and consistently followed. Implementing a QMS is a difficult task that can take food testing laboratories months or even years to complete as it involves all parts of the management structure, policies, and procedures needed to meet the standard (WHO, 2011).

To comply with the ISO/IEC 17025 standard, a food testing laboratory must have policies and procedures that cover many areas of concern from the accrediting body prior to attempting accreditation including: (ISO/ IEC 17025, 2005; ISO 17025, 2017)

1. **The organizational structure:** Descriptions of the roles and responsibilities of the laboratory, the management, and key personnel (WHO, 2009).
2. **Document control**: Procedures for all management system documents including policy statements, QM, QPs, DQMs, GQMs, PPs, CPs, SOPs, other needed procedures, specifications, textbooks, software, drawing, etc.
3. **Review of requests, tenders, and contracts** providing evidence that the laboratory has the capability and resources to perform agreed-upon test procedures, and that they are using appropriate approved methods.

4. **Purchasing services and supplies:** Policies and procedures for selecting external contract services and approved suppliers, and vendor lists and procedures for purchasing, receiving, and storing critical supplies.
5. **Service to the customer:** Proof of communication with customers as well as policies and procedures for a complaint and feedback system.
6. **Improvement and progress** showed through changes made due to audit findings, customer feedback, management reviews, and corrective/preventive action reports.
7. **Corrective and preventive action** procedures for handling issues of nonconformance to policies or procedures and to aid in the continual improvement of the laboratory.
8. **Control of records** to identify, collect, file, store, and dispose of quality and technical records.
9. **Internal audit:** Procedures and records for verifying that the laboratory's operations are compliant with the standards.
10. **Management reviews** to ensure that the management system policies and procedures are suitable for their intended purpose, reviewing corrective/preventive action reports, reviewing proficiency sample test results, reviewing customer feedback and complaints, and making recommendations for changes and improvement to the quality management system.

Putting these policies and procedures in place provides the necessary structure for the laboratory to be successful in obtaining ISO/IEC 17025 accreditation and maintaining ISO accreditation status into the future.

2.4 Technical Requirements

Along with developing a comprehensive quality management system to govern and provide structural support to the laboratory, food testing laboratories seeking ISO/IEC 17025 accreditation must also meet other requirements. These requirements involve the development and implementation of "technical requirements" that include everything from the generation of laboratory records, test methods, and SOPs to the implementation of support programs such as environmental monitoring, training, equipment, and temperature monitoring programs that affect sample results in the laboratory for methods that are part of the scope of accreditation (ISO 17025, 2005; ISO 17025, 2017). Some of these requirements (ISO 17025, 2005; ISO 17025, 2017) include:

1. **General:** Understand and monitor the factors (human error, environmental conditions, etc.) that contribute to the measurement uncertainty of a test result.
2. **Personnel:** The overall quality and performance of a laboratory are influenced by its personnel. Microbiological testing is done or supervised by an experienced person with at least a bachelor's degree in microbiology or an extensive relevant practical experience in microbiology (ISO/IEC 17025(E) clause 6.2, 2017). Personnel records, including training records, displaying the technical competence and abilities of staff to perform in-scope analyses as well as a training program with procedures on how to train personnel on laboratory procedures properly.

3. **Facilities and environmental condition** of the laboratory and facilities where specific microbiological testing and associated activities are carried out. Ensures that there are procedures to control the environmental factors or conditions that might impact the validity of the tests or calibrations results (Rowley, 2005). The laboratory should be arranged to minimize potential cross-contamination events. A temperature monitoring program for all laboratory supplies and samples must be in place to verify the accuracy of test parameters and proper storage of test items. The laboratory must demonstrate that the area where sample analysis is performed is suitable for that purpose.
4. **Test and Calibration methods:** Use of only approved, recognized methods for performing accredited tests and verifying test methods that are part of the scope of accreditation to demonstrate that the laboratory can perform them according to approved methods to a level of acceptability.
5. **Measurement uncertainty**: Obtaining the measurement of uncertainty for all test methods to verify the accuracy of test results (if applicable).
6. **Document control**: policies and procedures for document control and management records from creation through destruction, including retention requirements and document destruction policies. Control of all data generated by the laboratory and having procedures for the acquisition, processing, reporting, storage, and retrieval of all customer information and data/results.
7. **Equipment and calibration:** Laboratory equipment calibration procedures and policies to demonstrate that they are calibrated must be available prior to being used for any scope of an accredited test method. Implementing an equipment program ensuring all equipment is approved for use with procedures for operating and maintaining all equipment within the laboratory with records to track the cleaning and maintenance of the equipment.
8. **Reference material and reference culture:** Detailed procedures and policies for handling test items, including the receipt, storage, retention, and disposal of all test items using reference materials, reference cultures, and certified reference cultures to demonstrate that the integrity of test samples is not compromised – sample items must be traceable.
9. **Verification and validation of test methods:** The laboratory must have specified test methods and controls in place to verify the validity of test results, and the data from those controls should be trended to look for issues within the system.
10. **Reagent and cultural media:** Laboratories should ensure that the reagents' quality is suitable for the test involved. The proper performance of culture media, diluents, and other suspension fluids prepared in-house should be checked, where relevant, concerning:

- 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)

The laboratory must have SOPs for reagent and media qualification, preparation, and quality testing procedures.

11. **Quality assurance of results/Quality control of performance:** Internal quality control consists of all the procedures undertaken by a laboratory to evaluate its work continuously. The main objective is to ensure consistent results day-to-day and their conformity with defined criteria. A program of frequent checks is essential to demonstrate the variability between analysts and between equipment or materials is under control. All tests included in the laboratory's scope of accreditation need to be covered. The program may involve the following:
    - 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 proficiency testing scheme
    - replicate testing
    - replicate evaluation of test results

The internal quality control program 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 a particular instance, a laboratory may be accredited for a test that it is rarely called on to perform. It is recognized that an ongoing internal quality control program may be inappropriate in such cases. A scheme for demonstrating satisfactory performance in parallel with the testing may be more suitable. However, this does not stop the need to participate in proficiency testing schemes at an acceptable frequency. In any case, the laboratory should be aware of the inherent risk associated with such an approach and take all appropriate measures.

12. **Reporting of test results**: Results obtained by the laboratory should be reported to the client accurately, unambiguously, objectively, and according to the standards on a test report signed by the individual who authorized the sample analysis. The qualitative test results should be reported as "detected" or "not detected" in a defined quantity or volume. They may also be expressed as less than a specified number of organisms for a designated unit where the specified number of organisms exceeds the method's detection limit, which has been agreed with the customer. Laboratories may have to check if the standards used have specific requirements regarding the expression of results.

2.5 Difference Between ISO/IEC 17025:2005 and ISO/IEC 17025:2017

New information is constantly being generated, impacting the food industry, and leading to improvements in the way food products are being harvested, produced, and tested for foodborne pathogens. These modifications are sometimes large enough that updates to the ISO standards may be necessary to facilitate change and improvement across the entire industry. In 2017, ISO released an updated version of the ISO/IEC 17025 standard going from version ISO/IEC 17025:2005 to ISO/IEC 17025:2017 (ISO 17025, 2017). Although most of the content stayed the same, some notable changes include the structure of the document, changes to the scope of the standard, and a change in the explanation of standards from providing detailed steps to focusing on results (EURO, 2017).

The ISO/IEC 17025 standard structure has changed dramatically. It is no longer based on just two sections consisting of "Management" and "Technical" requirements. Instead, it is broken

into five main sections entitled: General Requirements, Structure Requirements, Resource Requirements, Process Requirements, and Management System Requirements (EURO, 2017; SADCAS, 2018). These new sections still contain all the original content from the previous version but in a different order to better facilitate the flow of information within the standard (EURO, 2017).

Some of the sections that were moved to new sections include: personnel moving from sections 4.1.5 f-h and 5.2 in the 2005 standard to 6.2 in the 2017 standard; accommodations of environmental conditions being moved from section 5.3 to section 6.3 and renamed to facilities and environmental conditions; equipment moving from section 5.5 to section 6.4 in the new standard; measurement traceability moving from section 5.6 to section 6.5 and being renamed to metrological traceability; and review of requests, tenders, and contracts moving from section 4.4 to section 7.1.1 in the new standard (EURO, 2017; SADCAS, 2018).

The scope of the standard has also changed along with the definition of what a laboratory is. In the 2017 standard, a laboratory is defined as "an organization that can perform testing, calibration and/or sampling associated with subsequent testing or calibration," (EURO, 2017). This new definition places an emphasis on sampling as being a part of laboratory activities instead of just testing and calibration which implies that all ISO/IEC 17025 accredited establishments will now need to address sampling whenever laboratory activities are mentioned within each section of the standard (EURO, 2017; SADCAS, 2018).

Finally, there is a new emphasis on obtaining results from each process instead of providing a detailed description of obtaining those results. The ISO/IEC 17025:2017 standard has been altered to remove descriptions of individual processes and instead focus on performance, making them more open to interpretation and leaving it up to each laboratory on how they will meet the requirement (EURO, 2017). With the performance-based requirements, laboratories will now have much more freedom to design and develop procedures and systems that meet their unique needs while still adhering to and meeting the requirements outlined in the ISO/IEC 17025 standard and maintaining their accreditation status.

3 QUALITY CONTROL DEPARTMENT OF NAFAA IN LIBERIA

The Quality Control department of NAFAA is a department under the research and statistics division of NaFAA. It is designated to support the Competent Authority in evaluating fish and fishery products (NaFAA, 2019). Established in 2019, the department has grown into an integrative resource that provides educational and technical knowledge and business development services to fish processing establishments in Liberia (NaFAA, 2018). The department's role is to verify the effectiveness and efficiency of Quality Assurance (QA) and the food safety management system of fish-related establishments in Liberia to ensure that they meet the requirements of local and international fish markets. In addition, it is responsible for studying and developing new analytical techniques in the field of quality control of fishery products (NaFAA, 2019). The department also conducts four quarterly inspections of cold storage facilities. This is done by assessing the product quality through sensual techniques and physical examination to ensure that the frozen fish are fit for human consumption (NaFAA, 2018).

In support of the Competent Authority, the department offers laboratory services for analyzing fish and fishery products. The quality control laboratory is well-equipped with microbiological testing equipment, which is yet to be set up. Currently, the quality control laboratory of NaFAA only performs sensory evaluation using the organoleptic method (smell, color, texture, and physical appearance). This evaluation process proves that some sanitary measures are in place. Still, the products' safety is not guaranteed because there are no testing procedures to conduct microbiological or chemical tests. Additionally, the Quality Control laboratory provides other services to the fish processing establishment, such as monitoring and issuing operational permits and certificates to trade fish and fishery products in and out of Liberia.

The quality control laboratory of the NaFAA management structure includes the director of research and statistics, fisheries research manager, research officer, quality assurance supervisor, quality assurance officers, and a laboratory technician. All these individuals play an essential role in the ability of the laboratory to function appropriately and help maintain the Quality Management System (QMS) daily. The quality control department has a fully functional training program. All new laboratory personnel undergo training prior to performing their respective duties. The success of the training sessions is demonstrated by providing evidence of the competency of the laboratory staff. In doing this, a master checklist is used to show all laboratory functions that each employee is trained on and when the training took place. The personnel training forms capture training activities and show that each technician has been fully trained.

3.1 Microbiological laboratory

The microbiology laboratory of the quality control department of NaFAA is still in the embryonic stage, and equipment will be installed within a few months. The laboratory under the Icelandic project is well equipped with microbiological testing equipment. Its role is to test and analyze seafood and water quality to detect and quantify pathogenic microorganisms to meet international trade quality system requirements, to ensure that quality fish products are safe for consumption. However, the laboratory lacks a quality manual to tailor all its management and technical system requirements, which must be met to qualify them for accreditation. However, once the microbiology laboratory is installed, the institution intends to adopt a quality manual detailing all aspects of the QMS including document and record control, employee policies, purchasing services and supplies, corrective and preventive action, internal audits, handling customer feedback and communication with clients, and management reviews. The manual will be adopted from an accredited food laboratory from any regional country.

4 ROAD-MAP FOR ESTABLISHING AN ACCREDITED LABORATORY AT NAFAA IN MONROVIA

Before applying for an accreditation, the laboratory needs to be established and become functional. To accomplish that task, the main activities will include setting up the structure of the laboratory and deciding the professional requirements needed, setting up the laboratory furniture and equipment, securing necessary training for personnel, setting up the laboratory budget plan and securing income, establishing necessary procedures and monitoring activities, establishing a Quality System based on ISO/IEC 17025 standards and obtaining accreditation within 6 months of setting up of a basic microbiology laboratory

4.1 The laboratory structure

The number of personnel, their education and experience levels depend on the analysis, methods chosen, and the number of samples to be analyzed daily. Figure 1 shows the ideal staffing structure required for the NaFAA laboratory to carry out analyses on about 240 samples per month, including a microbiological examination.

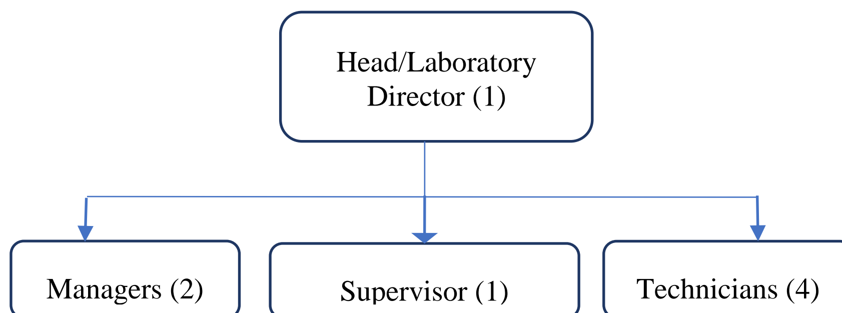

Figure 1. Suggested organization chart for NaFAA laboratory in Monrovia.

4.1.1 Laboratory Director

The Director of the laboratory will preferably have a master's degree in food science or related subjects with hands-on experience of 5-10 years in food analysis and Quality Management Systems with thorough and deep knowledge on other national and international standards. They will be dynamic with strong communication and interpersonal skills. They will be able to solve analytical problems and be well-versed in analytical procedures, instrumentation, and quality assurance. They will ensure that proper laboratory safety and housekeeping practices are adhered to and will review completed work reports. It will be their responsibility to ensure that the laboratory has a supply of necessary chemicals and instrumentation.

4.1.2 Laboratory Managers

There will be two essential managers heading the analytical sections: the Quality Control Manager and the Quality Assurance Manager. Having a manager assigned to specific units or areas of work permits the laboratory director to execute the total workload of the laboratory effectively. They will be responsible for the daily organization of the analytical processes, ensuring that daily and weekly deadlines for test results are met, quality control for each batch of testing meets ISO requirements and is recorded, staff training is up-to-date, and there is sufficient staff to meet the workload requirements. The minimum education required of them should be a post-graduate study or a master's degree in food science/food technology and related subjects. They should ensure that the group follows proper laboratory safety and housekeeping practices. They will be responsible for recommending the supply of new instruments or equipment to the laboratory director. They must be capable of answering questions and assisting in solving analytical problems posed by laboratory technicians. The managers should possess the ability to optimize methods, develop Standard Operating Procedures (SOPs), make independent decisions regarding peak identification and its area. They should be aware of all laboratory safety requirements and safety equipment when working with toxic and carcinogenic compounds.

4.1.3 Laboratory Supervisor

The supervisor should have a postgraduate or master's degree in chemistry/Biochemistry/Food Science or related subjects. Alternately, a bachelor's degree in chemistry or microbiology with three to five years of experience in food analysis will also be considered. The supervisor must have a deep knowledge of the basic microbiological principles involved in the methods that will be used. they must be aware of laboratory safety when working with solvents and strong acids and bases. They must be computer literate and know how to use manuals or specific equipment for proximate analysis. They must be capable of supervising all laboratory technicians on how to carry out their laboratory functions.

4.1.4 Laboratory Technicians

The minimum educational requirement of laboratory technicians will be a bachelor's degree in chemistry, biology, food science, and related subjects. There will be four laboratory technicians assigned in the laboratory. The laboratory technicians are the heart of every laboratory. They must be reliable, precise, competent, and motivated. Technicians will be trained in sample and media preparation, serial dilution, inoculation, autoclaving, etc., before being allowed to perform any laboratory operation. They should be familiar with the use of scales, pH meters, making reagents with an attention to detail such as chemical names, expiry date, purity of the chemical etc.

4.2 Suggested layout of the NaFAA microbiology laboratory

The microbiology laboratory will be laid out suitably to ensure that materials are appropriately stored and safety measures are in place. The laboratory will have two rooms: a wet room for media preparation, sterilization, and glassware washing, and the other room for inoculation, incubation, gram staining, counting of colonies etc., as shown in **Error! Reference source not found.**. A freezer and a refrigerator where samples will be stored while waiting for testing will be set up under the stairways.

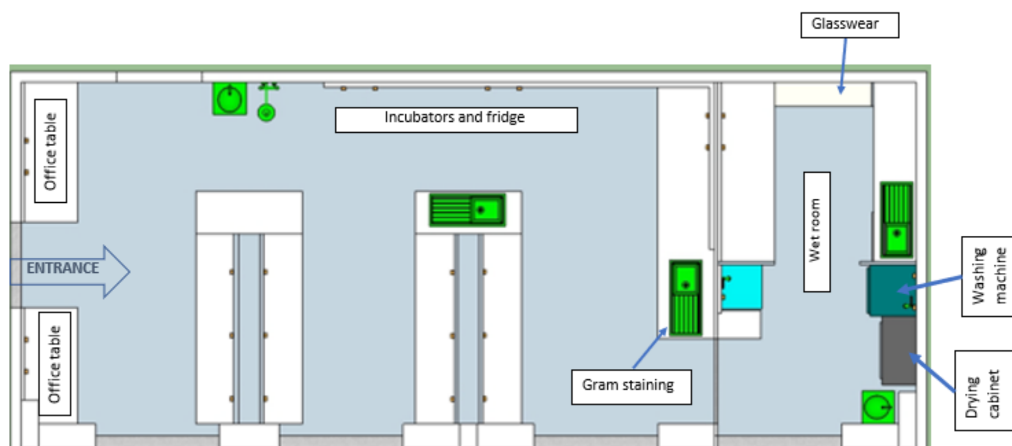

Figure 2. Suggested layout of NaFAA laboratory in Monrovia.

4.3 Personnel training

Personnel will receive specific basic training on test methods and equipment operation. Orientation and training for new personnel on the concept of quality control through an on-the-job training program and a performance appraisal for all personnel is an essential element of the quality assurance system.

4.4 Laboratory budget and financial security

The institution will allocate funds from its yearly budget to the laboratory for equipment maintenance, routine purchase of consumables, repairs, and contingency expenses to ensure the smooth running of the start-up activities. The laboratory income will be generated from the testing of microbiological samples. Fish processing establishments will pay a specific amount into the institution's account to test their products' safety and quality. The annual laboratory budget allocated from the income generated will be used to run the operations of the laboratory to ensure the smooth running of the start-up activities of the laboratory. However, if the required number of samples received per month is not met, the laboratory will then write the institution for financial support to cover the gap.

4.5 Procedures and monitoring activities

Standard operating procedures (SOPs) for ten suggested microbiological methods were developed and will be verified. These in-scope methods include the methods for the detection and enumeration of Total Plate Counts, Coliforms, E. coli, Salmonella, Listeria, Vibrio parahaemolyticus, Vibrio cholera, Vibrio Vulnificus, Yeast, and mold (Appendix 2). SOPs for laboratory procedures including media preparation, pipetting methods, measuring device verification, environmental monitoring, temperature monitoring, eyewash checks, housekeeping schedules, etc., were also created (Appendix 2). The formatting elements of the SOPs developed for microbiological methods include the following guidelines as shown in the Table 1.

Table 1. Standard Operating Procedure Sections and Content Requirements.

SOP Section	Description of Contents
Scope	What is the overall reason for having the document
Equipment	Any special equipment needed for performing the procedures
Media/reagents	Any special media and reagent needed to properly perform the test
Procedures	All the steps and information necessary to complete the procedure
Calculations	The process in obtaining the results
Expression of results	How to write the calculated results
References	Any external documents utilized for the procedure to be used effectively, or that were used in the creation of the SOP

The microbiology laboratory of NaFAA intend to train personnel to perform analysis on the above methods. A staff member from the microbiology laboratory has recently undergone practical training in Iceland on performing test analysis on these methods.

Monitoring the temperature of all areas that might affect client samples is crucial to the success of laboratory procedures. If the temperature of sample storage is excessively warm or cold, it could dramatically affect the results seen during analysis, so the microbiology laboratory intends to implement a temperature monitoring program following its setting up. The program will consist of monitoring activities for all incubators and refrigerators as well as the testing environment to demonstrate that all samples and testing supplies are stored or utilized in an appropriately controlled environment. To facilitate the temperature monitoring program, SOPs will be in place to determine what should be monitored, how often monitoring should take place, and what is an acceptable temperature range for all equipment or areas to be checked. Verification forms were developed to ensure the accuracy of each reading (Appendix 1). To record monitoring activities, the laboratory will use a temperature monitoring log which will be reviewed for temperature fluctuations or deviations, allowing the laboratory to make the appropriate adjustments to maintain the temperature of its test and storage environments (Appendix 1).

4.6 Laboratory accreditation

Once a functional laboratory has been established and all laboratory activities are implemented, the laboratory will start seeking accreditation. To accomplish that, the laboratory will purchase and review the ISO/IEC 17025 standard and any other documents required for the laboratory to meet ISO requirements for accreditation. Next, the laboratory will obtain the accordance checklist from the accreditation body, which will be completed to determine all parts of the ISO/IEC 17025 standard that need to be addressed within the microbiology laboratory. To accomplish that, the laboratory will develop a unique and fully functioning Quality Management System (QMS) from the procedures listed above to cover areas of concern from the accrediting body.

After taking the initial steps for accreditation, a draft scope of accreditation detailing what food analysis application they intend to become accredited for will be developed. Next, the unique and fully functional quality management system (QMS) will be developed to meet all the requirements of the ISO/IEC 17025 standard and the needs of the laboratory. An internal audit will be carried out against the standard checklist to verify that all the ISO/IEC 17025 requirements are met and certified. After the verification, the laboratory will conduct a management review meeting to discuss issues to show improvement and predict concerns. Once the laboratory quality management system is implemented and all required preliminary tasks are met, the laboratory will apply to the accrediting body of their choice to begin its accreditation process. After applying, an evaluator will assess the laboratory's documentation to determine if the laboratory will receive the ISO accreditation for its intended scope.

5 CONCLUSION

Laboratory accreditation generally changes the way a laboratory operates. The status, function and paperwork procedures of the laboratory will represent some of the changes. However, ISO accreditation is not a one-time endeavor. It is a living, breathing, ever-changing system that will require the involvement of personnel running and maintaining the laboratory. Preparing for ISO/IEC 17025 accreditation is exceptionally challenging. It takes a lot of hard work and dedication to develop and implement all necessary policies and procedures to meet ISO accreditation requirements. For the NaFAA laboratory to be fully established and prepared to obtain accreditation, there will be many challenges along the way that could include setting up the laboratory's quality structure, validation of test methods, renewing the media, training laboratory personnel, and setting up the financial structure of the laboratory.

Setting up and implementing a QMS based on ISO/IEC 17025:2005 standards is one of the challenges the NaFAA laboratory will face in preparing to obtain accreditation. Documentation development is the most time consuming and challenging aspect of setting up a quality management system. NaFAA laboratory lacks a Quality Plan, Quality Manual, Standard Operating Procedures (SOPs), and forms to support its accreditation process. The creation of these documents will require the knowledge of laboratory personnel as the development of the quality system will impact the workload. Therefore, the NaFAA laboratory will need to recruit additional personnel to maintain the QMS and the guidance of a consultant for the implementation process. To get familiarized with the ISO system implementation, a structured approach should be adopted while implementing the ISO/IEC 17025:2005 standard in the NaFAA laboratory. The appointment of a quality manager and the technical management of the laboratory by top management is one crucial step when planning the implementation of an ISO/IEC quality system. NaFAA does not have a quality manager and will therefore need to appoint one. The appointed quality manager should coordinate all the work and determine what resources will be needed to implement the system. The NaFAA laboratory should use gap analysis to devise a plan for the implementation of the standard. The plan should address the preparation of procedures and work instructions and how the work will be distributed among the staff. Implementation of the standard involves much work, and the role of the quality manager is to synchronize the laboratory work and the implementation program. Laboratory personnel should develop a new timetable for their work to accommodate space for the implementation program as they will develop the new procedures. The quality manager should guide them and inspect the appropriateness of the written documents.

The validation of test methods could be another challenge that will influence the NaFAA laboratory’s ISO/IEC 17025 accreditation preparation process. Ten standard testing methods were suggested for use by the NaFAA laboratory. Therefore, validation for accuracy and precision is required because there is no guarantee that operators' skills or performance of instruments are the same as those used to generate the standard validation. Additionally, personnel at the NaFAA laboratory are not trained to carry out microbiological testing on these methods. Therefore, laboratory staff need to receive adequate training on these methods before validating them for use. The laboratory should determine parameters like bias, selectivity, and detection limit before validation.

The old culture media packaged in the NaFAA quality control department could affect the laboratory preparation for ISO/IEC 17025 accreditation. The culture media has been packaged for two years in the department and may be outdated. Therefore, a quality verification test must first be performed to determine if the media is still suitable for its intended use. Several characteristics may be assessed when determining if the media, either liquid or solid, is suitable for its intended use. Characteristics that international organizations have recommended include drying and cracking, color changes, uneven filling or insufficient amount, growth, colony size and morphology, turbidity, volume, excessive moisture or dehydration, noticeable precipitants, biochemical responses, and contamination (CSLI, 2004; ASM, 2012). To evaluate for these characteristics in the NaFAA laboratory, the media should be separated into three distinct categories (nonselective solid, nonselective liquid, and selective liquid) and then assessed based on the category by specific characteristics for both quality and growth. The characteristics that should be evaluated for nonselective solid media (Tryptic Soy Agar – TSA) should include cracking, drying/thinning of agar, color, pH, and contamination. Acceptable growth for nonselective solid media must be evaluated based on the econometric method of growth determination utilizing absolute growth index (AGI) scores. The characteristics for evaluating the non-selective liquid media (Tryptic Soy Broth TSB and Buffered Peptone Water BPW) should include color, approximate volume loss, pH, and contamination. Finally, the characteristics for evaluating selective media should include color, volume loss, pH, and contamination.

Personnel at the NaFAA laboratory have not received any microbiological training. This could include another factor that could slow down the preparation process as it requires a lot of training and verification that all activities be performed according to the ISO standard in the laboratory. Therefore, the NaFAA laboratory needs to train its laboratory employees to operate laboratory procedures and estimate measurement uncertainty. A temperature monitoring training program must also be carried out. The laboratory should also ensure that newly recruited personnel work under the supervision of experienced staff.

Finally, the NaFAA laboratory could experience some issues regarding the financial structure or the income of the laboratory. In 2018, a memorandum of understanding was reached between the Liberian government and the government of Iceland that included a grant of 3.1 million dollars in revamping the Liberian fisheries sector. The fishery authorities planned to set aside about 35% of the grant to set up the microbiology laboratory and finalize its quality management system to prepare the laboratory for ISO/IEC 17025 accreditation. However, Liberia did not receive the funds, and the agreement has been terminated. Therefore, the NaFAA laboratory currently does not have the necessary funding to run a microbiology laboratory in line with ISO/IEC 17025 towards obtaining accreditation. There still could be a way for the laboratory to be financially structured to generate income to guide them through the accreditation process.

It is recommended that the NaFAA laboratory have its account outside of the institution account, and the laboratory director should manage the account to ensure direct access to funds and to be aware of its monthly income and expenditure. The laboratory could consider having fisheries inspectors/officers as its customers. The inspectors will collect samples from the fish processing establishments and bring them to the laboratory. The inspectors will charge the fish industries,

and the industries will pay for the services in the laboratory's account. The laboratory could also seek funds from other sources.

NaFAA microbiology laboratory will be laid out within a few months. Following that, testing procedures and the laboratory's quality system must be completed and activated to reach accreditation according to the ISO 17025 standard to fulfill international trade requirements. This road map analysis shows that the NaFAA laboratory will need to develop a QMS based on the ISO/IEC 17025:2005 standard and implement it. Some of the standard clauses are already being operated in the quality control department. However, they require modifications to comply fully with the requirements. The summary of the evaluation of the existing system can be used as a starting point to fill the gap between the existing conditions and recommended requirements.

With the implementation of ISO/IEC 17025:2005 at the microbiology laboratory of NaFAA, the laboratory, the personnel, and the clients will enjoy various benefits including better traceability, involvement of personnel in decision-making processes, acknowledgement of testing competence, a benchmark for performance, marketing advantages, international recognition, risk minimization, customer confidence and cost reduction. This feasibility study has shown that the accreditation process is time consuming and demanding. The guidelines developed in this report for implementing ISO/IEC 17025:2005 standards in the NaFAA microbiology laboratory highlight various requirements that need to be fulfilled before the laboratory will be prepared for assessment and accreditation. Laboratory accreditation is a challenging process. The financial commitment of top management and staff involvement will be necessary to develop and implement a QMS based on the ISO/IEC 17025:2005 standard in the NaFAA laboratory.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisors, Mr. Margeir Gissurarson and Ms. Halla Halldórsdóttir, for their enthusiasm, patience, insightful comments, helpful information, practical advice, and unceasing ideas that have helped me tremendously in this project.

I also wish to extend my sincere thanks to Ms. Mary Frances Davidson, Director of the GRO Fisheries Training Programme (GRO-FTP) and her entire staff for making this dream of attending this training program a reality and the moral and financial support for the time spent here in Iceland. I am also grateful to lecturers and staff at MATIS - Icelandic Food and Biotech Research & Development for transmitting relevant knowledge during the course's theoretical component.

I would like to express my special thanks of gratitude to Stefán Úlfarsson for his insightful comments and invaluable guidance. His vision, sincerity, and motivation have deeply inspired me.

I am deeply indebted to the National Fisheries and Aquaculture Authorities (NaFAA) for approving me and supporting my participation and showing confidence in me to represent the Institution in the GRO-FTP 2021 program. My profound acknowledgement goes on to Director William Y. Boeh, Deputy Director-General for Technical Services of NaFAA, my father, mentor, and motivational factor who granted me the opportunity to work in the Liberian fisheries.

I also want to acknowledge the GRO-FTP 2021/2022 fellows for the friendship developed over these months, which strengthened and helped us overcome all the difficulties and make our time in Iceland a memorable one. A special thanks to Mariam Oluwakemi Emmanuel, who became a very good friend.

Finally, to my wonderful family for their unconditional support, words of encouragement, and prayers throughout my time in Iceland.

REFERENCES

A2LA. (2015a). A2LA: I105 – Typical Steps in Preparing for the Accreditation Process, 1-6. *American Association for Laboratory Accreditation*.

A2LA. (2016). Food Microbiology Program Requirements. "AOAC INTERNATIONAL Accreditation Criteria for Laboratories Performing Food Microbiological Testing.”. *THE AMERICAN ASSOCIATION FOR LABORATORY ACCREDITION.* Retrieved from https://www.a2la.org/.

A2LA. (2016a). A2LA: C204 Specific Checklist - Combined ISO/IEC 17025 and Food and Pharmaceutical Testing Laboratory Accreditation Program Requirements. *The American Association for Laboratory Accreditation*.

Africa, A. (2019). *Fish-Drained Senegal to Exploit Liberian Waters Through New Agreement With Governmen.*

Allen, L. C. (2013). "Role of a Quality Management System in Improving Patient Safety – Laboratory Aspects. *Clinical Biochemistry*.

AOAC. (2015). AOAC International: Guidelines for Laboratories Performing Microbiological and Chemical Analysis of Food, Dietary Supplements, and Pharmaceuticals. *AOAC international journal*.

ASM. (2012). Guidelines for Assuring Quality of Medical Microbiology Culture Media,. (M. Arghyros, G. Douglass, M. Locher, P. Mugg, D. C. Myatt, T. Olma, . . . I. Wilkinson, Eds.) *. Australian Society for Microbiology, Inc.*

Bell, L. (2019). EssentialOmega 3 Fatty Acids Benefits from fish Oil and other Rich Sources. *EssentialOmega 3 Fatty Acids Benefits from fish Oil and other Rich Sources*.

Bureau of National Fisheries. (2014). Fisheries and Aquaculture Policy and Strategy. *Fisheries and Aquaculture Policy and Strategy*.

CSLI. (2004). Quality Control for Commercially Prepared Microbiological Culture Media Approved Standard M22-A3. *The National Commitee for Clinical Laboratory Standards*.

ECCBC. (2018). Annual Report. *Equatorial Coca Cola Bottling company*.

Eurachem. (2013). Accreditation of Microbiological Laboratories.

EURO. (2017). Handbook ISO/IEC 17025:2017,. *Eurolab. Belgium*, 1-32. Retrieved from http://www.eurolab.org/documents/.

FAO. (2020). Fish Seafood - Food Suppy quantity. *Journal of World fisheries and Aquaculture*. Retrieved from http://www.fao.org/faostat/en/#data/FBS

FAO. (2021). Liberia - Agricultural livelihoods and food security in the context of COVID-19. *Monitoring report, january 2021*.

FDA. (2011). Public Law 111-353, 111th Congress, an Act. U.S. Government Publishing Office. *FDA Food Safety Modernization Act*.

Grochau, I., Ferreira, C., Ferreira, J., & Caten, C. t. (2010). Implementation of a Quality Management System in University Test Laboratories. A Brief Review and New Proposal. *Accredition and Quality Assurance*, 681-689. doi:doi:10.1007/s00769-010-0713-6

Honsa, J. D., & McIntyre, D. A. (2003). ISO 17025: Practical Benefits of Implementing a Quality System. *Journal of AOAC International*, 1038-1044. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14632407.

ILAC. (2001). Why Become an Accredited Laboratory. *IALC Publications, International*.

ISO 17025. (2005). GENERAL REQUIREMENTS FOR THE COMPETENCE OF TESTING AND. *PECB-WHITEPAPER*.

ISO 17025. (2005). ISO 17025, GENERAL REQUIREMENTS FOR THE COMPETENCE OF TESTING AND CALIBRATION LABORATORIES. *PECB- WHITEPAPER*.

ISO 17025. (2017). General Requirements for the testing and a calibration Laboratoy. (3. edition, Ed.) *International Standard Organization*.

ISO/ IEC 17025. (2005). General requirements for the competence of testing and calibration laboratories. *Official Journal of the ISO*.

ISO/IEC 17025(E) clause 6.2. (2017). *General requirements for the competence of testing and calibration laboratories.*

NaFAA. (2018). Annual Report. Monrovia. *Annual Report. Monrovia*.

NaFAA. (2019). *The Fisheries and Aquaculture Management Act of Liberia.* Monrovia, Liberia: Ministry of Foreign Affairs .

Negri, S. (2009). Food Safety and Global Health. *An International Law Pespective, 3*.

PJLA. (2009). Steps to ISO/IEC 17025 Accreditation. *Perry Johnson Laboratory Accreditation, Inc. Troy, MI*.

Rippen, T. E., & Skonberg, D. I. (2012). Handling of Fresh fish. *Handling of Fresh fish*.

Rodima, Ako, Vilbaste, M., Saks, O., Jakobson, E., Koort, E., & Viljar Pihl, L. (2005). Quality System in a University Environment. *Accreditation and Quality Assurance*, 369-372. doi:doi:10.1007/s00769-005-0011-x.

Rowley, D. A. (2005). *Complying with ISO 17025.* United Nations Industrial Development .

SADCAS. (2018). Sadcas Policy – ISO/IEC 17025:2017 Transition. *Southern American Development Community Accreditation Service, TR14*.

Sampaio, P. P. (2009). "ISO 9001 Certification Research: Questions, Answers and Approaches. *International Journal of Quality Reliability Management*.

Scholaert, F. (2020). Marketing of and trade in fishery and aquaculture products in te EU. *Marketing of and trade in fishery and aquaculture products in te EU*.

Tavares, J., Martins, A., Fidalgo, L. G., Lima, V., Amaral, R. A., Pinto, C. A., . . . Saraiva, J. A. (2021). Fresh Fish Degradation and Advances in Preservation Using Physical Emerging Technologies. *US National Library of Medicines, National Institute of Health*.

Vlachos, N. A., Michail, C., & Sotiropoulou, D. (2002). Is ISO/IEC 17025 Accreditation a Benefit or Hindrance to Testing Laboratories? the Greek Experience. *Journal of Food Composition and Analysis*, 749-757. doi:doi:10.1006/jfca.2002.1097

WHO. (2007). Guidelines on Establishment of Accreditation of Health Laboratories. *World Health Organization*.

WHO. (2009). Laboratory Quality Management System. *Clinical and Laboratory Standards Institute, WHO Laboratory Center*.

WHO. (2011). Laboratory Quality Management System. *World Health Organization*.

Zapata-García, D., Llauradó, M., & Rauret, G. (2007). "Experience of Implementing ISO 17025 for the Accreditation of a University Testing Laboratory. *Accreditation and Quality Assurance*, 317-322. doi:doi:10.1007/s00769-007-0274-5

APPENDICES

Appendix 1: Verification forms

QA-SL-001-V-01 SAMPLE LOG FORM/BOOK

No.	Date	Customer/Client Name	Sample Description	Sample Code	Comment
1					
2					
3					
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12					
13					
14					
15					
16					
17					
18					
19					
20					

QA-ARC-001-V-01 AUTOCLAVES RUN CYCLE

Model: …………………………….

Serial No.…………………………

Last Date of Service: ……………

Date	**Time**	**Run No.**	**Content of Load**	**Cycle Type**	**Sterilization Temp (°C) & Time**	**Indicator**	**Comment**	**Signed**
			BPW; PCA; LST; BGLB; EC; BPA; NA;	Gravity, Vacuum, Liquid		Autoclave tape Ver. Indicator		

QA-ET-001-V-01: EQUIPMENT TEMPERATURE CHECK FORM

Equipment Name: ………………… **Parameter: …………………………**

Equipment No: ……………………. **Year: …………………………………**

Date	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
1												
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QA-CMP-001-V-01: CULTURE MEDIA PREPARATION FORM -1

QUALITY CONTROL SHEET

Month…………………. Year…………………

This form should be used each time culture media is prepared (with each batch). Its purpose is to maintain a record of Technologists who make media, plus associated QA/QC.

Date	**Technician Name**	**Media made**	**Manufacturer**	**Batch number**	**Batch expiry Date**

Reviewed by Section Head……………… Checked by QA/QC Officer………

Date………………… Date…………

NB: 1. This sheet is audible and therefore must be filled and archived on file all the time.

2. A list indicating the names and initials of all staff in the section should be available.

QA-CMC-001-V-01 **CULTURE MEDIA CONTROL FORM -2**
CONTROL OF CULTURE MEDIA

This form should be used each time media is prepared (with each batch) and used to grow organisms to check its quality and ability to support growth.

NAME OF MEDIUM………………. PREPARED BY……………..
DATE PREPARED………………… EXPIRY DATE……… LOT No………..

Date	Sterility of medium checked	Standard organisms set on each plate	Results of Gram -ve bacteria (Growth/No growth	Results of Gram +ve bacteria (Growth/No growth	PH	Technician Initial	Comments

Received by……… (Section head) Checked by………..(QA/QC Officer)

Date………… Date………………….

NB:1. This sheet is audible and therefore must be filed and archived on file All the time.

2. A list indicating the initials of all staff in the section should be available.

QA-THR-001-V-01: DAILY TEMPERATURE AND HUMIDITY RECORD SHEET

Temp. Limit……… **Relative Humidity………….**

Month……………... **Frequency:** Daily (Every four hour)

Equipment ID No… e.g. (9:00-10:00, 13:00-14:00, 17:00-18:00

Date	Time	Observed Temp.		Temp. Difference (℃)	Calculated RH (%)	Done by	Checked by	Remark
		Dry bulb (℃)	Wet bulb (℃)					

QA-PH-OO1-V-01: pH METER CALIBRATION CHECK LOG

EQUIPMENT No………………...

Date	Time	PH Reading in 7.0 Buffer Solution	PH Reading in 4.0 Buffer Solution	Meter Calibrated? (Yes/No)	Signed

QA-BC-001-V-01: BALANCE CALIBRATION CHECK LOG

EQUIPMENT No……………

Date	Level	Zero	+Ve weight	-Ve weight	Zero	Comment	Signed

QA-DOS-001-V-01 DRY OVEN STERILIZATION CHECK LOG

Date	**Glass-ware type**	**Start Time**	**Stop Time**	**Sterilization tape**	**Signed**
				Dry ster. tape	

Noah

QA-LA-001-V-01 LABORATORY ANALYSIS WORK SHEET

SAMPLE DESCRIPTION………………………… DATE………………………………

ANALYST………………………………………... TIME………………………………

HEAD OF LABORATORY………………………..

Sample	**pH**	**Total Plate Count 48h**	**Vibrio Spp. (37°C)**		**Total Coliform (37°C)**				**Coliforms (37°C)**				**Faecal (44°C)**			***Listeria monocytogenes* (37°C)**					**Salmonella spp. (37°C)**								**Yeast (22 °C)**	**Mould (22°C)**
Log No.	Te.	22°C () 30°C () 37°C ()	ASPW: 48h		LST: 24/48h				BGLB 48h				EC:24			Frazer		MOX	Ct	Gc	TT				RV/SEL				DRBC:	DRBC:
					0	1	2	3	0	1	2	3	0	1	2	24h	48h	48h	5 colonies		BSA, XLD, HE				BSA, XLD, HE				120h	120h
																					24		48		24		48			
																					1	2	1	2	1	2	1	2		
Control 1					E. coli (-c)				E. coli (-c)				E. coli (-c)			Listeria					BSA: XLD: HE									
Control 2					E. aerog.				E. aerog.				E. aerog.								TSI: B/S LIA: B/S									
Date																														
Time																														
Sign																														

LEGEND: (-C) ➡ Negative control; Ct ➡ Catalyst test; Gc➡Gram counting/ staining; MOX➡ Modified Oxford Agar

Date………………………………….

Signature………………………………………………………Time…………………………………

QA-MRM/A-001-V-01 **MANAGEMENT REVIEW MEETING FOLLOW UP ACTION**

DATE………………

1. **Status of actions from previous meeting**

Prior Action Items Review
Prior Meeting Minutes Review

2. **Changes in external and internal issues**

Discussion		
Conclusion		
Action items ☐ None	**Person Responsible**	**Deadline**

3. **Information on the performance and effectiveness of the Quality Management System (QMS)**

Discussion		
Conclusion		
Action items ☐ None	**Person Responsible**	**Deadline**

4. **Customer satisfaction and feedback from interested parties**

Discussion		
Conclusion		
Action items ☐ None	**Person Responsible**	**Deadline**

5. **Quality objectives and Key Performance Indicators (KPIs)**

Discussion		
Conclusion		
Objective	**KPI**	**Status**

Action items ☐ None	**Person Responsible**	**Deadline**

QA-IAS-001-V-01: AUDIT SCHEDULE

Area/Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Date
Management Requirement													
Technical Requirements													

QA-IAC-001-V-01 INTERNAL AUDIT CHECKLIST

Clause of ISO 17025	Title of element and Description	Compliance	Non-Compliance	Detail of Non-Conformance	Time fame for Corrective Action

Confirmation of the findings

Auditor: …………………… Signature: ……………… Date: ……………………

Head of Laboratory: …………… Signature: ……………. Date: ………………...

QA-TRN-001-V-01 TRAINING PROGRAM FOR NEW LABORATORY PERSONNEL

NAME OF TRAINEE: ………………………………………………………………………………………

AREA OF WORK: ……………………………………………………………………………………….

NAME OF TRAINER: ………………………………………………………………………………………

Date	Trainee	Trainer	Description of training	Training Period	Trainee's recommendation	Trainer's recommendation after training

Name of Trainee………………… Signature………………. Date………………….

Evaluation criteria: A trainee whose test results of the analysis do not differ from expected values shall be considered competent.

Verification by Head of Laboratory:

Name……………………… Signature……………… Date…………………

National Fisheries and Aquaculture Authority (NaFAA)
Department of Research and Statistics
Quality Control Laboratory

QA-FRM-001-V-01 **LABORATORY PERSONNEL DAILY LOG**

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National Fisheries and Aquaculture Authority (NaFAA)

Quality Control Department

Microbiology Laboratory

MICROBIOLOGY QUALITY LABORATORY
STANDARD OPERATING PROCEDURES (SOPS)

VERSION No: 01   DATE: March 04, 2018

SOP-TM/E-001-V-01

Prepared By______________________________________ Date______________________
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Authorized By____________________________________ Date______________________
NaFAA Director General

Approved By______________________________________ Date______________________
NaFAA Laboratory Director

Appendix 2: Standard Operating Procedures

MICROBIOLOGICAL METHODS FOR ANALYSIS OF FISH PRODUCTS AND WATER

Scope

This guide is developed within the GRO-FTP Fisheries Training Program framework in Iceland. It serves as a guide for the Quality Control Laboratory of the National Fisheries and Aquaculture Authority (NaFAA) for testing and analysing seafood and water quality for ten microbiological methods in meeting quality system requirements of local and international trade. The Quality Control Laboratory (NaFAA) ensures that quality fish and fishery products reach both the Liberian and international markets and that those products are fit for purpose and human consumption.

This manual provides step-by-step directions on laboratory best practices for the authorized control of water quality and fishery products. It gives detailed supervision on necessities for microbiological testing. It provides:

- Details on test analysis
- Apparatus functions
- Spike samples and reference materials
- Cultural media and its preparation
- Other quality assurance measures

It will enable management to make informed decisions on the practicality of the experiment and the scale of action. This guide intends to support laboratory technicians in carrying out their test analysis.

Disposal of Samples:

All liquid samples should be disposed of at an acid sink.

Dispose of solid samples in a trash container. All contaminated or used microbiological media and bacteria cultures shall be autoclaved at 121℃ prior to disposal in a trash container.

A2.1 Aerobic mesophilic plate count

Indicates microbial counts for quality assessment of fishery products

Equipment:

Refer to Appendix 4 - Equipment, Materials, and Glassware

Medium:

- Plate count agar (PCA)
- Peptone water 0.1%
- (Chapter 2 for composition of medium)

Procedure:

1. Sampling

A representative sample of fish products should be sent to the Laboratory. It should not be damaged or changed during transport storage.

2. Preparation of homogenate sample

Cut fish samples into pieces on a surface cleaned with ethanol to reduce the bacteria load and put them into a sterile blender jar. Blend for 2 minutes at low speed. Make a 1:10 dilution of the well mixed sample to the desired volume of diluent.

Weigh 20g of fish sample into a stomacher bag and add 180ml of diluent (peptone water). Mix in the stomacher for 60 seconds.

3. Serial dilution

If the count is expected to be more than $2.5x10^3$ per ml or g, prepare decimal dilutions as follows. Shake each dilution 25 times in 30 cm arc. For each dilution use fresh sterile pipette. Pipette 1ml of homogenate sample into a tube containing 9ml of the diluent (peptone water).

Repeat using a third, fourth or more tubes until the desired dilution is obtained.

4. Pour plating

Label all Petri plates with the sample number, dilution, date, and any other desired information. Pipette 1ml of the homogenate sample and such dilutions selected for plating into a petri dish in duplicate. Pour into each petri dish 10 to 12ml of the molten PCA (cooled to 42-45°C) within 15 minutes from the preparation of the original dilution. Mix the media and dilutions by swirling gently clockwise and anti-clockwise, to and fro thrice, and ensuring that the contents do not touch the lid. Allow setting.

Incubation

Incubate the prepared dishes, inverted at 35°C for 48±2 hours. (Or the desired temperature as per food regulation, e.g., in case of packaged drinking water).

Counting Colonies

Following incubation, count all the colonies on dishes containing 30-300 colonies and record the results per dilution counted.

Calculation

In dishes that contain 30-300 colonies, count the actual number in both plates of a dilution and as per the formula given below:

$$\boldsymbol{N} = \frac{\Sigma \text{C}}{(\text{N1} + 0.1\text{N2})\text{D}}$$

ΣC is the sum of colonies counted on all the dishes retained

N1 is the number of dishes retained in the first dilution

N2 is the number of dishes retained in the second dilution

D is the dilution factor corresponding to the first dilution

E.g.

At the first dilution retained (10^{-2}):165 & 218 colonies

At the second dilution retained (10^{-3}) 15 & 24

$$\boldsymbol{N} = \frac{165 + 218 + 15 + 24}{[2 + (0.1\text{x}2)\text{x } 10\text{x} - 2]}$$

$$\boldsymbol{N} = \frac{422}{0.022} = 19182$$

Rounding the result to first two digits gives 19000 CFU.

Expression of Result

Aerobic (mesophilic) Plate Count = 19000CFU/g or $1.9\text{x}10^4$ CFU/g

Or

If plates from all dilutions contain less than 30 colonies and inhibitory substances have not been detected, the result is expressed as less than 1 x 10^1 CFU per g or ml.

If plates from the lowest dilutions contain less than 30 colonies, record the actual number, and calculate as above but express results as CFU per g or ml.

Note: *This method has some limitations as all other methods. Microbial cells often occur as clumps, clusters, chains, or pairs in foods and may not be well distributed irrespective of the mixing and dilution of the sample. Moreover, the single agar medium used in the conditions of incubation, aeration, etc., is not conducive to the growth of various populations of the bacteria that may be present in a food sample.*

For statistical reasons alone, in 95% of cases, the confidence limits of this test vary from ± 12% to ± 37%. Even more significant variation may be found in practice, especially among results obtained by different microbiologists.
(Corvell and Morsettle, J. Sci. Fd. Agri., 1969, vol. 20p 573)

References

Official of Analysis of AOAC International (1995). 16th Edition. By patricia cuniff. Publish by AOAC International. Virginia. USA. Test 17.2.01 p.3-4.

Compendium of Methods for the Microbiological Examination of Foods. (1992) Carl Vanderzant and Splittstoesser Eds. Washington D.C. p. 75-87

Bacteriological Analytical Manual (1992) 6th Edition. Artington, V.A. Association of Official Analytical Chemists for FDA, Washington, D.C. p. 17-21.

Microbiology- General guidance for the enumeration of Microorganisms-Colony count technique at 35°C (first revision) IS5402-2002, ISO4833:1991. Bureau of Indian Standards, Manak Bhavan, 9 Bhadur Shah Zafar Marg, New Delhi 110002.

A2.2 Detection, determination and confirmation of coliforms, faecal coliforms, and Escherichia coli *in fish.*

Equipment

Refer to Appendix 4 - Equipment, Materials, and Glassware

Culture media and reagents as in A2.1

Procedure

Test for Coliforms

Coliforms in fish may be enumerated by the solid medium method or the Most Probable Number (MPN) method.

- **Solid medium method**
  1. Prepare fish homogenate as directed under 1.3.2
  2. Serial dilutions as directed under 1.3.3
  3. Pour Plating

  Pipette 1ml of the fish homogenate (prepared sample) and each dilution into each appropriately marked duplicate Petri dishes.

  Pour into each petri-dish 10-12ml of Violet Red Bile Agar (VRBA) cooled to 48°C and swirl plates to mix. Allow solidifying.

  Incubate the dishes, inverted at 35°C for 18 to 24 hours.

  4. Count colonies

  Following incubation, count all purple-red colonies in color, 0.5mm in diameter or larger, and are surrounded by a zone of precipitated bile acids. Optimally the plates should have 30 to 300 colonies.

  5. Calculations

  Multiply the total number of colonies per plate with the reciprocal dilution used and report as coliforms per g or ml.

- **Most Probable Number method**

This method is valuable in samples with low coliform density because a higher quantity of samples can be used for examination. It is based on the probability statistics wherein aliquots of decimal volumes/dilutions of the sample are transferred to several (1 to 5) tubes of a specific medium. Positive tubes are scored, and the MPN estimate is directly made using Table A2.

1. Prepare fish homogenate as directed under 1.3.2
2. Serial dilutions as directed under 1.3.3
3. Inoculation

Inoculate each of 3 tubes of LST broth (containing inverted Durham tubes) with 1ml of fish sample homogenate (1:10).

Carry out the same operation from the first (1 in 100) and the second (I in 1000) dilution tubes. Use a fresh sterile pipette for each dilution.

4. Incubate the LST tubes at 35±0.5°C for 24 and 48 hours.
5. Presumptive test for coliforms

Record gas production after 24 and 48 hours and incubate negative tubes for further 24 hours. Then record tubes showing gas production.

6. Confirmed test for coliforms

- Transfer a loopful from each gas positive tube of LST to a separate tube of BGLB broth.
- Incubate the BGLB broth tubes at 35±0.5°C for 48±2h. The formation of gas confirms the presence of coliform bacteria.
- Record the number of positive tubes that were confirmed positive for coliform.

7. Calculation - Note the MPN appropriate to the number of positive tubes from the (table 1).

   For example:

   3 in 1:10; 1 in 1: 100 and 0 in 1:1000. The table shows that MPN=43 coliforms per g or ml.
   Coliforms = present/absent per gram

Table A2. Most Probable Number (MPN) per 1g of sample, using 3 tubes with each of 0.1, 0.01, and 0.001 g portions.

Positive tubes				**Positive tubes**				**Positive tubes**				**Positive tubes**			
0.1	**0.01**	**0.001**	**MPN**	**0.1**	**0.01**	**0.001**	**MPN**	**0.1**	**0.01**	**0.001**	**MPN**	**0.1**	**0.01**	**0.001**	**MPN**
0	0	0	<3	1	0	0	9.1	2	0	0	9.1	3	0	0	23
0	0	1	3	1	0	1	14	2	0	1	14	3	0	1	39
0	0	2	6	1	0	2	20	2	0	2	20	3	0	2	64
0	0	3	9	1	0	3	26	2	0	3	26	3	0	3	95
0	1	0	3	1	1	0	15	2	1	0	15	3	1	0	43
0	1	1	6.1	1	1	1	20	2	1	1	20	3	1	1	75
0	1	2	9.2	1	1	2	27	2	1	2	27	3	1	2	120
0	1	3	12	1	1	3	34	2	1	3	34	3	1	3	160
0	2	0	6.2	1	2	0	21	2	2	0	21	3	2	0	93
0	2	1	9.3	1	2	1	28	2	2	1	28	3	2	1	150
0	2	2	12	1	2	2	35	2	2	2	35	3	2	2	210
0	2	3	16	1	2	3	42	2	2	3	42	3	2	3	290
0	3	0	9.4	1	3	0	29	2	3	0	29	3	3	0	240
0	3	1	13	1	3	1	36	2	3	1	36	3	3	1	460
0	3	2	16	1	3	2	44	2	3	2	44	3	3	2	1100
0	3	3	19	1	3	3	53	2	3	3	53	3	3	3	>1100

Test for Faecal Coliforms

1. Proceed as directed from 2.2.1.C.1
2. Transfer a loopful from each gas positive tube of LST to a separate tube of EC both.
3. Incubate the EC tubes at 45±0.2°C in a water bath for 24±2hours.
4. Submerge broth tubes so that the water level is above the highest level of medium. Record tubes showing gas production.
5. Calculations: As directed under the test for coliforms.

Test for Escherichia coli

1. Proceed as directed under test for faecal coliforms.
2. Streak one plates L-EMB from each positive BGLB tube to obtain discrete colonies.
3. Incubate inverted plates at 35 ± 0.5°C for 24±2 hours.
4. Examine plates for typical nucleated dark-centered colonies with or without sheen. If typical colonies are present, pick two from each EMB plate by touching a needle to the colony's center and transferring to a PCA slant.
5. Incubate slants at 35 ± 0.5°C for 18 to 24 hours
6. Transfer growth from PCA slants to the following broth for the biochemical test (view chapter 4 under Biochemical tests).

   Tryptone broth: Incubate for 24±2 hours at 35 ± 0.5°C and test for indole.
   MR-VP Medium: Incubate 48±2 hours at 35 ± 0.5°C. Aseptically transfer 1ml of culture to a 13x100 mm tube and perform the Voges Proskauer test.
   Incubate the remainder of MR-VP culture an additional 48h and test for methyl red reaction.
   Koser citrate broth: Incubate for 96 hours 35 ± 0.5°C as recorded as + or – for growth.
   LST broth: Incubate 48±2 hours at 35 ± 0.5°C and examine for gas formation.
   Gram stain: Perform the Grain stain in a smear prepared from 18 hours PCA slant. The presence of small, red-colored rods confirms *Escherichia coli*.

7. Compute MPN of E. coli per g or ml considering gram-negative, non-spore-forming rods producing gas in lactose and biochemical types as follows (IMViC) (table A3)

Table A3. Microorganism and IMViC.

Indole	MR	VP	Citrate	Type
+	+	-	-	Typical E. coli
-	+	-	-	Atypical E. coli
+	+	-	+	**Typical Intermediate**
-	+	-	+	Atypical intermediate
-	-	+	+	Typical Enterobacter aerogenes
+	-	+	+	Atypical Enterobacter aerogenes

8. Calculations as per MPN table
9. Interpretation: Escherichia coli= x MPN/g

References

Official of Analysis of AOAC International (1995). 16th Edition. By patricia cuniff. Publish by AOAC International. Virginia. USA. Test 17.2.02 p.4-5.

Compendium of Methods for the Microbiological Examination of Foods. (1992) Carl Vanderzant and Splittstoesser Eds. Washington D.C. p. 325-341

Bacteriological Analytical Manual (1992) 6th Edition. Artington, V.A. Association of Official Analytical Chemists for FDA, Washington, D.C. p. 27-31.

A2.3 Detection and confirmation of Salmonella *species in fish*

Equipment:

Refer to Appendix 4 - Equipment, Materials, and Glassware

Culture Media:

- Lactose broth
- Trypticase Soy Broth
- Trypticase Soy Broth Containing Potassium Sulfite at a final concentration of 0.5%.
- Reconstituted Non-Fat Dry Milk
- 1% aqueous Brilliant Green Dye Solution
- Selenite Cystine Broth
- Tetrathionate Broth
- Xylose Lysine Deoxycholate (XLD) Agar
- Hektoen Enteric Agar (HEA)
- Bismuth Sulphite Agar (BSA)
- Triple Sugar Iron (TSI) Agar
- Lysine Iron Agar (LIA)
- Urea Broth
- Phenol Red Dulcitol Broth
- Phenol Red Lactose Broth
- Tryptone Broth
- KCN Broth
- Malonate Broth
- Buffered Glucose (MR-VP) Medium
- Brain Heart Infusion (BHI) Broth
- Buffered Peptone Water

Procedure:

1. Preparation of sample and pre-enrichment
    - Aseptically open the sample container and weigh 25g sample into a sterile empty wide-mouth container with a screw cap or suitable closure.
    - Add 225ml of sterile lactose broth to the sample. Buffered peptone water, Trypticase soy broth, and nutrient broth can be used for pre-enrichment. Make a uniform suspension by blending if necessary. Cap container and let stand at room temperature for 60 minutes. Instead of lactose broth, the recommended pre-enrichment broth for the following food samples is as follows:
        - Non-Fat milk and dry whole milk – Sterile water. Add 0.45 ml of 1% aqueous brilliant green dye before incubation.
        - Dried active yeast- Trypticase soy broth containing potassium sulfite at a final concentration of 0.5%
        - Milk chocolate- Reconstituted non-fat dry milk.
    - Shake and adjust pH (if necessary) to 6.8±0.2 with sterile 1N NaOH or 1N HCl.
2. Incubate at 35°C for 24±2 hours
3. Selective enrichment - Gently shake the incubated sample mixture and transfer 1 ml to 10 ml of selenite cystine broth and 1 ml to tetrathionate broth. Incubate for 24±2 at 35°C.

4. Selective media plating
   Vortex - mix and shake 3 mm loopful of incubated selenite cystine broth on selective media plates of XLD, HEA, and BSA. Repeat with a 3mm loopful of incubated tetrathionate broth.
5. Incubate plates at 35°C for 24±2 hours and 48±2 hours.
6. Observe plates for typical Salmonella colonies
   - On XLD (after 24hours)- Pink colonies with or without black centers.
   - On HEA (after 24hours)- Blue-green to blue colonies with or without black centers.
   - On BSA (after 24hours)- Brown, grey, or black colonies, sometimes with a metallic sheen. The surrounding medium is usually brown at first, turning black with increasing incubation time.
7. Treatment of typical or suspicious colonies

   Pick with needle typical or suspicious colonies (if Present) from each XLD, HEA, and BSA plate. Inoculate the portion of each colony first into a TSI agar slant, streaking slant, and stabbing butt and do the same into an LIA slant.

8. Incubate TSI, and LIA slants at 35°C for 24±2 hours and 48±2 hours, respectively. Cap tubes loosely to prevent excessive H2S production.

Table A4. Typical Salmonella reactions. A culture is treated as presumptive positive if the reactions are typical on either or broth TSI and LIA slants.

	TSI	**LIA**
Slant	Alkaline (red)	Alkaline (Purple)
Butt	Acid (Yellow)	Alkaline (Purple)
H_2S production (blackening in butt)	+ or -	+

9. Biochemical Tests

   Using sterile needle, inoculate a portion of the presumptive positive culture on TSI slant into the following broths. Incubate at 35°C for the specified periods of days and read for Salmonella typical reactions.

Table A5. Biochemical tests. Note: Majority of S. arizonae are atypical for these reactions.

Broth/Media	**Time of incubation**	**Results**
Urea broth	24±2h	Negative (no change in yellow color of medium)
Phenol red lactose broth	48±2h	*Negative for gas and /or acid reaction
Phenol Red sucrose broth	48±2h	*Negative for gas and /or acid reaction
Phenol red dulcitol broth	48±2h	*Positive for gas and /or acid reaction
Tryptone broth	24±2h	Negative for indole test
KCN broth	48±2h	Negative (no Turbidity)
Malonate broth	48±2h	*Negative (green color unchanged)
MR-VP medium	48±2h	Negative for VP test but positive for MR test

Table A6. Criteria for discarding Non-Salmonella Cultures. * Malonate broth positive cultures are tested further to determine if they are *Salmonella arizonae*. ** Do not discard positive broth cultures if corresponding LI agar cultures give typical Salmonella reactions; test further to determine if they are Salmonella sp. (See Table A5).

Test(s) or Substrate(S)	**Results**
Urease test	Positive (purple red)
Indole	Positive (red)
Flagellar test (polyvalent or Spicer-Edward	Negative (no agglutination)
Lysine decarboxylase test	Negative (yellow)
KCN broth	Positive (growth)
Phenol red lactose broth*	Positive (acid and/or gas)**
Lysine decarboxylase test	Negative (yellow)
Phenol red sucrose broth	Positive (acid and/or gas)**
Lysine decarboxylase test	Negative (yellow)
KCN broth	Positive (growth)
Voges- Proskauer test	Positive (red)
Methyl red test	Negative (yellow)

10. Serological Tests

To reduce the number of presumptive positive cultures (TSI positive and urease negative) carried through biochemical identification tests, the following serological flagellar (H) screening test may be carried out:

- Transfer 3mm loopful of culture into 5ml of BHI broth and incubate at 35°C until visible growth occurs (About 4-6 hours).
- Add about 2.5ml formalized physiological saline solution.
- Test with Salmonella flagellar (H) antisera. Positive cultures show visible agglutination.
- Further confirmation can be made by using Salmonella Polyvalent (O) antiserum.

11. Expression of Result: Salmonella = Present/Absent per 25g

References:

Official of Analysis of AOAC International (1995). 16th Edition. By patricia cuniff. Publish by AOAC International. Virginia. USA. Test 17.9.01 p.55-62.

Compendium of Methods for the Microbiological Examination of Foods. (1992) Carl Vanderzant and Splittstoesser Eds. Washington D.C. p. 371-422

Bacteriological Analytical Manual (1992) 6th Edition. Artington, V.A. Association of Official Analytical Chemists for FDA, Washington, D.C. p. 51-69.

A2.4 Detection and determination of pathogenic Vibrio *in fish*

Equipment:

Refer to Appendix 4 - Equipment, Materials, and Glassware

Culture Medium

- Thiosulphate Citrate Bile Salts Sucrose (TCBS) Agar
- Gelatin Phosphate Salt Broth and Agar.
- Klingler Iron Agar
- T1N1 Agar

Procedure:

1. Enrichment

Weigh 25g sample and transfer to 225ml of GPS broth. Incubate at 35°C for 6 to 8 hours.

2. Plating:

Prepare dried plates of TCBS and GPS agar medium. Transfer a loopful of the surface growth of the broth culture to the surface of the two-plating medium and streak in a manner that will yield isolated colonies.

Incubate plating medium for 18 to 24 hours at 35°C.

3. Interpretation:

Typical colonies of *V. cholerae* on TCBS agar are large (2 to 3 mm in diameter), smooth, yellow (occasional slow sucrose fermenters are green), and slightly flattened with opaque centers and translucent peripheries. On GPS agar, the colonies have a cloudy zone around them that becomes more definite after a few minutes of refrigeration. The colonies appear iridescent green to bronze-colored and finely granular in oblique light.

Typical colonies of *V. parahaemolyticus* on TCBS agar appear round, opaque, green, or bluish colonies, 2 to 3 mm in diameter.

4. Confirmation:

Subculture all suspect colonies of *V. cholerae* onto T2N1 agar and incubate at 35°C for 24 hours. Stab streak a KIA slant with the culture and incubate the KIA slant overnight at 35°C. *V. cholerae* cultures have an alkaline (red) slant and an acid (yellow) butt, no gas, and no blackening in the butt. Also, perform the string test on suspect cultures as follows. Emulsify a large inoculum from the T1N1 agar culture in a large drop of 0.5% sodium deoxycholate in 0.85% saline solution. Within 60 seconds, a mucoid mass forms, and this material strings when a loopful is lifted (up to 2 to 3 cm) from the slide. Further confirmation is by serological reactions.
Stab streak suspects colonies of *Vibrio* on the TSI slant and incubate overnight at 35°C. The typical reaction of *V. parahaemolyticus* is an alkaline slant and acid butt but not as or H2S production

5. Results:

Test for pathogenic *Vibrios* = Positive/Negative

References

Official Methods of Analysis of AOAC International (1995). 16th Edition. By patricia cuniff. Publish by AOAC International. Virginia. USA. Test 17.11.01 p.108-110.

Compendium of Methods for the Microbiological Examination of Foods. (1992) Carl Vanderzant and Splittstoesser Eds. Washington D.C. p. 451-473

Bacteriological Analytical Manual (1992) 6th Edition. Artington, V.A. Association of Official Analytical Chemists for FDA, Washington, D.C. p. 111-121.

A2.5 Estimation of yeast and molds in fish

Equipment:

Refer to Appendix 4 - Equipment, Materials, and Glassware

Media

- Potato Dextrose Agar
- Mycophilic Agar
- Antibiotic Solution

Procedure:

1. Prepare fish sample homogenate and decimal dilutions as directed under 1.3.2 and 1.3.3 respectively.
2. Pour plating:

- Label all Petri plates with the sample number, dilution, date, and any other described information.
- Pipette 1ml of the fish homogenate sample of such dilutions was selected for plating into a Petri dish in duplicate.
- Acidify PDA or malt agar with sterile 10% tartaric acid to pH 3.5±0.1.
- Do not reheat the medium once the acid has been added. Pour 10-12 ml of the agar medium (cooled to 45°C)—mix by swirling and allow to solidify.

  (OR)

- Add 2ml antibiotic solution to 100ml of plate count, mycophile or malt agar. Mix and pour 10-12ml of the agar medium cooled to 45°C—mix by swirling and allow to solidify.

3. Incubation:

Invert plates and incubate at 20 or 25°C for 2 to 5 to 7 days. Discard plates after seven days of growth are not observed. Observe plates every day and mark the colonies because sometimes fungal growth spreads to the entire plate and masks the colonies. Do not open the plates which are showing fungal sporangia.

4. Count colonies, multiply by the inverse of the corresponding dilution and report as yeast or mold count per g or ml.
5. Reporting: Yeast and Mold count = x/g

References

Compendium of Methods for the Microbiological Examination of Foods. (1992) Carl Vanderzant and Splittstoesser Eds. Washington D.C. p. 239-249

Bacteriological Analytical Manual (1992) 6th Edition. Artington, V.A. Association of Official Analytical Chemists for FDA, Washington, D.C. p. 227-230.

A2.6 Detection and confirmation of Listeria *monocytogenes in fish*

Warning: While testing *L. monocytogenes*, a properly equipped laboratory under the supervision of a skilled Microbiologist is recommended. The material used during testing is carefully disposed off after sterilization. Pregnant personnel may be asked to avoid handling *L. monocytogens* cultures and undertaking the tests.

Equipment:

Refer to Appendix 4 - Equipment, Materials, and Glassware

Culture media and reagents:

- Phosphate buffered peptone water
- Half Frazer broth
- Modified Oxford Agar
- PALCAM Agar
- Tryptone Soya Yeast Extract Agar
- Tryptone Soya Yeast Extract broth
- Sheep Blood Agar
- Carbohydrate utilization broth (Rhamnose and Xylose)
- Motility Agar
- CAMP Medium and test organisms
- Hydrogen peroxide solution

Preparation of test sample:

1. Take 25g of a well-mixed sample into a stomacher bag and use 225 ml of Half Frazer broth. Mix the sample for 2 minutes in a stomacher. Pour the contents into a wide mouth bottle aseptically and incubate at 30°C for 24±2h (a black coloration may develop).
2. Take one ml of the above culture and transfer it to 9ml of Frazer broth.
3. Incubate the inoculated tube at 37°C for 48±2h at 35-37°C.
4. From 24hours of Half Frazer broth and 48hours of Frazer broth, streak out culture on Modified Oxford Agar and PALCAM agar so that well-separated colonies are obtained.
5. Invert the plates and incubate at 35 or 37°C for 24hours and, if required, an additional 18h if growth is slight or no colonies appear. Examine the plates for colonies presumed to be *L. monocytogenes.*

Appearance of colonies:

The colonies are small (1mm) greyish surrounded by a black halo on Modified Oxford agar.

After 48hours, the colonies turn darker with a possible green luster. They are about 2mm in diameter with black halos and a sunken center.

On PALCAM agar, after 24hours, the colonies appear 1.5 to 2mm in diameter greyish-green or olive green, sometimes with a black center and always surrounded by a halo and depressed center.

Confirmation of Listeria species:

1. Select five typical colonies from one plate of each medium. If presumed colonies are less than five on a plate, take all of them.

2. Streak the selected colonies from each plate onto the surface of a well-dried TSYEA to obtain well-separated colonies. Invert the plates and incubate at 35 or 37°C for 18 to 24 hours or until satisfactory growth.
3. Typical colonies are 1mm to 2mm in diameter, convex, colorless, and opaque with an entire edge.
4. Carry out the following tests from colonies of a pure culture on the TSYEA.

Catalase reaction:

- With the help of loop, pick up an isolated colony and place it in H_2O_2 solution on a glass slide. Immediate production of gas bubbles indicates catalase positive reaction.

Gram staining:

- Perform Gram staining on a colony Listeria are Gram positive slim short rods.

Motility Test:

- Take colony from TSYEA plate and suspend it TSYE broth. Incubate at 25°C for 8 to 24 hours until the cloudy medium is observed. Take a drop of culture and place it on a glass slide. Cover the top with a coverslip and observe under a microscope. *Listeria* is seen as slim rods with tumbling motility (cultures grown above 25°C fail to show this motion). Compare them with a known culture – *cocci* or large rods with rapid motility are not *Listeria.*
- As an alternative, stab motility agar tube with an isolated colony from TSYEA and incubate at 25°C for 48 hours. A typical umbrella-like appearance around the stab indicates positive motility culture. If growth is not positive, incubate up to five days and observe the stab again.

Confirmation of Listeria monocytogenes:

Heamolysis test:

- Take a colony from TSYEA and stab it on a well-dried Sheep Blood agar plate surface. Simultaneously stab positive (*L. monocytogenes)* and negative (*L. innocua)* control cultures. Invert the plates and incubate at 35°C or 37°C for 24±2hours. Examine the plates.
- *L. monocytogenes* show clear light zones of beta heamolysis.
- *L. Innocua* does not show any heamolysis. Examine the plates in bright light to compare test cultures with the controls.

Carbohydrate utilization:

- Inoculate each carbohydrate utilization broth (rhamnose and xylose) with culture from TSYE broth and incubate at 35°C or 37°C for up to 5 days. The appearance of yellow color indicates a positive reaction within 24 to 48 hours.

CAMP test:

- On a well dried surface of Sheep Blood Agar streak each of the *Staphylococcus aureus* and *Rhodococcus equi* cultures in single lines and parallel to each other and diametrically opposite, a thin even inoculum is required.
- Streak the test strain separated in a similar manner at right angles to these cultures as that the test strain and *S. aureus and R.equi* cultures do not touch but their closet are about 1mm or 2mm apart. Several test strains can be streaked on the same plate. Simultaneously

streak control cultures of *L. monocytogenes, L, innocua* and *L. ivanovii.* Incubate plates at 35℃ or 37℃ for 18 to 24hours.

- Observe plates against bright light. In *L. Monocytogenes* case there is enhanced zone of beta heamolysis at the intersection of *S. aureus.*
- *L. innocua* does not show any enhanced of haemolysis with *S. aureus* or *R. equi.*
- In case of *L. ivanovii* enhanced beta zone of haemolysis is seen on *R. equi* side.

Interpretation of results:

All Listeria species are small, Gram-positive rods that demonstrate motility and catalase positive reaction. *L. monocytogenes* are distinguished from other species by the characteristics listed in the table given below.

Species Haemolysis	**Production of acid with Rhamnose**	**Production of acid with Xylose**	**CAMP Test**	
			S. aureus	***R. equi***
L. monocytogenes	+	+	-	-
L. innocua	-	V	-	-
L. Ivanivii	+	-	+	+
L. seeligeri	(+)	-	+	-
L. welshmeri	-	V	+	-
L. grayi sub species gayi	-	-	-	-
L. grayi sub species murayi	-	V	-	-

Expression of results:

Based on the observations and interpretation of the results, the presence or absence *of L. monocytogenes* in the test portion specifies the mass in grams or milliliters of the sample taken.

L. monocytogenes = present or absent/ g or ml

A2.7 Bacteriological examination of water for Coliforms

Equipment:

Refer to Appendix 4 - Equipment, Materials, and Glassware

Culture Media:

Lauryl Sulphate Tryptose (LST) broth tubes of single and double strength with inverted Durham (10ml quantities in tubes and 50ml in bottles).

Procedure:

1. De-chlorination:
    - Samples collected in pre-sterilized bottles are mixed well. To every 100ml portion of chlorinated samples, 0.1 ml of a 10% sterile sodium thiosulphate solution is added.
    - Various combinations of sample volume are taken depending on the probable coliform load in the sample. Equal volumes of samples are added to 10ml or 50ml volume of double strength broths. To 10 ml of single strength broths, 1 ml or 0.1 ml is added.
2. Tubes/bottles are incubated at 35°C for 24 and 48 hours.

Interpretation

Record tubes showing gas production after 48 hours. The MPN index per 100ml sample is determined using the following statistical tables.

Table A7. MPN index and 95% confidence limits for various combinations of positive and negative results when five 10ml portions are used.

No. of tubes giving positive reaction out of five of 10 ml each	**MPN Index/100ml**	**95% confidence Limits (Approximate)**	
		Lower	**Upper**
0	<2.2	0	6.0
1	2.2	0.1	12.6
2	5.1	0.5	19.2
3	9.2	1.6	29.4
4	16.0	3.3	52.9
5	>16.0	8.0	Infinite

Table A8. MPN index and 95% confidence limits for various combinations of positive and negative results when ten 10ml portions are used.

No. of tubes giving positive reaction out of ten of 10ml	**MPN Index/100 ml**	**95% confidence Limits (Approximate)**	
		Lower	**Upper**
0	<1.1	0	3.0
1	1.1	0.03	5.9
2	2.2	0.26	8.1
3	3.6	0.69	10.6
4	5.1	1.3	13.4
5	6.9	2.1	16.8
6	9.2	3.1	21.1
7	12.0	4.3	27.1
8	16.1	5.9	36.8
9	23.0	8.1	59.5
10	>23.0	13.5	Infinite

Table A9. MPN Index and 95% confidence limits for various combinations of positive results when five tubes are used per dilution (10ml, 1.0ml, 0.1ml).

Combination of Positives	**MPN Index/100ml**	**95% confidence Limits (Approximate)**		**Combination of Positives**	**MPN Index/100ml**	**95% confidence Limits (Approximate)**	
		Lower	**Upper**			**Lower**	**Upper**
0-0-0	<2	-	-	4-2-0	22	9.0	56
0-0-1	2	1.0	10	4-2-1	26	12	65
0-1-0	2	1.0	10	4-3-0	27	12	67
0-2-0	4	1.0	13	4-3-1	33	15	77
				4-4-0	34	16	80
1-0-0	2	1.0	11	5-0-0	23	9.0	86
1-0-1	4	1.0	15	5-0-1	30	10	110
1-1-0	4	1.0	15	5-0-2	40	20	140
1-1-1	6	2.0	18	5-1-0	30	10	120
1-2-0	6	2.0	18	5-1-1	50	20	150

				5-1-2	60	30	180
2-0-0	4	1.0	17	5-2-0	50	20	170
2-0-1	7	2.0	20	5-2-1	70	30	210
2-1-0	7	2.0	21	5-2-2	90	40	250
2-1-1	9	3.0	24	5-3-0	80	30	250
2-2-0	9	3.0	25	5-3-1	110	40	300
2-3-0	12	5.0	29	5-3-2	140	60	360
3-0-0	8	3.0	24	5-3-3	170	80	410
3-0-1	11	4.0	29	5-4-0	130	50	390
3-1-0	11	4.0	29	5-4-1	170	70	480
3-1-1	14	6.0	35	5-4-2	220	100	580
3-2-0	14	6.0	35	5-4-3	280	120	690
3-2-1	17	7.0	40	5-4-4	350	160	820
4-0-0	13	5.0	38	5-5-0	240	100	940
4-0-1	17	7.0	45	5-5-1	300	100	1300
4-1-0	17	7.0	46	5-5-2	500	200	2000
4-1-1	21	9.0	55	5-5-3	900	300	3900
4-1-2	26	12	63	5-5-4	1600	600	5300
				5-5-5	≥1600	---	---

Expression of results:

Coliforms = x MPN/250 ml or 100 ml

Alternately take the desired volume of water (250 ml or 100 ml) and pass it through a microscope filter 0.2µ. Take the filter disk and place it on a well-dried surface of LST Agar. Incubate at 35°C for 24 to 48 hours. Count the typical colonies and express the result as: Coliform count = x cfu/g.

References

Bacteriological Analytical Manual (1992) 6th Edition. Artington, V.A. Association of Official Analytical Chemists for FDA, Washington, D.C. p. 28-31.

Standard Methods for Examination of water and wastewater. (1989). 17th Edition. Edited by Lenore. S. Clesceru; Arnold. E. Greenberg and R. Rhodes Trussell. Test 9221. P. 66-76

A2.8 Bacteriological examination of water for detection, determination and confirmation of Escherichia coli

Equipment:

Refer to Appendix 4 - Equipment, Materials, and Glassware

Procedure:

1. Take 250ml or 100ml (as per the requirement of standard). Pour 50ml of sample in 50ml of double strength LST broth in five bottles containing inverted tubes, or 20 ml of sample in 20 ml of double strength LST broth in sugar tubes containing inverted Durham tubes.
2. Incubate the tubes at 35°C for 24 to 48 hours. Note the number of bottles/tubes showing gas formation.
3. Refer to MPN tables from test No. 17 for calculation of the number of presumptive +ve E. coli. Proceed further as per A2.2 for confirmation of E. coli.

Calculation:

As in A2.7

Expression of Result:

Escherichia coli = present/ absent in 250ml or 100ml.

Filtration Technique:

Alternately filter the required volume through a 0.2µ micropore filter and place it on VRBA Mc Conkey agar plate and incubate at 35°C for 24 to 48 hours. Count typical colonies and select five such colonies for confirmation as per A2.2.

References:

Bacteriological Analytical Manual (1992) 6th Edition. Artington, V.A. Association of Official Analytical Chemists for FDA, Washington, D.C. p. 325-369.

Standard Methods for Examination of water and wastewater. (1989). 17th Edition. Edited by Lenore. S. Clesceru; Arnold. E. Greenberg and R. Rhodes Trussell. Test 9221. P. 66-76.

A2.9 Bacteriological examination of water for detection and confirmation of Clostridium perfringens

Equipment:

Refer to Appendix 4 - Equipment, Materials, and Glassware

Culture Medium

- Cooked Meat Medium
- Tryptone sulfite Cycloserine Medium

Procedure:

1. Take 50 ml of sample water and pour into five cooked Meat mediums (de-chlorinate the sample if required. Put the tubes in a water bath maintained at 80°C for 15minutes. Plug the tubes with vespar and incubate at 35°C for 18 to 24 hours. The formation of a gas bubble below the wax seal indicates anaerobic growth.
2. Take a loopful of culture and streak from each positive tube to a TSC medium. Overlay a thin layer of TSC agar. Incubate the inverted plates at 35°C for 18 to 24 hours. Appearances of black colonies surrounded by a black zone are clostridium perfringens colonies.

Expression of Results

Clostridium perfringens = present or absent/50 ml

References

Official Methods of Analysis of AOAC International (1995). 16th Edition. By Patricia Cuniff. Published by AOAC International. Virginia. USA. Test 17.7.02 p.48-50.

Compendium of Methods for the Microbiological Examination of Foods. (1992) Carl Vanderzant and Splittstoesser Eds. Washington D.C. p. 623-635

Bacteriological Analytical Manual (1992) 6th Edition. Artington, V.A. Association of Official Analytical Chemists for FDA Washington D.C. p. 209-214.

Appendix 3: Culture Media

Bismuth Sulfite Agar

Peptone………………………………………………………… 10.0g
Beef extract…………………………………………….…… 5.0g
Dextrose……………………………………………………….. 5.0g
Disodium phosphate………………………………………… 4.0g
Ferrous sulfate………………………………………….…… 0.3g
Bismuth ammonium citrate………………………….……… 1.85g
Sodium sulfite…………………………….………………… 6.15g
Agar………………………………………………………… 20.0g
Brilliant green……………………………………….…… 0.025g
Distilled water……………………………………………… 1.0 liter

Dissolve ingredients in distilled water by boiling approximately for 1 minute. Adjust to pH 7.7±0.2, cool to 45 to 50°C, suspend precipitate with gentle agitation, and pour plates without sterilizing medium. Let plates dry with covers partially open caution: Plates lose selectivity after 72 hours.

Brain Heart Infusion Broth

Calf brain, Infusion from………………………………………….. 200.0g
Beef heart, infusion from…………………………………………. 250.0g
Proteose peptone or poly-peptone………………………………… 10.0g
Dextrose………………………………………………………… 2.0g
Sodium chloride…………………………………………………… 5.0g
Disodium phosphate………………………………………………. 2.5g
Distilled water………………………………………………….. 1.0 liter

Dissolve ingredients in distilled water by bringing them to a boil. Dispense into tubes and autoclave for 15mins at 121°C. The final reaction should be 7.4±0.2.

Brilliant – Green Lactose Bile Broth 2%

Peptone……………………………………………………. 10.0g
Lactose…………………………………………………….. 10.0g
Ox-bile……………………………………………………… 20.0g
Brilliant-green…………………………………………… 0.01g
Distilled water……………………………………………… 1.0 liter

Dissolve the peptone and lactose in 500ml of distilled water, add the ox bile dissolved in 200ml of water, mix and make up to 975ml, and adjust pH to 7.4±0.1. Add 13.3ml of 0.1% aqueous solution of brilliant green. Add distilled water to bring the total volume to 1 liter. Dispense in 10ml portions into 20 x 50 mm test tubes containing inverted Durham tubes. Sterilize for 15 minutes at 121°C.

Buffered Peptone water

Peptone………………………………………………………….. 10.0g

Sodium chloride……………………………………………… 5.0g

Disodium hydrogen phosphate……………………………….. 9.0g

Potassium dihydrogen phosphate…………………………….. 1.5g

Distilled water………………………………………………… 1.0 liter

Adjust pH to 7.0, dispense in a portion of 225ml into bottles of 500ml capacity and 9ml in tubes. Sterilize for 20 mins at 121°C.

Cooked Meat Medium

Beef heart……………………………………………………… 454.0g

Proteose peptone……………………………………………… 20.0g

NaCl…………………………………………………………… 5.0g

Glucose………………………………………………………… 2.0g

Finely chop beef heart. Add approximately 1.5g of heart particles to test tubes. Add remaining components to distilled water and bring volume to 1.0 L. Mix thoroughly. Distribute into tubes in 10 ml volumes—autoclave for 15 mins at 121°C.

Decarboxylase Test Media

Basal for use with Lysine, Arginine, Ornithine Moeller method (1954, 1955)

Basal medium

Peptone………………………………………………………….. 5.0 liter

Beef extract……………………………………………………. 5.0g

Bromocresol purple (1.6%)…………………………………… 0.625ml

Cresol red (0.2%)……………………………………………… 2.5ml

Glucose………………………………………………………… 0.5g

Pyridoxal……………………………………………………… 5.0mg

Distilled water………………………………………………… 1.0liter

The basal medium is divided into four equal portions, one of which is tubed without adding any amino acids. These tubes of basal medium may be REUSED FOR CONTROL PURPOSES. One of the remaining portions of basal medium is added 1% of L-lysine dihydrochloride; to the second, 1% of L- arginine monohydrochloride and the third portion, 1% of L-ornithine dihydrochloride. If DL amino acids are used, they should be incorporated into the medium 2% concentration since the microorganisms are active against the L forms only. The pH of the fraction to which ornithine is added should be readjusted prior to sterilization. The amino acid medium may be tubed in 3, or 4ml amounts in small (13x100mm) screw-capped tubes and sterilized at 121°C for 10minutes. A small amount of floccular precipitate may be seen in the ornithine medium. This does not interfere with its use.

Inoculation: Inoculate lightly from a young agar slant culture. After inoculation, add a layer (about 10mm in thickness) of sterile mineral (paraffin) oil to each tube, including the control. A control tube should always be inoculated with each culture under investigation. Incubate at 37°C; examine daily for 4days. The alkalization of the medium indicates positive reactions with a color change from yellow to violet. Weakly positive reactions may be bluish gray.

Dextrose Tryptone Agar

Agar………………………………………………………… 15.0g

Pancreatic digest of casein…………………………………. 10.0g

Glucose…………………………………………………….. 5.0g

Bromocresol purple………………………………………… 0.04g

pH: 6.9±0.2 at 25°C.

Add components to distilled water and bring volume to 1.0 L. Mix thoroughly. Gently heat and bring to boiling. Autoclave at 121°C for 15 minutes.

Pour into sterile tubes or Petri dishes.

EC Broth

Trypticase or Tryptone…………………………………………. 20.0g

Bile salt No. 3…………………………………………………… 1.5g

Lactose………………………………………………………….. 5.0g

Dipotassium hydrogen phosphate………………………………. 4.0g

Potassium dihydrogen phosphate……………………………….. 1.5g

Sodium Chloride………………………………………………… 5.0g

Distilled water…………………………………………………… 1.0 liter

Egg yolk tellurite enrichment

Soaked eggs in aqueous mercuric chloride 1:1000 for not less than one minute. Rinse in sterile water and dry with a sterile cloth.

Aseptically crack eggs separate whites and yolks. Blend yolk and sterile for 5 seconds. Mix 50.0ml blended egg yolk to 10.0ml of filter-sterilized 1% potassium tellurite. Mix and store at 2 to 8℃.

Preparation of plates

Add 5.0 pre-warmed (45 to 50℃) enrichment to 95ml of melted basal medium adjusted to 45 to 50℃. Mix well (avoiding bubbles), pour 15.0 to 18.0 ml into 15 x100mm Petri dishes. Plates can be stored at 2 to 8℃ in plastic bags for four weeks. Immediately prior to use, spread 0.5ml per plate of 20% solution of Millipore filter-sterilized sodium pyruvate and dry plates at 50V for 2 hours or 4 hours at 35℃ with agar surface uppermost.

If complete medium plates were prepared from commercial or laboratory prepared medium containing sodium pyruvate to adding Egg yolk tellurite. These plates must be used within 48 hours while being stored at 2 to 8℃. These plates should also be dried as indicated above prior to inoculating with the sample

L-EMB Agar

Peptone………………………………………………….   10.0g

Lactose…………………………………………………..   10.0g

Disodium hydrogen phosphate………………………….   2.0g

Agar……………………………………………………..   15.0g

Distilled water…………………………………………..   1.0 liter

Make an (a) solution and adjust the pH to 7.1 to 7.2. Dispense in 100ml portions. Sterilize for 15min at 121℃. Before use melt, and to each 100ml portion add 2.0 ml of aqueous 2% eosin Y solution and 1.3 ml 0f 0.5% aqueous methylene blue solution.

Gelatin Phosphate salt Broth

Gelatin…………………………………………………………….   10.0g

NaCl……………………………………………………………….   10.0g

K_2HPO_4……………………………………………………………...   5.0g

pH 7.2±0.2 at 25℃

Add components to distilled water (1L). Autoclave at 121℃ for 15min.

Gram Negative (GN) Broth

Glucose…………………………………………………………   1.0g

Glucose D. mannitol……………………………………………….   2.0g

Sodium Citrate……………………………………………………..   5.0g

Sodium deoxycholate………………………………………………   0.5g

Dipotassium phosphate……………………………………………….	4.0g
Monopotassium phosphate……………………………………………	1.5g
Sodium chloride………………………………………………………	5.0g
Tryptose………………………………………………………………	20.0g
Distilled water……………………………………………………..	1.0 liter

Dissolve ingredients into distilled water by heat. Dispense in convenient amounts and sterilize at 116°C for 15 minutes. The final pH is 7.0±0.2. Avoid excessive heating.

Hektoen Enteric Agar

Proteose peptone………………………………………………………	12.0g
Yeast extract…………………………………………………………	3.0g
Lactose………………………………………………………………	12.0g
Sucrose………………………………………………………………	12.0g
Salicin……………………………………………………………….	2.0g
Bile complex………………………………………………………..	9.0g
Sodium chloride……………………………………………………...	5.0g
Sodium thiosulfate……………………………………………………	5.0g
Ferric ammonium citrate…………………………………………….	1.5g
Bromthymol blue…………………………………………………….	0.065g
Acid fucasin…………………………………………………………..	0.1g
Agar…………………………………………………………………..	14.0g
Distilled water………………………………………………………..	1.0liter

Suspend ingredients in distilled water. Boil with frequent stirring. Do not overheat or autoclave. When completely in solution, cool to 55 to 60°C and distribute into plates. Allow plates to solidify with lids ajar to provide a dry surface for inoculation. Plates may be refrigerated for future use—final pH 7.5±0.2.

Klingler Iron Agar

Peptone……………………………………………………	20.0g
Agar………………………………………………………	12.0g
Lactose…………………………………………………….	10.0g
NaCl………………………………………………………..	5g
Beef extract……………………………………………….	3g
Yeast extract………………………………………………	3g

Glucose………………………………………………………… 1.0g

Ferric citrate……………………………………………………. 0.3g

$Na_2S_2O_3$……………………………………………………….. 0.3g

Phenol …………………………………………………………. 0.05g

pH 7.4±0.2 at 25°C.

Add components to 1 L of distilled water.

Distribute into tubes and autoclave at 121°C for 15 mins. Make slants deep butts.

Koser's Citrate Broth

Sodium ammonium hydrogen phosphate…………… 1.5g

Monopotassium hydrogen phosphate……………….. 1.0g

Magnesium sulphate…………………………………. 0.2g

Sodium citrate………………………………………… 3.0g

Distilled water………………………………………… 1.0liter

Adjust pH 6.7±0.1, dispense in 10ml portions in test tubes. Sterilize for 15 mins at 121°C.

Lactose Broth

Beef extract…………………………………………… 3.0g

Peptone……………………………………………………….. 5.0g

Lactose…………………………………………………. 5.0g.

Distilled water……………………………………….. 1.0liter

Adjust pH to 6.8 into fermentation tubes. Sterilize for 15 minutes at 121°C. Allow temperature in the autoclave to drop slowly below 75°C before opening.

Lactose Gelatin medium

Gelatin…………………………………………………. 120g

Tryptone Lactose……………………………………. 15g

Yeast extract…………………………………………. 10g

Phenol red…………………………………………... 10g

pH 7.5±0.2 at 25°C.

Add gelatin to 590 ml distilled water. Gently heat while stirring and bring to 50 to 60°C. Add phenol red. Add the rest of the components to 400ml of distilled water and mix with gelatin solution. Dispense 10ml volumes in the tubes. Autoclaves for 10 minutes at 121°C.

Lauryl Sulphate Tryptose Broth

Tryptose, tryptone or trypticase……………………………….. 20g

Lactose……………………………………………………… 5g

Dipotassium mono-hydrogen phosphate……………………….. 2.75g

Sodium chloride……………………………………………… 5g

Sodium lauryl sulphate………………………………………. 0.1g

Distilled water……………………………………………….. 1.0liter

Potassium dihydrogen phosphate……………………………… 2.75g

Adjust pH to 6.8±0.1, dispense in 10ml portions with inverted Durham tubes. Sterilize for 15 minutes at 121°C.

Lysine Iron Agar (Edwards and Fife)

Peptone……………………………………………………. 5.0g

Yeast extract………………………………………………. 3.0g

L-lysine……………………………………………………. 1.0g

Ferric ammonium citrate…………………………………... 10.0g

Sodium thiosulfate…………………………………………. 0.5g

Bromocresol purple………………………………………… 0.02g

Agar………………………………………………………… 15.0g

Distilled water……………………………………………. 1.0liter

Dissolve ingredients in distilled water and adjust to pH 6.7±0.2.

Dispense in 14ml amounts in 100 x 13mm tubes and sterilize at 121°C for 12 minutes. Slant tubes to obtain a deep butt and a short slant.

Lysozyme Broth

Preparation A – Nutrient Broth: Prepare nutrient broth and dispense 99ml amounts in bottles or flasks. Autoclave for 15 minutes at 121°C.

Preparation B – Lysozyme solution: Dissolve 0.1g of lysozyme in 65 ml of sterile 0.01N hydrochloric acid. Heat to water boiling for 20 minutes and dilute to 100ml with sterile 0.01N hydrochloric acid. Alternatively, dissolve 0.1g of lysozyme chloride in 100ml water and sterilize by filtration. Test solution for sterility before use.

Add 1.0ml of sterile 0.1% lysozyme solution to each 99ml of nutrient broth. Mix thoroughly and aseptically dispense 2.5ml of the complete medium into sterile 13 x 100 tubes.

MacConkey Agar

Peptone…………………………………………………..	20.0g

Lactose…………………………………………………..	10.0g

Bile salts…………………………………………………	1.5g

Sodium chloride…………………………………………	5.0

Agar……………………………………………………..	15.0g

Neutral red………………………………………………	0.03g

Crystal Violet……………………………………………	0.001g

Distilled water…………………………………………..	1.0 liter

Adjust pH to 7.1 sterilize for 15 minutes at 121°C. Pour in Petri-dishes.

Malonate Broth

Yeast extract……………………………………………………	1.0g

Ammonium sulfate……………………………………………..	2.0g

Dipotassium phosphate………………………………………	0.6g

Monopotassium phosphate…………………………………..	0.4g

Sodium chloride……………………………………………..	2.0g

Sodium malonate…………………………………………….	3.0g

Glucose………………………………………………………	0.25g

Bromthymol blue…………………………………………….	0.025g

Distilled water……………………………………………….	1.0 liter

Dissolve ingredients in distilled water by heating, if necessary.

Dispense into tubes and autoclave for 15 minutes at 121°C. Final pH 6.7±0.1.

Malt Agar

Malt Extract…………………………………………………….	30.0g

Agar…………………………………………………………….	15.0g

Distilled water…………………………………………………..	1.0 liter

Dissolve the ingredients in 1.0-liter distilled water with occasional agitation and boil gently for one minute.

Dispense into suitable containers and sterilize at 121°C for 15 minutes.

Malt Agar (Acidified)

Malt agar acidified with 10% sterile tartaric acid to pH 3.5±0.2.

Prepare an acid solution by weighing 10.0g of tartaric acid into a beaker and bringing up to 100ml with water.

Dissolve and sterilize at 121℃ for 15 minutes. Acidify the sterile and tempered medium with a predetermined quantity of acid solution immediately before pouring the plates.

Do not attempt to reheat the medium once acid has been added. Determine the accuracy of adjusted pH by pouring an aliquot of the medium into a small beaker, cooling to temperature, and placing a recently standardized pH directly into the solidified medium.

Malt Agar (With Antibiotic)

Solution A

Prepare malt agar.

Solution B

Add 500mg each, of chloro-tetracycline HCL and chloramphenicol to 100ml sterile distilled water and mix. (Not all material dissolves. Therefore, the suspension must be evenly dispersed to pipetting into the medium).

To prepare mixture:

Melt medium (solution A above), temperature to 45±1℃, and add 2ml of antibiotic solution per 100ml medium.

Motility Test Medium (Motility Agar)

(Tittsler and Sandholzer)

Tryptose………………………………………………… 10.0g

Sodium chloride………………………………………… 5.0g

Agar…………………………………………………….. 5.0g

Distilled H_2O…………………………………………… 1.0 liter

Suspend ingredients and heat to boiling to dissolve the medium completely. Sterilize by autoclaving for 15 minutes at 121℃. Final pH 7.2.

MR-VP Broth

Peptone…………………………………………………. 7.0g

Glucose…………………………………………………. 5.0g

Dipotassium hydrogen phosphate……………………… 5.0g

Distilled water………………………………………….. 1.0 liter

Adjust pH to 6.9±0.2 and dispense in 10ml portions in tubes. Sterilize for 15 minutes at 121℃.

Mycological (Mycophil) Agar

Phytone or Soytone……………………………………….. 10.0g

(papaic digest of soya meal)

Dextrose………………………………………………… 10.0g

Agar…………………………………………………….. 18.0g

Distilled water…………………………………………….. 1.0 liter

Dissolve ingredients in distilled water with heat and autoclave for 12 minutes at 118°C (12 lb steam pressure for 10 minutes).

For yeast and mold counts of carbonated beverages, sugars, and other similar materials, adjust the pH from 4.5 to 4.7 by adding up to 15ml of sterile 10 percent lactic acid to each liter of the melted medium prior to plating. Do not reheat after acidification.

Mycophil Agar + Antibiotic

Preparation of antibiotic solution: Add 500mg of chloramphenicol to 100ml sterile phosphate-buffered distilled water and mix. (Not aa material dissolves, therefore the suspension must be evenly dispersed before pipetting into the medium); 2ml of this solution is added per 100ml of tempered agar, giving a final concentration in the medium of 100mg/l of each antibiotic. After swirling, the medium is ready for use.

Nutrient Broth

Beef extract………………………………………………… 3.0g

Peptone…………………………………………………… 5.0g

Distilled water……………………………………………… 1.0 liter

Suspend ingredients in distilled water and melt agar by gently boiling.

Dispense into suitable flasks, and autoclave for 15 minutes at 121°C. Final pH, 7.3.

Peptone Water Diluent

Peptone………………………………………………….. 1.0g

Distiller water…………………………………………… 1.0 liter

pH………………………………………………………. 7.0

sterilize for 15 minutes at 121°C.

Plate Count Agar (PCA) (Standard Methods Agar (TGE Agar)

Dehydrated yeast extract………………………………….. 2.5g

Pancreatic digest extract………………………………….. 5.0g

Glucose…………………………………………………… 1.0g

Agar……………………………………………………… 15-18g

Distilled water……………………………………………. 1.0 liter

Adjust pH to 7.0±0.1 dispense in 15ml portions in tubes or flasks.

Sterilize for 15min at 121°C. Before use, melt the medium completely in boiling water and keep the tubes or flakes in a water bath at 45 to 48°C.

Potassium Cyanide (KCN) Broth

Basal Broth:

Proteose peptone No. 3 or Polypeptone…………… 3.0g

Disodium phosphate………………………………... 5.64g

Monopotassium phosphate …………………………. 0.225g

Sodium chloride………………………….................. 5.0g

Distilled water………………………………………. 1.0 liter

Dissolve ingredients in distilled water with stirring. Autoclave 100ml portions 15 minutes at 121°C. The final pH should be 7.6. Prepare 0.5% potassium cyanide by weighing 0.5g into 100ml sterile distilled water using a pipette filter. Transfer 1.5ml cold potassium cyanide solution to 100ml basal broth (precooled). DO NOT PIPETTE BY MOUTH. Mix.

Distribute 1.0ml portions to sterile 13 x 100mm tubes and stopper immediately with No. corks impregnated with paraffin. (Prepare corks by boiling in paraffin for 5 minutes). Store medium at 5 to 10°C. Storage life is two weeks. Exercise caution because potassium cyanide is lethal.

Potato Dextrose Agar (Acidified)

Infusion from white potatoes……………………………… 200ml

Dextrose…………………………………………………… 20g

Agar……………………………………………………….. 15g

Distilled water……………………………………………… 1.0 liter

Suspend ingredients in distilled water and heat mixture to boiling to dissolve. Distribute into tubes or flasks, and autoclave for 15 minutes at 121°C (15 lb pressure). When used as plating medium for yeasts and molds, melt in flowing steam or boiling water, cool, and acidify to pH 3.5 with sterile 10% tartaric acid solution. (For use in cultivating yeasts and molds, adjust to the desired pH if different from pH 3.5). Mix thoroughly and pour into plates. To preserve the agar's solidifying properties, do not heat medium after the addition of tartaric acid. To prepare potato Dextrose Agar with an Antibiotic, add antibiotics described under Mycophil agar with Antibiotic.

Selenite Cystine Broth

Tryptone…………………………………………………………… 5.0g

Lactose…………………………………………………………….. 4.0g

Disodium hydrogen phosphate…………………………………. 10.0g

Sodium selenite…………………………………………………. 4.0g

L – cystine………………………………………………………. 0.01g

Distilled water………………………………………………… 1.0 liter

Dissolve by boiling for 5 minutes. Dispense 10ml portions into sterile 16 x 150 mm test tubes. Heat 10 minutes in flowing steam. Do not autoclave. Final pH 7.0±0.2. Medium is not sterile. Use the same day as prepared.

Tetrathionate Broth

Basal medOium*:*

Polypeptone or proteose peptone……………………………………. 5.0g

Bile salts………………………………………………………………… 1.0g

Calcium carbonate…………………………………………………… 10.0g

Sodium thiosulfate…………………………………………………… 30.0g

Distilled water………………………………………………………... 1.0 liter

Iodine solution:

Iodine…………………………………………………………………… 6.0g

Potassium Iodide…………………………………………………….. 5.0g

Distilled water……………………………………………………….. 20.0ml

Heat the ingredients of the basal medium in distilled water to boiling temperature, cool to less than 45°C, add 2.0ml of iodine solution to each 100ml of the base. Add 1.0 ml of 1:1000 solution of brilliant green per 100ml of the basal medium. With or without added brilliant green, the basal medium may be tubed, sterilized at 121°C for 15 minutes, and stored. In this case, an iodine solution is added (0.2ml per 10 ml of medium) prior to use.

Sulfathiazole (0.125 ml of medium) may be added to present excessive growth proteus.

Thiosulfate-Citrate-Salts-Sucrose Agar (TCBS)

Yeast extract………………………………………….. 5.0g

Polypeptone or Proteose Peptone No. 3……………… 10.0g

Sucrose……………………………………………….. 20.0g

Sodium thiosulfate ($5H_2O$)…………………………… 10.0g

Sodium citrate ($2H_2O$)………………………………..	10.0g
Sodium chlorate………………………………………	3.0g
Oxgall…………………………………………………	5.0g
Sodium chloride………………………………………	10.0g
Ferric citrate………………………………………..	1.0g
Bromthymol blue………………………………………	0.04g
Thymol blue……………………………………………	0.04g
Agar……………………………………………………	15.0g
Distilled water……………………………………….	1.0 liter

Distilled ingredients in a distilled water by bringing to a boil. Adjust pH to 8.6±0.2. This medium should not be autoclaved.

T_1N_1 Agar

Trypticase (pancreatic digest of casein)…………………..	10.0g
Sodium chloride………………………………………..	10.0g
Agar……………………………………………………..	15.0g
Distilled water………………………………………….	1.0 liter

Dissolve ingredients in distilled water by bringing them to a boil. Dispense in tubes and sterilize at 121°C for 15 minutes. Allow solidifying in an inclined position (long slant). The final reaction should be pH 7.2±0.2.

To prepare T1N1 broth, omit the agar.

Urea Broth

Yeast extract………………………………………………….	0.1g
Monobasic potassium phosphate……………………………..	0.091g
Dibasic sodium phosphate……………………………………	0.095g
Urea……………………………………………………………..	20.0g
Phenol red………………………………………………………	0.01g
Distilled water……………………………………………….	1.0 liter

Mix ingredients in the distilled water. This medium is filter-sterilized and tubed in sterile tubes in 3.0ml amounts. The basal medium (without urea) may be prepared in 900ml of distilled water and sterilized at 121°C for 15 minutes. After cooling, 100ml of 20% sterile urea solution is added and the medium dispensed in sterile tubes in 3.0ml amounts.

Inoculation

Three loopful (2mm loop) from an agar slant culture are inoculated into a tube with a culture medium and shaken to suspend the bacteria.

Incubation

Tubes are incubated in a water bath at 37°C, and the results are read after 10 minutes, 60 minutes, and 2 hours.

Violet Red Bile Agar (VRBA)

Yeast extract………………………………………………… 3.0g

Peptone or Gelysate…………………………………………… 7.0g

Sodium chloride……………………………………………….. 5.0g

Bile salts or Bile salts No. 3…………………………………… 1.5g

Lactose………………………………………………………… 10.0g

Neutral red…………………………………………………….. 0.03g

Crystal violet………………………………………………….... 0.002g

Agar…………………………………………………………… 15.0g

Distilled water………………………………………………… 1.0 liter

Suspend the ingredients in distilled water and stand for a few minutes. Mix thoroughly and adjust to PH 7.4±0.2. Heat with agitation and boil for 2 minutes. Do not sterilize. Prior to use, cool to 45°C and use as a plating medium. After solidification, add a cover layer above the agar of approximately 3.0 to 4.0ml to prevent surface growth and spreading of colonies.

Violet Red Bile Agar + Glucose

Prepare 1000ml VRBA Add 10g of glucose. Heat with agitation and boil for 2 minutes. Do not autoclave.

Xylose Lysine Deoxycholate Agar (XLD)

Yeast extract…………………………………………. 3.0g

L-lysine……………………………………………… 5.0g

Xylose………………………………………………… 3.5g

Lactose……………………………………………….. 7.5g

Sucrose……………………………………………….. 7.5g

Sodium chloride……………………………………… 5.0g

Phenol red……………………………………………. 0.08g

Agar………………………………………………….. 13.5g

Distilled water ………………………………………. 1.0 liter

Heat mixture in distilled water to boiling temperature to dissolve the ingredients. Sterilize at 121°C for 15 minutes, then cool to 55 to 60°C.

Aseptically add 20.0mlof sterile solution containing:

Sodium thiosulfate………………………………………. 34.0g

Ferric ammonium citrate………………………………… 4.0g

Distilled water…………………………………………… 100ml

Mix well to obtain a uniform suspension.

Appendix 4: Equipment, Materials, and Glassware

1. Autoclaves of sufficient size and number: Used for sterilization of media and for discarded plates/ used media, etc. (with calibrated thermometer and pressure gauge). The autoclave shall maintain sterilization temperature (121℃) during a cycle and complete entire cycle with 45 minutes when 15minutes sterilization period is used.
2. Balance sensitive to 0.1g with 200g load
3. Blenders with steel jar and lid / stomacher.
4. Bunsen burners.
5. Colony counter (Quebec or equivalent)
6. Dilution and media storage bottles. 120, 300, 600, and 1500ml in capacity
7. Durham's tubes
8. Glass test tubes 16 x 150mm. Rimless
9. Plastic caps for test tubes
10. Serological test tubes
11. Hot air ovens used for sterilization of glass and metal ware. They should have a thermostat range between 150-185℃.
12. Hockey sticks: Glass bent rods (or suitable plastic make) with fine polished edges, 3-4mm diameter, 15-20 cm long with angled spreading surface 45-55cm long or disposable plastic material.
13. Howard Mold Counting Chamber
14. Haemocytometer.
15. 4 Incubators, to be adjusted at 30℃, 37℃, 44.5℃, and 55℃ of proper size. B.O.F. incubator for temperature less than ambient temperature.
16. Inoculating loops and wires (3-5mm diameter of nichrome or platinum or plastic).
17. Magnetic stirrer
18. Membrane filtration apparatus, for sterilizing fluids which are affected by heat, e.g. Seitz filter operationalized membrane filters.
19. Microscope binocular with 900 x and higher magnification.
20. Microscopic slides and cover slips.
21. Non-absorbent cotton.
22. Petri plate (glass or plastic)
23. Petri plate containers. (Stainless steel or aluminum, with covers) for hot air sterilization of glass petri plates
24. Pipettes (Glass or Plastic). Graduated, with 1, 5, and 10ml total flow type / Automatic pipette with error < ±5 % with sterilizable or Auto pipettor with pre-sterilized plastic tips.
25. Pipette containers (Stainless steel plastic tip containers boxes)
26. pH meter. Electronic pH meter with accuracy of 0.1 pH unit shall be used.
27. Refrigerator and deep freezer.
28. Test tube racks and baskets to hold test tubes
29. Thermometers.
30. Vortex- mixer.
31. Water bath for holding media at 44-46℃.
32. Serological water bath
33. Laminar flow chamber
34. Biological safety cabinet level II