UNIT 14 REQUIREMENTS SPECIFIC TO FOOD TESTING LABORATORIES - BIOLOGICAL PARAMETERS

Structure

14.0 Objectives

14.1 Introduction

14.2 Quality and Safety Requirements of Food Products

14.3 Biological Testing Requirements of Food Products

- 14.3.1 Foods of Plant Origin
- 14.3.2 Foods of Animal Origin
- 14.3.3 General Category (Some of these may be Processed Foods from Mixed Origin)

14.4 Application of Laboratory Quality Management Requirements

14.5 Management Requirements (Clause 4 of ISO 17025)

- 14.5.1 Organisation and Management
- 14.5.2 Document Control
- 14.5.3 Review of Requests, Tenders and Contracts
- 14.5.4 Subcontracting of Tests
- 14.5.5 Purchase of Services and Supplies
- 14.5.6 Reagents
- 14.5.7 Dehydrated Ready to Use Media
- 14.5.8 Kits
- 14.5.9 Other Consumables
- 14.5.10 Control of Records
- 14.5.11 Internal Audits

14.6 Technical Requirements (Clause 4 of ISO 17025)

- 14.6.1 Personnel
- 14.6.2 Competence for Opinions and Interpretation
- 14.6.3 Accommodation and Environmental Conditions
- 14.6.4 Layout and Laboratory Design
- 14.6.5 Environment Monitoring
- 14.6.6 Laboratory Hygiene Maintenance
- 14.6.7 Test Method
- 14.6.8 Equipment

14.7 Traceability of Measurement

- 14.7.1 Autoclave
- 14.7.2 Ovens, Water Baths and Incubators
- 14.7.3 Laminar Flow/Bio-safety Cabinet
- 14.7.4 Other General Equipments
- 14.7.5 Reference Cultures

14.8 Sampling

14.9 Handling Test and Calibration Items

- 14.9.1 Examination on Receipt
- 14.9.2 Sample Registration

14.9.3 Sub-sampling Prior to Testing
14.9.4 Sample Retention and Storage
14.10 Assuring the Quality of Test and Calibration Results
14.10.1 Internal Quality Control
14.10.2 External Quality Control, Proficiency Testing Programs
14.11 Reporting of Results
14.12 Let Us Sum Up
14.13 Key Words
14.14 Answers to Check Your Progress Exercise
14.15 Suggested Reading

14.0 OBJECTIVES

After reading this unit, we shall be able to:

- understand the Biological Parameters/Requirements of Food Products;
- identify the Biological testing requirements of food products; and
- apply Laboratory Quality Management System requirements to a laboratory involved in testing biological parameters in food products.

14.1 INTRODUCTION

Micro-organisms are present every where on earth, in all living creatures like Plants and Animals, including in Humans and the atmosphere – soil, water and air. They also have potential for entering the food supply chain which consists of plants and animals and products derived from them. Once entered, they use food as a source of nutrients for their growth. This can result in deterioration of the food and consequent spoilage. This generally happens through, utilization of nutrients, production of enzymatic changes, breakdown of a food component or /synthesis of new ones, etc. while the micro-organism multiplies in the substrate matter. When the micro-organisms involved are pathogenic in nature, their association with food is critical from human health point of view. Many of the foods are capable of supporting the growth of pathogenic micro-organism or at least are capable of serving as vector for them.

Fruits and vegetables generally harbor micro-organisms on surface, with their type and level varying with soil conditions, type of chemicals used (fertilizers and other chemical nutrients, pesticides, and other chemicals); quality of water and air. Micro-organisms like Molds, Yeast, Lactic acid bacteria, bacteria from genera *Pseudomonas, Alcaligenes, Micrococcus, Clostridium* and *Enterobacter* are generally found in plant source. Pathogens specially of enteric type can be present if the soil is contaminated with untreated sewage.

Foods of animal origin including milk and milk products may harbor pathogens of genere *Salmonella & Shigella, Escherichia coli (Ecoli), Staphylococcus aureus, Compylobacter, Listeria monocytogenes*, etc. and micro-organisms like yeast and mould, etc. Water may be contaminated with micro-organism like *Ecoli*, Yeast & mould, Fecal Streptococci, Staphylococcus aureus, Sulphite-reducing anaerobes, Pseudomonas aeruginosa, Salmonella & Shigella, Vibrio Cholerae & Vibrio

Parahaemolyticus, etc., due to contamination coming from raw water sources and subsequent handling.

These micro-organism may subsequently be carried forward from the source or added in the subsequent stages of food chain, due to improper processing, handling or storage of food products at various stages of processing, making the food unfit for human consumption. In order to ensure that this does not happen and that the food remains safe till the stage that it is consumed, it is very essential that the food is tested for microbial load at initial stage and all subsequent stages of processing as felt appropriate. Various types of decisions would be based on the results of such tests:

a) Is the food fit as a material for direct consumption or as raw material for further processing?
b) What types of process control, handling, packaging, storage and other activities are required to make it safe through the stages of processing?
c) Is the processed food fit for human consumption? For how long? What should be its shelf life? etc.

Hence the reliability and accuracy of testing for Biological/microbiological parameters plays a very important role in ensuring over all food quality and safety.

14.2 QUALITY AND SAFETY REQUIREMENTS OF FOOD PRODUCTS

For details of various quality and safety requirements in food products, please refer Unit 13.

14.3 BIOLOGICAL TESTING REQUIREMENTS OF FOOD PRODUCTS

Testing requirements for biological parameters in food products would generally cover following categories of food products:

14.3.1 Foods of Plant Origin

Cereals and cereal products; Edible oils, fats and derived products; nuts, fruits, vegetables and derived products; sauces, herbs, spices and condiments; sugar and sugar confectionery; jams, juices, sauces & concentrates; genetically modified foods and agricultural products; other unspecified fresh foods and Other unspecified preserved/canned/processed foods, made predominantly from plant material.

14.3.2 Foods of Animal Origin

Milk and other dairy products; meat, poultry and derived products; fish and fish products, eggs and egg products, other unspecified fresh foods and preserved/canned/processed foods, made predominantly from animal products.

14.3.3 General Category (Some of these may be Processed Foods from Mixed Origin)

Alcoholic beverages; non-alcoholic beverages; food additives, preservatives and supplements; nutritional supplements; essential nutrients including vitamins; animal feeds and drinking and processing waters.

The biological testing requirements of food products from safety point of view cover microbiological parameters and genetically modified organisms (GMO). In addition certain chemical parameters are also tested using test methods based on biological techniques. These are vitamin assay using microbiological test methods, identification and estimation of bacterial and viral pathogens in food products using techniques like ELISA, PCR or using immunological assay based test kits; antibiotic residue analysis using ELISA based test kits, etc. This unit provides specific guidance on application of ISO 17025 for laboratories carrying out all the above biological tests in food products.

14.4 APPLICATION OF LABORATORY QUALITY MANAGEMENT REQUIREMENTS

Compliance to the requirements of ISO/ IEC 17025: 2005 by any laboratory carrying out testing and/or calibration activities provides assurance regarding their competence to carry out on a consistence basis the specific the tests and/or to which these requirements are applied. However, ISO/IEC 17025 covers general requirements which are generic in nature. Hence specific guidance is generally required for applying various clauses of ISO 17025, specially concerning the technical requirements to different technical areas of testing like chemical and biological testing of Food products. This unit makes us familiar with application of the generic requirements specified in ISO 17025 for biological) testing of Food products, which would cover the requirements detailed at 14.3 above.

14.5 MANAGEMENT REQUIREMENTS (CLAUSE 4 OF ISO 17025)

While applying the various management system elements as specified in Clause 4 of ISO 17025:2005 to a laboratory engaged in biological/ microbiological parameters in food products, specific guidelines are required to be kept in mind as applicable to specific applications. While describing these the numbering of the clauses have been aligned with the numbering of ISO/ IEC 17025.

The numbering of the clauses below refers to the numbering of ISO/ IEC 17025. Where clause numbers from that standard are omitted no further clarification is felt necessary for food testing laboratories.

14.5.1 Organisation and Management

The requirements prescribed in this clause are generic in nature and are applicable to all laboratories engaged in all types of testing activities including the Food testing. As per the requirements of Clause 4.1.5 (h) the laboratory is required to have technical management which has overall responsibility for technical operations. A laboratory engaged in biological parameters of food testing is required to provide person (s) with appropriate competence commensurate with types of chemical parameters of food related testing carried out, which would constitute the technical management. In case laboratory is engaged in diverse testing activities like microbiological testing, testing for genetically modified food products, etc., then the laboratory may need to consider more than one persons as constituting the technical management.

14.5.2 Document Control

All the requirements of document control as specified under this clause are applicable. In case if the laboratory is using any sophisticated, computer controlled equipment like real time PCR for GMO estimation, which may make use of proprietary software's, then the document control procedure would require to be extended to these softwares also. In case if the laboratory makes use of Laboratory Information Management System (LIMS) data acquisition and for the purpose of calculations, then again the software used will require to be suitably addressed for its control and validation.

14.5.3 Review of Requests, Tenders and Contracts

Each laboratory is required to determine the testing scope which will denote its range of activities – the range of tests and/or analyses. The scope of a laboratory engaged in testing of food products for biological parameters can be defined in terms of:

a) The type of food products tested/analysed,

b) The types of tests carried out – Individual tests under a broad category,

c) The test method(s) used – reference to a specification, inhouse method number along with technique if relevant; and

d) The range of analysis normally defined as range of concentration and the accuracy / precision / uncertainty of measurement as applicable. In case of microbiological testing, which are designed to give results as present or absent, it is desirable to indicate the same clearly in the scope along with indicating the quantity of sample taken for test. In cases where the biological tests are qualitative in nature like GMO testing, it is desirable to indicate the same in the scope along with information on the Limits of Detection/Quantification.

The above scope is required to be carefully arrived at after considering the laboratories capabilities in terms of equipment and infrastructure available, level of competence of manpower at a given point of time. When ever a request for conduct of tests is received it needs to be reviewed in terms of capability of the laboratory. It is important at this point to understand precisely the requirements of the customer, especially in terms of specification or expected range because this would generally govern the type of test method used. For example for a given microbiological parameter, there may be a need to decide whether, a plate count method is required to be used, MPN method or a method designed to yield results in terms of present/absent. For taking the decision a consultation with the customer may also be required. Further this information would also need to be communicated appropriately among all the testing sections involved in the analysis of product received for testing.

14.5.4 Subcontracting of Tests

In case for few of the parameters in a range of tests required to be carried out on a food product the laboratory does not have testing facilities or its testing facility is temporarily out of order, there is a provision that these tests may be sub-contracted to a competent sub-contractor. As per ISO 17025 a competent sub-contractor is the one who complies with the requirements of ISO 17025. When a Food testing laboratory decides to sub-contract it will need to ensure that besides the above the sub-contracted laboratory also has the testing

capability for the tests and range and accuracy/precision equivalent to its own for the food product under question before taking the sub-contracting decision.

14.5.5 Purchase of Services and Supplies

The laboratory should have a policy and procedure(s) for the selection and purchasing of services and supplies. Quality and grade of reagents including detergent should be appropriate for the tests concerned. In a biological test laboratory, one of the primary consideration is that the purchased products should not contain any impurities that may inhibit bacterial growth.

14.5.6 Reagents

While purchasing the reagents and media the laboratory should ensure that these are of specification/quality suitable for the tests to be performed. It should also verify the suitability of reagents on receipt before use and during its shelf life, through using positive and negative reference culture.

14.5.7 Dehydrated Ready to Use Media

While procuring these the laboratory should ensure that the same are suitable for use and that it has the knowledge of or experience of the performance characteristics. These should essentially cover adequate knowledge of the manufacturer's quality specifications list of components, including any supplements, shelf life and the acceptability criteria applied, storage conditions, sterility checks and check of growth of target and non-target control organisms used and acceptability criteria, physical checks and the acceptability criteria, and date of manufacture. Further these performance characteristics should be verified on receipt.

14.5.8 Kits

All Serological and biochemical kits used by the laboratory should be of appropriate specification. Chemicals and reagents involved in sample preparation for PCR testing should be of molecular biology grade or equivalent and free from contaminating nucleic acids or nucleases (both DNase and RNase).

14.5.9 Other Consumables

Apart from reagents, the laboratories are required to ensure that consumables such as culture dishes, culture tubes, sample containers, sample bags, spatula, pipettes and pipette tips membrane filtration devices, etc. are of appropriate specification. membrane filtration units should be made of stainless steel, glass, or autoclavable plastic, not scratched or corroded and should not leak. Diameter and pore size of membrane filters, and diameter and absorption capability of absorbent pads should meet the requirements specified in the test standards. Sterile metal or disposable plastic loops, wood applicator sticks, sterile swabs, spreaders etc. used as inoculating equipment, of appropriate specification should be procured. The metal inoculating loops should be made of alloys that do not interfere with any biochemical tests.

The sources and history of consumables having an effect on the validity of tests such as media, antisera, biochemical kits and membrane filters should be recorded. It is a good practice to maintain a logbook to record receipt of all

such materials received at laboratories. This logbook should include information such as supplier, lot number, date received, date put in use, date of verification and date of expiration.

In addition to the above, requirements stated under 4.6 in Unit 13, as applicable to a biological test laboratory should also be considered.

14.5.10 Control of Records

All the relevant provisions, as specified in ISO 17025 for this clause are applicable. In addition records specific to biological / microbiological testing are required to be maintained.

14.5.11 Internal Audits

The Internal audit system of a food laboratory engaged in testing of food products is required to cover both management requirements (Clause 4) and technical requirements (Clause 5) of ISO 17025. A broad checklist for technical requirements specific to a food testing laboratory as covered in Unit 13 may be used with few changes in respect of use of culture media, cultures strains; records to be maintained, sample handling, etc. as detailed in this unit.

14.6 TECHNICAL REQUIREMENTS (CLAUSE 4 OF ISO 17025)

14.6.1 Personnel

The Food Testing Laboratory Management is required to define its staff competence requirements for different levels of operation and activities within the laboratory. These typically cover all the activities that directly or indirectly affect the quality of testing operations. In a biological testing laboratory, these would include the laboratory personnel involved in following activities:

a) Sampling, if applicable,

b) Sample preparation activities, Sample analysis activities, including all stages like – Media preparation and sterilization, sample preparation (including, sample enrichment and extraction, where applicable), inoculation/plating, operation of equipment, enumeration and interpretation of test results, biochemical tests, operation of specialized equipment like Real time PCR, etc., where applicable.

d) Test method development and validation, where applicable, Interpretation of data, supervision of testing and other operations like authorized signatory for test reports, giving opinions on test results, etc., and technical management,

e) Subsidiary functions like maintenance and calibration of equipment, sample entry, purchase, etc.,

f) Quality assurance functions, if separately provided, and

g) Laboratory management.

Laboratory needs to deploy competent manpower for all the functions/activities as stated above. Competence is defined in terms of capability to do an assigned job competently. In actual terms competence is

required to be defined in terms of combination of educational qualification, experience, skill and training. Hence the laboratory needs to define these competence criteria for various functions as above. For a biological testing section of a Food testing laboratory, these need to be defined in suitable terms. The educational qualification for functions specified at c), d), e), f) and g) (could be excluded incase the lab management functions are purely administrative) should be graduate or postgraduate in Microbiology or equivalent. In exceptional cases alternate qualification in biological science may be acceptable if the personnel have considerable experience in the testing activities corresponding to the laboratory's scope of testing and their competence is evaluated and found adequate.

Personnel involved in testing activities should receive adequate training before they are assigned testing activities independently. This should include training in basic techniques, e.g. plate pouring, counting of colonies, aseptic technique, etc. On-going competence should be monitored objectively with provision for retraining where necessary. Where a method or technique is not in regular use, verification of personnel performance before the testing is undertaken may be necessary. The critical interval between performance of tests should be established and documented. The interpretation of test results for identification and verification of micro-organisms is strongly connected to the experience of the performing analyst and should be monitored for each analyst on a regular basis.

For certain specialized testing activities like pathogen testing, GMO testing, PCR based microbiological testing, supervisory and other activities, it may be desirable to prescribe a higher level of qualification. The experience component would also vary depending upon the activities carried out. Certainly a person involved in method development, supervisory or technical manager functions / role would be expected to have about 5 years of relevant experience and at times even higher degree in qualification.

At any point of time (entry level or subsequently) because of technological advancement in testing activities if a gap is created, the same can be bridged up through training. The laboratory is expected to have an on going system for identifying such gaps and providing suitable training. The need to periodically retrain staff should be considered where a method or technique is not in regular use of one person is shifted from one activity to other. In each case, the critical interval should be established and documented. In addition to test methods, in some cases, it may be more appropriate to relate competence to a particular technique or instrument, for example use of approved biochemical, serological kits or microbial identification kits.

The training is expected to be considered effective only when it is suitably evidenced through demonstration. Analysts may only perform tests on samples if they are either recognised as competent to do so, or if they do so under adequate supervision. Continued competence should be monitored, for example, through using quality control techniques or participation in proficiency testing schemes, etc.

14.6.2 Competence for Opinions and Interpretation

If the laboratory is required to include opinions and interpretations in the reports, based on the test results, then the responsibility for the same should be clearly defined, based on the required competence criteria. The person

authorised for doing the same should not only be competent in terms of suitable qualification and testing experience but should also have relevant knowledge of the specific application, including, for example, legislative and technological requirements and acceptability criteria. In certain cases the authority for these type of activities in food testing laboratories is defined in the regulation itself. For example, the authority for giving opinions regarding "Fitness for Consumption" for food products has been defined as local Municipal corporations in the food legislation. This requirement would override all others.

14.6.3 Accommodation and Environmental Conditions

Following distinct operations are required to be carried out in a biological testing section of food testing laboratory:

a) Sample receipt and storage;

b) Media preparation, equipment preparation and sterilization;

c) Maintenance of reference organisms;

d) Sample/used media disposal and decontamination;

e) Washing;

f) Sample preparation (It would be a good idea to use a segregated area for preparation of powdery products likely to be highly contaminated);

g) Actual testing/Plating using Laminar flow / Bio-safety cabinet;

h) Incubation and plate examination;

i) Ancillary activities, requiring - documentation room, dress change rooms and toilets, storage room; doors, corridors, etc; and

j) Biotechnology – PCR, GMO Testing.

The layout, environmental conditions and access control requirements would be different for the different activities as listed above, in a biological testing laboratory. In any case, irrespective of what activity takes place there must be appropriate segregation, and space for various tests to minimise potential contamination and to ensure protection of personnel. The important things to be kept in mind are:

14.6.4 Layout and Laboratory Design

a) The laboratory layout should be such that the appropriate segregation of activities minimizes the risks of cross-contamination where these are significant to the type of activity being performed. The layout should be such that the activities/procedures should be organized in a sequential manner with appropriate sequence of material and personnel movement. Ideally it would be best if all the areas as listed are physically separated. However, the need for physical separation should be judged on the basis of the activities specific to the laboratory (e.g. number and type of tests carried out). In case a laboratory is engaged in Microbiological tests and Biotechnology (molecular biology) related tests (GMO), then it is a good idea to physically separate the areas, where these two types of tests are carried out, since the risk of contamination is much more significant in Biotechnology test area. Also it is good idea to segregate within Biotechnology work area the low, medium and high DNA, working

environments and also to maintain dedicated equipment like pipettes, tips, centrifuges, tubes, etc.

b) The space provided for different sections in a Biological test laboratory should be adequate to allow work areas that can be kept clean and clutter free. The space allowed should be commensurate with the volume of analyses carried out, number of testing and other personnel working in the test laboratory and the overall internal organisation of the laboratory.

c) The testing areas should be appropriately lighted and ventilated and temperature controlled as appropriate. The ventilation may be provided by natural or forced mechanism or by use of an air conditioner. The areas like plating and incubation and media/sample preparation areas the source of ventilation should be provided with appropriate filters, which are maintained to ensure performance. In modern biological laboratory, the entire ventilation and air conditioning system appropriate to biological testing facility is achieved through specially designed air handling units.

d) The testing area construction should be such that it ensures reduction of contamination. This can be ensured by having smooth, non-absorbent and easy to clean and disinfect surfaces for walls, ceilings, floors and benches; concave joints between the floor, walls and ceiling; appropriate air locks for entries to plating and other similar areas; non-utilization of wood; separate hand-washing arrangements, preferably non-manually controlled; etc.

e) For procedures that involve handling of pathogens and reference stock cultures, they shall be operated within a safety cabinet of a class commensurate with the risk level of the micro-organism handled.

f) In case of an inhouse laboratory of a food product manufacturing unit, it is desirable that area involved in Pathogen culture handling/testing should be physically separated and located as far away from the manufacturing unit as possible. Personnel involved in testing should also be aware of the potential for contamination of production areas, and should demonstrate that they have taken appropriate measures to avoid any such occurrences.

14.6.5 Environment Monitoring

An appropriate environmental monitoring programme should be devised by the laboratories. The monitoring system should include monitoring of airborne contamination through use of exposure plates, swab testing of critical surfaces such as sampling and testing benches, utensils, balances, stomachers, etc. An acceptable limit for background counts should be documented and there should be a procedure in existence for dealing with situations in which these limits are exceeded.

14.6.6 Laboratory Hygiene Maintenance

The Biological testing laboratory is expected to have documented cleaning programme for laboratory fixtures, equipment and surfaces. It should take into account the results of environmental monitoring and the possibility of cross-contamination. There should be a procedure for dealing with spillages. Measures should be taken to avoid accumulation of dust. A procedure should also be available for maintenance of personnel hygiene within the laboratory, which should include use of appropriate clothing including, if necessary protection for hair, beard, hands, shoes, etc., while working in the test area.

This is particularly important in the biotechnology (molecular biology) laboratory, where for example, movement from an area of high DNA load to one of low DNA load may unwittingly introduce cross-contamination. In many laboratories a laboratory coat may suffice.

a) Disposal of Contaminated Waste

As a matter of good laboratory management and in view of maintenance of safety of environment, correct disposal of contaminated materials – Used media, tested plates, contaminated food samples, etc., is very essential. This assumes greater importance when an inhouse laboratory of a food manufacturing unit is engaged in testing of pathogenic/hazardous micro-organism. Suitable procedures for decontamination, - autoclave sterilisation or incineration, etc., as applicable should be available. Guidance on these aspects can be drawn from ISO 7218.

b) Test Method Selection and Validation

As per the requirements specified in ISO 17025, Clause 5.4, the Biological testing laboratories are free to use any test method – Standard method (method published in International, Regional or National standards), Laboratory developed method or Non-standard method, as long as it is appropriate for required application and to meet the customers requirement of range and accuracy. Although full validation is not required, a laboratory must verify that it can properly operate the method, and can demonstrate (where specified) that the limits of detection, selectivity, repeatability and reproducibility can be obtained.

Laboratory Developed Methods could include, but not restricted to :

i) Method developed in the laboratory,

ii) Method supplied by a customer,

iii) Method developed by Industry level association,

iv) Amplification or modification of a standard method, and

v) Method published in a scientific book or journal, but not validated, however it is preferable that a laboratory uses standard methods, because these are generally collaboratively tested/pre-validated methods. In case the laboratory is engaged in testing on behalf of a regulatory body then it is imperative that it should use the test method referred in the relevant regulation. In all other cases, the laboratory should ascertain that the test method used is appropriate and fit for the purpose. The most common way of ascertaining the same is through the process of Method Validation. The details of Method validation principle and theory are covered in Unit 15. Most important consideration of all is that it is the laboratory's responsibility to ascertain from customer about the testing requirements and use a method from within its defined scope which is suitable for the purpose intended, be adequately validated and documented and provide results that are traceable to stated references at an appropriate level of uncertainty.

c) Standard Methods for Biological Testing

Generally standard methods for biological testing of food products are available in ISO Standards or Standards produced by most of the national standards bodies. Besides these most well known texts (particularly in

bacteriology) offer an array of internationally recognised standard test methods. These are methods published by AOAC, APHA, US FDA (Bacteriological Analytical Manual), etc. These methods are generally appropriate and can be employed in the analysis of particular sample types as such, without any deviations.

Need for Laboratory developed Methods and Method Validation in Biological Testing Laboratories -Still there may be certain instances where these methods may not be applicable for certain matrices or certain special/new types of matrices may not be covered in the standard methods. For example for certain types of inhibitory foodstuffs such as spice samples the recovery of bacteria using standard test procedures may not be possible. Yet another type of processed food samples may contain chemical preservatives which would prohibit the growth of the organisms, especially at low dilutions. The standard test method may need to be modified to include neutralisers or dilution techniques, etc., to remove or to reduce the inhibitory influences and to allow the recovery of stressed cells. Another example is a customer requirement for testing of bacteriological contamination in Clay dough used as Children's play item. None of the Standard test methods would probably include this matrix. In all these cases the laboratory would require to develop it's own methods, may be based on certain standard methods available and making use of literature data available, it's own experience and certain amount of experimentation. Subsequently the fitness for purpose would need to be verified and demonstrated through the process of method validation.

Method validation could be done through comparison of the modified method against the original method by analyzing samples before and after spiking with a known low number of the appropriate target organism(s), and then checking the recovery. If the expected sample is likely to contain stressed organisms, then the use of a stressed control organism in the recovery checks should be considered. Validation of test methods should be performed under the same conditions as those of a real assay, by using a combination of naturally contaminated products and spiked products. Proficiency testing or a Inter-laboratory comparison program can be used to check the validity of the laboratory method. In fact this is one of the best ways of validation. Analysis of series of samples by the proposed new method and any existing methods for the same determination may also be done. The validation may require to be repeated periodically to ascertain continued fitness.

After the validations are completed and the fitness of the developed method is established, the complete method should be documented as an inhouse method. and all validation data must be recorded and stored for at least as long as the method is in force and as long as necessary to ensure adequate traceability of raw data and results.

d) Use of KITS for Analysis of Biological Parameters

In many cases, laboratories use kits for estimation of biological/microbiological parameters. These kits have certain specific advantages that these are much faster than the conventional methods of analysis. Generally the suppliers of Kits of repute provide validation data. These validation data may be obtained through collaborative testing, from the manufacturers and subjected to third party evaluation, say by AOAC.

However, the commercially available kits will require further validation in case the laboratory is unable to source the validation data. When the manufacturer of the test kits supplies validation data, the laboratory will require to perform only the verification activities. If the validation data is not available or not applicable, the laboratory shall be responsible for completing the primary validation of the method.

e) Validation of Test Methods for Biological/Microbiological Analysis

The microbiological test methods generally yield two types of test results. First are those, which are qualitative in nature and are expressed in terms of detected/not detected (present/absent), in which case the tests are performed through identification and confirmation procedures. The second category are the quantitative tests which are performed through enumeration of colonies present on the exposed plates. In these cases, the results are expressed in terms of colony forming units (CFU's) in a specified quantity of sample size.

Both the type of test methods may require validation in case of laboratory developed methods or modified standard methods. The validation of microbiological test methods may be carried out using naturally contaminated products or products spiked with a predetermined level of contaminating organisms obtained from reference cultures. The extent of validation would depend on the type of method – whether it is based on standard method/technique or is totally an inhouse developed one. As stated above the laboratory should also validate standard methods used beyond its stated application – say when applied to matrices not specified in the standard procedure.

The microbiological test methods which are qualitative in nature can be validated by determining the method performance characteristics like specificity, relative trueness, positive deviation, negative deviation, limit of detection, matrix effect, repeatability and reproducibility, as appropriate. For quantitative estimations, in addition to the above, limit of determination/limit of quantification should also be determined. The differences due to the matrices must be taken into account when testing different types of samples. The results should be evaluated with appropriate statistical methods. If a modified version of a standard method is required to be used, then the simplest form of validation would be comparison of the modified method with the original method, performed using replicates, repeated under different sets of test conditions, as appropriate.

f) Kits

In addition to the requirements specified above, the laboratory shall demonstrate their capability to achieve the limit of detection quoted by the manufacturer or if the laboratory has to establish its own limit of detection, to minimise false positive and false negative results. In some cases, (e.g. veterinary microbiological testing) it is observed that a specific test kit performs differently under local environmental conditions, to that of the original environmental conditions when it was subjected to primary validation. In such cases, the laboratory should conduct the validation to prove that the kit performs under local environmental conditions.

Documentation requirements - The validation methods need to be documented and the records of validation need to be maintained. Subsequently as a part of internal control mechanism, the laboratory would

require to verify on a regular basis that the performance documented at the time of method validation is being met. This can be done through the use of spiked samples or reference materials incorporating relevant matrices. The general principles of method validation are covered in detail in Unit 15.

g) Uncertainty of Measurement for Biological testing Laboratories

As defined above the biological tests can be classified under two categories– Qualitative and Quantitative. The Measurement of uncertainty is not applicable to the qualitative tests and even for quantitative tests, because of the empirical nature of biological tests, a rigorous, metrologically and statistically valid calculation of uncertainty of measurement may not always be plausible. It is considered appropriate to base the estimation of uncertainty on repeatability and reproducibility data alone. The factor of bias obtained from results of proficiency testing programs participated by the laboratory may also be considered. Further for both qualitative and quantitative tests the laboratory should identify the individual components of uncertainty to verify and demonstrate that these are under control and their contribution to the variability of results/uncertainty is negligible. Some of the components like uncertainty due to volume, mass and dilution effects can easily be estimated to ascertain that these are under control. The other factors like sample stability and sample preparation, temperature of incubation, consistency of reagent performance and analyst's interpretation, though not directly measurable, their contribution could be assessed estimated through analysis under variable conditions.

For tests where the limit of detection is an important indication of suitability, the uncertainty associated with the inoculate used to determine the limit should be estimated and its significance evaluated. Laboratories should also be aware of the incidence of false positive and false negative results associated with the qualitative tests they use.

One of the most important factors which generally contributes towards variability in results is the uneven distribution of organisms within the sample matrix and its effect on the sub-sampling required for conduct of actual test. However, this factor is generally not considered while estimating uncertainty since the uncertainty due to distribution of organisms within the product matrix is not a function of the laboratory's performance and would generally be unique to individual products tested. Further it is expected that the test method specifies the sample size to be used for the conduct of individual tests, taking into account the factors of poor homogeneity.

14.6.7 Test Method

The test methods covered in a biological test laboratory should include procedure for preparation of media, cultures, their validation procedures, etc. These should cover the following**:**

1) **Laboratory Prepared Media –** In case the media is prepared by the laboratory itself, then it is required that the quality of prepared culture media, diluents and other suspension fluids prepared in-house should be checked, where relevant, for the performance characteristics like recovery or survival maintenance of target organisms, inhibition or suppression of non-target organisms, physical parameters like pH, volume and sterility. Guidance for these procedures/methodologies can be obtained from ISO

11133 Part 1 & 2. For preparation purposes, distilled, deionised, or reverse osmosis produced water, free from bactericidal, inhibitory or interfering substances, suitably tested, should be used.

The basic reagents/ingredients used for preparation of the media should be stored under appropriate conditions as specified by the manufacturer. Appropriate shelf life of prepared media should be established based on past experience or literature data.

All media recipes and procedures for preparation should be documented and the records of all relevant details of each batch of medium prepared should be kept. Guidance on the preparation, sterilisation of media and recommended storage times is available in ISO 7218: 1996 “Microbiology of food and animal feeding stuffs – General rules for microbiological examinations” and American Public Health Association (APHA) “Standard Methods for the Examination of Water and Wastewater”.

2) **Dehydrated Media –** For Media (diluents and other suspension fluids) procured from external sources, either in ready to use form or partially prepared form, it is required that the user laboratory validates the quality of the same before use to establish the performance characteristics as stated above. These validations should also be performed at a predefined interval during subsequent uses within the shelf life stated by the manufacturer.

   Serological and biochemical kits shall be verified with positive and negative strains with typical and negative characteristics, if applicable.

3) **Storage** - All the reagents (including stock solutions), media, (including prepared ones), diluents, and other suspending fluids should be stored in appropriate containers with proper sealing; under suitable storage conditions; properly labelled indicating identity, concentration, preparation date, expiry date etc.

   The detailed procedure for preparation or use of reagents, media, diluents, suspension liquids including guidance on precautions should be documented, and followed in actual practice and the persons responsible for their preparation should be identifiable from records.

14.6.8 Equipment

A food testing laboratory engaged in testing of biological/microbiological parameters is required to be furnished with all the items of equipment required for activities like sampling (if applicable), sub-sampling/preparation of test items, media preparation and sterilization, plating, incubation, enumeration of result, processing, PCR related test equipment, etc., commensurate with the scope of biological testing of the laboratory. These should generally cover the different types of tests as listed in 14.6.7 above, all or in part. These should also cover all bio-safety and air handling unit related and personnel hygiene related equipment and facilities, as applicable, if not already covered at 14.6 above. The choice of equipment in terms of capacity, range, least count, sensitivity, etc., should be commensurate with the scope of testing of the laboratory. All equipment used in food testing laboratory should be of a specification sufficient for the intended purpose and should be kept in a state of maintenance and calibration consistent with its use.

The laboratories are required to maintain a documented program for the maintenance, calibration and performance verification of its equipment. Details of the same are covered in Clause 5.6 of ISO 17025:2005.

Commonly used equipment in a biological test laboratory include volumetric glass ware, balances, thermometers, pH meter, timer, ovens, refrigerators and other cold chambers, incubators, autoclaves (at least two in number, one for sterilizing clean equipment and media and the other for decontaminating the used equipment), water bath, laminar flow chamber, bio-safety cabinets, colony counter, sample digester – Stomacher, etc.; GMO testing equipment like thermocycler, PCR/Real time PCR equipment, etc.

Cross-contamination – One of the most important considerations in a biological testing laboratory is the avoidance of cross-contamination arising from equipment. This can be achieved by any of the following means:

a) Disposable equipment should in actual terms be clean and sterile;

b) Re-used glassware should be properly cleaned and sterilized; and

c) Laboratories should have a separate autoclave for decontamination.

The following equipment are required to be maintained through the process of cleaning and servicing, inspecting for damage, general verification and where relevant, sterilizing for ensuring that cross-contamination does not take place. The effectiveness of cleaning, where appropriate may be verified through swab tests.

a) Equipment like water baths, incubators, microbiological cabinets, autoclaves, homogenisers/blenders, refrigerators, freezers, etc;

b) Measuring instruments - thermometers, timers, balances, pH meters, colony counters;

c) Filtration apparatus, glass or plastic bottles, test tubes and petri-dishes, sampling instruments, wires or loops of Pt, Ni/Cr or disposable plastic; and

d) Volumetric equipment - pipettes, automatic dispensers.

Consumables - Apart from reagents, laboratories should ensure that procured lab ware such as culture dishes, culture tubes, sample containers, sample bags, spatula, pipettes and pipette tips shall be pre-sterilised or sterilisable. Membrane filtration units should be confirmed of their sterility prior to release for use.

14.7 TRACEABILITY OF MEASUREMENT

The laboratory is required to establish a program for the calibration and performance verification of equipment which has a direct influence on the test results. The frequency of such calibration and performance verification is required to be decided by the user. Laboratory based on variety of factors like the type of equipment and its influence over the test results, frequency of usage, manufacturer's recommendations, past experience, previous experience data or literature data, etc. Many of the guidance documents issued by the accreditation bodies provide information on calibration intervals and performance checks for the guidance of laboratories and the same can be referred while deciding. Typically a equipment requiring calibration/ performance checks are balances, thermometers, pH meter, timer, ovens, incubators, autoclaves, water bath, laminar flow chamber, bio-safety cabinets, thermocycler, real time PCR and volumetric equipment.

The requirements with respect to calibration/verification and maintenance for above equipments are described briefly as below:

14.7.1 Autoclave

The verification of autoclaves is required to be done to ensure that the autoclaves are capable of meeting specified time and temperature tolerances. Pressure measurements alone cannot guarantee that appropriate temperature are attained through the sterilization cycle. Measurement of temperature is essential for each autoclave cycle. Sensors used for controlling or monitoring operating cycles and the temperature recording devices, are required to be calibrated periodically and the performance of timers verified. In addition to monitoring the temperature, the effectiveness of sterilization need to be checked with biological indicators and chemical indicators. Temperature sensitive tapes or indicator strips should be applied for each load. However, they are used only to show that the load has been processed but not as a monitor of the actual process applied. Monitoring of temperature during the use may be carried out either through direct observation and recording of maximum temperature achieved and time at that temperature or through use of recording device.

14.7.2 Ovens, Water Baths and Incubators

The heating/cooling equipment like Ovens, water baths and incubators are required to be calibrated, especially for the temperature ranges of utilization. The temperature gradient in an incubator/oven should also be verified to ensure that the equilibrium conditions can be achieved with respect to typical usage conditions, say while stacking the petri dishes at various heights.

14.7.3 Laminar Flow/Bio-safety Cabinet

The calibration of laminar flow would essentially involve verification/ calibration of airflow rate, verification of HEPA filters. Particle count shall also be checked on a routine basis to comply with relevant standard. The laminar flow should also be subjected to periodic servicing and maintenance. Routine cleanliness and disinfection as appropriate, before and after every use or even during prolonged use should also be part of maintenance. This should also be monitored through swab testing method. During operation, the aerial microbial contamination should also be routinely checked using agar plates or air sampler.

For bio-safety cabinet, which is primarily used for personnel safety when testing for hazardous micro-organisms/pathogens, its efficiency should be ensured through periodic maintenance, which may be done monthly, quarterly or annually depending on the class of cabinet. Parameters such as final filter and exhaust filter integrity, air velocity and uniformity, air barrier containment, induced air leakage, UV radiation, light intensity and noise level should be checked/calibrated and monitored.

a) **PCR Related Equipment:** The performance of the PCR equipment such as thermocycler and the built in spectroscopic components of real time PCR equipment should be calibrated/ verified periodically using reference samples.

b) **Weighing Equipments:** Analytical and top loading balances and weights in a weight box used for verification purposes should be calibrated through a competent external calibration agency initially and at preplanned frequency. Acceptance criteria should be defined based on the type of

weighing operation performed (Media weighment, sample weighment, etc.) and the required accuracy.

c) **Volumetric Equipment:** Volumetric equipment generally in use in a biological/microbiological testing laboratory are glass pipettes, disposable pipettes, automatic pipettes, automatic dispensers, etc. Laboratories are required to carry out Initial calibration/verification of volumetric equipment before use and subsequently at regular intervals, as considered appropriate for the type of volume measures. The volumetric equipment should be checked for the accuracy of the delivered volume against the set volume (for several different settings in the case of variable volume instruments) and the precision of the repeat deliveries should also be measured. This calibration/verification may be performed by the laboratory, inhouse, using calibrated balance and thermometer, through the density route, or at a outside calibration laboratory. In case of disposable volumetric equipment, since it is not possible to verify each and every unit, laboratory should ensure that these are procured from reliable suppliers with proven track record. This should be followed by initial verification, on a random sample basis, of the suitability and accuracy of the equipment.

14.7.4 Other General Equipments

Other equipment which may be used in a biological/microbiology lab are :pH meter for checking and maintaining pH of medias, etc.; Conductivity meters for checking conductivity of water used for testing; thermometers used for verifying temperature of sample, heating equipment, etc.; humidity meter used for checking humidity of laboratory environment where appropriate; timers and centrifuges; etc. Depending upon their impact on the test results, appropriate calibration and maintenance programs should be drawn up for these equipment. Yet another set of equipment like refrigerators and freezer, generally in use in a biological testing laboratory should be periodically verified for their fitness for use.

14.7.5 Reference Cultures

In a biological/microbiological testing laboratory reference cultures are required for establishing acceptable performance of media (including test kits), for validating test methods and for assessing / evaluating on-going performance. Traceability is necessary, for example, when establishing media performance for test kit and method validations.

To demonstrate traceability, laboratories must use reference strains of micro-organisms obtained directly from a recognized national or international collection. In India reference cultures obtained from following sources are accepted as valid materials:

a) American Type Culture Collection (ATCC),

b) IMTECH, Chandigarh (MTCC),

c) National Collection of Industrial Micro-organisms (NCIM) - National Chemical Laboratory (NCL), Pune,

d) Christian Medical College (CMC), Vellore,

e) National Institute of Communicable Diseases (NICD), Delhi, and

f) Central Research Institute, Kasouli, Himachal Pradesh.

1) The **Reference Strains/Cultures** obtained from above sources may be sub-cultured within the laboratory to provide **reference stocks**. However this process should not be repeated more than once, for maintaining the purity of the strain. The reference stocks are required to be stored in aliquots under appropriate conditions, say frozen (freeze dried, liquid nitrogen storage, deep frozen) conditions or in lyophilized conditions.

2) **Working Cultures** used for routine use should be primary sub-cultures from the reference stock. While carrying out this process of sub-culturing certain precautions should be taken. These are – i) If reference stocks have been thawed, they must not be re-frozen and re-used; ii) Working stocks should generally not be sub-cultured; iii) Working stocks should not be sub-cultured to replace reference stock; and iv) Commercial derivatives of reference strains may only be used as working cultures. Under certain special circumstances, when it is required by test method standards or when documentary evidence is available to demonstrate that there has been no loss of viability, no changes of biochemical activity and/or no change in morphology, working stocks may be sub-cultured up to a defined number of generations. Normally more than five passages from the original national collection culture is not considered appropriate. At various stages in the above process – on receipt and during their life in the laboratory, the desired characteristics of the strains should be verified by serological, biochemical and/or morphological tests. While performing these activities, appropriate guidance may be taken from information provided in the standard ISO 11133-1. Procedures for maintenance of reference cultures, preparation and verification of working stocks and working cultures, should be documented. Further following records should be kept :

   a) details of organisms held in the laboratory; their sources, lot numbers, dates of receipt and expiry, dates when put in use, conditions of packaging of reference cultures;

   b) verification records of cultures at all stages as applicable;

   c) history of sub-culture from reference stocks - dates of preparation and expiry, personnel responsible; and

   d) preservation procedures, record of monitoring storage conditions, etc.

3) **Reference Standards -** Reference materials and certified reference materials provide essential traceability in measurements and are used to demonstrate the accuracy of results; calibrate equipment; monitor laboratory's performance; validate methods and enable comparison of methods. If possible, reference materials should be used in appropriate matrices.

   The mechanisms to ensure traceability of reference material are not well developed when the reference standard is biochemical or immunological in nature. Hence in such cases the biological testing laboratories are expected to source their reference materials from best possible industry known sources or use in-house produced and verified reference standards. Reference material supplied by reputable chemical suppliers (particularly kit manufacturers and for pure biochemical standards or reagents) may also be used in such cases.

   Laboratories shall have a policy and procedures for purchase, handling, storage, maintenance and use of certified reference materials and stocks.

14.8 SAMPLING

In case of food products, biological tests are generally required to establish safety parameter of a lot/batch of food product to verify if the lot/batch conforms to the product specific requirements and whether it is fit for consumption. However in a laboratory only a small quantity is required for conduct of various types of tests, say 25 grams or 25 ml per test for pathogens. Based on the tests on such a sample portion a decision is required to be taken regarding the lot quality which may be any thing from few kilograms to few tons. If the test portion is not representative of the original material, it will not be possible to relate the analytical result obtained to that in the original material, no matter how good the analytical method is nor how carefully the analysis is performed. Hence it is very essential that the samples drawn for laboratory testing should be representative of the lot/batch. In case of biological parameters few other dimensions get added. Firstly, the biological substances – microorganisms, GMO, etc., would generally not be uniformly distributed through the matrix. Secondly sampling itself, if not done properly can be source of major contamination.

All food testing laboratories are not required to carry out the sampling activities prior to testing. In most cases organisations/individuals outside of the test laboratory are responsible for sampling and as far as the laboratory is concerned its responsibility is restricted to the sample received.

Sampling: However if the laboratory is responsible for drawal of sample (sampling), then following systems are essential to be maintained.

a) Documented procedure for sampling should be based on established procedures as given in any standard methods or as specified in regulation and the persons responsible for sampling shall be aware of it. Sampling procedure can form part of the test method and shall include procedures for sterilization of sampling equipment and precautions in performing aseptic techniques.

b) Sampling should only be performed by trained personnel. It should be carried out aseptically using sterile equipment. Environmental conditions for instance, air contamination and temperature, should be monitored and recorded at the sampling site. Time of sampling should also be recorded.

c) Transport and storage should be under conditions that maintain the integrity of the sample (e.g. chilled or frozen where appropriate). The conditions should be monitored and records kept. Where appropriate, responsibility for transport, storage between sampling and arrival at the testing laboratory shall be clearly documented. Testing of the samples should be performed as soon as possible after sampling and should conform to relevant standards and/or national/international regulations.

d) In case laboratory receives customer drawn samples, it may be in lab's own interest to make the customers aware of sampling procedures, need for appropriate care for ensuring sterile atmosphere and use of appropriate containers, precautions to be taken while drawing the samples, proper storage and transportation conditions. Customers should be informed if the sample received is too small for meaningful analysis.

14.9 HANDLING TEST AND CALIBRATION ITEMS

Following specific aspects are required to be ensured while handling samples of food products received for testing of Biological parameters. These should also be addressed in the laboratory's documented procedure for sample handling:

14.9.1 Examination on Receipt

a) Microbiological organisms would generally be sensitive to factors such as temperature or duration of storage and transportation. Hence the laboratory should as a good practice, check and record the condition of the sample on receipt.

b) The samples should be verified for any damages to outer container, torn packaging, deficient labelling, signs of physical deterioration, incorrect temperature on receipt or during transportation, leakages, etc., which would render the sample unfit for testing for microbiological parameters. The laboratory may consult with the client before deciding whether to test or discard the sample. In any case, the condition of the sample should be indicated on the test report.

14.9.2 Sample Registration

a) The laboratory should record all relevant information - particularly the date and, where relevant, the time of receipt; condition of the sample on receipt and, when necessary, temperature; characteristics of the sampling operation (sampling date, sampling, conditions, etc.).

b) The samples should be appropriately identified on receipt. The procedure for identification should be performed with minimum delay and without causing any sample contaminating action.

c) Samples awaiting test should be stored under suitable conditions to minimise changes to any microbial population present. Storage conditions should be defined in the documented procedure for sample handling and appropriate records should be kept.

d) The outer packaging and labels on the samples may be highly contaminated and should be handled and stored with care so as to avoid any spread of contamination. Sometimes it may be a good idea to wipe them to get rid of outer contamination or store such packages with visible contamination separately. In any case such samples should not be taken into the sample preparation or plating area without removing the outer covering.

14.9.3 Sub-sampling Prior to Testing

Sub-sampling by the laboratory immediately prior to testing is considered as part of the test procedure. It should be performed according to national or international standards, where they exist, or by validated in-house methods. Sub-sampling procedures should be designed to take account uneven distribution of the micro-organisms in the sample matrix. The general guidance given in ISO 6887 and ISO 7218 may be used. It is essential that procedures are available for preventing spread of contamination, delivery of samples including special transportation such as refrigeration and exclusion of light,

disposal and decontamination processes and unbroken chain of identification of the sub-samples/samples shall be provided.

14.9.4 Sample Retention and Storage

a) The sample handling procedure should include procedure for storage, retention and disposal of samples. Samples should generally be stored until the test results are obtained or longer if required by the regulation. Laboratory sample portions that are known to be highly contaminated should be decontaminated prior to disposal. The procedure for decontamination could be similar to that used for used media/plates, etc., and should be as per documented procedure.

b) Conventional, biological and hazardous waste should be removed and disposed off regularly and safely. For large laboratories, systems for on site incineration, landfill, neutralization and sterilization before disposal or pick-up by a licenced contractor as applicable, may be considered subject to local regulations. Adequate number of properly labeled waste containers should be strategically placed throughout the laboratory, they should be leak proof with tight fitting lids and disposable liners to collect the waste. Waste storage area should be marked and kept free from insects and pests till final disposal.

14.10 ASSURING THE QUALITY OF TEST AND CALIBRATION RESULTS

Microbiological/Biological test methods are generally empirical in nature. The result obtained (and its associated traceability and uncertainty) is dependent on strict adherence to the method used, including the test method conditions specified. For methods like these, the ultimate way of ascertaining that the results are under control would be through revalidating the test method conditions every time the test is conducted (e.g. by the quantitative assessment of the recovery of a reference organism from the sample matrix for each sample tested).

However this is not practicable and the alternate to this would be that the laboratories implement a quality control programme that ensures the test method conditions are adequately controlled. This control procedure will also ensure that the test method conditions (or parameters contributing to the measurement uncertainty) operate within defined parameters and thus have a predictable and consistent contribution to the measurement uncertainty in the results. In other words, it means that all possible inputs into the testing system need some level of quality control to ensure consistency to the quality of the results produced. Some of the key inputs common to most microbiological testing and which need to be subjected to such a quality control programmes are:

a) Personnel involved in all activities related with testing;

b) Use of valid test methods, exactly as they are prescribed or validated;

c) Laboratory accommodation and environment appropriate to the type of test conducted;

d) Equipment, its calibration with valid traceability of it’s measurement;

e) The authenticity and maintenance of reference organisms to ensure their validity and viability; and

f) Quality of consumables used in the conduct of the tests; including media, reagents and diluents and their preparation;

These quality control requirements are very much part of the ISO 17025 requirements and have been described in detail in this Unit. However additional Quality Control requirements may be required by method specifications, or general principles of good laboratory practices for a biological testing lab. These would also depend on the types of tests performed and the size of laboratory and the scale of operations. The quality control measures can be achieved through Internal Quality control means or External Quality Control means.

14.10.1 Internal Quality Control

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

Laboratories are required to plan their activities with respect to internal quality control and implement the same. The plans should include types of quality control checks, their frequency and acceptance criteria, and actions to be taken when results fall outside the defined acceptance criteria. The Internal Quality Control means for a biological test laboratory should include:

a) Sterility controls - Uninoculated samples should be run at a minimum of once for every test run. Sterility controls are used to detect the presence or absence of possible laboratory contamination.

b) Split samples (Duplicates) for quantitative tests - Split samples comprise a sample divided into 2 sub-samples. Analyses of spilt samples are normally expected to be conducted at a frequency of once per test run.

c) Confirmation/verification of presumptive positive samples - Positive and negative characteristic strains, if applicable, should be tested concurrently with any biochemical and serological tests for confirmation of presumptive micro-organisms. The number or percentage of colonies that are stipulated in test standard required for confirmation process should be followed. Laboratories can also define the minimum number of colonies for confirmation if such requirements are not specified.

d) Establishment of precision of Test Method/Use of spiked samples -The laboratories should establish the precision of test methods. Acceptance limits for precision can be established by running spiked samples of cell suspension in duplicate or triplicate, using two or more operators. Criteria used to set acceptance limits for precision (for example relative standard deviation or range) should be based on statistical principles and clearly presented for each test method. Recommendations given in APHA section 9020B “Intra laboratory Quality Control Guidelines” and ISO 5725-6 : 1994 “Accuracy (trueness and precision) of measurement method and results Part 6: Use in practice of accuracy values”, should be followed, if appropriate. The laboratory should also document the application of the precision criteria in monitoring acceptance of daily test results.

e) Verification of continuing competence - Laboratories should establish schedules, in compliance with the verification frequency stipulated in test standards, for checking the continuing competence to perform positive tests for each test method if no positive samples are encountered. Reference stocks shall be maintained for all tests conducted, and suitable suspensions of fresh sub-cultures should be spiked into appropriate matrix and run through each entire test procedure. The analyst is required to make parallel analyses with another analyst. Criteria should be set for maximum allowance difference between the counts based on precision of test methods.

f) Control charts should be used, where appropriate, to monitor the performance of the laboratory. Furthermore, full confirmation of few suspected colonies should be conducted using appropriate biochemical or serological tests.

g) For a GMO testing laboratory Quality Control measures would generally include extraction negative (or blank) control, negative PCR control, detection limit control using CRM, positive PCR amplification control using reference DNA extracted from CRM or known positive GMO sample. These means also help in periodically validating the method. In addition as a good practice the laboratory should carry out every analysis in duplicate using two different primers.

The frequency of quality control activities, both internal and external, for a laboratory should be governed by factors like the number of tests conducted on a routine basis, number of employees involved in testing activities, tests which are carried rarely, etc. generally 5-10% of the tests carried out as QC samples is considered fairly adequate. Where tests are performed infrequently, the laboratory should carry out regular performance checks to demonstrate its continuing competence to perform them, or have in place a system for demonstrating proficiency prior to performing the test on a customer sample. In fact incorporating the relevant mechanisms to control performance of the tests in the individual test method itself is a good practice, especially when dealing with empirical test methods like biological tests.

14.10.2 External Quality Control, Proficiency Testing Programs

The most important mechanism of external quality control is through participation in independently organised Inter-laboratory comparison programs, also known as proficiency testing programs. A laboratory is required to regularly participate in the proficiency testing programs relevant to the scope of laboratory as defined under 14.4 above. Participation in proficiency testing is a very good mechanism for assessing laboratory bias and also to check the validity of the whole quality system.

14.11 REPORTING OF RESULTS

All the requirements, as specified in ISO 17025 Clause 5.10, as relevant to a laboratory engaged in sampling and/or testing are applicable. However it is important to note that in many instances the product or test standards and regulatory requirements will determine the report format and content. In addition for food testing laboratories following requirements are applicable:

Microbiological test laboratory: For an analysis which is quantitative in nature, while reporting the results where the result of the enumeration is negative, it should be reported as per the requirements specified in the test method standard used – "Less than 10 CFU per one gram" for solid samples or "Less than one CFU for liquid samples per gram". The result should not be given as "zero for a defined unit" unless it is a regulatory requirement. In cases where it is regulatory requirement all the requirements of the same should be followed. Qualitative test results should be reported as "detected/not detected in a defined quantity or volume" or "present or absent in a defined unit".

Biotechnology (GMO) testing: laboratories carrying out GMO testing activities with PCR should accurately describe both the primer sets used and the results obtained. The specificity of the target sequence should be reported, i.e. '35S promoter: detected', or NOS terminator not detected'' instead of a general statement 'does not contain GMO'. The latter wording would imply that primer sets covering all potential GM varieties had been run. Similarly, quantitative results shall be reported as 'x.x % of 35 S promoter, instead of 'x.x % GM material. Further the sample preparation procedure should be given if it is required for the proper interpretation of test results in GMO testing laboratory's test reports.

Check Your Progress Exercise 1

Note: a) Use the space below for your answers.
b) Compare your answers with those given at the end of the unit.

1) Name the micro-organism that may contaminate water, food of animal origin and fruits and vegetable?

……

……

……

2) Explain sub-contracting of tests?

……

……

……

3) Which points should be kept in mind while planning layout and design for biological sample?

……

……

……

4) Explain procedure for sample retention and storage of biological samples?

……

……

……

14.12 LET US SUM UP

While applying the various management system elements as specified in Clause 4 of ISO 17025:2005 to a laboratory engaged in testing biological/ microbiological parameters in food products specific guidelines are required to be kept in mind as applicable to specific applications. In case laboratory is engaged in diverse testing activities like chemical testing, microbiological testing and testing genetically modified food then the laboratory may need to consider more than one person as constituting the technical management. If the biological food testing laboratory is using sophisticated equipments like real time PCR for GMO estimation which may make use of proprietary software then the document control system must be extended to these softwares also. Preparation for PCR testing should be of molecular biology grade or equivalent and free from contaminating nucleic acids or nucleases (BOH, DNAse and R Nase).

In laboratory testing biological parameters of food the layout, environmental conditions and access control requirements would be different for different activities like sample receipt and storage, media preparation, maintenance of reference organisms, washing, sample preparation, actual testing using laminar flow and bio-safety cabinet, incubation and plate examination and biotechnology PCR, GMO testing. In any case irrespective of what activity takes place there must be appropriate segregation and space for various tests to minimise potential contamination and to ensure protection of personnel. The laboratory layout and design is the most important aspect of a food microbiological laboratory. It should be such that the appropriate segregation of activities minimize the risks of cross-contamination. It would be best if all the areas as listed above are physically separated. The testing areas should be appropriately lighted and ventilated and temperature controlled as appropriate. In areas like plating and incubation and media/sample preparation the source of ventilation should be provided with appropriate filters. The testing area construction should be such that it ensure reduction of contamination. This can be ensured by having smooth, non-absorbent and easy to clean and disinfect surface for walls, celling, floors and benches, concave joints between the floors walls and ceiling, appropriate air locks for entries to plating and other similar areas. For procedures that involve pathogen handling and reference stock cultures these should be handled within bio-safety cabinet.

Similar to layout and design of the laboratory the sampling is also a very important aspect of food microbiology laboratory. In case of food products biological test are generally conducted to establish safety parameters of a lot/ batch hence it is very essential that the sample drawn for laboratory testing must be representative of the lot/batch. Sampling for microbiological testing of food must be done by trained person. Further, the sampling equipments must be sterilized and aspeptic techniques must be followed to avoid contamination. The transportation and storage should be under conditions that maintain the integrity of the sample.

14.13 KEY WORDS

AOAC : Association of Official Agricultural Chemists, now known as AOAC INTERNATIONAL, a not-for-profit scientific association engaged in providing and facilitating in the development, use, and

harmonization of validated analytical methods and laboratory quality assurance programs and services.

AOCS US FDA : United States Food and Drug Administration.

APHA : American Public Health Association.

GMO : Genetically Modified Organism.

Limit of Detection : Applied to qualitative microbiological tests- the lowest number of micro-organisms that can be detected, but in numbers that cannot be estimated accurately.

Limit of Determination : As applied to quantitative microbiological tests - The lowest number of micro-organisms within a defined variability that may be determined under the experimental conditions of the method under evaluation.

Negative Deviation : Occurs when the alternative method gives a negative result without confirmation when the reference method gives a positive result. This deviation becomes a false negative result when the true result can be proved as being positive.

Positive Deviation : Occurs when the alternative method gives a positive result without confirmation when the reference method gives a negative result. This deviation becomes a false positive result when the true result can be proved as being negative.

Proficiency Testing : Determination of laboratory testing performance by means of inter-laboratory comparisons" (ISO Guide 43:1996).

Reference Cultures : Collective term for reference strain, reference stocks and working cultures.

Reference Method : Thoroughly investigated method, clearly and exactly describing the necessary conditions and procedures, for the measurement of one or more property values that has been shown to have accuracy and precision commensurate with its intended use and that can therefore be used to assess the accuracy of other methods for the same measurement, particularly in permitting the characterization of a reference material.

Reference Stocks : A set of separate identical cultures obtained by a single sub-culture from the reference strain (ISO 11133-1:2000).

Reference Strains : Micro-organisms defined at least to the genus and species level, catalogued and described according to its characteristics and preferably stating its origin (ISO 11133-1:2000). Normally obtained from a recognised national or international collection.

In India, strains obtained from National Chemical Laboratory (NCL), Pune; Institute of Microbial Technology (IMTECH), Chandigarh; Central Research Institute (CRI), Kasouli, Himachal Pradesh; National Institute of Communicable Diseases, Delhi; Christian Medical College (CMC), Vellore; etc.; are considered appropriate for use as reference strains.

Sensitivity : As applied to microbiological tests - The fraction of the total number of positive cultures or colonies correctly assigned in the presumptive inspection (ISO 13843:2000).

Specificity : Applied to microbiological tests - The fraction of the total number of negative cultures or colonies correctly assigned in the presumptive inspection (ISO 13843:2000).

Working Culture : A primary sub-culture from a reference stock (ISO 11133-1:2000).

Note 1: Most of the Definitions included above are based on Standard definitions as defined in publications like VIM, EURACHEM, ISO, IUPAC, etc.

Note 2: The definitions as included above are for terms specific to biological testing laboratory as included in this unit. In addition to the above the general definitions as covered in units 2 and 4 will also apply.

14.14 ANSWERS TO CHECK YOUR PROGRESS EXERCISE

Your answer should include following points:

Check Your Progress Exercise 1

1) Water may be contaminated with micro-organism like *Ecoli*, Yeast & mould, Fecal Streptococci, Staphylococcus aureus, Sulphite-reducing anaerobes, Pseudomonas aeruginosa, Salmonella and Shigella, Vibrio Cholerae & Vibrio Parahaemolyticus, etc., due to contamination coming from raw water sources and subsequent handling.

   Foods of animal origin including milk and milk products may harbor pathogens of genera *Salmonella & Shigella, Escherichia coli (Ecoli), Staphylococcus aureus, Compylobacter, Listeria monocytogenes*, etc. and micro-organisms like yeast and mould, etc.

   Fruits and vegetables generally harbor micro-organisms like molds, yeast, lactic acid bacteria, bacteria from genera *Pseudomonas, Alcaligenes, Micrococcus, Clostridium* and *Enterobacter*.

2) Sub-contracting of tests: In case for few of the parameters in a range of tests required to be carried out on a food product, the laboratory does not have testing facilities or its testing facility is temporarily out of order, there is a provision that these tests may be sub-contracted to a competent sub-contractor. As per ISO 17025 a competent sub-contractor is the one who

complies with the requirements of ISO 17025. When a Food testing laboratory decides to sub-contract, it will need to ensure that besides the above the sub-contracted laboratory also has the testing capability for the tests and range and accuracy/precision equivalent to its own for the food product under question before taking the sub-contracting decision.

3) Layout and Laboratory Design

   a) In case a laboratory is engaged in Microbiological tests and Biotechnology (molecular biology) related tests (GMO), then it is a good idea to physically separate the areas, where these two types of tests are carried out, since the risk of contamination is much more significant in Biotechnology test area. Also it is good idea to segregate within Biotechnology work area the low, medium and high DNA, working environments and also to maintain dedicated equipment like pipettes, tips, centrifuges, tubes, etc.

   b) The space provided for different sections in a Biological test laboratory should be adequate to allow work areas that can be kept clean and clutter free.

   c) The testing areas should be appropriately lighted and ventilated and temperature controlled as appropriate.

   d) The testing area, construction should be such that it ensures reduction of contamination. This can be ensured by having smooth, non-absorbent and easy to clean and disinfect surfaces for walls, ceilings, floors and benches; concave joints between the floor, walls and ceiling; appropriate air locks for entries to plating and other similar areas; non utilization of wood; separate hand-washing arrangements, preferably non-manually controlled; etc.

   e) For procedures that involve handling of pathogens and reference stock cultures, they shall be operated within a safety cabinet of a class commensurate with the risk level of the micro-organism handled.

   f) In case of an inhouse laboratory of a food product manufacturing unit, it is desirable that area involved in Pathogen culture handling/testing should be physically separated and located as far away from the manufacturing unit as possible.

4) a) The sample handling procedure should include procedure for storage, retention and disposal of samples. Samples should generally be stored until the test results are obtained or longer if required by the regulation. Laboratory sample portions that are known to be highly contaminated should be decontaminated prior to disposal. The procedure for decontamination could be similar to that used for used media/plates, etc, and should be as per documented procedure.

   b) Conventional, biological and hazardous waste should be removed and disposed off regularly and safely. For large laboratories, systems for on site incineration, landfill, neutralization and sterilization before disposal or pick-up by a licensed contractor as applicable, may be considered subject to local regulations. Adequate number of properly labeled waste containers should be strategically placed throughout the laboratory, they should be leak proof with tight fitting lids and disposable liners to collect the waste. Waste storage area should be marked and kept free from insects and pests till final disposal.

14.15 SUGGESTED READING

Draft ISO/FDIS 11133-2, *Microbiology of Food and Animal Feeding Stuffs. Guidelines on Preparation and Production of Culture Media. Part 2- Practical Guidelines on Performance Testing on Culture Media.*

Draft ISO/DIS 16140, *Food Microbiology*. Protocol for the validation of alternative methods.

EA-04/10:2002 - *Accreditation in Microbiological Laboratories*. Published by European Accreditation.

EN 12741, *Biotechnology- Laboratories for Research, Development and Analysis – Guidance for Biotechnology Laboratory Operations*.

ISO/IEC 17025:2005, *General Requirements for the Competence of Testing and Calibration Laboratories*.

ISO 5725-6:1994 *Accuracy (Trueness and Precision) of Measurement Method and Results* Part 6: Use in practice of accuracy values.

ISO 6887-1:1999 *Microbiology of Food and Animal Feeding Stuffs - Preparation of Test Samples, Initial Suspension and Decimal Dilutions for Microbiological Examination.* Part 1 - General Rules for the Preparation of the Initial Suspension and Dilution.

ISO 7218, *Microbiology of Food and Animal Feeding Stuffs - General Rules for Microbiological Examinations.*

ISO 11133-1, *Microbiology of Food and Animal Feeding Stuffs. Guidelines on Preparation and Production of Culture Media*. Part 1- *General Guidelines on Quality Assurance for the Preparation of Media in the Laboratory*.

LAB C 1, February 2004 – *Specific Criteria for Accreditation – Biological Testing*. Published by a International Accreditation New Zealand(IANZ).

NABL 102 - *Specific Guidelines for Biological Testing Laboratories*.

Technical note: c&b-002, February 2005 "*Quality Assurance of Equipment commonly used in Chemical and Biological Testing Laboratories*", Issued by Singapore accreditation council - Singapore laboratory accreditation scheme (sac-singlas).

VIM: 1993, *ISO International Vocabulary of Basic and General Terms in Metrology*.