FAO FOOD AND NUTRITION PAPER 14/12

manual of food quality control

12. quality assurance in the food control microbiological laboratory

FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS ROME

FAO
FIAT PANIS

FAO FOOD AND NUTRITION PAPER 14/12

manual of food quality control

12. quality assurance in the food control microbiological laboratory

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 1991

The designations employed and the presentation of material in this publication do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

M-82
ISBN 92-5-103053-7

FOREWORD

The control of food safety and quality is an integral part of national programmes for development. National food control systems are designed to protect the health and welfare of the consumer, to promote the development of trade in food and food products, and to protect the interests of the fair and honest food producer, processor or marketer against dishonest and unfair competition. Emphasis is placed on the prevention of chemical and biological hazards which result from contamination, adulteration or simple mishandling of foods. Also important are the maintenance of general food quality.

An integral part of a national food control system is the analytical laboratory service. The typical food control laboratory has both chemical and microbiological analytical capabilities. Each of these technical areas have differing requirements and procedures to establish and maintain high quality analytical work.

This manual is a practical handbook on the establishment of a Quality Assurance (QA) Programme for a Food Control Microbiological Laboratory. Its ultimate aim is to assure that a microbiological laboratory produces reliable high quality analytical results with a continuity of documentation providing a clear, accurate and indisputable history of the analysis with all segments of documentation in agreement. A similar QA manual for the chemistry laboratory is in preparation.

The manual is designed for microbiology laboratory management and analytical staff, but regulatory authorities or any other concerned persons can gain useful information and insights into the problems involved in establishing and operating a quality assurance programme in a Food Control Microbiology Laboratory.

FAO wishes to acknowledge the efforts of Dr. Wallace Andrews, Division of Microbiology, Food and Drug Administration (FDA), Washington DC, USA, who prepared the text for this manual. The contribution of other FDA staff including Ms Lois Tomlinson for technical editing and Ms Donna Alesia Newman for typing the draft manuscript, are also gratefully acknowledged. Portions of the text were provided by Mr. Peter Martin of Lynn, Martin and Radford, Public Analysts, Reading, Berkshire, England.

The manual in draft form was reviewed by Dr. B. Amla, Director, Central Food Technological Research Institute, Mysore India; Dr. T. Karki, Director, Central Food Research Laboratory, Ministry of Agriculture, Kathmandu, Nepal; Dr. H. Leonhardt, Director, International Scientific Cooperation, Robert Von Ostertag Institute, Berlin, Germany; and Dr. H. Mol, retired Director, National Food Inspection Service, Utrecht, The Netherlands. FAO is grateful for those reviews.

This publication is available to persons and organizations. A list of publications and papers on mycotoxins published by FAO is given at the end of this Manual. Comments on suggestions for possible future editions of this publication should be sent to:

The Chief
Food Quality and Standards Service
Food Policy and Nutrition Division
Food and Agriculture Organization of the United Nations
00100 Rome, Italy

SPECIAL NOTE

The Laboratory procedures described in this Manual are designed to be carried out by properly trained personnel in a suitably equipped laboratory. In common with many such procedures, they may involve hazardous materials.

For the correct and safe execution of these procedures, it is essential that laboratory personnel follow standard safety precautions.

While the greatest care has been exercised in the preparation of this information, FAO expressly disclaims any liability to users of these procedures for consequential damages of any kind arising out of or connected with their use.

The quality assurance procedures are also not to be regarded as official because of their inclusion in this Manual.

QUALITY ASSURANCE IN THE FOOD CONTROL MICROBIOLOGICAL LABORATORY

TABLE OF CONTENTS

ANNEXES

ANNEXES

QUALITY ASSURANCE IN THE FOOD CONTROL MICROBIOLOGICAL LABORATORY

1. MANAGEMENT

1.1 Objectives of the laboratory

The objectives of the laboratory should be clearly defined and expressed as simply as possible. Their clear definition is of fundamental importance because it is on this that all laboratory activities are based. The Laboratory Director should define the objectives after taking such advice as he sees fit and such instructions as he may have received from those directing his activities. The statement should include reference to the quality of results, their timeliness and their cost-effectiveness. The objectives may be divided into a number of statements. All aspects crucial to the laboratory operation should be included but detail should be avoided.

The principal objective of a laboratory is to produce reliable results, which is therefore the activity to which due attention must be paid. Indeed, a laboratory producing unreliable results to a significant extent is unlikely to be acceptable as part of any government machinery. The assurance of the quality of those results is not an extra burden or an extra activity that can be taken or left, it is one of the fundamental management tools for the director and his staff, to ensure they achieve whatever they set out to do.

The objectives in relation to quality must be as realistic as any other objectives. The general objective of the laboratory might be defined as producing analytical data of adequate accuracy and reliability within an acceptable time and at an acceptable cost.

The quality objective might be considered as being as sure as possible that approximately the right answer had been obtained. This statement requires examination. What is 'as sure as possible'? It is being so sure that if subsequently shown to be erroneous, the reasons would not reflect on the integrity, probity or technical competence of laboratory staff. And what is meant by 'approximately'? It means obtaining a result which is good enough for the purpose for which it will be used. If a sample is seriously deficient in a particular analyte, the precise amount of the deficiency probably will not be of great practical importance in, for example, court proceedings or refusal of a consignment. As the amount of analyte approaches the legal limit, the accuracy of the analysis becomes more crucial until a point is reached when the result is closer to the limit than the precision of the method. Thus both accuracy and precision have to be greater for marginal samples than those for which results are far from any standard or limit.

Quality Assurance (QA) is that part of the management function that ensures the quality of results. It is a function that should be carried out to the extent necessary, no more and no less, and should be a fully integrated part of managerial duties on a daily basis. It is important to note that it involves not only getting the right answer but being able to prove the right answer has been obtained as well as maintaining documentary evidence of this. Introduction of written QA procedures into a laboratory for the first time may require

considerable changes in attitude. When introduced in the right way, QA leads to improved morale as people gain confidence in their results and pleasure at being able to prove the veracity of those results. Quality assurance focuses attention on relevant aspects of daily activities and training needs and helps staff to develop their skills and further their careers.

1.2 Quality assurance and quality control defined

Although the term "quality assurance" may seem self explanatory, it is often confused and used interchangeably with another term, "quality control." Garfield (8) defines quality control as "a planned system of activities whose purpose is to provide a quality product." In the case of a food control laboratory, this quality product would be a valid analytical result. He defines quality assurance as "a planned system of activities whose purpose is to provide assurance that the quality control program is actually effective." Garfield uses the term "quality assurance" to encompass both definitions.

Somewhat different terms and definitions are used by Taylor (6,9), who states that the objective of a quality assurance programme is to reduce errors to acceptable levels and provide assurance that the data have a high probability of being of an acceptable quality. Further, two individual concepts are involved: "Quality control," which Taylor defines as "the mechanism established to control errors," and "quality assessment," which he defines as "the mechanism to verify that the system is operating within acceptable limits."

Yet another term is "quality system," defined by a task force of the International Laboratory Accreditation Conference (7) as "the organization structure, responsibilities, activities, resources, and events that together provide organized procedures and methods of implementation to insure the capability of the organization to meet quality requirements." The task force states that the quality system encompasses all elements of quality control and quality assurance.

Quality control, then, may be considered the combination of systems, procedures, activities, instructions, and management reviews that control and improve the quality of work performed. Quality assurance, however, is the system of activities that gives management confidence that the quality control systems are in place and are effective in producing analytical results of the highest quality.

1.3 Advantages of a quality assurance programme

A quality assurance programme that is working properly has several advantages. First, it provides a tracking record to ensure sample integrity, with documentation to verify that laboratory instruments are functioning properly and that laboratory data were generated according to approved written protocols. Such documentation is especially important in regulatory laboratories where analytical findings must withstand the scrutiny of legal proceedings.

A second advantage is a savings of analytical time and costs. Although the quality assurance programme may initially seem to reduce a laboratory's productivity, it may actually

save analytical time and costs over a long period, since analyses would tend to be done correctly the first time.

A third advantage of a quality assurance programme is its aid in identifying training needs of analysts. This training would not be restricted to new employees; it would also apply to present employees whose performance may be deficient or needs updating.

A fourth advantage would be an increase in analyst confidence derived from knowing that results are reliable. This increased confidence, in turn, would lead to improved morale and performance.

Other advantages of a quality assurance programme include:

- Ensuring errors are minimised or eliminated. It is impossible to eliminate all errors but it is possible to ensure that very, very few serious errors are made without discovery before the results are transmitted outside the laboratory.

- Ensuring forensic credibility. There is usually a strong legal tradition about the test applied to evidence in court. The criteria for the development of scientifically valid evidence are just as rigid but this does not necessarily mean that the evidence will comply with court rules or be understandable to a court. For example, if the legal test is 'beyond reasonable doubt', the court may have difficulty in equating this to statistical information about probability.

- Ensuring, in the event of enquiry or dispute, that management has confidence in the results produced. This confidence derives from the body of evidence that gradually accumulates about the performance of the laboratory in the range of analyses it carries out.

- Ensuring, in the event of enquiry, dispute or error that records are available to resolve the issue. Records should be kept for a considerable length of time. Six years is often chosen.

- Providing a review of deficiencies, errors and complaints so that remedial action can be systematic and lead to intrinsic improvement.

- Ensuring resource utilisation is optimal. This often a slow process, but as more information accumulates about analytical performance within the laboratory, it becomes easier to evaluate the effectiveness with which the resources of the laboratory are being used. For example, it is easier to ensure that reagents are available and still within a 'use-by' date.

- Providing results of sufficient certainty for use in databases for the purposes of food control, public health, nutrition and other food-related local, national or international policies. These databases form an extremely valuable resource for monitoring food products over a period of time. This leads to

identification of changes in products over time and the ability to compare analytical results very easily. If databases do not contain reliable information, false conclusions may very easily be drawn.

1.4 Definition of responsibilities

Responsibilities of management

Unfortunately, the quality assurance component of a laboratory's operations often receives inadequate attention, or may not be properly balanced. For instance, it may be so detailed that practically every analytical function is covered. Although seemingly attractive and advantageous, this type of programme can be overwhelming and frustrating for laboratory personnel. Consequently, what begins as an admirable undertaking, may end in discouragement and the absence of any meaningful quality assurance programme whatsoever. At the other extreme is a programme so abbreviated that it really has little, if any, meaning. It is the responsibility of management to decide upon the scope and priority of the quality assurance programme for a particular laboratory, basing its decision on a calculation of the cost/benefit ratio. When a particular programme has been selected, management must fully support its implementation, maintenance, and concept. If management is perceived as not being supportive of the programme, the staff can be expected to make something less than a full commitment.

Once a quality assurance programme is included as a part of daily operations, management must commit resources to reinforce its support and establish a quality assurance unit to carry out its specifications. Management must project a positive image for the quality assurance programme. The programme should not be viewed as threatening, confrontational or as something extra that must be done. Instead, it should be projected as an instrument to improve employee performance and as a mechanism to document and reward exceptional work.

Many analysts started their working lives in laboratories where quality assurance as we now know it, was not practiced. There are still laboratories working in this way, even though they are diminishing in number. Thus at some point, an analyst has to change his style of working and needs to develop a positive attitude to quality assurance. There are many obstacles to this.

The analyst may feel all his past work is being called into question because it was not undertaken under the new system. Quality assurance is time-consuming initially. The analyst will probably feel sure he already fills his day and nothing he does is inessential. Thus, the new way of working is unachievable and also unnecessary and more time-consuming. Management can easily compound these negative feelings by poor personnel skills, lack of commitment and poor understanding. Unfortunately, quality assurance is often introduced due to external pressure, because of a need for accreditation or because the reputation of the laboratory has suffered when an error came to light.

The concept of quality assurance may have to be sold both inside and outside the laboratory. In the first place, it may have to be sold to those with a part to play in the decisions that lead to funding for the laboratory. There can be difficulty in persuading administrators and legislators, who may have limited technical knowledge, to accept the need for an additional overhead. It may be argued that the laboratory appeared to operate satisfactorily for a long time on the old basis and further costs cannot be justified.

The changing circumstances must often be explained. The risk of questioning laboratory results has always existed but that risk increases as other laboratories introduce quality assurance, better technology and better management. Decision makers have to address changing concepts in the use of technology. Increasing automation and computerisation is diverting attention from the manipulative skills required for often slow and tedious methodology to the administrative and management skills required to assure the quality of large amounts of data. Thus there is a change in the internal laboratory environment too, from haphazard sampling to sampling programmes, from single or very few samples to optimum batches of the same type of product, from analytical virtuosity to quality-assured results, from reacting to events to a food law enforcement policy. The systematic management approach, which brings benefits in cost-effectiveness and best use of resources, cannot operate without the quality assurance aspect.

The nature and size of the quality assurance unit are management decisions. In large multi-discipline laboratories the quality assurance unit may consist of two or more persons whose sole responsibility is to monitor the effectiveness of the quality assurance programme. In smaller laboratories, management may not wish to have a separate quality assurance unit. Instead, an analyst may be assigned to spend part of his or her time ensuring laboratory compliance with an approved quality assurance programme.

The frequency of formal audits to ensure compliance with the approved quality assurance programme is also determined by management. Some managers may elect to have quarterly, or even monthly, inspections. Others may decide to have abbreviated quarterly inspections and a comprehensive annual inspection. Regardless of the frequency of inspections, management reviews the findings of the quality assurance unit and acts on its recommendations. Management may decide to reward employees who have excelled in compliance with the quality assurance programme. For example, the concept of "merit pay" is increasingly used to rate an employee's performance. One consideration for merit pay could be quality assurance, thus enabling management to motivate employees to excel at the approved quality assurance programme. Where there is noncompliance with the programme, management may need to use disciplinary action to ensure compliance.

In addition to reviewing the recommendations of the quality assurance unit, management should periodically review both the quality assurance policy and the programme itself. Although the policy and the provisions should be strictly enforced, there should be enough flexibility to allow for reasonable deviations that may result when provisions of the original quality assurance programme are too specific or not specific enough. If the deviations become too specific, the programme itself may need to be modified. Management

should review the quality assurance policy and programme continually and make appropriate modifications.

In summary, quality assurance does not become an integral part of an organisation's activities without commitment and effort by management. There is a great deal of work to be done initially in writing a quality manual, generating quality control data and commissioning the quality system. Management support at this stage is essential and can be demonstrated by encouragement, counselling and provision of adequate resources. Quality assurance is brought into disrepute if the manager himself is prepared to lapse into an old way of working to achieve some short-term objective, such as reporting a sample very quickly because there is pressure to do so. That is typically the time when mistakes are made. Management can also express their commitment to the programme very effectively by insisting on strict adherence to the schedule of audits and reviews and taking a keen interest in any follow up action. The audits and actions resulting from them represent the mechanism of change built into quality assurance and thus largely reflect the effectiveness of the programme.

Responsibilities of the quality assurance unit

The first step in the formation of a quality assurance unit will generally be to obtain budgetary approval for the appointment of a Quality Assurance Officer and any staff he may need, or agreement to divert existing personnel and other resources into this activity. The QA Officer should preferably have formal qualifications in quality assurance but this is not always possible. More likely, an analyst will be appointed to this position who will have to self-train, attend courses and so on. This person needs to be a competent analyst of some seniority, as he/she must command the respect of colleagues for his/her analytical skills. The QA Officer must be keen to understand the principles of QA and to apply them correctly.

The QA Officer needs auditors and these will generally be appointed from among the analysts. Teams of two are sufficient in a small laboratory, auditing any section including administration but their own. In a larger laboratory, the QA Officer may need his own staff but the QA unit does not usually become large. One QA person for every ten to twenty analysts is probably adequate. The wider the range of analyses undertaken and the lower the overall level of experience, the more will quality assurance be required.

The quality assurance unit is responsible for formulating the written quality assurance plan or manual and assuring adherence to the programme by the laboratory staff. The quality assurance unit acts as a liaison between management, which has committed resources to ensure the success of the project, and the laboratory staff, which is directly responsible for actual maintenance of the programme. The quality assurance unit is dependent upon the staff, specifically the laboratory bench scientist, the team leader, to provide technical expertise in formulating the written quality assurance plan.

The quality assurance unit reports reviews directly to management. In addition to scheduling and conducting reviews, quality assurance personnel should make recommendations to management regarding the results of those reviews; they should also

recommend quality assurance policy to management and assist in its formulation, identify staff training needs, and provide guidelines for adhering to all aspects of the quality assurance programme.

Keen attention to reviews and follow-up action is one way in which management provides support to the quality assurance function. This regular and fairly formal system provides the necessary discipline to keep the programme in operation. The Quality Assurance Officer and, where appropriate, members of his unit must have direct access to the Laboratory Director or his delegated subordinate. A review report or an irregularity can then be discussed promptly and remedial action taken quickly.

There should be review meetings once or twice a year. At this time, in consultation with the Quality Assurance Officer and senior analysts, management may decide on any changes in policy and programme. Need for change will become apparent as a result of quality audits.

Responsibilities of the analyst

The analyst plays an essential role in the operation of the quality assurance programme. The trained bench scientist is responsible for the quality of the data and related laboratory activities, and is the first with an opportunity to detect malfunctions of the analytical system. Analytical personnel must be able to distinguish what is a normal, random fluctuation and what constitutes an abnormal deviation.

Both management and the quality assurance unit expect the analytical staff to provide technical input and advice in the formulation of the quality assurance programme. Selected analysts may be asked to write a portion of the quality assurance plan, subject to review and approval by the quality assurance unit and management. Being included in formulating the quality assurance plan can be a motivation factor, giving staff personnel a feeling of being a creative part of the quality assurance programme.

The laboratory staff is responsible for adherence to the provisions of the approved plan. The success or failure of the plan ultimately depends on the performance of the analyst. The bench scientist, who actually forms the first level of "management" of any quality assurance programme, is responsible for doing the work properly, documenting it, and having the work reviewed to ensure that it meets acceptable standards.

Thus, each of the three groups (analytical staff, quality assurance unit, and management) must provide the appropriate input to ensure the success of a quality assurance programme. The analytical staff provides the technical expertise needed to prepare the quality assurance plan and is responsible for the daily adherence to it.

The quality assurance unit monitors the staff's adherence to the plan and, based on its reviews, makes recommendations to management. Management, in turn, reviews the reports of the quality assurance unit and acts on its recommendations.

1.5 References

The following non-text references provide general background on management of Quality Assurance programmes:

1. NAMAS Executive 1989. General criteria of Competence for Calibration and Testing Laboratories. National Physical Laboratory, Teddington, TW11 OLW, U.K.

2 Uelner, A.F. 1984. The Watchdog of the Industry, Concepts, Toxicol. 1 (93-102) Karger, Basel.

3. Kilshaw, D. Quality Control & Assurance, MLW, June 1986, pp 25 and 26.

4. Loftus, P. 1986. Quality Assurance. Water Bulletin Supplement 21.3.86, pp 3 and 4.

5. Waddell, A. 1988. The Importance of Quality, International Good Laboratory Practice Conference, Stratford, England.

6. Taylor J.K. The Quest for Quality Assurance, American Laboratory, October 1985, 67-75.

7. Anonymous. 1984. Report of Task Force "D" at the International Laboratory Accreditation Conference, London, U.K. Department of Trade and Industry, London, U.K.

8. Garfield, F.M. 1984. Quality Assurance Principles for Analytical Laboratories. Association of Official Analytical Chemists, Arlington, VA.

9. Taylor, J.K. 1987. Quality Assurance of Chemical Measurements, Lewis Publishers, Inc., Chelsea, MI.

10. Weatherwax, J., and P.G. Martin. 1986. Manuals of Food Quality Control. 1. The Food Control Laboratory, 2nd ed. Food and Agriculture Organization of the United Nations, Rome, Italy.

2. THE QUALITY ASSURANCE PROGRAMME

2.1 Definition

A quality assurance programme may be defined as a mechanism used to ensure that data generated by a laboratory are of the highest quality. It makes this assurance by determining that all laboratory functions are performed as intended. Moreover, documentation exists to permit reassessment of the data as needed.

2.2 Preparation

To prepare a quality assurance programme, one must consider the various elements of the programme. The U.S. National Institute for Occupational Safety and Health (1) has identified more than 20 elements that potentially may be covered in a quality assurance programme:

a. Statement of objectives
b. Policy statements
c. Organization
d. Quality planning
e. Standard operating procedures
f. Recordkeeping
g. Chain of custody procedures
h. Corrective action
i. Quality training
j. Document control
k. Calibration of instruments
l. Preventive maintenance
m. Reagent and reference standards
n. Procurement and control
o. Sample identification and control
p. Laboratory analysis and control
q. Interlaboratory and intralaboratory testing programmes
r. Handling, storage, and delivery of samples
s. Statistical quality control
t. Data validation
u. System audits

Element (e), above, is termed Standard Operating Procedure (SOP). A Standard Operating Procedure may be considered a document describing any procedure which is not a method of analysis. It may describe an administrative routine, a non-analytical laboratory procedure such as starting up an instrument, or any other procedure used in the laboratory. An SOP will normally describe an activity in sufficient detail to enable it to be performed without supervision and in some cases without training. A method of analysis can be written in SOP format but is best regarded as a different type of document. Quality assurance covers all the working activities in the laboratory, not just analysis. All these activities are

controlled and can only be controlled if there is a written record of them (or perhaps a computer record, but in that case a hard copy will normally be kept). That written record is the Standard Operating Procedure.

Because of the diverse nature of food control laboratories, a single uniform programme cannot cover all laboratory activities. Instead, a quality assurance programme must be tailored to cover the functions of a particular laboratory. A quality assurance programme, however, need not be so specific that it can be used only by a single laboratory. A sound quality assurance programme is flexible enough so that, with minor modifications, it may cover the activities of different laboratories performing essentially similar tasks.

An effective quality assurance programme is simple. The programme should be clear, concise, and uncomplicated, not tedious or unduly long and cluttered with unnecessary details or trivia. A complex programme is likely to generate analyst resentment, which may lead to less than a full commitment.

An effective quality assurance programme must be practical from the standpoint of the analytical time and costs involved. If a disproportionate amount of the analyst's time is needed to maintain it, the quality assurance programme is not properly balanced. An effective programme should result in a savings of analyst time and costs because repeat analyses are seldom required.

Not all of the 21 elements listed above are needed in the preparation of a given quality assurance programme. Each element may be emphasized to a different extent in various quality assurance programmes. Garfield (2) provided a somewhat simpler formulation of a quality assurance programme, proposing three essential components:

a. Prevention, which requires an orderly programme of planning and positive actions before or during analyses to ensure that all analytical systems are performing appropriately (e.g., calibration and maintenance of instruments, use of microbiological standard cultures, and training).

b. Assessment, a form of control that includes periodic checks on analyst performance (e.g., analysis of check samples and validation of methodology).

c. Correction, an action taken to determine cause(s) of quality defects and to reestablish proper functioning of analytical operations (e.g., trouble-shooting to correct malfunctioning equipment, reevaluation of methodology, and retraining).

The final form of the quality assurance programme should be both a scientific and a management decision. The day-to-day analytical operations of the food control laboratory should determine which segments are needed in the programme. Management must then prioritize these segments and determine the extent to which analytical resources will be allocated to the programme.

2.3 The quality assurance manual

Each laboratory with a quality assurance programme should have a manual that documents the operations of the laboratory. The U.S. Environmental Protection Agency (3) defines the quality assurance manual as a written document that describes the policies, organization, objectives, functional activities, and specific quality assurance activities designed to achieve the objectives of quality desired by the laboratory. A typical manual might consist of the following:

a. Title page with signatures of all approving officials
b. Table of contents
c. Organizational structure and exactly where the laboratory fits into this structure
d. Objectives of the quality assurance programme
e. Inclusion of essential elements of the quality assurance programme as listed previously
f. Documentation forms
g. Performance and frequency of audit
h. Corrective and follow-up action

A statement of quality assurance policy, both general and specific, is needed in the QA manual. For example, the U.S. Food and Drug Administration's Bureau of Foods Quality Assurance Manual (4) includes a general policy statement: "The Bureau of Foods Quality Assurance Program is intended to serve in maintaining the highest level of quality and integrity of the Bureau data. The policies, procedures and instructions contained in this manual establish a quality assurance program uniformly applicable to all Bureau laboratory facilities. This manual covers nonclinical laboratory studies, other laboratory studies and regulatory samples. All laboratory personnel who are associated with the supervision or conduct of laboratory work are responsible for following the instructions in this manual."

In addition to this general policy statement, specific policy statements appear throughout the manual, for example, a definition of the responsibilities of various organizational levels in implementing the programme, a list of laboratories (regardless of location) to which the quality assurance programme applies, reference(s) to recommended laboratory methodology, ownership of or property rights to laboratory data, and any exceptions to policy statements.

An example and discussion of some of the elements which could be included in a QA manual are contained in Annex 1.

2.4 Implementation

Actual implementation of the quality assurance programme is a cooperative effort involving management, members of the quality assurance unit, and analysts. Management decides the amount of resources to be allocated to the quality assurance programme. This decision determines the nature and size of the quality assurance unit. In formulating the quality assurance plan, this unit receives technical input from the analysts. Once formulated

by the quality assurance unit and approved by management, the quality assurance plan becomes operational. Thereafter, analysts are responsible for day-to-day maintenance of the programme. The quality assurance unit periodically monitors this adherence and makes its report and recommendations to management, which then acts on these recommendations to achieve compliance with the programme.

2.5 References

1. National Institute of Occupational Safety and Health. 1976. Specification for Industrial Hygiene Laboratory Quality Program Requirements. National Institute for Occupational Safety and Health, Cincinnati, OH.

2. Garfield, F.M. 1984. Quality Assurance Principles for Analytical Laboratories. Association of Analytical Chemists, Arlington, VA.

3. U.S. Environmental Protection Agency. 1980. Guidelines and Specifications for Preparing Quality Assurance Project Plans. U.S. Environmental Protection Agency, Cincinnati, OH.

4. U.S. Food and Drug Administration. 1982. Bureau of Foods Laboratory Quality Assurance Manual. U.S. Food and Drug Administration, Washington, DC.

3. LABORATORY FACILITIES

3.1 Design of the laboratory

Even though the final design of the laboratory is made by architects and engineers, the analytical staff should be involved in some of the decisions that will ultimately affect their working environment and conditions. This discussion, then, presents several points for analysts to consider if, and when, they are asked to provide input into the design of their laboratory.

Weatherwax and Martin (1) have provided a comprehensive discussion of the details involved in establishing the food control laboratory. The food control laboratory may have several functions: analysis of foods for trace metals, additives, nutrients, and toxicants as well as basic food microbiology. The discussion presented here will cover those details of concern in designing food microbiology laboratories.

General considerations

Laboratory layout should be devised with efficiency in mind. For example, the distances staff have to walk for the different steps of the analytical processes they undertake should be as short as possible.

The design must include space for support facilities. These will include a workshop unless reliance can be placed on outside contractors for all the aspects of laboratory maintenance, including plumbing, electrical supplies, servicing the electronics and electrics of items of non-analytical equipment.

There is a need for offices for clerical staff, and toilet and washing facilities, as well as a canteen, however simple. There must be stores, for samples, for equipment and for chemicals and glassware. The sample storeroom must be constructed so that it can be kept vermin-free.

The microbiology laboratory

Under ideal conditions, the microbiology laboratory should not be an all-purpose room, but rather a suite consisting of separate rooms for storage of glassware, storage of dehydrated media, media preparation and sterilization, housing of animals (if any), decontamination of pathogenic or hazardous materials, and staff functions. Regulatory laboratories need separate areas for storage of incoming samples to be analyzed and for storage of reserve portions of samples already analyzed. In reality, however, this requirement cannot always be met, and a compromise must be made. Many microbiology laboratories may consist of a single room which contains a central workbench where media and reagents are prepared and where the microbiological analyses are performed. This room may also contain storage areas of various capacities for media and glassware. However, additional functions, e.g., decontamination of pathogenic materials, storage of incoming samples, storage of sample reserves, and animal housing, should not be attempted in a single room.

Media, reagents, and glassware that must be stored in the same room where microbiological analyses are performed should be kept in tightly closed containers and all items should be stored in dust-free cabinets, preferably with sliding glass doors. Except when opened to provide access, these doors should be kept closed at all times.

A side-loading autoclave is perhaps the most expensive acquisition for many microbiology laboratories. Even so, it is recommended that separate autoclaves be used for media sterilization and for decontamination of pathogenic materials in order to minimize the possibility of cross-contamination. The two autoclaves should ideally be housed in separate rooms or, at the very least, at distant sites from each other if they must be located in the same room.

Whereas large microbiology laboratories may have their glassware washed for them in a centralized facility altogether separate from the microbiology laboratory, the personnel in the smaller microbiology laboratory may be responsible for washing their own glassware. In that situation, glassware may be washed in the same room containing the autoclave for decontaminating pathogenic materials or, if need be, in the room containing both autoclaves, i.e., for media sterilization and for decontamination of pathogenic materials.

A separate staff room, however small, deserves consideration since it not only provides a greater degree of safety to laboratory personnel but also helps to ensure sample integrity. Eating, drinking, and smoking are always discouraged, and often prohibited, in the laboratory proper; it is the responsibility of management to provide an appropriate alternative area for these activities.

To provide for a prompt exit in the event of fire or other emergency, at least two entrances/exits must be provided for each room whenever possible. Entrances should be designed to minimize pedestrian traffic.

The possible expansion of laboratory staffs and work loads should be considered in designing a new laboratory. Most laboratory staffs seem to outgrow their facilities soon after they have moved in and have begun working. Thus, managers should keep in mind future staff projections, numbers and types of samples, and equipment needs.

Walls should be covered with a waterproof, mildew-resistant paint that provides a smooth, impervious surface for easy cleaning.

In many microbiology laboratories the wall space is under-utilized. If possible, this wall space should be used for additional shelving, protected by glass-enclosed cabinets to provide a dust-free environment for storage of media, chemicals, and other materials.

Because microbiologists may have to stand for several hours during a normal working day, floors should be relatively comfortable. A heavy duty, impervious tile that can be readily cleaned is recommended. To increase comfort, rubber mats can be strategically placed throughout the laboratory. Linoleum squares laid over a concrete slab are not recommended because the crevices between the squares cannot be cleaned thoroughly. In

time, the linoleum itself will develop cracks, providing additional areas for bacteria to proliferate.

If possible, the microbiology laboratory should be located away from any building exhaust or fume outlets. The microbiology laboratory has unique contamination problems and should have a central air conditioning system if possible. There are several advantages of central air conditioning. First, the incoming air is filtered, thereby reducing the risk of environmental contamination of the laboratory. Second, closed windows minimize drafts and other air currents which could cause cross-contamination. Third, closed windows reduce the possibility of flies and other flying insects contaminating laboratory samples and/or surfaces. Fourth, air conditioning controls the humidity (50% is optimal), which reduces problems with hygroscopic media and chemicals, particularly in countries with tropical climates. Moreover, excessive humidity over a prolonged period may promote growth of mildew and mould on laboratory surfaces. Spores may eventually become airborne, affecting analytical results. Finally, air conditioning stabilizes the room temperature, enabling incubators to function more efficiently. Because many air incubators have no built-in refrigeration system, they can maintain temperatures only as low as room temperature, which is generally understood to be 21-23°. In tropical countries, however, ambient temperatures may be above 30 or even 35°. Thus, these incubators will not function efficiently if the room temperature is above 23°.

Failure to maintain a relatively constant room temperature may also affect the performance or cause malfunctioning of pH meters. An elevated temperature may also cause a change in the composition or integrity of heat-sensitive media and reagents or a loss of viability of stock cultures normally maintained at 21-23°.

Fans are not recommended as a replacement for an efficient ventilation system. Fans stir up dust and could be a significant source of cross-contamination in the microbiology laboratory.

Even with central air conditioning, soot and other fine particles will pass through ventilation system outlets. Thus, filters should be placed on these outlets. These filters should be changed at least annually, or more often if needed. Laboratory personnel should keep a written record indicating when these filters should be replaced.

In providing input for the laboratory design, microbiologists may consider the need for a ventilation or fume hood. Each hood should have its own supply of gas, water, compressed air, and electricity. Strong acids or solvents and similar materials should be used under this type of hood. For maximum efficiency, the hood sash should be lowered to the level indicated by the manufacturer. The efficiency of ventilation should be checked annually by a factory representative or by building maintenance personnel. The laboratory staff, however, should maintain a written record of this servicing. Hoods should not be used for long-term storage of materials. Instead, only a 3-day supply of any chemical should be stored under the hood.

Although the inclusion of windows in the laboratory design should not be discouraged, media, chemicals, and reagents should be stored in areas protected from direct exposure to

sunlight, which may alter their performance. Likewise, analytical work must not be performed in direct sunlight since final results may be affected.

The workbench is the center of activity of the microbiology laboratory. It should be kept clear for actual microbiological analyses and should not be used to store laboratory equipment, media, or other items. Preferably, it should not support additional shelving. The workbench itself should be made of nonporous, impervious material free of cracks, exposed seams, or other defective areas where microorganisms could grow. The space under the workbench may be used to accommodate cabinets and drawers, but alcoves at least 90 cm wide should be included to allow easy access to the workbench when the analyst is seated. The bench should be adequately equipped with sources of gas, vacuum, compressed air, electricity, distilled water, and hot and cold tap water.

Separate from the central or main workbench should be one or more auxiliary side benches. Some pieces of laboratory equipment, e.g., water baths, generate vibrations, and therefore must not be on the same bench that is used to support delicate instruments, such as microscopes and analytical balances.

3.2 Environmental monitoring

General considerations

The environment to which the samples, extracts from them, personnel and equipment are subjected must be checked to ensure that quality of results is not affected. Thus records will be checked to show that:

1. samples are received, stored, handled and analysed under environmental conditions that will not adversely affect analyses;
2. temperature, humidity and light controls are adequate in sensitive areas to protect samples, extracts from them, personnel and equipment.
3. the results of environmental sampling in laboratory areas are recorded.

The microbiological laboratory

Normally, microbiological monitoring of the environment involves analysis of laboratory surfaces and air for the presence of microorganisms. Laboratory surfaces may be tested to determine cleanliness of the same work area over a prolonged period or of different work areas at any given time, the needed frequency of housecleaning, effectiveness of disinfectants on workbenches and needed frequency of workbench disinfection, and efficiency of laminar flow hood. Monitoring of air is performed to determine efficiency of air filters and needed frequency of changing them, and any possible sources of environmental contamination of samples.

The enumeration of microorganisms on laboratory surfaces may be determined either by the swab method (Annex 2) or the replicate organism direct agar contact (RODAC) method (Annex 3). The RODAC method is especially adapted for sampling flat, impervious surfaces. It should not be used on irregular surfaces or on surfaces with cracks or crevices. It is optimally used on flat surfaces that have been cleaned and sanitized or disinfected. Heavily contaminated surfaces will result in overgrowth on the RODAC plates.

Microbiological quality of the air should be monitored at least biweekly to be certain that the laboratory environment is not a significant source of contamination. One simple, yet effective, approach for monitoring air quality is referred to as the sedimentation procedure or the "fallout" plate technique. Plates of a nonselective medium, e.g., plate count agar, are exposed to the environment at various sites throughout the laboratory. The actual choice of sites may be based on such factors as flow of pedestrian traffic or relative magnitude of analytical activity. After a 15-minute exposure, plates are closed and incubated at 35° for 48 $\pm$ 2 hours. Plates are counted and results are written in a hard-bound record book. Plates exhibiting more than 15 colonies indicate that the microbiological quality of the air may be unsuitable for performing laboratory analyses. In this event, laboratory work should be suspended, all laboratory surfaces disinfected, and the microbiological quality of the air reevaluated before normal laboratory operations are resumed. For laboratories desiring a more sophisticated approach, there are various types of environmental air sampling devices, e.g., sieve samplers, split samplers, and centrifugal samplers, discussed in detail in the American Public Health Association's (APHA) Compendium of Methods for the Microbiological Examination of Foods (2).

3.3 Housekeeping services

The building floors and benches and other surfaces have to be cleaned. It is also necessary to clean fume cupboards, dust extraction units, equipment and glassware. Freezers and refrigerators must be emptied and cleared from time to time without jeopardizing the integrity of the contents. There may be hesitation on the part of cleaning staff without technical training to clean analytical equipment for fear of damage. For the same reason analysts may not welcome their attentions. In this case it must be stipulated by management that the analysts adhere to a cleaning schedule in relation to the equipment, while the building cleaning staff is responsible for the other areas. Thus, there should be a cleaning schedule for both analysts and cleaning staff.

All surfaces should be cleaned with a moistened rag frequently. Proper housekeeping is indicated by a lack of dust build-up on shelves. Floors should be wet-mopped and disinfected on a regular basis to prevent the accumulation of debris where bacteria could survive and proliferate. Routine waxing results in a wax/dirt build-up, particularly at baseboards, and is therefore not recommended.

Records of cleaning should be maintained and reviewed to ensure cleaning has been carried out in accordance with the schedule. The laboratory should also be checked physically from time to time to see if the level of cleanliness is acceptable.

If teak benches are waxed, the wax has to be scraped off periodically to prevent accumulation of dirt.

A preventive pest control program is needed to keep the population of flies, roaches, and other insect pests in check. These insect pests are attracted particularly to bulk quantities of stored foods. Long term storage of bulk amounts of food, although not generally recommended, may be unavoidable in regulatory laboratories where samples involved in litigation may have to be retained for a long period. Pest control can be done by the laboratory staff or a commercial firm. A written record should be maintained, indicating the dates of pest control servicing.

3.4 References

1. Weatherwax, J., and P.G. Martin. 1986. Manuals of Food Quality Control. 1. The Food Control Laboratory, 2nd ed. Food and Agriculture Organization of the United Nations, Rome, Italy.

2. American Public Health Association. 1984. Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., M. L. Speck (Ed.). American Public Health Association, Washington, DC.

4. PERSONNEL

In a typical food microbiology laboratory, there are two general types of technical personnel: analysts, who perform the actual analyses, and support personnel, who, with adequate training and supervision by the analysts, prepare media and solutions, clean glassware and instruments, and weigh test portions for analysis.

Support personnel should be advised of the importance of their duties and the need to report to their supervisor any condition beyond their skill, knowledge, or control. The training of support personnel is not discussed here because they are trained directly by their supervisor and their duties are usually well defined. This discussion will focus on the analyst who, in turn, may impart certain aspects of this training to support personnel.

4.1 Selection and qualifications

The selection of personnel should be based on matching the best qualified applicant with the position. Each position should be fully described in a document such as a position description. The position description itself should contain at least three elements. First, an introductory paragraph giving a summary description of the position, indicating exactly how the position fits in the overall organizational structure. Second, detailing all the duties and responsibilities of the analyst. Third, describing the overall degree of supervision by the supervisor and the extent of the analyst's work-related independence.

Some analyst positions in a microbiology laboratory do not necessarily require a formal education at the university level. In selecting for these positions, credit should be given for job experience as a substitute for formal education, particularly if the experience has been in the specific area to be filled.

In addition to education and experience, the applicant must be physically able to perform the tasks required by the job. The candidate selected for a position must be able to manipulate flasks, tubes, plates, and other glassware in a safe and expeditious manner; operate and maintain laboratory equipment and stand and/or sit for prolonged periods.

The personality of the analyst must also be considered. Some analyst positions require a team effort, and in those situations, the analyst must be able to work successfully with his peers to achieve a common goal. Other analyst positions require independent performance with little or no team work. The position description should explicitly define the work situation to avoid any personality conflicts.

The immediate supervisor, who is most familiar with the nature of the position and will be working with the analyst on a daily basis, should have input into the final selection of the analyst.

4.2 Training

Training should be organised to achieve defined objectives that relate to the laboratory as a whole. A senior member of staff should assume responsibility for training. Training needs can be identified from the defined objectives of the laboratory. There will be a need for training in specific analytical methodology, usually given in-house, as nearby organisations are unlikely to be undertaking identical work. There is also a need to ensure that analytical staff have adequate fundamental understanding of the science underlying the processes that they use. This knowledge should have been obtained during formal study but if there are gaps, these need to be identified and filled by attendance at short courses and seminars, by reading or by in-house training. The highest level of training relates to interpretation of data. This will probably be largely in-house and relatively informal, as it may be feasible to pursue only as particular examples arise.

Overall general training objectives might be:

1. to ensure analysts are skilled in the techniques of analysis.

2. to ensure analysts practice the investigational skills of analysis and develop interpretive skills.

3. to ensure analysts produce analytical data of known accuracy which are meaningful and assist in achieving the objectives of the laboratory.

A new employee should be given a general orientation of his or her new surroundings. The following matters should be discussed: work schedules, work loads, introduction to co-workers and management personnel, location of library, eating facilities, leave policy, pay schedule, locations of laboratories, procedures for disposing of waste or contaminated material, safety program, and appropriate laboratory attire.

After the general orientation, the new analyst is ready to begin a scientific training programme. A supervisor or senior analyst should be responsible for training the analyst. This training should be direct, i.e., "one-on-one" between trainer and analyst. The training of the analyst should be conducted in phases. During the first phase, the analyst is familiarized with all aspects of the quality assurance programme which can include operation and maintenance of equipment, documentation of sample accountability, preparation of analyst worksheets, etc. Depending upon the extent and/or detail of the quality assurance programme, this period of training may require 2-4 weeks.

The second phase of training could involve reviewing general analytical techniques, use of equipment and preparation of solutions, standards, media, etc. This phase of training requires about 2 weeks.

The third phase of training is the most extensive. It is concerned with specific analytical techniques used in the laboratory. As an absolute minimum, procedures for the identification and/or enumeration of the following organisms or groups of organisms should

be taught in all food microbiology laboratories: total aerobic microflora (standard plate count, aerobic plate count, or mesophilic plate count), total coliforms, fecal coliforms, Escherichia coli, Salmonella, and Staphylococus aureus. Depending on the commodities examined by the laboratory, additional training may be needed for the following: Campylobacter (poultry, pork, and raw milk), Yersinia enterocolitica (refrigerated meats, raw vegetables, seafood, and dairy products), Listeria monocytogenes (meats, dairy products, and vegetables), Vibrio cholerae and Vibrio parahaemolyticus (seafoods), Shigella (raw vegetables), yeasts and moulds (nuts, spices, grains, and canned foods), Bacillus cereus (rice, wheat, and other grains), and Clostridium perfringens (meats). If the laboratory is responsible for the analysis of canned foods, the analyst should be trained in techniques for the identification of spoilage organisms in canned foods. The duration of this third phase of training is dependent on the extent of coverage of the various topics.

In teaching these analytical techniques, the trainer should do more than just demonstrate a procedure. Precautions to be taken in using a method should be mentioned as well as the need to analyze certain products immediately, requirement of storing laboratory samples before analysis at a particular temperature, use of a supplement for a commercially available medium, unusual incubation temperature and/or periods, and the potential occurrence of atypical strains on a plating agar.

In addition to precautions associated with a method, the trainer should emphasize its limitations. For example, a method may not be applicable for the analysis of all types of foods; it may not be sufficiently specific, or may have a limited sensitivity.

During all phases of the training period, the trainer and the trainee should have frequent daily conversations. The training period should demonstrate "how" and "why" for each analytical step. If the analyst does not ask questions during the training period, the trainer may have to generate discussion by questioning the analyst about his understanding of the various training segments. A training record should be kept such as noted in Annex 1.

In summary, training is the means by which the analyst produces results of acceptable quality. Training is therefore an extremely important laboratory activity and training assessments are valuable in determining the effects and the effectiveness of training. The assessment should be carried out for each person by individual interview and review of the training records. The assessment may be done by a supervisor or a member of the quality assurance team. Once or twice a year is usually sufficient frequency unless training has been particularly intensive. The results of assessments should be made available and discussed with management and used to identify needs for changing the training programme, retraining individuals and reviewing the means of assessment.

4.3 Staff performance

General considerations

The quality assurance programme can be used as part of the formal mechanism to review and evaluate staff performance at least annually or semi-annually. The reviewer sets

the tone of the review, which must not be perceived as negative or threatening by the analyst. The purpose of the review is not just individual accountability; it should provide the following: determination of both immediate and long-range training needs; a forum, which may otherwise not be available, for the analyst to discuss particular work-related problems; opportunity for supervisor to recognize, commend, and document efficient analyst performance; and the opportunity for both parties to suggest improvements.

Check sample programmes

A check sample programme is a type of interlaboratory testing to determine the analytical proficiency of individual participants. A reference laboratory prepares homogeneous test samples with theoretically identical levels of microbiological analyte(s). It is essential to the validity of the programme that these test samples be homogeneous. The test samples are sent to the participants in the programme, who are asked to begin their analyses on a certain day.

In an effort to make a favorable impression, some laboratory directors may assign the "best" analyst to examine these check samples. Knowing that these are check samples, the analyst will usually exert his or her best effort. For a more accurate assessment of the analyst's proficiency, the supervisor and/or laboratory director may decide not to identify these samples as check samples. Analysts should be advised during their introductory period that they should expect to analyze such samples as part of the quality assurance programme to demonstrate their proficiency in particular analyses. It is important that during the course of the year, as many analysts as possible be given the opportunity to analyze check samples.

Results of the check sample analyses (either quantitative or qualitative) are forwarded to the originating laboratory for statistical evaluation.

The originating laboratory calculates the mean of each laboratory's individual results; it then calculates the mean of the means for all laboratories and determines the standard deviation. Based on a normal distribution, 95% of the means for any set of analytical results should be between +2 and -2 standard deviations of the mean of the means. Five percent or 1 in 20 of the laboratory means may fall outside this limit. If all means are within ± 2 standard deviations, the standard deviation may be too wide. Only an occasional mean should be beyond ± 3 standard deviations.

For procedures involving only qualitative data there is a somewhat different approach. The originating laboratory sends a set of test samples, some positive and some negative, to each participating laboratory. One of several levels of analyte (high, medium, and low) may be present in each of the positive test samples. However, at least one positive test sample should contain a level of the analyte at which the test is expected to discriminate between positive and negative results, i.e., the lower limit of determination. Participating analysts examine the test samples and report their results to the originating laboratory as either positive or negative.

The originating laboratory collates the data from all the participants and categorizes the reactions as follows:

a. In agreement. A reaction in which the analyst finds the analyte in positive samples or does not find it in negative samples.
b. False positive. A reaction in which the analyst reports the presence of the analyte in a negative sample.
c. False negative. A reaction in which the analyst fails to find the analyte in a positive sample.

The occurrence of discrepant reactions, i.e., false-positive and false-negative reactions, is a cause for concern. For example, the microbiological identification of pathogens in foods intended for human consumption is usually qualitative since their presence at even very low levels could pose a serious threat to human health. In such a case the reporting of a false-negative reaction would be much more serious than a false-positive result.

Extenuating circumstances beyond the control of the analyst, however, may explain the occurrence of a false-negative reaction. If the false-negative reaction occurred in a microbiology sample containing a low level of analyte, it is possible that the analyte became nonviable between the time of inoculation and the initiation of analysis. Unlike chemical analytes, microbiological analytes are relatively unstable and their behavior in a food is more difficult to predict.

A false-negative reaction may also be caused by a non-uniform distribution of the analyte within the sample. Although the use of homogeneous samples in a check sample programme would be ideal, it is less than a reality in many cases. Uniform distribution of an analyte is far easier to achieve in a liquid than in a solid matrix. It is even conceivable that the analyte may not have been present in a sample receiving a low level of inoculation, thus leading to the false-negative result.

False-negative results in samples receiving a high, or medium, level of analyte inoculation, however, should be cause for concern by both the analyst and the supervisor. If a logical explanation for such results cannot be given, then additional training of the analyst is indicated.

Although false-positive reactions are less serious than false-negative reactions, their significance should not be underestimated. Whenever the false-positive rate exceeds 10%, analyses become uninterpretable from a lack of confidence in the presence or absence of the analyte (1).

Another type of interlaboratory testing programme is the collaborative study, which measures the performance of a method. The originating laboratory prepares homogeneous test samples and distributes them to analysts in participating laboratories. Working independently, the participants analyze the test samples with the method to be validated and forward the results to the originating laboratory. The data are statistically analyzed and the performance of the method is expressed in terms of accuracy, reproducibility, and

repeatability. Each analyst is furnished a copy of the results so that his or her performance, relative to that of other participants in the study, may be known.

If deficiencies are noted in an analyst's participation in a check sample or other proficiency testing programme, the supervisor should schedule additional training for the analyst.

4.4 References

1. Committee on Interlaboratory Studies. 1988. Guidelines for collaborative study procedure to validate characteristics of a method of analysis. J. Assoc. Off. Anal. Chem. 71:160-162.

5. SAMPLES

There are several different types of samples, each having its own purpose. Survey samples provide information about industry practices regarding a particular issue. A survey may be conducted to determine if there is a specific microbiological risk associated with a particular food or group of foods. Food standards samples provide information on which to base food standards. Official samples are those which, if violative, serve as a basis for specific legal actions. This discussion is concerned exclusively with official samples.

5.1 Accountability

From a legal or regulatory standpoint, a written record providing accountability of the sample is essential. That is, there must be an official record accounting for the integrity of the sample between the time of its collection and its ultimate disposition. Actually, in larger facilities the official sample is not delivered to the analyst directly but to a sample custodian who is responsible for its handling and storage. In smaller laboratories, however, the analyst may receive the samples directly.

The form this record takes can be devised to fit circumstance. It can be relatively elaborated such as the sample accountability record used by the U.S. Food and Drug Administration (see Annex 4) or simpler. If a computerized system is not used, it is recommended that a card record system be chosen rather than log books as cards permit greater flexibility in grouping, filing and accessing. There are certain minimum data which the record card should collect. These data are those which may or may not exist on other sample documents (e.g. sample collection report, analytical worksheet, etc.) and which establish a means to track the sample from the date of collection to the date of disposal. Minimum data and information for the record card would be:

1. Sample number.]
   ] (to identify the card)
2. Product name.]
3. Date sampled.
4. Date received at the laboratory (important to show custody).
5. Method of storage (dry, refrigeration, freezing, etc.).
6. Storage location (coded for easy finding).
7. Date assigned to an analyst (necessary as it may differ from date of analysis).
8. To whom assigned (the analyst should initial to show receipt).
9. Date returned (from analyst).

10. From whom returned (may be different from the original analyst).

11. Reserve storage method and location.

12. Final disposition or disposal of sample, method and date.

Whoever receives the sample should note the number, type, and condition of the sample containers, e.g., paper bag, plastic pouch, or glass jar, etc. If any sample units are damaged or the containers opened, the laboratory management should be notified immediately.

During the entire period that the sample is in the laboratory, its physical security must be maintained, whether it is in locked storage or in the hands of the analyst.

5.2 Identification and integrity

Each sample is singularly identified by a multidigit number that is only used with that sample. Normally, a sample may consist of several sub-units or "subs." Each sub is identified by a number or letter written on waterproof tape. This same series of numbers or letters may be used to identify subs in a different sample. When multiple subs are taken from cases or boxes in the lot, arabic numerals and letters in combination may be used to identify the subs. For example, if two cans (a and b) are taken from each case in a lot, they may be marked as sub numbers 1a, 1b, 2a, 2b, etc., to identify them as subs taken from cases 1 and 2, etc.

The integrity of the sample between the time of its collection and its delivery to the sample custodian or analyst in the microbiology laboratory is ensured by some form of seal. An example of a paper seal is shown in Annex 5. On this type of seal the inspector or collecting official can write the sample number, the date applied and his signature. Such paper seals are glued to the sample package in such a way that it may not be opened at any point without giving evidence that it has been tampered with or opened.

When the sealed package arrives at the laboratory, the person receiving the sample notes the condition of the seal. If the seal is broken or indicates possible tampering, management is notified immediately.

After the analyst has removed the portion of the sample needed for analysis, the reserve portion should return under seal to storage. It may be necessary to use more than one seal to secure the sample reserve. If the original broken seal is still present, the analyst should ensure that the broken seal remains visible when the new seal is applied. This ensures continuity of integrity of the sample.

5.3 Sampling for analysis

Before removing the test portion(s) for analysis, the analyst must be certain that all records are in order, sample integrity has been maintained, sample containers are intact, and

appropriate sampling controls (discussed below) have been collected and included by the inspector.

When microbiology samples are collected using aseptic technique, the inspector or collecting official should include five types of controls with each sample submitted for analysis:

a. Sterile container (unopened). If sterile containers were used, one unopened container, sterilized under the same conditions as those used for samples, should be submitted.

b. Sterile container (opened). At least one empty container, sterilized under the same conditions as those used for samples, should be opened and closed in the sampling area and submitted along with the sample.

c. Sterile disposable gloves. If sterile disposable gloves were used to handle the product sampled, one unused glove in a sterile container should be submitted.

d. Sterile sampling equipment (unopened). If presterilized sampling tools (spoons, spatulas, scissors, knives, etc.) were used, at least one unopened sampling tool of each type used should be submitted.

e. Sterile sampling equipment (opened). At least one sampling tool of each type, which was opened but not used in the sampling area, should be placed in a sterile container and submitted.

These controls are to be examined microbiologically in the same manner as the test portions and will demonstrate whether or not the aseptic sampling procedure influenced the analysis result.

For the analysis, the analyst first removes a test portion from each sample subdivision. If the food is powdered, ground, or comminuted, the sub-unit should be mixed thoroughly with a sterile utensil before the test portion is removed. Sub-units of liquid or semiliquid foods in containers that are full can be mixed by rapidly inverting the container 25 times before removing test portions. Sub-units of liquid or semiliquid products in containers that are 1/2 to 3/4 full should be shaken 25 times over a 30-cm arc within 7 seconds. After mixing liquid or semiliquid sub-units, test portions for analysis should be removed immediately.

It is preferable not to thaw frozen subs before analysis. However, if a frozen sub must be thawed or tempered to obtain test portions, it may be thawed within 18 hours at 2-5°. If more rapid thawing is needed, the sub may be thawed below 45° for 15 minutes. Rapid thawing must be done with continuous agitation in a thermostatically controlled water bath. The product must not be removed from its container to promote thawing.

After the test portions are obtained, but before beginning the microbiological analysis, the analyst must decide whether to analyze the test portions individually or collectively as a composite. However, with few exceptions, test portions for microbiological analysis are usually examined individually, particularly if microorganisms are to be enumerated or quantitated.

5.4 Sample storage and disposal

Between the time of arrival at the laboratory and initiation of analysis, samples of perishable, unfrozen food must be maintained between 0 and 4°, and frozen food must be kept frozen. All perishable and frozen samples should be examined within 36 hours of collection. Perishable samples that cannot be examined within 36 hours after being sampled should be frozen. However, freezing refrigerated microbiology samples may cause microbial cell injury and even death. Thus, this practice should be performed only as a last resort and not routinely.

For microbiological samples of unfrozen shellfish, special holding provisions apply (1). Samples of unfrozen shellfish should be examined within 6 hours after collection. Samples held at 0-4° for more than 24 hours should not be examined.

Canned or dry, nonperishable foods may be stored at room temperature before analysis.

After the test portions have been removed from the sample, the sample reserve is returned to storage. Depending upon the type of food, samples should be stored under conditions recommended in Annex 6.

Reserve portions of microbiology samples containing pathogenic microorganisms and/or microbial toxins should be autoclaved before disposal. Large masses of dry food should be autoclaved in smaller (0.5 - 1kg) amounts to ensure that adequate penetration of steam during autoclaving will kill all viable pathogenic organisms. About 1 liter of water should be added to each 500 g amount of dry food to ensure adequate generation of steam during the sterilization cycle. If necessary, the dry reserve sample may be kneaded with added water to dissolve or disintegrate large clumps of solid material.

5.5 Packaging and shipment

Usually, the inspector or collecting official is responsible for the packaging and shipment of a food sample. Occasionally, however, the analyst will have to send a sample to another laboratory for confirmational analysis or for some other reason. Samples must be packaged and shipped in a way which maintains their integrity. The container, with the food in it, can be placed in a plastic bag and sealed with a lead seal or metal clasp. Alternatively, rigid containers such as glass jars may be sealed with sealing wax, which can be stamped with an official, identifying stamp.

Frozen samples that are to be shipped should be packed with Dry Ice in insulated cartons. Special precautions are necessary for using Dry Ice: Do not handle Dry Ice with unprotected hands, do not transport it in a car without adequate ventilation, do not place it inside tightly closed containers that may explode from the accumulation of excessive pressure, and clearly indicate on the shipping container that Dry Ice is contained therein so that carriers may take necessary precautions. Freezing by Dry Ice is not effective for more than 48 hours. Never use less than 15 kg of Dry Ice for overnight shipment. For samples weighing less than 15 kg, use 15 kg of Dry Ice; for each additional 0.5 kg of sample, use an additional kg of Dry Ice. The amount of Dry Ice should be increased for shipments requiring longer periods or during unusually warm weather. In all packages where Dry Ice is used, the Dry Ice should be equally distributed as nearly as possible. If sub-units have been collected in plastic bags, the Dry Ice must be wrapped in paper to prevent direct contact with the plastic. The extreme cold generated by Dry Ice makes the plastic brittle and subject to rupture.

As a temperature control to demonstrate that microbiology samples did not thaw in transit, a container, identical to the containers with the test sub-units, may be half filled with ethylene glycol. Upon arrival at the laboratory, the temperature of this control may be observed and recorded. Alternatively, a leak-proof bag of chipped ice may be included as an indicator of melting.

To ship perishable samples under refrigeration, either wet ice or a commercial "Ice Gel" pack may be used. These packs, which contain a chemical, should be placed in sealed plastic bags to protect the sample from possible contamination in the event that the coolant container ruptures during shipment. Insulated containers or chests should be used. As a temperature control to demonstrate that the maximum desired temperature was not exceeded during shipment, water collected in the same type of container as the test sub-units may be included. Upon arrival the water temperature may be observed and recorded.

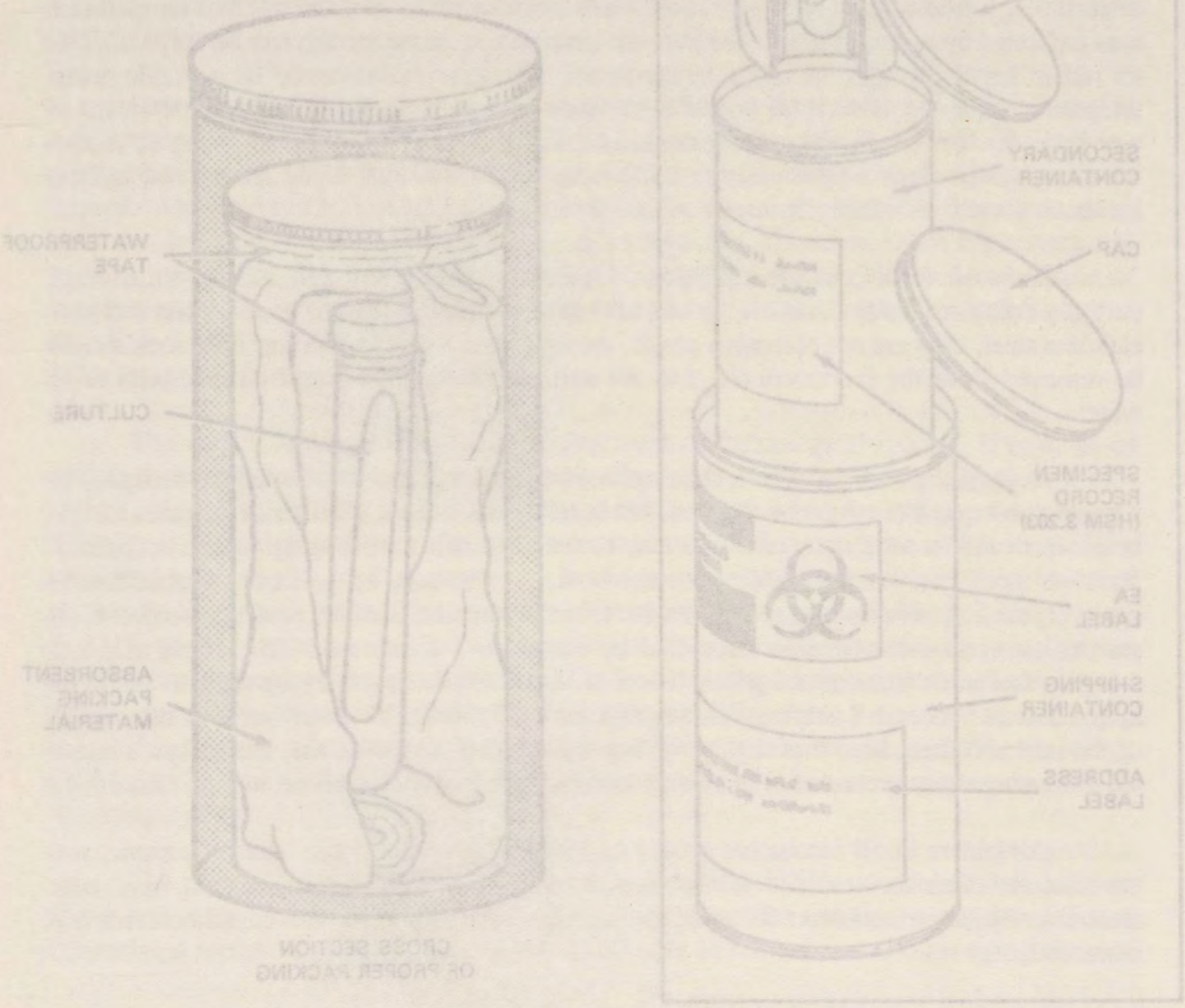
Dry, nonperishable samples should be packed in a sturdy cardboard box with appropriate packing material to prevent breakage during shipment.

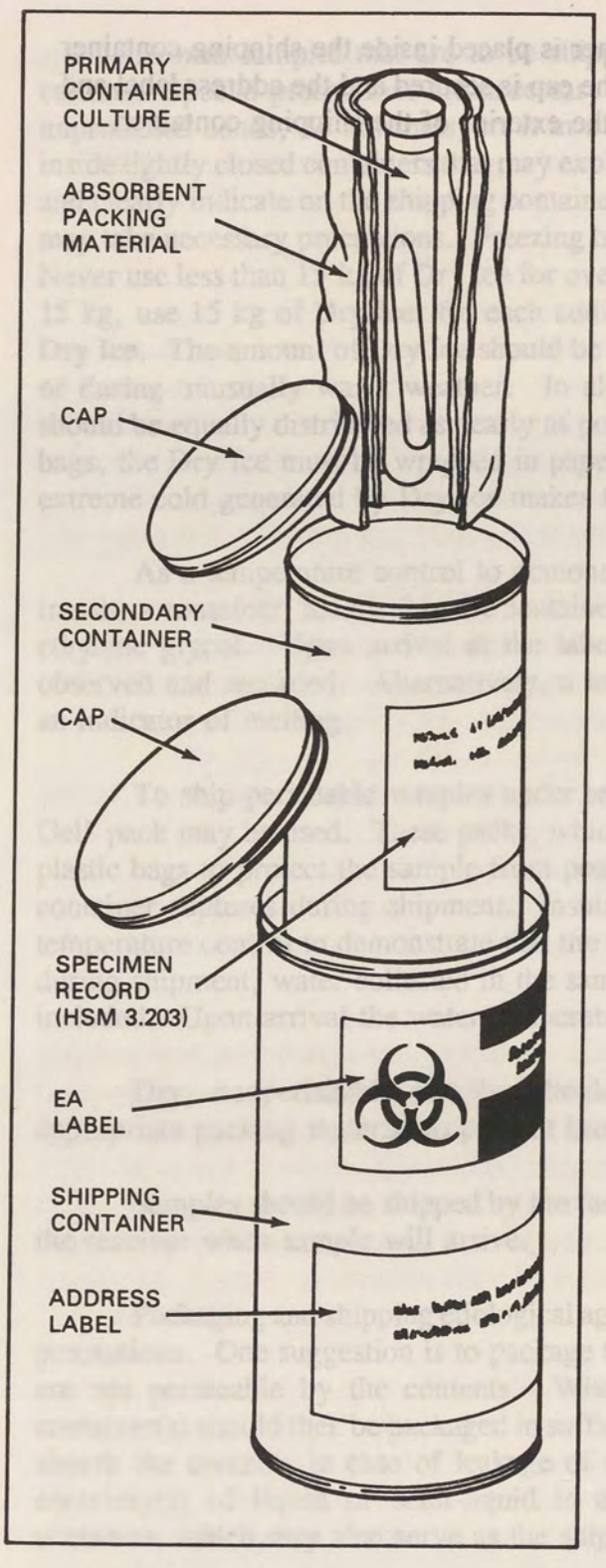
Samples should be shipped by the fastest mode possible and the shipper should advise the receiver when sample will arrive.

Packaging and shipping etiological agents and other biological material involves special precautions. One suggestion is to package these items in waterproof inner containers which are not permeable by the contents. When other than dry items are mailed, the inner container(s) should then be packaged in sufficient absorbent cushioning material to completely absorb the contents in case of leakage of the inner container. The cushioning and inner container(s) of liquid or semi-liquid is then enclosed in a sealed nonpermeable outer container, which may also serve as the shipping container.

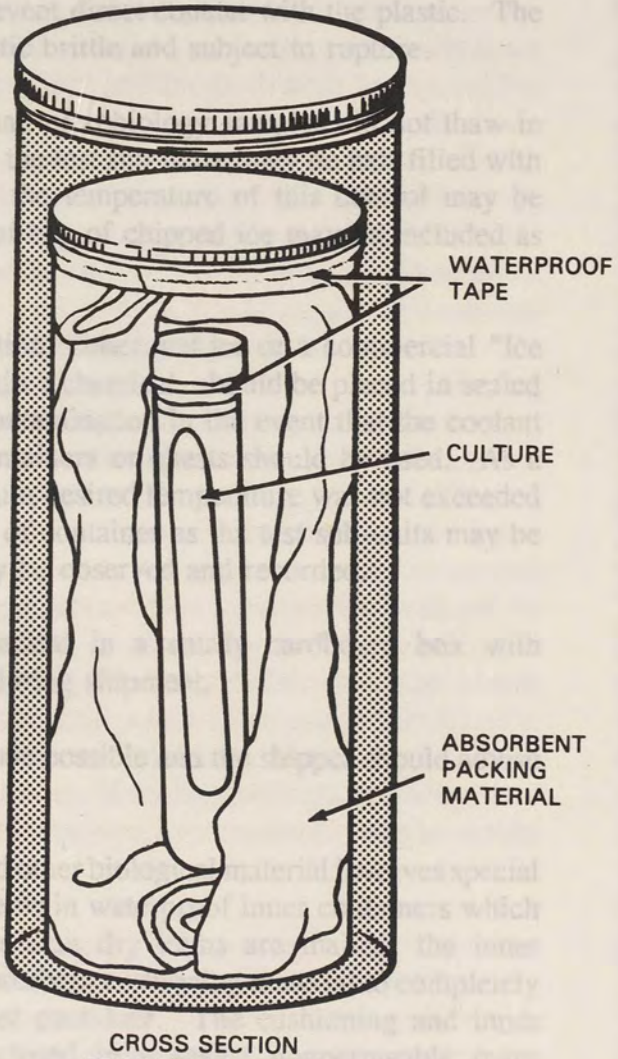
The shipping procedure for cultures recommended by the American Type Culture Collection (2) meets the following requirements (see Figure 1). The culture is on a slant contained in a screw-cap tube (primary container) that is sealed with tape. This tube is well-cushioned with absorbent packing material inside the secondary container with a screw-cap

lid that is sealed with tape. The secondary container is placed inside the shipping container and cushioned with absorbent packing material. The cap is secured and the address label and an etiological agents warning label is attached to the exterior of the shipping container.





PACKAGING AND LABELING OF ETIOLOGIC AGENTS



CROSS SECTION OF PROPER PACKING

Figure 1

5.6 References

1. American Public Health Association. 1985. Laboratory Procedures for the Examination of Seawater and Shellfish, 5th ed., A.E. Greenberg and D.A. Hunt (Eds.). American Public Health Association, Washington, DC.

2. ATCC Quality Control Methods for Cell Lines. 1985. R.J. Hay (Ed.). American Type Culture Collection, Rockville, MD.

6. EQUIPMENT

6.1 Maintenance and repair

Properly functioning equipment is essential for the production of reliable microbiological data. A list of equipment needed to perform basic microbiological analyses follows, along with guidelines for their appropriate maintenance and repair.

Incubators

The air incubator is the most commonly used piece of equipment in the food microbiology laboratory. Although most manufacturers claim that the internal temperature is not affected by normal fluctuations in room temperature, some models can be very sensitive to rather small changes in room temperature. Because maintenance of a stable room temperature may not always be within the analyst's control, the analyst should be aware of any large fluctuations in the room temperature, especially during seasonal changes, so that necessary precautions may be taken. The temperature intended to be maintained by any incubator should be clearly indicated on the exterior.

Some air incubators are equipped with a relative humidity unit using a softened or partially deionized water. Although many of these units are constructed of corrosion-resistant stainless steel, they are not corrosion-proof. At least once a month, the wet-bulb sock should be removed from the thermocouple, and the unit should be drained and cleaned with fresh water.

Any spillage within the incubator should be cleaned and disinfected immediately to prevent subsequent cross-contamination. At least once a month, all interior surfaces should be cleaned with a mild detergent solution, rinsed, and dried thoroughly with a soft cloth. Stainless steel wool, and not ordinary steel wool, may be used on particularly troublesome spots. Grease or oils may be removed with toluene, naphtha thinner, or similar solvent. If stainless steel surfaces become discolored by iron rust, a solution of 20% nitric acid and 1.5% hydrofluoric acid or a 2-5% solution of warm oxalic acid may be used to swab the affected area. After 1-2 minutes the area should be flushed with clean water to remove all of the acid and then dried thoroughly. When using these acid solutions, the analyst's hands must be adequately protected with rubber gloves, and the room must be well ventilated.

Containers in all incubators should be labeled clearly with the analyst's name, and the date and time the material was placed in the incubator. Materials which have been inadvertently left in incubators for an unusually long time should be removed, autoclaved, and discarded.

Water baths

Thermostatically controlled circulating water baths should be used whenever a temperature must be maintained within a tolerance of 0.1°. The cover should fit securely on the water bath to prevent excessive moisture evaporation.

The primary maintenance task is to prevent or retard damage caused by corrosion. The baths should be inspected frequently since uncontrolled corrosion products will eventually damage the water pump as well as other components. If more than 2 weeks will intervene between uses, the baths should be drained, washed with a mild detergent, and dried thoroughly with a soft cloth. When the baths are in use, a commercial corrosion inhibitor may be used in the water. Only distilled water should be used in the baths.

Refrigerators and freezers

A food microbiology laboratory should have a refrigerator maintained at 4° and a freezer maintained at -20° for storage of samples, specimens, media, sera, reagents, and cultures. The temperature to be maintained by refrigerators and freezers should be indicated on the exterior.

Exteriors should be cleaned with a damp cloth at least monthly. The freezing compartment of the refrigerator should be defrosted every 3 months and all interior surfaces should be cleaned. The freezer should be defrosted every 6 months and all interior surfaces cleaned. An alarm system indicating excessively high temperatures should be kept in working order for both refrigerators and freezers. All containers placed in the refrigerators and freezers should be labeled with the following information: identity of material, name of person responsible, and date that the material was placed in the refrigerator or freezer.

Autoclaves

The autoclave is essential for sterilizing most microbiological media. It must be of sufficient size to sterilize media without crowding. The use of a vertical type, or a top-loading, autoclave is not generally recommended because of unavoidable crowding and difficulty in adjusting and maintaining an adequate sterilization temperature. The use of a horizontal type, or side-loading, autoclave is always preferable, particularly in laboratories that handle large work loads. The autoclave must be able to maintain an internal temperature of 121° under a pressure of 1 bar (15 psi); it should be equipped with a calibrated thermometer to measure the temperature within the sterilizing chamber; it must be equipped with a pressure gauge and safety valves that are connected directly to a saturated steam supply line; and it must be able to reach the desired temperature within 30 minutes. The autoclave should also be equipped with a temperature recorder to provide a permanent record of the sterilizing cycle.

The autoclave should be used primarily for sterilizing media and solutions. Sterilizing pipets and other calibrated glassware in the autoclave is not recommended because accumulated moisture will affect the delivery capacity of the calibrated glassware.

Repairs or adjustments of the autoclave made by inexperienced personnel could result in serious personal injury and/or costly repairs. These repairs should be made only by professionals who are fully acquainted with the equipment. Routine maintenance, however, can and should be performed by laboratory personnel.

Because of the variation among different autoclave models, only general maintenance guidelines can be given here. Maintenance tasks may be categorized according to their frequency of execution: daily, weekly, monthly, and quarterly.

Two tasks should be performed on a daily basis. First, the daily temperature record should be removed from the printer mechanism and stored or filed appropriately. Second, the interior of the sterilizing chamber must be cleaned daily. The interior surfaces are cleaned with a mild detergent solution, rinsed with tap water, and dried with a lint-free cloth. The chamber drain strainer should be removed so that all lint and sediment may be collected. The strainer is then reverse-flushed with running water and placed back into the chamber drain.

Three tasks should be performed on a weekly basis. First, the chamber drain should be flushed, the strainer removed, and the drain rinsed with a hot solution of trisodium phosphate (2 tablespoons to 1 quart of water). After 5 minutes, the drain should be flushed with hot water and the strainer placed back in the chamber drain. The second weekly task involves checking the control and status signals. With the chamber empty, the thumbwheel switches are set for 1 minute. As the sterilizer operates through each phase, the control panel should be observed closely. If any lights are out, a qualified service technician should be contacted. The third weekly task involves flushing the steam generator. This procedure will vary with the model, and the instructions provided in the manufacturer's manual should be followed.

On a monthly basis a few drops of heavy machine oil should be placed on the chamber door hinge pins.

Several tasks are performed on a quarterly basis. First, the door post grease fitting is lubricated with a high-temperature grease. Second, the door gasket should be examined. If it is brittle or cracked, it should be replaced. Third, the chamber and steam generator safety valves should be checked. Each safety valve is examined for accumulations of rust, scale, or other substances which would prevent the valve from opening. A water supply with a maximum of 5 grains hardness is recommended for steam generators to minimize scale accumulation. Each try lever (or pull ring) should be operated several times. The lever should move freely and return to its closed position after each operation. After allowing the chamber and steam generator to reach operating pressure, safety valves should be checked for leakage. A qualified technician should replace the leaking safety valve. The fourth task involves the cleaning and descaling of the steam generator according to the manufacturer's instructions.

Hot air ovens

The hot air oven is used for sterilizing most laboratory glassware. It should be of sufficient size to prevent crowding; it should be able to give uniform temperatures and should be equipped with a calibrated thermometer capable of registering accurately in the range of 160-180°. It is also preferable to have a temperature recording device to keep a permanent record of the sterilizing cycle. Maintenance of the hot air oven is minimal. On a monthly

basis the interior surfaces should be cleaned with a mild detergent solution, rinsed with tap water, and dried.

Balances

The microbiology laboratory should be equipped with two top-loading balances, one with a capacity of 2,000 g and a sensitivity of 0.1 g, and a second with a capacity of 100-200 g and a sensitivity of 1 mg. Maintenance by the analyst is minimal, limited to replacing a defective bulb and cleaning the protective cover and weighing pan. If two or more balances are being cleaned simultaneously, the analyst should be certain not to interchange the pan supports and weighing pans of different balances.

pH meters

Unless otherwise specified, the pH of diluents, rehydrated media, and other materials should be adjusted by using a pH meter rather than pH indicator paper. Maintenance of the pH meter is minimal and is restricted to keeping the casing and protective cover clean.

Unlike the pH meter, the electrodes need special care. All electrodes require an initial conditioning. The glass bulb of a new sensing electrode must be soaked for at least 1 hour in demineralized or slightly acidic water or in a pH 4 buffer. This soaking hydrates the pH-sensitive glass membrane.

Electrodes should always be rinsed when being transferred between standard and sample solutions. Although it is preferable to rinse with a portion of the next solution, demineralized water may be used. A beaker is held under the electrodes as a stream of rinse solution from a wash bottle is directed over the electrodes. After thorough rinsing, the electrodes are blotted with soft tissue paper.

Storage procedures are determined by the type of electrode. Glass electrodes should be stored in a pH 7 buffer or in a slightly acidic solution. Reference electrodes should be maintained in a 0.1 M KCl solution to keep the junction moist and free-flowing. The level of filling solution in the reference electrode should always be maintained above the level of both test and soaking solutions to provide a positive head pressure, thereby forcing filling solution out through the junction. Combination electrodes should be maintained in a mixture of 50% pH 7 buffer and 50% 0.1 M KCl.

Blenders

Blending is perhaps the most common procedure in test sample preparation. Often, an unavoidable consequence of blending food is spillage. Whenever spillage occurs, the exterior of the blender base should be disinfected immediately to prevent contamination of other test samples or work areas. Following disinfection, the exterior of the blender base should be washed with a warm detergent solution and rinsed to prevent accumulation of dried material.

Laminar flow hood

All microbiological procedures involving pathogenic organisms, dispensing of sterile media, or the analysis of canned foods for sterility should be conducted under a laminar flow hood, preferably a vertical flow type. Main and exhaust high energy particulate air (HEPA) filters should be 99.99% efficient in removing particles 0.3 μm, or larger, in diameter. Standard equipment should include a splash-proof electrical socket, solenoid gas inlet, service connection for vacuum line, exhaust adapter, elapsed time meter, and an externally mounted fluorescent light. Contrary to what may be a common practice, hoods should not be allowed to "run" indefinitely or for long periods of time, e.g., over the weekend. Hoods should be operational only when they are going to be used. The use of a Bunsen, Fisher, or other laboratory burner within the hood also is not recommended. The use of these burners may generate a rising vertical column of air that is stronger than the downward flow of sterilized air through the HEPA filter, thereby negating the efficiency of the hood.

The filters should be checked monthly for plugging or obvious dirt accumulation and replaced as needed. The plug should be removed from the outlet and the fluorescent lamps cleaned every 2 weeks with a soft cloth moistened with ethanol. Every 3 months the ultraviolet lamps should be tested with a light meter. If the lamp emits less than 80% of its rated output, it should be replaced. Because ultraviolet rays do not penetrate, hoods must be completely empty for the ultraviolet lamp to be effective. The ultraviolet lamp should be turned on for 10 minutes before the hood is used and for 10 minutes after analytical procedures in the hood are completed.

In addition to using the ultraviolet lamp to disinfect interior hood surfaces, the hood interior should be wiped before and after each use with a liquid disinfectant. The use of different "before" and "after" disinfectants is recommended to maximize bactericidal effectiveness. The efficiency of disinfection may be monitored by using replicate organism direct agar contact (RODAC) plates. The RODAC plate method is performed as described in Annex 3. The written results of these RODAC plates should be maintained in a bound record book containing the following information: date, site or sampling area within hood, disinfectant used before hood operations, disinfectant used after hood operations, microbiological counts, and name of analyst.

Microscopes

Much of the taxonomic work performed in a microbiological laboratory is based on microscopic examination of morphological features and staining reactions. Both a compound microscope and a stereoscope are recommended. The compound microscope should preferably be binocular with a 1.8 mm oil immersion objective, a substage actuated by a rack and pinion carrying an Abbe condenser with a numerical aperture of at least 1.25, an iris diaphragm, a flat mirror if the light is not an essential part of the microscope or mounted on the base, a mechanical stage, and oculars providing magnifications of 100X, 400X, and 1,000X. The stereoscope should preferably be binocular, with its own light source, and a magnification of 60X.

Microscopes should be positioned on a vibration-free surface and maintained at one location. Movement of microscopes from location to location is not recommended. When not in use, microscopes should be protected by a dust cover. The microscope stand should be cleaned, as needed, with a piece of linen or chamois leather. Enamel surfaces may be cleaned with a moistened cloth. Light patches on the object stage can be removed with liquid paraffin or acid-free petrolatum. Lenses must be cleaned after each use with lens paper and a commercial lens cleaner. Objectives should not be dismantled for cleaning.

A maintenance contract should be in effect so that all microscopes are serviced annually by a company representative. No repairs of microscopes should be attempted by laboratory personnel.

Maintenance and repair programme

For all major equipment in the microbiology laboratory, a formalized preventive maintenance and repair programme should be established. A principal user should be designated as responsible for maintenance and repair of each particular piece of equipment. The servicing record of each major piece of equipment may be maintained on separate index cards. This record should include the following information: date of service, name(s) of person(s) and affiliation providing service, nature of malfunction (if applicable), brief description of the nature of service or repair and, if desired, cost of service. Larger institutions may have their own in-house repair shop. Operating instructions or manuals for all major equipment should be maintained in a central location that is readily accessible to all laboratory personnel. Before using any equipment, laboratory personnel must be thoroughly familiar with its proper operation and maintenance.

6.2 Calibration

Calibration involves measuring, comparing, and adjusting the performance of equipment relative to that of an accepted standard. Certain tolerances usually are applicable to the performance of the standard, and the performance of the equipment being calibrated must be within this tolerance to be acceptable.

The following discussion relates to the calibration of basic equipment found in a food microbiology laboratory.

Incubators

The internal temperature of any incubator should be monitored by one or more thermometers, the actual number being determined by the size of the incubator. There are two general types of thermometers: partial immersion and total immersion. Each type must be used according to its design, and both types should be frequently inspected for breaks in the mercury column. A partial immersion thermometer is used to measure the internal temperature of air incubators. Graduations should not exceed 0.1°. A partial immersion thermometer should be supported in an upright test tube containing water at least up to the level of the circumferential marking. Putty or caulking material may be used to provide a

tight seal at the mouth of the test tube to minimize evaporation. It is recommended that in large floor-model incubators, a partial immersion thermometer be placed on the top, middle, and bottom shelves.

Before being used, partial immersion thermometers must be calibrated against a thermometer certified by the National Institute of Standards and Technology, formerly the National Bureau of Standards (NBS), or equivalent standardizing organization. One approach for calibrating partial immersion thermometers is given in Annex 7. All partial immersion thermometers should be calibrated annually.

Water baths

The second main type of thermometer, the total immersion thermometer, is mostly used to monitor the temperature of water baths. Unlike the partial immersion thermometer, there is no circumferential marking on the total immersion thermometer because it is always used in a completely submerged, horizontal position in the water bath. Graduations for this type of thermometer should not exceed 0.1°.

The calibration procedure is simpler for total immersion thermometers than for partial immersion thermometers. The uncalibrated and reference thermometers are completely submerged in proximity to each other in a circulating, thermostatically controlled water bath. The temperature of the water bath is set at or very near the temperature at which the thermometer will be used. After an equilibration period of at least 1 hour, the temperatures of the two thermometers are read. The correction certificate for the reference thermometer is consulted to determine the true temperature. The correction factor (the amount to be added to or subtracted from the reading of the thermometer being calibrated to obtain the correct or true temperature) is determined. This correction factor and a number to identify the thermometer are written on a piece of tape and attached to or close to the newly calibrated total immersion thermometer. These calibration data must also be recorded in the hard-bound book containing the temperature log. All total immersion thermometers should be calibrated annually.

In addition to having the appropriate type and number of thermometers for each incubator or water bath, it is advisable to include a temperature recorder to provide a continuous 24-hour record of the temperature. Like the mercury stem thermometers, the temperature recorder must be calibrated against a suitable reference thermometer. For this calibration, the recorder and the reference thermometer are placed side-by-side in a location where the temperature is not likely to fluctuate. After a 3-hour stabilization period, the corrected temperature of the reference thermometer and the temperature given by the recorder are observed. This difference is the correction factor. Unlike the procedure used with partial and total immersion thermometers where the correction factor is noted, written on a piece of tape, and, if appropriate attached to the thermometer, a correction factor is not attached to the temperature recorder. Instead, a manual adjustment is made on the recorder. On the rear of the recorder is a calibration adjustment knob, which is turned so that the temperature pen records the same temperature as the corrected temperature of the reference thermometer.

Whenever a temperature recorder is used, care must be taken to change the temperature recording sheets before overprinting occurs.

Refrigerators and freezers

The internal temperature of refrigerators and freezers should be monitored with partial immersion thermometers calibrated against a suitable reference thermometer of an appropriate range. Graduations of these low-temperature thermometers should not exceed 1°. The calibration procedure is the same as that described earlier for partial immersion thermometers. These calibration data must be recorded in the hard-bound record book containing the temperature log. All low-temperature thermometers should be calibrated annually.

Autoclaves

Except for routine cleaning and maintenance by laboratory personnel as described previously, any needed calibrations or adjustments should be performed only by experienced service personnel. It is recommended that the autoclave be serviced annually under contract.

Hot air ovens

The internal temperature of hot air ovens must be monitored with special high-temperature thermometers capable of measuring temperatures up to 200°. Graduations for this type of thermometer should not exceed 1°. These thermometers should be calibrated against a reference thermometer at or near the temperature of use. Alternatively, a dial scale thermometer may be used, provided that is has been calibrated against a reference thermometer. These calibrations should be repeated on an annual basis. Hot air overs should be monitored for "hot spots" by checking temperatures at various points in the oven.

Balances

Accuracy of all high-precision analytical balances should be checked at least every 3 months with a series of calibrated weights. A balance having an approximate capacity of 2000 g should have at 200 g an accuracy of 0.1 g and a balance of a capacity of 100-200 g an accuracy of 1 mg, respectively.

It is further recommended that all high-precision analytical balances be under contract for annual cleaning and calibration by a company representative. Records of annual balance calibrations under contract should be maintained in a hard-bound book or filed appropriately if the company provides certificates of calibration.

pH meters

Before each use, pH meters must be standardized. Certified buffer solutions are commercially available for this purpose. Although these buffers are available in 1.00 increments for pH values from 1.00 to 11.00, buffer solutions at pH values of 4.00, 7.00, and 10.00 are most commonly used in the food microbiology laboratory. Either a single

buffer standardization, where maximum precision is not required, or a 2-buffer standardization may be performed. Because the procedures for these standardizations vary from model to model, detailed instructions for making these calibrations cannot be provided. However, certain precautions should be followed in standardizing a buffer or determining a pH value on any model of pH meter: adequate level of reference filling solution should be ensured in the electrode, all samples and buffers should be allowed to reach the same temperature (unless an automatic temperature compensator is used) before any measurements are taken, and both the buffer and test solutions should be stirred with a magnetic stirrer while a measurement is being made.

Blenders

A commercial type blender capable of blending at one of several speeds is recommended. Although rotating speeds in revolutions per minute (rpm) are usually specified by the manufacturer, blenders should be calibrated quarterly with a tachometer, preferably photoelectric, to measure the actual number of rpms for any designated speed level. Calibration data should be maintained in a hard-bound record book with the following information: date, blender number, rpms measured for each level of speed, and name of analyst.

Laminar flow hood

No calibration by the analyst is required.

Microscopes

See Annex 8.

6.3 Performance checks

Incubators

Measurements of temperature and humidity are used to determine the performance of air incubators. The internal temperature of incubators is monitored by reading calibrated thermometers twice daily, first thing in the morning before analytical work is started and last thing in the afternoon. If a temperature recorder is being used, a single daily reading is sufficient. Both the uncorrected and corrected temperatures are recorded in a hard-bound book. Additional information to be recorded includes the incubator number, shelf position, date, time, thermometer number, and name of analyst. If a temperature recorder is being used, the record sheets should be maintained in a separate file for at least 3 years. These recorder sheets should be initialed and dated when inserted into and removed from the temperature recorder. Air incubators should maintain an internal temperature within a tolerance of $\pm 2^{o}$, and preferably $\pm 1^{o}$.

The amount of humidity in the incubator is indirectly determined every 3 months by calculating the percentage of weight loss of agar plates incubated under prescribed conditions.

A simple approach consists of placing 15 x 100 mm covered plates containing 20 ml of solidified plate count agar at various sites throughout the incubator. After incubation at 35° for 48 ± 2 hours, the percentage of agar weight loss is calculated. The following information should be recorded in the same hard-bound record book containing the temperature readings: incubator number, shelf position, date and time of initiation of incubation, weight of 20 ml of agar before incubation, date and time of completion of incubation, weight of 20 ml of agar after incubation, amount and percentage of agar weight loss. If this percentage exceeds 15%, added moisture is needed. If the incubator is not equipped with a built-in humidifier, an exposed tray of water may be kept in the incubator.

Anaerobic incubators and anaerobic chambers should contain appropriate anaerobiosis indicators. Indicator strips are available commercially and should be changed daily.

Water baths

The primary function of a thermostatically controlled, circulating water bath is to maintain precisely a specified elevated temperature within a relatively small tolerance, usually 0.1°. Although this type of bath may be used at one of several elevated temperatures, it is most commonly used either at 44.5 or 45.5° to confirm the fecality of coliform organisms. Because the precise maintenance of either of these two elevated temperatures is more critical than that of temperatures usually associated with air incubators (e.g., 25, 35, or 37°), it is recommended that water baths be equipped with calibrated temperature recorders to provide continuous monitoring. If this is not possible, the performance of water baths is determined by observing temperatures measured with calibrated total immersion thermometers first thing in the morning, last thing in the afternoon, and 3-4 times during normal working hours. Both the uncorrected and corrected temperatures are recorded in ink in a hard-bound record book along with the water bath number, date, time, thermometer number, and initials of the analyst.

If a temperature recorder is not used, assurance that the temperature did not extend beyond the accepted tolerance during nonworking hours is made by using appropriate microbiological cultures. Cultures of Escherichia coli and Enterobacter aerogenes are included with the unknown (test) cultures. The water bath is performing properly if the E. coli culture produces gas and if the E. aerogenes culture does not produce gas in tubes of inoculated EC media incubated at 44.5 ± 0.1° or 45.5 ± 0.1° (depending on specification of the method being used) for 24 ± 2 hours. Failure of E. coli to produce gas indicates that the temperature of the water bath has risen to an unacceptably high level. Production of gas by E. aerogenes indicates that the temperature has gone too low. A fresh set of these two cultures must be used for each 24-hour interval that the baths are operational. The reactions of these cultures must be recorded in the hard-bound record book containing the temperature log.

In addition to monitoring the temperature itself, the uniformity of this temperature throughout the water bath must be ensured. At least once daily when the baths are being used, the temperature at several sites within the water bath should be determined. If the temperature at one or more sites is beyond the accepted tolerance, the circulation of the water

throughout the bath should be checked. Occasionally, input and output hoses from the water pump become impinged under the metal shelf or platform used to support the incubated test tubes, causing inadequate flow of water throughout the bath, which, in turn, may result in non-uniform temperatures. Thus, the position of the water pump input and output hoses should be checked daily whenever the baths are operational.

Refrigerators and freezers

Although the sounding of a high-temperature alarm may signal a malfunctioning refrigerator or freezer, the temperatures must be monitored by reading calibrated thermometers once daily. Large capacity refrigerators should have thermometers on the top, middle, and bottom shelves, whereas one thermometer should suffice for domestic-sized refrigerators and for freezers. Temperature readings should be recorded in a hard-bound record book containing the temperature log. Each entry line should contain information analogous to that required for reading temperatures of air incubators, e.g., refrigerator/freezer number, date, and time, and should be initialed by the responsible analyst.

Autoclaves

The proper functioning of the autoclave may be ensured by physical and microbiological means. It is recommended that a service contract specialist make thermocouple readings at various sites within the sterilizing chamber on an annual basis. The laboratory analysts, however, should also use a microbiological indicator, such as a Bacillus stearothermophilus spore ampule, or a maximum registering thermometer to certify sterility conditions of the autoclave with each use. Commercially available tape, indicating sterility, should also be used with each load.

Hot air ovens

The performance of hot air ovens is determined by measuring the temperature with a calibrated thermometer or temperature recorder. A log, contained in a bound record book, should be maintained to indicate the date, time, load number, duration of sterilization, temperature of operation, and operator for each use. The use of commercially available indicator tape with each load is also recommended.

Balances

The accuracy of balances should be checked at least every 3 months with calibrated weights. If a balance fails to meet manufacturer's specifications, a company representative or other authorized service technician should make necessary adjustments.

pH meters

With continued use or age, the efficiency of electrodes is reduced, as evidenced occasionally by a sluggish or electrically noisy response, or by off-scale meter readings. This

reduction in efficiency may be caused by contamination or leaching of the membrane glass, or by clogging of the porous junction.

To restore electrode efficiency, the tip and other contaminated surfaces should be cleaned. Pepsin or 0.1 M HCl may be used to remove protein layers. To remove inorganic deposits, the electrode tip may be washed with ethylenediamine tetraacetic acid. To remove grease and oily films, the electrode tip may be cleaned with acetone, methanol, or diethyl ether.

If simple cleaning fails to restore electrode performance, a blocked or clogged junction may be responsible for erratic pH meter readings. A junction may be unclogged by one of the following procedures: replacing the filling solution, soaking the junction overnight in 0.1 M KCl solution, applying pressure to the filling hole or vacuum to the junction tip, boiling the junction for 10 minutes in a dilute KCl solution, or, as a last resort, sanding the junction tip with No. 600 emery paper.

If these two approaches fail to restore efficiency, the sensing membrane of glass electrodes should be reconditioned or rejuvenated by alternately immersing the tip in 0.1 M HCl (15 seconds) and 0.1 M KOH (15 seconds) for 3-4 cycles. If this cycling procedure does not restore performance, the electrode tip can be immersed in 20% ammonium bifluoride solution for 3 minutes or in 10% hydrofluoric acid not more than 15 seconds. Caution: These fluoride solutions are extremely hazardous, and appropriate safety precautions must be taken when handling them. After fluoride treatment, the electrode should be rinsed in a stream of tap water, dipped only for a moment in 5 N HCl to remove fluorides, and rinsed again with tap water. The treated electrode is then soaked for 3-4 hours in a pH 4 buffer solution before its performance is tested. If the pH meter does not respond appropriately, the electrode must be replaced.

Blenders

Unfortunately, various compendia of microbiological methods for foods differ in their recommended blending speeds. There is even a lack of agreement concerning the specifications for high speed and low speed blending. For instance, the American Public Health Association (APHA) recommends blending foods at low speed (8,000 rpm) for a standard plate count determination (1). The Association of Official Analytical Chemists, however, defines low speed as 10,000-12,000 rpm (2). The Bacteriological Analytical Manual of the U.S. Food and Drug Administration recommends blending foods at high speed (18,000 - 21,000 rpm) for analysis for Bacillus cereus (3), yet many laboratories regard a speed of 14,000 - 18,000 rpm as high speed blending. If a blending speed is specified for the particular method being used, that speed must be used. If a specific blending speed is not recommended, the food should be blended at the lowest speed that results in complete homogenization within a reasonable period (1-2 minutes). Blending at excessively high speeds and/or for excessively long periods could be harmful to heat-sensitive microorganisms and could therefore affect the analytical results.

The performance of the blender is determined by visual observation of the homogenate after blending. Presence of large chunks of unblended food indicates that either the blending speed and/or time may have to be increased for that particular food, or that the positioning of the blender blades may have to be readjusted. However, if the exterior of the blender jar feels warm to the touch, the blending speed/time may be excessive and should be reduced for that particular food.

Laminar flow hood

The performance of the laminar flow hood is monitored monthly by exposing blood agar plates to the air flow for 1 hour. Plates are incubated at 35° and examined at 24 and 48 hours. No colonies should be present on the plates. If one or more plates have colonies, the test must be repeated to determine if contamination is due to a malfunctioning hood or to analyst technique. If three individual trials consistently indicate contamination, it is assumed that the hood is malfunctioning, and a service technician should be contacted to check the air flow rate and determine if there are any holes in the filters or in the hood itself. Results of microbiological monitoring should be recorded in a bound record book containing the following information: date and time of plate exposure, exact sites sampled within the hood, microbiological counts (if any), and name of analyst. Results of the service technician's findings should be recorded in a bound record book.

Whenever a new ultraviolet lamp is installed and every 3 months thereafter, the effectiveness of the ultraviolet lamp should be determined. This procedure is outlined in Annex 9.

Microscopes

Microscopes are operating properly when the resolution of the focused image is sharp and of sufficient contrast. Occasionally an image may appear blurred because the objective lens is not properly mounted or there is dust or immersion oil on the lenses. The mounting of the objective lens should be checked and the lenses cleaned with soft tissue paper or lens paper moistened with a commercial lens cleaner. If the image is still not sharp, an authorized factory representative should be contacted.

Fluorescent microscopes are used in several microbiological procedures. The purity of fluorescein isothiocyanate, commonly used in fluorescent antibody tests, varies from 30 to 100% in commercial products (4). Ideally, dyes of 100% purity should be used. Dyes of less purity may be used if the weight used to label the protein component of the serum makes allowance for the impurities, and if the impurities do not increase nonspecific staining. Using a scale of - to ++++ (see performance specifications for sera, section 7.4), the reactions of known fluorescent-positive and -negative cultures should be determined with each use. Reactions of less than ++ with a positive culture indicate either a malfunctioning fluorescent microscope or a defective lot of fluorescent-labeled antisera. Positive reactions of any degree with negative cultures indicate undesirable nonspecific reactions with the antisera.

6.4 Glassware

Laboratory glassware should be made of low alkali borosilicate glass. With each use, glassware should be examined, and items with chipped edges or etched inner surfaces should be discarded. In particular, the tips of pipets should be carefully examined since a chipped tip will result in inaccurately delivered volumes. The mouths of dilution bottles should also be inspected for chips that could lead to leakage or spillage of bottle contents while the analyst is making dilutions.

Plastic caps or closures for dilution bottles or test tubes must be treated, when new, to remove toxic residues. They should be autoclaved twice while they are submerged in distilled water, or exposed to two successive washings in a warm detergent solution.

Because acid or alkali residue may remain on glassware after cleaning, the pH of random batches of glassware should be checked by adding a few drops of 0.04% bromthymol blue and observing the color reaction. This indicator dye is yellow (acid) to blue-green (neutral) to blue (alkaline) in the pH range of 6.5 to 7.3. The 0.04% bromthymol blue solution is prepared by adding 16 ml 0.01 N NaOH to 0.1 g bromthymol blue and diluting to 250 ml with distilled water.

In addition to the pH reaction, washed glassware should be checked annually for any bacteriostatic or bactericidal substances which may have adhered to the surface. The procedure is detailed in Annex 10. Results should be reported in a bound record book containing the following information: date, name of detergent used for washing glassware, source and lot or control number of presterilized petri dishes, manufacturer and lot number of plate count agar, microbiological counts, and name of analyst.

Sterility of laboratory glassware should be tested on a routine basis. Sterilized petri dishes may be spot-checked by pouring plate count agar into randomly selected plates, incubating the solidified plates, and examining them for growth. Items such as sampling utensils, dilution bottles, and pipets may be checked for sterility by rinsing with Butterfield's phosphate buffer and filtering the buffer rinsings through a membrane. The membrane filter is placed on a nonselective medium and incubated under conditions prescribed by the method. Sterilized test tubes may be checked by adding fluid thioglycollate broth and observing growth after incubation.

All volumetric glassware should meet the specifications established by the American Public Health Association (APHA) (5). Pipets and dilution bottles should meet the requirements for accuracy of the NBS or equivalent standardizing organization. The etched calibration marks on dilution bottles, and the markings on graduate cylinders should be checked with a cylinder certified by the NBS. Disposable plasticware may be used, provided that toxicity testing and accuracy of graduated markings are randomly, but routinely, determined.

6.5 References

1. American Public Health Association. 1984. Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., M. L. speck (Ed.). American Public Health Association, Washington, DC.

2. Association of Official Analytical Chemists. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, 15th ed., K. Helrich (Ed.). Association of Official Analytical Chemists, Arlington, VA.

3. U.S. Food and Drug Administration. 1984. Bacteriological Analytical Manual, 6th ed. Association of Official Analytical Chemists, Arlington, VA.

4. Bordner, R., J. Winter, and P. Scarpino. 1978. Microbiological Methods for Monitoring the Environment. U. S. Environmental Protection Agency, Cincinnati, OH.

5. American Public Health Association. 1985. Standard Methods for the Examination of Dairy Products, 15th ed. G.H. Richardson (Ed.). American Public Health Association, Washington, DC.

7. CHEMICALS/MEDIA/REAGENTS

7.1 Specifications and ordering

The use of commercially available dehydrated media is preferred for method uniformity and analyst convenience. However, if a commercial medium is not available, the medium must be made from its individual ingredients.

Methods are usually written in generic (noncommercial) terms so that a particular commercial brand of medium is seldom recommended. Occasionally, however, a particular brand name is recommended when its superiority to competing brands has been demonstrated. In those instances the specified brand must be used.

No more than a year's supply of media should be ordered at one time. Climates with high humidities may rapidly cause caking of opened dehydrated media which may affect media performance. In addition, alkaline media may absorb carbon dioxide and change its pH. Whenever possible, media should be ordered with an expiration date specified on the bottle. For all dehydrated media, the following information should be indicated on the label: date received, date opened, and expiration date. If an expiration date is not specified, the media should not be used beyond 1 year after its receipt in the laboratory. Media that have become visibly altered by discoloration, clumping, or moisture accumulation, should be discarded.

Serological reagents, such as typing sera, are commonly used as an aid in identifying various microorganisms. Almost invariably, these sera are provided with an expiration date. For most laboratories, it would be appropriate to order no more than a 6- month supply at one time.

Chemical compounds are widely used as media components, selective agents, indicators, and stains in various microbiological procedures. Because chemical impurities can either inhibit or stimulate microbial growth, or can otherwise produce an undesirable reaction, only chemicals meeting the specifications of the American Chemical Society, or equivalent organization, should be used. Dyes from commercial sources should receive special attention because of the variability from lot to lot in percentage of dye, dye complex, insolubles, and inert substances present. Only dyes certified by the Biological Stain Commission, or equivalent organization, should be used. Unlike most dehydrated media, chemicals are seldom provided with an expiration date. Thus, it is recommended that no more than a 2-year supply be ordered at one time.

An inventory of dehydrated media, lyophilized sera, and chemicals should be conducted every 3 months. Materials that have passed their indicated expiration date should be discarded. Under no circumstances should expired materials be used for such critical investigations as analysis of official regulatory samples, analysis of quality control samples, or accumulation of publishable research data.

7.2 Preparation and use

One of the most critical factors in preparing microbiological media and reagents is the quality of water used (1). Either distilled or preferably deionized water should be used in all cases. As an example it is worthwhile to recognize that fluorides, in some cases intentionally added to tap water, are not eliminated by distillation.

The American Public Health Association (APHA) has published guidelines (2) for conducting tests to determine the suitability of water to be used in preparing microbiological media and reagents. These guidelines are detailed in Annex 11.

Whenever a bottle of dehydrated medium or chemical compound or a vial of lyophilized serum is opened, the analyst should initial and date the bottle/vial. Details of preparation of all rehydrated media and chemical reagents should be maintained in a bound notebook. For rehydrated media, this information should include: name of dehydrated medium, commercial source, lot number, name of preparer, date of preparation, amount of dehydrated medium weighed, volume of water added, final adjusted pH before autoclaving, and autoclave "run." For preparation of chemical reagent solutions, the following information should be recorded: listing of all chemical compounds used, their commercial sources and lot numbers, name of preparer, date of preparation, amount(s) of chemical compound(s) weighed, and volume of water added.

In preparing rehydrated media, the analyst should use containers with the capacity of at least twice the final volume of the medium being prepared. To prevent the dehydrated medium from sticking to the bottom of the preparation container, about one-fourth of the total volume of water should be placed in the container before the dehydrated medium is added. The contents are swirled to wet the dehydrated medium and to eventually form a slurry. The remaining volume of water is added to this slurry in 2 or 3 equal volumes. Most dehydrated media readily dissolve when subjected to stirring and gentle heating; however, some media, e.g., tetrathionate broth or bismuth sulfite agar, contain one or more ingredients which are insoluble. Care should be taken not to overheat media because they may be susceptible to degradation when exposed to high temperatures over a prolonged period. Frequent, or even constant, stirring may be required to avoid scorching. If available, a magnetic stirrer-hot plate should be used.

For most microbiological media, a final pH value is specified. The pH of the rehydrated medium should be determined with a pH meter, not pH paper, both before and after sterilization. It may be necessary to adjust the pH with 1 N HCl or 1 N NaOH before autoclaving to achieve the final pH after autoclaving, as specified by the method or the manufacturer.

In preparing chemical reagents, the analyst should make all solutions to volume in volumetric flasks. After preparation, the reagents are transferred to a borosilicate glass bottle for storage. Each bottle should bear a label with the following information: name of solution, concentration, name or initials of preparer, date of preparation, and expiration date.

Most microbiological media are sterilized by autoclaving for 15 minutes at 121° under a pressure of 1 bar (15 psi). Some heat-sensitive substances, such as carbohydrates or "sugars," may be especially heat-sensitive and are autoclaved at a reduced temperature, or for a reduced period, or both. Rehydrated media normally should not be autoclaved in volumes over 1 liter. For media dispensed in tubes, snap cap closures may be used. Larger containers such as bottles or flasks should either have a screw cap or should be loosely plugged with cotton to allow steam to penetrate during the sterilization cycle. As an added safeguard against post-sterilization contamination, cotton plugs should be protected with a porous paper covering before autoclaving. Screw cap closures must be loose before autoclaving to allow steam penetration of contents. Care should be taken not to overload the autoclave, which would prevent adequate circulation of steam during the sterilization cycle. As soon as the chamber pressure reaches zero following sterilization, the media should be removed from the autoclave. Screw cap closures of bottles and flasks should be tightened as soon as possible.

After autoclaving and cooling, tubes of media with inverted fermentation vials should be examined to be certain there are no false-positive air bubbles. Autoclaved agar media to be used in pour plates must be allowed to temper to 44-46° before use. This temperature can be determined and monitored by placing a thermometer in a bottle of agar exposed to the same conditions of heating and cooling as other bottles of the same type of agar. Melted agar maintained at 44-46° for more than 3 hours should not be used.

An autoclaving log should be maintained in a separate bound notebook with the following information: autoclave "run" (to be cross-referenced to this same designation used in the media preparation log), autoclave number, period and temperature of sterilization, operator, and date. Separate autoclaves should be used for media sterilization and for decontamination of pathogenic materials.

Some heat-sensitive materials cannot be autoclaved but must be filtered. Filters must not contain any substances that would either be inhibitory or stimulatory to microorganisms and must not contain any materials that would interfere with bacterial indicator systems in the medium. At least 70% of the filter must contain pores with a mean diameter of 0.45 ± 0.04 μm. The flow rate through the filter should be at least 55 ml/min/cm^2 at 25°. Filters should be able to withstand autoclaving at 121° for 10 minutes. Whenever possible, filtration should be performed either in a laminar flow hood or in a safety cabinet.

Because some reagents autosterilize (i.e., are self-sterilizing), neither autoclaving nor filtration is required. Some dye solutions, for example, are prepared by adding sterile water to the dye in a sterile container and allowing the solution to autosterilize.

7.3 Shelf life and storage conditions

The shelf life of dehydrated media and lyophilized sera is determined by the expiration date; however, the shelf life of organic chemicals is not so clearly determined. A practice of not using chemicals 2 years beyond the date of receipt seems both scientifically valid and cost-effective. However, if the chemical changes visibly in appearance before that time, it

should be discarded. Stocks of dehydrated media, lyophilized sera, and chemicals should be rotated to minimize the amount of expired or old materials that must be discarded. The contents of opened bottles of dehydrated media should be used within 6 months or discarded. In humid climates, it may be necessary to store opened bottles of dehydrated media in a desiccator.

Dehydrated media and chemicals should be stored in a cool, dry place that is protected from sunlight or direct artificial illumination. If space allows, the bottles of dehydrated media should be kept in their original box for added protection against exposure to illumination and dust. Lyophilized sera are normally stored under refrigeration temperatures as recommended by the manufacturer.

The guidelines for determining the shelf life of rehydrated media, sera, and chemical reagent solutions are not as definite as those for dehydrated or lyophilized reagents. Several factors determine the length of time that rehydrated media may be stored: volume of dispensed medium, storage temperature, type of container and container closure, and the specific nature of the medium itself. Agar or broth that has been dispensed in tightly closed screw-cap tubes may be stored up to 3 months at 4°. Agar or broth that has been dispensed in tubes with snap cap closures may be stored up to 1 week at this same temperature. Refrigerated tubes containing inverted fermentation vials must be examined at room temperature for false-positive gas bubbles before use. Moisture loss in tubes of broth is determined by marking the original fluid level in several tubes of each batch of freshly prepared media. If the estimated loss exceeds 10% of the original volume, these tubes should be discarded. Poured plates with loose-fitting covers in sealed plastic bags may be stored up to 2 weeks at 4°, whereas large volumes of agar in tightly closed screw-cap flasks or bottles may be stored up to 3 months at this temperature. Without exception, all rehydrated media should be protected from exposure to light.

The holding guidelines discussed above will not apply in all situations. For instance, some selective media may lose their selectivity upon aging and must be made relatively fresh, or on the day of use, whereas other media are required to be made a certain number of days before use. In these instances the general guidelines recommended for holding prepared media are superceded.

Rehydrated sera should not be kept for more than 30 days. Occasionally rehydrated sera may become cloudy, or a precipitate may form. These sera should not be used unless they have been clarified and shown to react properly with control cultures. Rehydrated sera should not be exposed to room temperature for prolonged periods. During storage, they should not be subjected to repeated freezing and thawing, which are detrimental to the antibody titer.

There are no uniform guidelines for the storage and shelf life of chemical reagent solutions. Some reagents must be prepared only on the day of use while others may be prepared and stored a few days, or even several weeks, before use; some reagent solutions must be stored under refrigeration, while others may be stored at room temperature (24-26°); some solutions must be stored in glass-stoppered, air-tight bottles, whereas this precaution

would not be critical for other reagent solutions. The one precautionary measure, however, which all of these reagent solutions have in common is that they should be protected from exposure to excessive heat and light. Thus, one should follow the storage conditions recommended by the method being used. If these conditions are not specified, adequate controls must be included to ensure proper performance of the chemical reagent solutions.

7.4 Performance specifications

Whenever a new lot of dehydrated medium is received by the laboratory, its performance relative to specifications for physical standards and productivity must be determined. With respect to physical standards, dehydrated media should be dry and free-flowing. Most media should be completely soluble in distilled water, but some media such as tetrathionate broth and bismuth sulfite agar are notable exceptions. Upon rehydration, most broths should be transparent or translucent but, again, there are exceptions. The unadjusted pH should be relatively close ($\pm$ 0.1 pH units) to the final pH specified by the manufacturer.

The second performance specification, productivity, is determined by comparing the performance of the new (test) lot with that of a reference lot. The American Public Health Association (APHA) furnishes a reference lot of plate count agar for use in determining if the test lot meets APHA specifications. A test lot is acceptable if it yields counts with $\pm$ 10% of those obtained with the reference lot of plate count agar (3).

Unfortunately, reference lots of other types of agar are not available from APHA and the laboratory will have to use another approach. In the case of nonselective agars, parallel pour plates for enumeration of the appropriate analyte(s) by the test lot and the lot in current use (reference lot) are performed on a minimum of five positive test samples. A single source, batch, or lot of pure water, glassware, and other necessary materials should be used to minimize the effect of variables. After the plates are incubated under conditions recommended by the particular method being used, they are examined for size and appearance of colonies, regardless of the actual counts. Any atypical size or appearance of colonies should be recorded. The colonies are then counted, and the values are converted to logarithms. The difference, $\underline{d}$, between the two logarithmic values for each sample is calculated, including the + or - sign. The mean, $\underline{d}$, and the standard deviation, $S_{\underline{d}}$, of these differences are then determined. The $S_{\underline{d}}$ is determined by the following formula:

$$S_{\underline{d}} = \sqrt{\frac{\Sigma\ (x - \bar{x})^2}{n-1}}$$

where x is any observed value; $\bar{x}$ is the mean of all observed values; and $\underline{n}$ is the number of determinations. The Student's $\underline{t}$ value is then calculated using the following formula:

$$\underline{t} = \frac{\bar{d}}{S_{\underline{d}}/\sqrt{n}}$$

If the t value does not exceed 2.78, then the 2 lots do not produce significantly different counts, and the test lot is acceptable, provided that the size and appearance of colonies are typical. If the t value exceeds 2.78, then counts given by the test lot and the reference lot are significantly different, and the test lot is not acceptable. Liquid nonselective media may be similarly tested by using dilution-to-extinction procedures to compare new and standard lots of media.

The acceptability of test lots of selective media is determined somewhat differently from that of nonselective media. Liquid media are inoculated with organisms that are expected to grow and with organisms that are supposed to be inhibited. After incubation under prescribed conditions, the count of each organism is determined. A lot is acceptable if there are high counts of organisms that are expected to proliferate and low counts of organisms that are supposed to be inhibited. The acceptability of solid selective media is similarly determined. The target analyte should be recovered in numbers that are comparable to those on an appropriate nonselective agar. Organisms that are supposed to be inhibited are present in very low numbers, if at all.

If membrane filters are used in the laboratory, two microbiological specifications should be met. First, the arithmetic mean of five counts given by the filtration technique must be within $\pm$ 10% of the arithmetic mean of the counts of five agar spread plates using the same test sample volumes and agar media. Second, the filter must be able to retain a 100-ml suspension of Serratia marcescens containing 1×10^3 cells/ml (2).

Specifications for commercial sera relate to the physical appearance of the rehydrated material and to the intensity of agglutination reactions with known control cultures. Rehydrated sera should be a clear amber to straw-colored fluid, with no precipitate. Occasionally, a vial of rehydrated sera will have a very offensive odor, but it will otherwise appear normal. If this serum reacts strongly with known cultures, it may be used.

Depending upon the organism being tested, serological reactions may involve flagellar antigens, somatic (body wall) antigens, or both. Flagellar serology is normally performed in a test tube, and the precipitate from a positive reaction is fine, delicate, and "snowy." Somatic serology is usually performed on a glass slide, and the precipitate from a positive reaction is coarse and grainy. Flagellar serological reactions may require up to 1 hour, whereas somatic serological reactions are relatively prompt (1-2 minutes, depending on the particular organism). Agglutination reactions are normally quantitated as follows:

+ + + +	all of the cells agglutinate
+ + +	75% of the cells agglutinate
+ +	50% of the cells agglutinate
+	25% of the cells agglutinate
$\pm$	< 25% of the cells agglutinate
-	none of the cells agglutinate

Rehydrated sera that give agglutination reactions of less than + + with known control cultures are unacceptable.

Specifications for chemical reagents may be applied to selective agents and stains used in microbiological procedures. Dyes, surfactants, antibiotics, sulfa drugs, and metallic ion solutions are commonly used as selective agents in many types of media. Purity and, thus, the toxicity of these selective agents vary from manufacturer to manufacturer and even from lot to lot of the same selective agent from the same manufacturer.

Two approaches are used to determine the relative toxicity of various selective agents. In the first approach, 1 ml of a 10^{-5} dilution of a preenriched culture of the target analyte is inoculated into separate tubes containing 10 ml of selective enrichment made with the standard lot and the test lot of selective agent. After incubation under prescribed conditions, the number of target analyte organisms in the selective enrichment media is determined either by the most probable number technique or the surface plating technique. If counts obtained with the test lot are at least 90% of those with the standard lot, then the test lot is acceptable.

The second approach involves a dilution to extinction procedure. From an 18-24 hour preenriched culture of the target analyte, 1 ml of a series of 10-fold dilutions is inoculated into 10 ml volumes of selective enrichment media containing either the standard lot or the test lot of selective agent. The inoculated tubes of selective enrichment media are incubated under prescribed conditions and then streaked to appropriate selective plating agars. A test lot is acceptable if the target analyte is recovered from the selective enrichment made with the test lot at a level comparable to or greater than the selective enrichment containing the standard lot of selective agent.

Dyes may be used to stain bacterial spores, flagella, and cell walls and to facilitate the observation of certain enterotoxin production reactions. Unlike performance specifications for selective agents, specifications for dyes are not quantitative but are somewhat subjective. Control cultures with known staining reactions are stained with the appropriate dye(s). If the morphological feature to be discerned (e.g., spores) is readily visible, the dye is acceptable. Because a variation in staining techniques can lead to variability in staining reactions, any dye giving an initially unacceptable reaction should be retested by the same analyst and, preferably, by at least one other analyst. It is also advisable to retest the stain using additional control cultures.

7.5 References

1. Geldreich, E.E., and H. F. Clark. 1965. Distilled water suitability for microbiological applications. J. Milk Food Technol. 28:351-355.

2. American Public Health Association. 1985. Standard Methods for the Examination of Water and Wastewater, 16th ed., A.E. Greenberg, R.R. Trussell and L.S. Clesceri (Eds.). American Public Health Association, Washington, DC.

3. American Public Health Association. 1985. Standard Methods for the Examination of Dairy Products, 15th ed., G. H. Richardson (Ed.). American Public Health Association, Washington, DC.

8. STANDARDS

Primary reference standards are homogeneous materials with properties such as identity, purity, and potency that have been measured and certified by the National Institute of Standards and Technology, the U.S. Pharmacopeial Convention, the American Society for Testing and Materials, or other equivalent organization. Chemists use these standards to prepare working standards for chemical and drug analyses.

Even though microbiology is a relatively less exacting science than chemistry, reference standards are used in the food microbiology laboratory. Microbiologists use reference cultures to determine qualitatively that the media are performing properly.

8.1 Specifications and ordering

The primary source of reference cultures in the U. S. are:

1. American Type Culture Collection
   12301 Parklawn Drive
   Rockville, MD 20852

2. U.S. Department of Health and Human Services
   Public Health Service
   Centers for Disease Control
   Atlanta, GA 30333

3. Difco Laboratories
   P.O. Box 1058
   Detroit, MI 48232

4. BBL Microbiology Systems
   P.O. Box 243
   Cockeysville, MD 21030

By far, the American Type Culture Collection (ATCC) is the largest source of these cultures. Difco Laboratories and BBL Microbiology Systems are essentially distributors who primarily obtain their cultures from ATCC. Cultures are ordinarily lyophilized or freeze-dried, and each ATCC culture strain has its own identification or reference code number. Cultures are commonly freeze-dried in glass ampules and stored under refrigeration (4-8°) before use.

In addition to lyophilized powders, commercial distributors may supply ATCC cultures that are impregnated on filter discs. Both the ATCC lyophilized and the ATCC disc cultures are provided with an expiration date beyond which they should not be used. When ordering these cultures, the analyst should rotate stock. Moreover, an inventory of these commercially available reference cultures should be taken every 3 months. Cultures that have passed their expiration date should be autoclaved and discarded.

8.2 Preparation and use

Lyophilized cultures are rehydrated by aseptically breaking the neck of the ampule, adding the powder to a nonselective medium appropriate for the growth of the organism, and incubating the rehydrated culture under conditions recommended for that organism. A loopful of the culture is streaked to an agar to obtain isolated colonies. The plate is incubated under prescribed conditions. The analyst then proceeds as desired with the isolated colonies.

To rehydrate disc cultures, sterile forceps are used to aseptically remove a disc from the vial and to place it into a tube containing brain heart infusion or Trypticase soy broth. The broth is vortexed until the disc is completely dissolved. A loopful of the dissolved culture is streaked to an appropriate agar to obtain isolated colonies, and the plate is incubated under conditions prescribed by the method. The analyst then proceeds as desired with the isolated colonies.

8.3 Shelf life and storage conditions

After the lyophilized or disc cultures have been rehydrated, the analyst must maintain these stock cultures. Conditions for maintenance vary with the microorganism; maintenance procedures for those organisms of greatest interest to the food microbiologist are given in Annex 12.

The analyst should also maintain a log containing the following information: name of culture (genus and species), strain designation, source of culture (both commercial and original, e.g., type of food or clinical specimen from which the culture was originally isolated), date of receipt, date of rehydration, all dates of serial subculturing of the rehydrated stock culture, all media used (growth, purification, and storage), incubation period and temperature used for growth and purification, temperature used for storage, location of culture, and initials of analyst performing any particular segment of the maintenance procedure.

8.4 Performance specifications

Bacterial reference cultures must meet criteria for purity, morphology, biochemical reactions, and serological reactions.

Purity

The purity of microbiological cultures is determined by streaking a broth culture to an appropriate selective, nonselective, and/or differential plating agar. The selective agar contains one or more ingredients to inhibit the growth of nonanalyte, or competing, organisms; the non-selective agar does not. The differential agar merely indicates certain biochemical information about the inoculated culture by a color change in the agar. To ensure the purity of an organism, it is preferable to use both a selective and a differential agar. If a differential agar is not available for a particular organism, a nonselective agar may be substituted.

The appearance of more than one morphological type of colony indicates that the culture may be contaminated. However, different morphological types may be due to mutants, stressed or damaged organisms, or defective media. In this event, one or more colonies of typical morphology must be purified. There are two approaches:

In the first approach, the picked colonies are inoculated into an appropriate broth, incubated under prescribed conditions, and restreaked onto plating agars. The advantage of using a broth, especially a nonselective one, is that it gives the organism an opportunity to resuscitate, or revive, and to reach a high population density. One disadvantage, however, is that it may give competing organisms the opportunity to overgrow the target analyte. In any event, if all the colonies on the plating agar are morphologically similar, it may be reasonably assumed that the culture has been purified.

In the second approach, the picked colonies are restreaked directly onto appropriate plating agars. The advantage of restreaking plating media directly, rather than inoculating a broth and then restreaking the plating media, is the saving of time. However, it is sometimes more difficult to obtain isolated colonies with this second approach. Moreover, it may be very stressful for the organisms picked from a selective agar plate to be streaked directly to another selective agar plate without any resuscitation. In any event, if all the colonies on the plating agar are morphologically similar, it may be reasonably assumed that the culture has been purified.

Morphology

In addition to observing colonial morphology to determine culture purity, morphology can also ensure that the media are performing properly. Organisms produce colonies that have a characteristic, or typical, appearance on various agars. An appearance that is not typical may indicate a defective medium or a defect in medium preparation. The analyst should be aware of the appearance of colonies streaked from a pure culture that may have undergone one or more mutations. This type of culture may contain organisms that produce colonies that are quite dissimilar. Severely stressed or damaged organisms in an otherwise pure culture can also produce morphologically different colonies.

Biochemical reactions

A series of biochemical reactions may be used to confirm the identity of an organism. Several known reference cultures should be included in the identification procedure to ensure that the biochemical tests are performing properly. Individual test reactions of these reference cultures have been documented (1-3).

Typical biochemical reactions of the reference cultures (either conventional or rapid) indicate that the reference cultures are pure and the biochemical tests are reacting properly. One or more atypical biochemical reactions for a particular reference culture indicates that the reference culture is contaminated or has mutated, the biochemical test is defective, or any combination of the above.

The analyst should attempt to determine the cause of atypical biochemical reactions by obtaining and testing different copies of the same reference culture strains (e.g., another subculture of P. vulgaris ATCC 13315); altogether different reference cultures (e.g., Klebsiella pneumoniae ATCC 13883 or Enterobacter cloacae ATCC 13047 instead of Proteus vulgaris ATCC 13315); different lots of conventional biochemical test substrates; and different lots or manufacturer's code numbers of rapid diagnostic kits.

Serological reactions

Serological reactions are a useful, indispensable adjunct to biochemical reactions for identifying organisms. When used for definitive serotyping, both somatic (body wall) and flagellar antigens, if applicable, are serologically identified. In both cases reference cultures are included to determine the intensity and specificity of the immunological reaction.

If a scale of 0 to ++++ (see section 7.4) is used to quantitate the intensity of the agglutination reaction between a known reference culture (antigen) and its homologous antiserum (antibody), reactions of less than ++ indicate either that the culture is contaminated, the culture is pure but may contain damaged flagellar and/or somatic antigens, or the antisera have insufficient titer or are otherwise defective.

In addition to the intensity of reaction between a known reference culture and homologous antiserum (e.g., Salmonella antigen and Salmonella antiserum), the specificity of the reaction must be determined. The reactivity of a known reference culture is determined with nonhomologous antiserum (e.g., Escherichia coli antigen and Salmonella antiserum). Reactions of any degree of activity, i.e., + or greater, indicate that the reference culture is contaminated, or is pure but contains "rough" or autoagglutinable antigens, or that the antiserum is defective.

To ensure that rough known reference cultures, as well as unknown test cultures, are not used, a saline culture control should accompany each serological determination. For this control, the culture itself is mixed with a small amount of saline solution either on a glass slide or in a test tube, depending on the type of serological determination being made. A positive reaction of any degree indicates that the culture is rough and non-typable without additional special treatment.

8.5 References

1. Ewing, W.H. 1986. Edwards and Ewing's Identification of Enterobacteriaceae, 4th ed. Elsevier Science Publishing Co., Inc., New York, NY.

2. Krieg, N.R. 1984. Bergey's Manual of Systematic Microbiology, Vol. 1. Williams and Wilkins, Baltimore, MD.

3. Sneath, P.H.A. 1986. Bergey's Manual of Systematic Microbiology, Vol. 2. Williams and Wilkins, Baltimore, MD.

9. METHODOLOGY

9.1 Choice of method

Methods have to be chosen on their attributes, the most important technical ones being:

1. Accuracy or lack of bias, or lack of systematic error; how near they get to the true answer.
2. Precision or lack of scatter around the mean.
3. Specificity and lack of matrix dependence.
4. Practicality (usage range, relevance, applicability).
5. Reliability, ruggedness, reproducibility.
6. Sensitivity (response per unit concentration).
7. Limit of detection.

There are also the practical considerations of quickness, economy and simplicity.

There is no shortage of methods for the microbiological analysis of foods. The abundance of microbiological methods means that the microbiologist must choose the most appropriate method, because the use of different methods to analyze a particular food may, and often does, lead to different analytical results.

The microbiologist should be aware of the purpose and function of the major compendia for the microbiological analysis of foods. Perhaps the most familiar is the Official Methods of Analysis (OMA) of the Association of Official Analytical Chemists (AOAC) (1). This volume is a compilation of microbiological and chemical methods that have been subjected to collaborative study. A successful collaborative study is one in which several analysts, working independently, obtain equivalent results when using a particular method to analyze identical test samples for a particular analyte. A more detailed discussion of the collaborative study is given in section 9.3. Microbiological methods that have been subjected to a successful collaborative study are published in OMA, Chapter 46.

Another manual of microbiological methods, and one that should not be confused with OMA, is the Bacteriological Analytical Manual (BAM) of the U.S. Food and Drug Administration (USFDA) (2). The main purpose of the BAM is "to provide the field laboratories of the USFDA with methods that have been found effective for the detection of microorganisms and their products in food." There is no claim that these methods are indeed the best, but they are used by the FDA to analyze official regulatory samples. In several instances the AOAC and BAM methods for analyzing a particular food for a specific analyte may be identical. However, what distinguishes the two compendia is that all AOAC methods, by definition, have been subjected to a collaborative study, whereas the BAM contains some methods that have not been collaboratively studied in addition to those that have. Moreover, BAM methods are applicable to a wider range of foods than are methods recommended by the AOAC.

The American Public Health Association (APHA) has published a Compendium of Methods for the Microbiological Examination of Foods (3) that is similar to the AOAC and BAM compendia. Like the BAM, some of the methods in the APHA compendium have been collaboratively studied and some have not. A major distinction between the APHA compendium and the AOAC and BAM is the editorial style. Both the AOAC and BAM contain little, if any, explanatory narrative of the analytical steps of the methods. In contrast, the APHA compendium includes background information about the various procedures, precautionary measures and limitations associated with the procedures, aids in interpretation of results, and numerous related references.

Like the BAM, a series of methods for the microbiological analysis of foods has been published by Health and Welfare Canada (4). These methods provide for the identification and/or enumeration of a wide variety of microorganisms whose presence in food may be significant.

In the fourth of a series of manuals on food quality control, the Food and Agriculture Organization of the United Nations (FAO) has published a manual for microbiological analysis of foods (5). Its purpose is "to provide a single source book on methods and information useful in the microbiological examination, quality control and monitoring of foods in developing countries." The manual was designed for daily use in food quality and safety programmes and emphasizes the use of practical methods. It is presently being revised.

Several other international organizations are concerned with microbiological criteria of foods as related to public health safety throughout the world. They are also concerned with developing, studying, and standardizing microbiological methods used by analysts in different countries engaged in international commerce. Among these organizations are the International Commission on Microbiological Specifications for Foods, the Codex Alimentarius Commission, the Expert Committee on Food Hygiene and Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Hygiene, the International Dairy Federation, and the International Association for Cereal Science and Technology.

Thus, there is no scarcity of methods; the problem is deciding which one(s) to use. This decision is usually made by management after consideration of such factors as reliability, speed, and cost, as discussed in section 1.1. The methods selected for use must be practical. However, what is practical in one laboratory may not be practical in another. Thus, the practicality and the ultimate acceptability of any method must be judged on an individual basis.

Managers of laboratories concerned with the microbiological certification of exports must consider the particular analytical method used by the country to which the food is being exported. It is always advisable to use the same method as that used by the regulating authority of the country receiving the food shipment. Thus, the microbiology laboratory may need to use different methods for the same type of food exported to different countries.

9.2 Controls

The use of proper controls in a microbiological laboratory is frequently overlooked. Proper controls are necessary to support the validity of the analytical results.

Sampling controls

See section 5.2.

Positive analytical controls

Two types of positive analytical controls should be considered.

The first type, the media or culture control, ensures that the media are performing properly. It is prepared by inoculating the initial medium with the analyte and proceeding through the entire analytical protocol as used with the test samples. This control should also demonstrate the typical appearance of the analyte on any plating agars that may be used in the method.

The second type of positive analytical control, the test sample control (not to be confused with sampling controls), serves the same purpose as the media or culture control. Food comprising this control is inoculated with the analyte and is carried through the entire analytical procedure as used with the test samples. This control may encompass the media or culture control and is actually preferable because the effect of food matrix material on the performance of the media is considered.

The level of analyte in these positive analytical controls need not approach the sensitivity level of the procedure, but it should be high enough to ensure that viable analyte cells are present on the day that analyses are initiated.

Negative analytical controls

Three types of negative controls should be considered; however, the actual number used depends upon the type of microbiological analysis.

The first, the negative media control, ensures that the analytical media are not contaminated with the analyte. The initial uninoculated medium is carried through the entire analytical protocol just as are the test samples.

The second type of negative analytical control, the culture control, demonstrates either the appearance of the nonanalyte(s), e.g., competing organisms, on various media, or it shows that the nonanalyte(s) will not grow on media used in the test procedure. This control is prepared by inoculating the initial medium with the nonanalyte and carrying the medium through the entire procedure in the same manner as the test samples.

The third type of negative analytical control, the environmental control, offers reasonable assurance that the environment is not a source of the analyte. With a few exceptions, this control is prepared by exposing the initial medium to the open air environment during all analytical operations on the initial day of analyses. For example, this control may be an open flask of lactose broth if the procedure is intended for the identification of Salmonella in dried egg powder; an open flask of Trypticase soy broth for identifying Salmonella in dried active yeast; an uncapped tube of lauryl tryptose broth for the enumeration of coliforms; or a plate of exposed Baird-Parker medium for the enumeration of Staphylococcus aureus. However, if the procedure is intended for the enumeration of total aerobic microflora in a food, exposing a petri dish of plate count agar to the open atmosphere during the entire analysis would not be appropriate. An appropriate environmental control in this instance would be to expose a 15 x 100 mm petri dish containing 20 ml of plate count agar to the open atmosphere for 15 minutes. Growth of no more than 15 colonies on the incubated plate would ensure that the laboratory environment is suitable for performing a total plate count determination. (See section 3.2, Air Monitoring). After the initial day of analysis, all negative environmental controls are treated in the same way as the test samples. The negative environmental control may encompass the negative media control.

Glassware sterility controls

See section 6.4.

Water bath culture controls

See section 6.3, Water Baths.

9.3 Method validation

Before a method is routinely used in a laboratory, it must be properly validated by an outside organization and by the laboratory itself. Perhaps the largest organization responsible for the validation of analytical methods is the AOAC, whose primary objective is to "obtain, improve, develop, test, and adopt uniform, precise, and accurate methods for the analysis of foods" (6). Before a method is accepted as official by the AOAC, it must meet three criteria.

First, the method must give data of a predictable degree of precision and accuracy when it is used by qualified analysts. Precision measures the variability of the method within an individual laboratory and among different laboratories. Accuracy measures how well the method determines the true level of analyte.

The second criterion for validating a method is that of practicality. To meet this criterion, the procedure must be as simple and rapid as possible, while still meeting the requirements for reliability. Sometimes the only reliable method available may be impractical, in the sense of being expensive and time consuming. In this case, the method may be accepted by AOAC because there is a need for it. In the meantime, however, analysts should continue to search for a method that is both reliable and practical.

The third criterion is the availability of a method. The method must not contain a "trade secret" nor part of a confidential document that would be unavailable to all interested analysts. Insofar as possible, AOAC encourages the description of a method in generic or noncommercial terms.

Any method meeting these criteria must be subjected to a successful collaborative study before it is recognized by AOAC as an official method. The collaborative study is the mechanism used to officially validate a procedure. It is an interlaboratory study in which competent, experienced analysts, working independently in different laboratories, use a specific method to analyze homogeneous test samples for a particular analyte. Its purpose is to demonstrate that a particular method can be used in several independent laboratories to obtain essentially equivalent results. Guidelines for conducting a collaborative study are given elsewhere (7).

A laboratory may have to conduct its own validation of a procedure that is being considered for routine use if no procedure has yet been validated for a particular analytical determination. In other instances a laboratory may choose to revalidate a method that has already been validated by AOAC or some other international organization. There are several reasons for revalidation. First, the laboratory may want to be assured that the method is applicable to the specific foods which it examines. Many validated methods may apply to the analysis of only one or a few specific food types. If the method does not apply to the food(s) analyzed by a particular laboratory, then that laboratory should conduct its own validation of the method for analyzing the foods in question.

Second, a laboratory may wish to determine the specificity of the method. For example, if a collaborative study has evaluated a method for identifying three of more than 1700 Salmonella serotypes in selected foods, a laboratory may want to establish that the method can be used to identify additional serotypes, or it may want to document the behavior of non-Salmonella organisms with the method.

Third, it may be beneficial to determine whether any problems could arise when the method is used in a particular laboratory. A collaborative study is conducted under very controlled laboratory conditions, and although the method may have been demonstrated as reliable, rapid, and cost-effective, for some unknown reason it may just not be adaptable for routine use by a particular laboratory.

Fourth, the laboratory may wish to test the analyst's proficiency in using the method. If acceptable results are not obtained, the laboratory should use an equally acceptable alternative method or consider additional training for the analyst.

Whenever possible, the method to be validated should be compared with an existing or standard method. In performing the actual validation, or revalidation, of a method, the analyst should use foods that are naturally contaminated with the desired analyte(s). If these foods are not available, the analyst must artificially contaminate the foods with the target analyte(s). In preparing test samples for a validation study, the analyst should consider use of stressed versus nonstressed analyte cells; type and degree of stress (freezing, drying,

heating, radiation, and chlorination); levels of inoculation; inclusion of nonanalytes; use of atypical analytes; and numbers of test samples. Andrews (8) has provided details for preparing samples for AOAC collaborative studies of microbiological procedures for foods. However, these same guidelines are equally adaptable for preparing test samples for a noncollaborative validation study by a single laboratory.

9.4 Reference samples

Instead of using reference samples, microbiologists use positive analytical sample controls, as described in section 9.2. These controls not only provide assurance that the media are working properly, but also serve as a reference, i.e., the analyst may compare the reactions of unknown samples with those of the "reference" sample.

In analyzing the "reference" and unknown or test samples, the analyst should follow the recommended method verbatim. It is often tempting to "improve" or otherwise modify an official or standard method. However, modifications that are seemingly minor may compromise the integrity of the analytical results, thereby negating the official or standard method.

9.5 References

1. Association of Official Analytical Chemists. 1990. Official Methods of Analysis, 15th ed., K. Helrich (Ed.). Association of Official Analytical Chemists, Arlington, VA.

2. U.S. Food and Drug Administration. 1984. Bacteriological Analytical Manual, 6th ed. Association of Official Analytical Chemists, Arlington, VA.

3. American Public Health Association. 1984. Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., M.L. Speck (Ed.). American Public Health Association, Washington, DC.

4. Health Protection Branch. 1989. Compendium of Analytical Methods for the Microbiological Analysis of, and the Detection of, Extraneous Materials in Foods. Poly Science Publications, Inc., Montreal, Canada.

5. Refai, M.K. 1979. Manuals of Food Quality Control. 4. Microbiological Analysis. Food and Agriculture Organization of the United Nations, Rome, Italy.

6. Association of Official Analytical Chemists. 1982. Handbook for AOAC Members, 5th ed. Association of Official Analytical Chemists, Arlington, VA.

7. Committee on Interlaboratory Studies. 1988. Guidelines for collaborative study procedure to validate characteristics of a method of analysis. J. Assoc. Off. Anal. Chem. 71:160-172.

8. Andrews, W.H. 1987. Recommendations for preparing test samples for AOAC collaborative studies of microbiological procedures for foods. J. Assoc. Off. Anal. Chem. 70:931-936.

10. USE OF ANIMALS IN TESTING

A variety of animals (mice, rats, hamsters, guinea pigs, rabbits, cats, dogs, monkeys, and chickens) are used in laboratory procedures. Of these, however, the mouse is the most widely used because of its relatively low cost to purchase, breed, and maintain. Although larger animals, such as rabbits, may be used to produce antibodies or determine toxic dermal or ocular responses, mice are useful in basic microbiological techniques, such as determining the presence of Clostridium botulinum toxin or paralytic shellfish poison toxin. Thus, this discussion will focus exclusively on the use of mice in laboratory testing.

10.1 Personal hygiene

Analysts must take special precautions with regard to personal hygiene while working with animals. Hands should be thoroughly washed before handling mice and again before leaving the animal room. Disposable plastic gloves should be worn whenever feeding, watering, handling, or removing infected mice to prevent skin contact. During and after handling mice, the analyst should never touch his or her face, nose, eyes, or mouth. When handling syringes, the analyst must wear hard-toed, as opposed to open-toed, shoes as a precaution against accidents. Eating, drinking, smoking, or food storage in the animal room must be prohibited. Persons entering the room containing infected animals should wear surgical-type masks and disposable gowns that can be removed before the analyst leaves the animal room. These articles should be placed in a plastic biohazard bag for subsequent autoclaving.

10.2 Ordering and quarantine

The analyst ordering the mice should specify the species, strain, sex, age, weight, carton type, number of animals, and date of arrival. Arrival of mice should be scheduled during normal working hours.

Upon arrival, the mice should be inspected by a veterinary medical officer (VMO) or his designated representative. Mice should be quarantined for a minimum of 1 week, or longer, as desired by the VMO, under the same environmental and nutritional conditions to be used in the study. Mice are quarantined in a room separate from the room housing the other mice. Mice resulting from in-house breeding need not be quarantined. After the quarantine period has ended, the VMO should transfer animals to the investigator with the animal health certificate (shown in Annex 13).

10.3 Animal room facilities

Separate rooms, under a negative pressure, should be set aside for housing animals. Not more than one animal species must be maintained in a single room. Thus, the room set aside for mice must not be used to house any other types of laboratory animals. The room should have a sink with sources of hot and cold tap water. Floors must be swept and mopped daily with a disinfectant solution, and the walls cleaned and disinfected at least monthly. Bench tops, shelves, glass in doors, and sink area must be disinfected before any experiment is started.

A hygrothermograph provides continuous monitoring of the temperature and humidity of the room. Ideally, the temperature should be between 18 and 26°, and the humidity between 40 and 70%.

The hygrothermograph for temperature readings is calibrated as described for the temperature recorder in section 6.2. The hygrothermograph is calibrated for humidity readings by placing it inside a polyethylene bag with a bowl of water. After 6 hours of exposure, the relative humidity is reasonably assumed to be 96%, which is within the sensitivity range of the instrument. If necessary, the pen arm may be adjusted to reflect this humidity on the recording chart.

The hygrothermograph recording charts should be changed weekly before overprinting occurs. The analyst who inserts and removes the charts should initial and date these records. After the charts are removed from the hygrothermograph, they should be taped in a record book.

The analyst should control the room lighting. The room should be supplied with an electrical timer to maintain normal day/night cycles, with alternating 12-hour periods of light and darkness.

10.4 Cage facilities

Mice should be maintained in cages made of plastic or stainless steel that are easily cleaned. At least twice a week the cages, cage covers, and bedding should be changed.

Cages should be spacious enough to prevent crowding, with a height of 13 cm. The amount of floor area required by each mouse depends upon its weight: 39 sq cm for mice weighing up to 10 g; 52 sq cm for mice between 10 and 15 g; 77 sq cm for mice between 15 and 25 g; and 97 sq cm for mice weighing over 25 g.

Each cage should be labeled with the following information: project and/or experiment number, type of toxin or bacteria used for inoculation, date of inoculation, inoculation dose, and name of investigator. This information should also be contained in a hard-bound record book.

10.5 Care and feeding

Mice should be provided with a nutritionally balanced commercial feed. Pregnant mice should be fed a specially formulated type of commercial feed. Animal feed for all mice should be changed twice a week, and water bottles three times a week. A log should be maintained documenting the frequency of feeding and watering.

General health and appearance of the mice should be monitored daily. Symptoms of illness in mice include weight loss, decrease in activity, and hair loss resulting in exposed patches of skin, especially around the neck and on the back. Mice that appear to be unhealthy should be quarantined until symptoms disappear. Mice whose symptoms do not disappear should be euthanized, autoclaved, and disposed of, as described in section 10.8.

10.6 Selection for test

To minimize the possibility of bias in selecting mice for laboratory studies, the following randomization procedure may be used. All the mice of a particular shipment which have passed the quarantine period and which have become acclimated are weighed and separated by weight into boxes. Weights of individual mice in each box must not vary more than 1 g.

As an example of randomization of selection, suppose that there are 10 mice: 5 in box of weight class "x" and 5 in box of weight class "y." If there are two experimental groups to be tested, one mouse from box "x" will be placed in each group. Then one mouse from box "y" will be placed in each group. This procedure will be continued until each experimental group has 5 mice assigned to it. It may be necessary to rearrange a few of the mice so that the mean weights of each group do not deviate more than 3-6 g.

After the mice have been assigned to a particular experimental group, individual mice can be labeled or identified by using dyes to color-code their tails. Colors of red (safranin O), purple (crystal violet) or green (brilliant green) can be obtained by dissolving 0.1 g of dye in 100 ml of a 50:50 mixture of acetone and alcohol. These reagents should be identified and labeled as described in section 7.2. A cotton swab is used to apply the dye to the tails of the mice.

10.7 Restraint and injection

The procedures for restraining and injecting laboratory mice is given in Annex 14. Although there are several injection routes (intraperitoneal, intramuscular, subcutaneous, intradermal, intravenous, and gavage), intraperitoneal injection is the most commonly used in basic microbiological procedures.

10.8 Disposal

All injected mice, even those that survive the experimental inoculations, must be euthanized (Annex 15), if necessary, and autoclaved. Uninoculated mice that have died from apparently natural causes, as well as from unexplained causes, must be autoclaved. In addition to the mice themselves, their bedding and cages must be autoclaved. It is recommended that all materials be autoclaved for at least 30, and preferably 45 minutes, at 121°. Autoclaved animal carcasses must be incinerated.

11. DOCUMENTATION

There must be a systematic and documented record of all the information that has any practical relevance to the analysis performed. Documentation may be needed to reconstruct an analytical situation long after the actual event has occurred. For example, in the case of a regulatory sample, an extensive period may lapse between the time analytical findings are reported and the time these findings may be discussed in a court of law.

The records should also be such that if the need for reanalysis arose, it could be done under the same conditions and in the same way as before. All observations and calculations should be clearly and permanently recorded preferably with ink at the time they are made. If mistakes do occur in recording data or making calculations or interpretations, the mistaken data should be crossed out but remain legible, and the correct value entered immediately above.

The records must be retained for a minimum time, often specified in the national legislation and they must be protected from misuse, loss or damage. In the case of computer-held records, this will include security codes.

11.1 Traceability

Documentation provides a mechanism to trace the location of a laboratory sample from its initial collection until its final disposition. A flow chart showing the relationship among the various documentation items or steps is shown in Annex 16.

Under most conditions the sample is collected by an inspector or investigator rather than by the laboratory analyst. The sample is sealed and sent to the laboratory for analysis. Accompanying the collected samples are the sample collection records. When the sample arrives at the laboratory, the sample custodian prepares the sample accountability record, which documents the identity and storage location of the sample, the arrival date and name of the individual responsible for its storage. The laboratory supervisor assigns one or more analysts to examine the sample. The sample accountability record also contains the dates and signatures of individuals (sample custodian and analysts) involved in custody exchange of the sample.

All analytical results from the sample examination are recorded on the appropriate analyst worksheets and a laboratory report containing a condensed version of the analytical results that appear on the worksheet is prepared. This report and the analyst worksheet are appropriately filed for potential retrieval. The dates and signatures of individuals who withdraw and return records in storage must be recorded.

After laboratory analysis, it may be necessary to store the sample for a lengthy period pending legal action. Storage location and conditions, names of individuals responsible for storage, and dates and signatures of individuals (sample custodian and analysts) involved in

custody exchange during this storage period are recorded on the sample accountability record.

The sequence of records (sample collection report, official sample seal, sample accountability record, and analyst worksheet) should form a continuity of documentation to provide a clear, accurate, and indisputable history of the sample with all segments of documentation in agreement. It should be possible to choose a sample at any point in its passage through the laboratory, find any documents relating to it and trace the history of the sample back to its arrival and any information that arrived with it.

11.2 Sample collection records

The sample collection record or report is usually completed by the inspector or investigator. However, the analyst must ensure that the information provided on the collection report accurately reflects the identity of the sample submitted for laboratory analysis and that the information on the analyst worksheet (Chapter 11.3) is compatible with that on the collection report.

One example of a collection report is shown in Annex 17. This form is used by the U.S. Food and Drug Administration to meet its specific needs. Because some items do not apply to other agencies or organizations, only those of general or common interest or applicability are discussed here.

a. Item 1, Flag. Any special instructions related to the condition of the lot or special handling of the sample are given.

b. Item 2, Type Sample. Several different types of samples may be recognized. Official samples are those which, if violative, serve as a basis for specific legal actions. Investigation samples are collected to document observations or support regulatory or other findings and include factory samples (raw materials and finished products to demonstrate manufacturing conditions); survey samples (to provide information about industry practices regarding a particular issue); and complaint samples (injury and poisoning investigation samples). Food standards samples provide information on which to base microbiological food standards.

c. Item 3, Sample Number. Each sample is identified by a unique, nonrepeatable number.

d. Item 7, Date of Collection. The date of collection of the sample is given. If the sample collection took more than 1 day, then the inclusive dates (e.g., April 1-3, 1989) should be recorded.

e. Item 12, Related Samples. The numbers of other samples from the same shipment or of others that may be related to the shipment are indicated.

f. Item 17, Product Name and Identification. The specific product name is given.

For identification, the type of container (bulk, paper bag, Whirl-Pac™ bag, plastic jar) is indicated as well as pertinent portions of the label (brand name, generic name, quantity of contents, name and address of manufacturer or distributor, and code).

When the product is packaged in a carton, shipping case, or similar container, the pertinent labeling from the container should be recorded.

When quoting from a label, the exact spelling, capitalization, punctuation, and arrangement found on the original label must be used.

g. Item 18, Reason for Collection. The complete reason for collecting the sample is furnished along with the suspected violation or analysis desired. Interdistrict, regional, or headquarters-initiated memos, letters, or other assignment documents are referenced in sufficient detail to facilitate location of any document.

h. Item 19, Manufacturing Codes. All codes, lot numbers, and batch control codes shown on labels, cartons, and shipping containers are given.

i. Item 20, Manufacturer. The name, street, address, city, state, and zip code of the manufacturer are entered.

j. Item 21, Shipper. The name, street address, city, state, and zip code of the shipper are indicated.

k. Item 22, Dealer. This space contains the name, street address, city, state, zip code, and complete telephone number of the dealer from whom the sample was obtained.

l. Item 23. Size of Lot from Which Sampled. The amount of goods on hand before sampling, as determined by the collector's inventory of the lot, is indicated. This entry includes the number of shipping cases and the size of the components, e.g., 250/50kg bags.

m. Item 27, Description of Sample and Method of Collection. The sample is described and the number and size of sampled units or subs are given to show how each was taken, e.g., "Three cans from each of 20 previously unopened cases selected at random."

n. Item 28, How Prepared. An explanation is given of how the sample was prepared before its submission to the laboratory, how the sample units or subs were identified, and how the sample was wrapped and sealed. The form in which the sample was delivered to the laboratory (e.g., paper bags, original carton) is also indicated.

o. Item 29, Collector's Identification on Package and/or Label. The sample collector quotes the identification placed on the packages, labels, e.g., "79-180-121 10-15-78 SHR."

p. Item 30, Collector's Identification on Seal. The sample collector quotes the identification used on the final official seal applied to the sample, e.g., "79-180-121 10-15-78 Sylvia H. Rogers."

q. Item 31, Sample Delivered To. The person to whom the sample was delivered is indicated. If the sample was delivered to the sample custodian under seal, a designation such as "In person to Sample Custodian John Doe" should be given. If the sample was delivered to an analyst, a designation such as "In person to Analyst John Doe" should be furnished. If the sample was shipped, the name of the carrier to whom the sample was delivered and the bill of lading number, if used, should be indicated.

r. Item 32, Date Delivered. The date that the sample was delivered to the laboratory or for shipment is indicated.

s. Item 33, Laboratory. The name of the laboratory to which the sample was sent is given.

t. Item 35, Original Collection Report and Records To. The name of the laboratory to which the original collection report and records are sent is given.

u. Item 36, Records Obtained. All documents and records obtained by the inspector or investigator are listed by type, number, and date, e.g., invoice number and date, shipping record, affidavits.

v. Item 37, Remarks. If the "Remarks" space is used for continuation of information from other blocks, the involved block number(s) should be listed.

w. Item 41, Collector. The collector should type or print his name and then sign the collection report. The only person who signs the report is the one who identified and sealed the sample, whose initials are on the subs and whose name is on the seal.

11.3 Analyst worksheets and reports

The analyst worksheet(s) provides a written account of the laboratory's analytical results. Although the analyst worksheet may appear in one of several forms, certain requirements apply to all types of worksheets:

a. All the basic information must be recorded directly on the worksheet before analysis is begun. As soon as the worksheet is obtained, it should be initialed. As many entries as possible should be filled in at the onset.

b. All entries should be clearly legible and made with permanent ink.

c. No entry should be erased or "overwritten." If an incorrect entry is made, the analyst should draw a line through the incorrect entry, write above it the correct figure or work, and then date and initial the corrected entry.

d. Data should not be discarded without explanation. If it becomes necessary to discard data, the analyst should cross out the entry, initial, date, and explain why the data have been discarded.

e. The exact analytical method used should be referenced clearly and completely. If the method has not been published, it should be written in full on the worksheet or as an attachment to the worksheet. Many methods do not provide details for sample preparation. If the method cited is not specific as to sample preparation, the analyst should describe completely how the sample was prepared.

f. If the analysis has been made in duplicate, triplicate, etc., the result of each analysis, as well as the summary of all results, must be recorded.

g. The proper number of significant figures should be used. A larger number of figures than warranted by the limitations of the method gives a false impression of the accuracy of the method.

h. If more than one analyst is involved in the analysis, the worksheet must clearly indicate which analyst broke the seal and which analyst performed each segment of the analysis.

i. Any continuation sheets that accompany the analyst worksheet should be numbered in a consecutive series, e.g., 1 of 8, 2 of 8, ...8 of 8 pages.

One example of an analyst worksheet is shown in Annex 18. Information on this particular worksheet is furnished as follows.

a. Item 1, Product. The analyst should be as consistent as possible with the collection report (Annex 17, Item 17) in the terminology for this entry. Whenever possible, the common name of the product (e.g., frosting mix, milk chocolate, or frozen frog legs) should be used. If there is no common name for the product, a descriptive term such as "brown powder in plastic bag" should be used.

b. Item 2, Sample Number. The analyst should carefully copy the sample number that appears in the collection report (Annex 17, Item 2).

c. Item 3, Sample Seals. If the sample contains a seal, the appropriate box should be checked to indicate whether the seal is intact or broken. If the sample has no seal, this should be indicated.

d. Item 4, Date Received. The date that the analyst received the sample for analysis is entered.

e. Item 5, Received From. The full name of the person who actually handed the sample to the analyst is given. If the analyst obtains the sample from storage for himself, the source, e.g., freezer, is indicated.

f. Item 6, District or Laboratory. The laboratory performing the analysis is specified.

g. Item 7, Description of Sample. A complete description of the sample should be given as consistently as possible with the collection report (Annex 17, Item 27). The number and types of package(s) (e.g., cardboard carton, paper bag) should be indicated as well as the number and type of individual container(s) in the package. If the individual container(s) have no net contents declaration, a description of the size and type (e.g., "paper bag containing 5 pounds of product") should be given.

The seal inscription and inspector's identification should be quoted verbatim, stating where seals, identification, and sub numbers are found.

If applicable, a positive statement should be made that a sample is hard-frozen when received or that it had thawed or had apparently thawed and had been refrozen, as the case may be. Any unusual odors should be recorded. If any portions of the sample are damaged, the condition and the sub numbers for the damaged units should be specified.

h. Item 8, Net Contents. This section is used to provide information on the product label declaration of net contents and to record results of a routine examination for net contents. The appropriate box is checked.

i. Item 9, Labeling. Enter in the blank spaces preceding "ORIGINAL(S) SUBMITTED" and "COPIES SUBMITTED" the number of each type of label submitted by the analyst and/or the inspector with his records. When the label is submitted by the inspector, record under Item 10, Labeling, that the label was submitted with the collection report. Check "NONE" in space provided when no labeling is submitted with sample or collection report.

j. Item 10, Summary of Analysis. The following information should be recorded:
CONTAINER- The size, type, colour, and closure(s) of the immediate commercial container holding the product and the retail container(s) enclosing

it are described. Any abnormalities or unusual conditions associated with the container (e.g., opened can, leakage, broken commercial seal) should also be described as should containers used by the inspector or investigator.

LABELING- This information indicates how and where the container is labeled (e.g., printed paper wrap around, front panel sticker). The analyst describes all labeling associated with the sample, including shipping cartons, inserts, and wrappers attached to sample units. All original labeling submitted by the analyst must include the sample number, date, and initials on the label itself and on the paper on which the label is mounted.

CODE- Code(s) must be quoted if present, giving type and location. If code is not present on the sample submitted but is given on the collection report, code must be quoted from the collection report (Annex 17, Item 19).

PRODUCT- A complete and accurate description of the product is given, including colour, odor, and general appearance, if applicable. Under no circumstances should a sample for microbiological analysis be tasted. An objective description in layman's language should be given. Common names of easily recognizable products (e.g., frozen frog legs, canned green peas, or fresh mushrooms) and any abnormalities (e.g., putrid odor, discoloration, or swollen cans) should be used.

ANALYSIS- The results of the microbiological analyses should be summarized and the analyst should furnish a reference to the method used or details of the method if it has not been published. An accurate description of how the analytical sample was selected from the total sample and any preparative steps performed before a portion was taken for analysis (e.g., mixed, composited, ground) should be provided. The types of controls used and the results of these controls should be indicated.

k. Item 11, Reserve Sample. A clear description of the reserve sample should be provided, and the seal that the analyst puts on the reserve should be quoted. The number of subs and the quantity in each sub should be specified. If the reserve sample is not returned to the sample custodian, the analyst must state how and where the reserve is stored.

l. Item 12, Analyst(s) Signature(s). The analyst signs his full name. When more than one analyst was involved, the analyst who broke the seal signs in space 12a and checks the box "BROKE SEAL."

m. Item 13, Worksheet Check. Worksheets are checked for accuracy, completeness, and compatibility with other documents by the supervisor or his designated representative. The person who checks the worksheet is the individual who signs and dates this section. If an error is found, the

supervisor does not make the correcton. Instead, he brings the error to the attention of the analyst, who then corrects it.

n. Item 14, Date Reported. The date on which the worksheet was submitted to the supervisor by the analyst is indicated.

The total number of pages, the individual page numbers, and the number of attachments are indicated at the bottom of the worksheet.

The back of the worksheet is used as follows:

a. The sample number is indicated in the upper right hand corner before any entry is made there. This ensures the analyst that he is entering data on the correct worksheet (without having to refer to the front) when more than one sample at a time is being analyzed.

b. The exact method used is referenced. Any modifications to the referenced method are stated and the reasons for these modifications are given.

c. All calculations are clearly shown, with the proper number of significant figures used.

d. For all weighings, the gross, tare, and net weights are given.

e. An explanation is provided of how dilutions were made.

f. The use of controls and their results are specified.

g. Data and calculations from any attachments or continuation sheets are summarized on the back of the worksheet.

Several types of continuation sheets may be used for specific microbiological analyses. Some examples are as follows:

a. Summary of Bacteriological Results (Annex 19)
b. Bacteriological Record (Annex 20)
c. Salmonella Record (Annex 21)
d. Shigella Record (Annex 22)
e. Canned Food Continuation Sheet (Annex 23)
f. Botulism Continuation Sheet (Annex 24)
g. Shellfish Bacteriological Record (Annex 25)

Because of the diversity of these continuation sheets, it would not be practical to discuss each of them item-by-item. Instead, they are furnished to provide the reader with one approach for recording specific microbiological information. On the reverse of the

appropriate continuation sheets, the analyst should list all media, chemical compounds, and sera used, as well as their source (manufacturer) and lot number.

11.4 Other documents

Hard-cover bound notebooks with prenumbered pages are used to record analytical data and observations exclusive of those generated during an official sample analysis for which sample analysis data must be recorded on the analyst worksheet and not in notebooks.

As stated previously, all quality assurance data must be maintained in notebooks. Examples of these data include calibration of weights and equipment, maintenance and repair of equipment, preparation of media and reagent solutions, monitoring of the microbiological quality of the laboratory air, and incubator temperature monitoring. It is useful to reserve the first several pages of the notebook for a Table of Contents.

In addition to containing quality assurance data, notebooks may be used for research data. The general guidelines for recording data on analyst worksheets also apply to research data to be recorded in notebooks.

Proficiency testing of analysts was discussed in sector 4.3. Each analysis of a check sample should be recorded and a file maintained on the results. Analysts should be encouraged to review the files periodically to assess their performance. Supervisors should review the files periodically to determine if frequency of check sample analyses should be increased or if additional training is needed.

12. AUDITS AND QUALITY ASSURANCE REVIEWS

Audits and quality assurance reviews provide management with an assessment of the effectiveness of the quality assurance programme. Based on the results of these audits and reviews, management decides on such factors as future training needs, reallocation of personnel, budget adjustments, need for remedial action, and commendations. Over time, these audits and reviews should demonstrate a steady increase in compliance with the quality assurance programme, analyst proficiency, and quality of data. This should be the case even with a steady flow of new personnel into the system, if the quality assurance programme is valid and well managed.

12.1 Review of current work

The supervisor should review the analyst's work on a routine, or even daily, basis. The supervisor may use the worksheet, a main end product in the analytical laboratory, to monitor the quality of the laboratory work. Each analyst worksheet should provide a space for the supervisor's signature, signifying that he has checked the worksheet for accuracy and completeness. Before signing the worksheet, the supervisor should determine that the following requirements have been met.

a. Worksheet completely and clearly describes the laboratory sample and its condition when received by the analyst.

b. The information on the worksheet is comparable to that on the sample collection report. Both the sample collection report and the analyst worksheet may be used to completely document the continuity and integrity of the sample.

c. The use of appropriate reference control cultures, other controls, media, media lot numbers, and reagents is fully described on worksheet.

d. Mathematical calculations are accurate and easy to follow.

e. If more than one analyst participated in the analysis, each analyst has initialed the segment(s) for which he or she is responsible.

f. The location of the sample reserves or the final disposition of the laboratory sample is indicated on the worksheet.

In addition to reviewing the analyst's worksheet, the supervisor may request an oral review of the worksheet. During this review, the analyst, using his worksheet, is asked to discuss all the steps of the analysis. Essentially, the analyst is asked to verbally reconstruct the entire analytical procedure and explain the results.

Besides the worksheet review, the supervisor should frequently observe such quality control features as the maintenance and calibration of equipment, storage conditions for reference stock cultures, preparation of media, care and feeding of animals, etc. He should determine that these and all other quality control aspects are properly documented according to the provisions of the approved quality assurance programme.

12.2 Retrospective reviews

In addition to the daily reviews discussed previously, the operations of the laboratory should be monitored by a more detailed retrospective review. This review is conducted by the quality assurance unit, and its frequency is decided by management. Some laboratories are audited every 3 months, whereas others are reviewed annually.

As discussed in section 1.4, the nature and size of the quality assurance unit are determined by management. In large laboratories, the quality assurance unit may consist of two or more individuals who are employed exclusively to monitor the quality assurance programme. In smaller laboratories, one or more microbiologists may be responsible for monitoring the quality assurance programme on a part-time basis. In the latter case, the person should be as objective as possible and should not review his own work or work subject to his direct supervision. In any event, the auditor(s) should have enough scientific knowledge and experience to understand the nature of the scientific work being audited.

An audit by the quality assurance unit is usually announced beforehand. Management and first-line supervisors are notified of the exact date(s) of the audit to give the facility ample time to prepare and to minimize disruption of the laboratory's normal operations. However, under certain circumstances, it may be advantageous to conduct an unannounced audit. This type of audit would permit the auditor(s) to observe how the laboratory operates on a day-to-day basis, without the benefit of time to prepare for the audit. However, unannounced audits should be kept to a minimum.

The nature and duration of the audit visit may vary considerably. Some visits may be as brief as 15 minutes, whereas others may last all day. An audit checklist, for use by the analyst and the auditor, is furnished in Annex 26. The analyst may use this checklist to prepare for the audit, whereas the auditor would use it for the actual inspection. If the audit is to be brief and still meaningful, perhaps one or two sections of the checklist could be used for any particular audit. For example, one audit may cover only maintenance of equipment, whereas a subsequent visit may be concerned with documentation. If management decides to expend the required resources, the audit may be lengthy and comprehensive, covering all points on the checklist.

To alleviate apprehension and provide for more receptive responses, a staff member should accompany the quality assurance unit auditors during the review. The auditors should ask questions about anything that is not clear, giving the staff ample opportunity to clarify any misunderstandings.

After the audit visit, members of the quality assurance unit should meet with the laboratory director and staff member who accompanied them during the review. The auditors should discuss their findings and offer recommendations for improvement of specific problem areas.

Finally, the quality assurance unit should prepare a written report. It is important that the report be prepared in a timely manner, normally within 1 week of the review. Before submitting the report to management, the laboratory director and staff should be given the opportunity to review it so that any errors or misunderstandings may be corrected. The report itself should be written in language that is objective, impersonal, and nonprovocative.

It should identify the deficiencies, if any, according to room number. If these deficiencies are recurring or have not been corrected since the last review, they should be so identified. The relative significance of the various deficiencies should be listed in order of seriousness. The report should be constructive in making specific recommendations for correcting deficiencies, particularly those that are major.

12.3 Accreditation

There are many accreditation schemes around the world operated by government departments, professional bodies and other organisations, usually of national or international standing. A scheme, mandatory or voluntary, will operate for a particular country, region, industry or technical area. They require participant laboratories to achieve certain quality standards and the accrediting body will carry out assessments to ensure the standards are met. Assessments are carried out by professional assessors, who will usually be expert in the same area of work as the laboratory, and will have undertaken a course in laboratory assessment.

There are two critical elements for accrediting or certifying a laboratory: performance of the analyst in a proficiency testing program and the on-site visit by the certifying officer(s).

The proficiency testing programme is administered at least annually and all analysts must participate. The certifying organization sends "split" samples to all participants, who are required to perform certain analyses, e.g., aerobic plate count or total coliform MPN. All analyses are conducted under strictly controlled conditions: all analysts initiate analyses at the same time, a single uniform method is used, media are prepared according to specific directions, plates and tubes are counted or interpreted similarly, etc. After the analyses, the participating analysts promptly forward their results to the certifying laboratory or organization. The results are statistically analyzed and participants are notified of the results.

The second critical element for receiving accreditation is based on the results of the on-site visit by the certifying official(s). The format, style, and approach of this visit are similar to that of the quality assurance audit or review. Adherence to a sound quality assurance programme is an integral part of the on-site visit for accreditation. The certifying officials use a checklist to determine adherence to such key elements of the quality assurance programme as design of the laboratory, personnel training and qualifications, sample

accountability and integrity, maintenance and calibration of equipment, preparation of media, use of appropriate methods and, most importantly, documentation.

The International Organization for Standardization (1) and the Department of Trade and Industry of the United Kingdom (2) have established guidelines for auditing the analytical competence of testing laboratories. The latter organization promoted a quality assurance manual which clearly describes the elements of the programme and their implementation in the testing laboratory.

Laboratory accreditation programmes offer several advantages. First, they promote reliability of analytical results. Second, they result in a financial savings since the results of one laboratory can be accepted by another without the added expense and time involved in retesting. Third, accreditation increases the credibility, recognition, and status of approved laboratories. Fourth, they provide feedback on the adequacy of test methods to standards-producing groups. This, in turn, could result in an ultimate improvement in analytical methods.

12.4 Follow-up activities

After the quality assurance unit review, a follow-up review should determine if, and how, any deficiencies were corrected. This review enables members of the quality assurance unit to determine the extent to which their recommendations were followed. By its very nature, the follow-up review should be brief, usually not requiring more than a few minutes.

Ideally, not more than 2-4 weeks should intervene between the initial quality assurance review and the subsequent follow-up. It should be realized, however, that certain conditions beyond the control of the analyst, e.g., repair of malfunctioning ventilation system or presence of insect pests, may require a longer period for correction.

12.5 References

1. International Organization for Standardization. 1982. General requirements for the technical competence of testing laboratories, ISO/IEC Guide 25-1982(E). International Organization for Standardization, Switzerland.

2. Anonymous. 1984. Report of Task Force "D" at the International Laboratory Accreditation Conference, London, U.K. Department of Trade and Industry, London, U.K.

ANNEX I

An example of QA manual

QUALITY ASSURANCE MANUAL FOR THE FOOD ENFORCEMENT LABORATORY, COUNTRY OR REGION

This manual is issued under the authority of the Laboratory Director

.....

(signed)

EDITION NO. (OR ISSUE NO.):
DATE OF ISSUE:
COPY NO:
ISSUED TO:

TABLE OF CONTENTS

This details the content of the Manual by section and paragraph.

QUALITY POLICY STATEMENT

The policy statement must be meaningful, not just a platitude, but does not need to include specific statements. It should be direct and include identification of the person responsible for implementing that policy.

CHECK SAMPLING

The laboratory will benefit from taking part in external check sample programmes. The details of the activity should be given in the QA manual. Where check sample programmes are not available or too expensive and a number of laboratories are reasonably close geographically, it is worthwhile to initiate a group check sampling programme.

RESPONSIBILITY FOR THE MANUAL

The person(s) responsible for the compilation, distribution, amendment and maintenance of the Quality Control Manual should be named here. (Often termed the Quality Assurance Officer).

AMENDMENT PROCEDURE

There should be a system to record and track amendments which are made, from time to time, in the Manual. This will ensure that holders of the Manual have a current document. The system can be formalized with a tracking document such as Figure 1. Amendments can be inserted and old pages removed from each Manual by the QA Officer himself, or he can send the amendments to Manual holders and ask them to insert. The outdated sheets must be destroyed with a single copy kept for reference. Part of the auditing process will be to check that manual copies are complete and up-to-date.

LABORATORY ORGANIZATION

Prepare a statement of the Laboratory organization both internally and as part of a larger organization. Organograms may be included (see Figure 2). The statement should include responsibilities and any delegation of those responsibilities.

QUALITY ASSURANCE UNIT - TERMS OF REFERENCE

This will define the terms of reference responsibilities of the QA unit in relation to quality assurance. If a QA Officer exists, his terms of reference should be included.

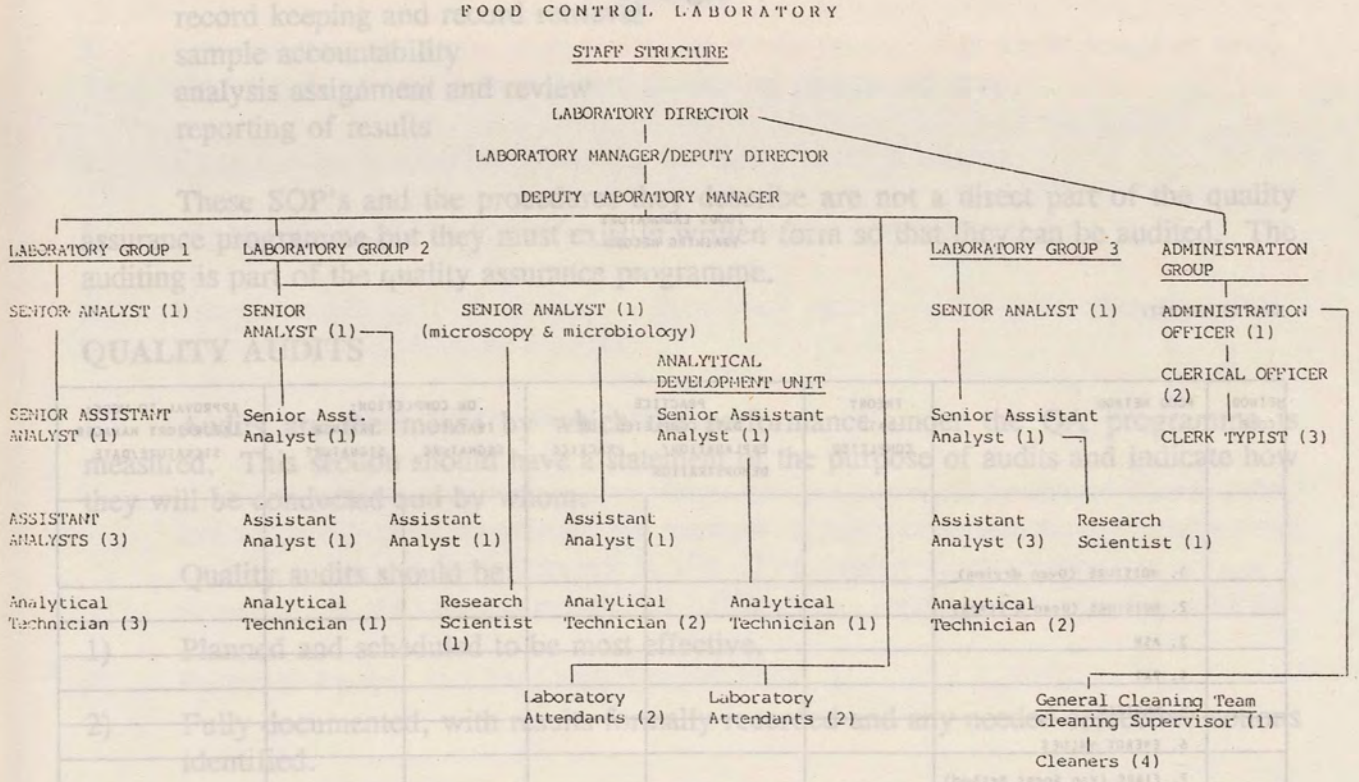
Figure 1

QUALITY ASSURANCE MANUAL FOR THE FOOD ENFORCEMENT LABORATORY	AMENDMENT SHEET SHEET: a of b ISSUED BY: Mr. Q.A. Person ISSUE DATE:
AMENDMENT SHEET	COPY NO: c of d

All amendments to the Quality Assurance Manual will be entered in the table below by the Quality Assurance Officer.

Amendment		Discard		Insert		Q.A. Officer's
Number	Date	Section	Sheet	Section	Sheet	Signature

Figure 2
Example Organogram



The Manual should describe the objectives of training and how these are achieved. Training in quality assurance must be included, at least for staff above a certain level. A training record sheet such as in Figure 3 is convenient and may be referred to in the Manual with a specimen copy as an Appendix. The Manual should also state how that training record is maintained and what is the general training policy.

(note that training is discussed further in Chapter 4)

Figure 3

FOODS LABORATORY
TRAINING RECORD

NAME OF ANALYST:

METHOD CODE	FOOD METHOD	THEORY DATE COMPLETED	PRACTICE DATE COMPLETED FOR EXPLANATION/ DEMONSTRATION	PRACTICE	ON COMPLETION: TRAINEE SIGNATURE	TRAINER SIGNATURE	APPROVAL TO WORK: LABORATORY MANAGER SIGNATURE/DATE
	1. MOISTURE (Oven drying)						
	2. MOISTURE (Dean & Stark)						
	3. ASH						
	4. FAT						
	5. PROTEIN						
	6. ENERGY VALUES						
	7. FIBRE (Van Soest Method)						
	8. SUGARS						
	9. STARCH						
	10. SODIUM						
	11. FATTY ACIDS						

STANDARD OPERATING PROCEDURES

All of the work activities in the laboratory may be described as operations. These operations can all be described and documented in Standard Operating Procedures (SOP's). Administrative procedures should be written up as SOP's. Under this section, the Manual can refer to a separate SOP master file or to a list of SOP's in an appendix. The latter may prove unsatisfactory if procedures are frequently amended or new ones introduced.

There should be SOP's for such administrative procedures, as:

record keeping and record retrieval
sample accountability
analysis assignment and review
reporting of results

These SOP's and the procedures they describe are not a direct part of the quality assurance programme but they must exist in written form so that they can be audited. The auditing is part of the quality assurance programme.

QUALITY AUDITS

Audits are the means by which the performance under the QA programme is measured. This section should have a statement of the purpose of audits and indicate how they will be conducted and by whom.

Quality audits should be:

1) Planned and scheduled to be most effective.

2) Fully documented, with results formally recorded and any needed corrective actions identified.

3) Carried out in accordance with a pre-planned programme and in a positive manner.

The person doing the audit should not be a member of the laboratory being audited. He may be a supervisor or analyst in another section. It is essential to avoid self-auditing where possible.

The Manual should include the Audit Programme which is decided upon by management.

AUDIT REVIEW

This is when line management and the quality assurance team get together and review audit reports and other activities relating to quality. The Manual should detail the areas reviewed and the review system to be used.

OTHER CONSIDERATIONS

This would include other parts of the QA programme not already covered, as well as documentation requirements. Much of this is discussed in Chapters 5 through 11.

ANNEX 2

Surfaces Monitoring - Swab Contact Method

1. Cut cellulose sponges into pieces approximately 5 x 5 cm and autoclave in individual paper bags.

2 Moisten sterilized sponge with approximately 10 ml of nutrient broth or 0.1% peptone water.

3. Using aseptic technique, hold moistened sterile sponge with sterile tongs or sterile gloves and vigorously swab 1 square meter of designated area.

4. Place sponge in a sterile plastic bag and add 100 ml of diluent.

5. Vigorously massage sponge for 1 minute to release microorganisms.

6. Transfer duplicate 1-ml aliquots to plate count agar using the pour plate procedure. Make further dilutions as required.

7. Incubate plates at 35° for 48 $\pm$ 2 hours.

8. Calculate number of microorganisms based on area swabbed, amount of diluent used, and volume of inoculum plated. For example, if 80 colonies are counted from a 1-ml inoculum obtained from a sponge in 100 ml of diluent which swabbed an area of 1 square meter, the count is reported as 8,000 colony forming units per square meter.

9. Record these data in a hard-bound record book.

ANNEX 3

Surfaces Monitoring - Replicate Organism Direct Agar Contact (RODAC) Method

1. RODAC plates may either be obtained commercially or prepared in the laboratory. To prepare in the laboratory, fill 15 x 100 mm petri plates with plate count agar so that meniscus of agar medium is above the rim of the plate.

2. Remove cover of the petri plate and press agar surface to the surface area to be sampled. A rolling pressure on the back of the plate is needed to be certain that the entire agar meniscus contacts the sampling area.

3. Replace the cover of the petri plate and incubate plates at 35° for 48 $\pm$ 2 hours.

4. Count colonies and report as number of colonies per sq cm of surface area.

ANNEX 4

Example Sample Accountability Record

A. Delivery from and return to sample custodian

1. STORAGE LOCATION		2. NAME OF PRODUCT	3. SAMPLE NO.
A ~~Freezer B~~ C		Frozen Raw Shrimp	89-79-880
B Freezer A D		4. NAME AND ADDRESS OF RESPONSIBLE FIRM: Johnson Fish Co. Tampa, Florida	☐ CRx/DEA SPL ☐ SPLIT SAMPLE

5. DATE SAMPLE RECEIVED	6A. BY WHOM RECEIVED	6B. DIST/DIV	7. DATE RECORDS REC'D.
1/6/89	Joseph P. Smith (Joseph P. Smith)	DEN-DO	1/6/89

8. METHOD OF SHIPMENT		
A. PERSONALLY FROM		C. SHIPPED FROM
B. VIA (Check one) ☐PP ☐BUS ☐FREIGHT ☒AIR		D. B/L NO. R-001,451-B

9. DESCRIPTION OF SHIPMENT				
A. SHIPPING CONTAINERS	NUMBER 1	TYPE Dry Ice Carton		CONDITION OK
B. SAMPLE PACKAGES	NUMBER 2	SIZE, TYPE, ETC. Cardboard Carton		CONDITION OK
C. SEAL INSCRIPTION	COPY IN FULL 89-79-880 1/5/89 Sidney C. Rogers			CONDITION Intact

10. SAMPLE DELIVERY				11. SAMPLE RETURNED			
DATE	AMOUNT	FROM	TO	DATE	AMOUNT	TO	FROM
1/10/89	2 cartons	JPS	BSW	1/10/89	1 carton	JPS	BSW

12. SAMPLE DISPOSITION	A. DATE SDN	B. DATE DESTROYED	C. DESTRUCTION METHOD	D. AMOUNT DESTROYED	E. BY WHOM	F. REASON

Continue on reverse; also record on reverse details for which space is lacking above

SAMPLE ACCOUNTABILITY RECORD

B. Personal delivery from inspector to analyst, return to sample custodian

1. STORAGE LOCATION		2. NAME OF PRODUCT	3. SAMPLE NO.
A ~~JAF~~ C		Fresh Spinach	89-01-505
B Freezer B D		4. NAME AND ADDRESS OF RESPONSIBLE FIRM: Southern Vegetable and Fruit Co. Waco, Texas	☐ CRx/DEA SPL ☐ SPLIT SAMPLE

5. DATE SAMPLE RECEIVED	6A. BY WHOM RECEIVED	6B. DIST/DIV	7. DATE RECORDS REC'D.
1/19/89	Janet A. Frances (Janet A. Frances)	CIN-DO	1/19/89

8. METHOD OF SHIPMENT		
A. PERSONALLY FROM	Dorothy R. Hughes	C. SHIPPED FROM
B. VIA (Check one) ☐PP ☐BUS ☐FREIGHT ☐AIR		D. B/L NO.

9. DESCRIPTION OF SHIPMENT				
A. SHIPPING CONTAINERS	NUMBER	TYPE None		CONDITION
B. SAMPLE PACKAGES	NUMBER 2	SIZE, TYPE, ETC. Brown paper bag		CONDITION ok
C. SEAL INSCRIPTION	COPY IN FULL 89-01-505 1/18/89 Dorothy R. Hughes			CONDITION Intact

10. SAMPLE DELIVERY				11. SAMPLE RETURNED			
DATE	AMOUNT	FROM	TO	DATE	AMOUNT	TO	FROM
				1/19/89	3 - 1 gt. carton	BCH	JAF

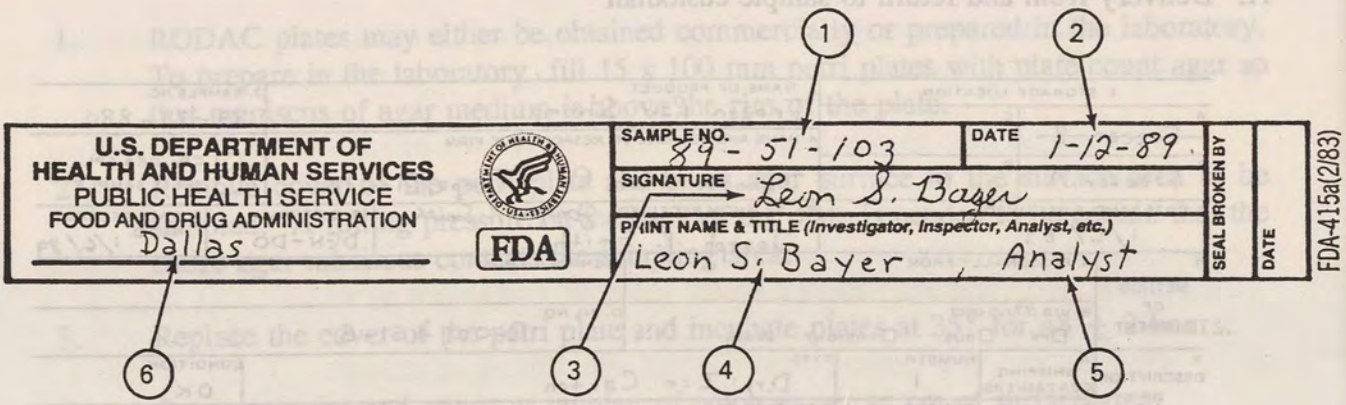
12. SAMPLE DISPOSITION	A. DATE SDN	B. DATE DESTROYED	C. DESTRUCTION METHOD	D. AMOUNT DESTROYED	E. BY WHOM	F. REASON

Continue on reverse; also record on reverse details for which space is lacking above

SAMPLE ACCOUNTABILITY RECORD

ANNEX 5

Example of a Sample Seal



(1) Insert Sample Number. When applicable, use prefix e.g., "INV.", "F.S.", "Doc.", "P.S.", etc.

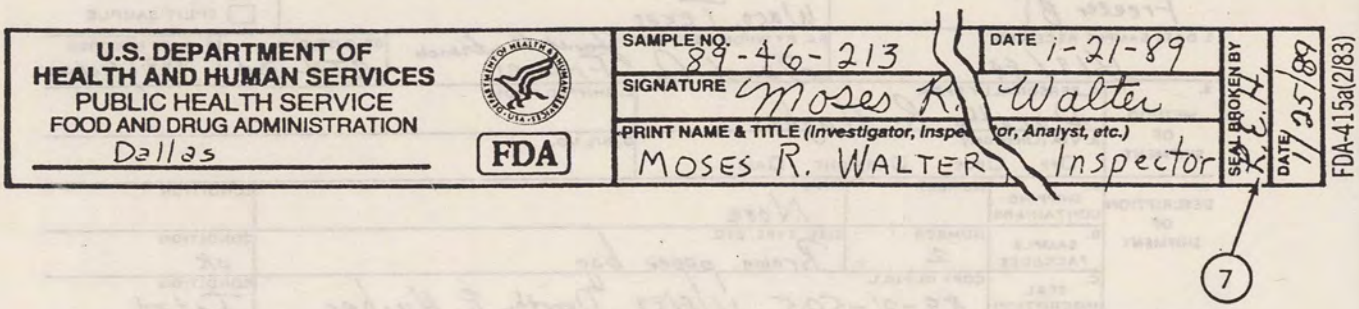
(2) Insert date sealed. Use figures, month, day, year, (see #7 below when seal is broken for any purpose).

(3) Sign your usual signature.

(4) Print your name same as signature. (A rubber name stamp may be used if desired but use it carefully and do not smear.)

(5) Print your title.

(6) Print your district — spell out — do not use district abbreviations or symbols. (A rubber stamp may be used).



(7) When seal is broken for any purpose, initial here and enter the date broken.

ANNEX 6

Food Sample Storage

Food	Storage[a]
Baked Goods	
Ready-to-eat bread, rolls, and buns	Freeze
Refrigerated or frozen unbaked breads, rolls, and buns	Freeze
Frozen sweet goods and cookies	Freeze
Ready-to-eat pies	Freeze
Refrigerated or frozen dough	Freeze
Crackers, biscuits	Freeze
Other bread and bread products	Freeze
Custard and cream-filled sweet goods	Freeze
Beverages and Beverage Materials	
Water	Refrigerate
Soft drinks	Refrigerate
Coffee, instant	Refrigerate
Coffee beans	Refrigerate
Tea	Refrigerate
Tea, instant	Refrigerate
Confectionery Products	
Chocolate and cocoa products	Room
Candy and confectionery products	Room

Food	Storage[a]
Chewing gum	Room
Syrups and molasses	Refrigerate
Honey	Refrigerate
Sugar, liquid	Freeze
Sugar, dry	Freeze
<u>Dairy Products</u>	
Butter	Refrigerate
Butter products (oil)	Refrigerate
Churning cream	Refrigerate
Cheese	Freeze
Cheese products	Freeze
Fluid whole milk	Freeze
Fluid milk products	Freeze
Concentrated liquid milk products	Freeze
Imitation dairy products	Freeze
Dried whole milk	Room
Nonfat dry milk	Room
Casein	Room
Ice cream	Freeze
Ice milk	Freeze
Frozen ices and sherbet	Freeze

Food	Storage[a]
Ice cream mix	Freeze
Ice milk mix	Freeze
Eggs and Egg Products	
Liquid and frozen eggs and egg products	Freeze
Dried eggs and egg products	Room
Shell eggs	Refrigerate
Fish, Shellfish, and Seafoods	
Frozen fish	Freeze
Fresh fish	Freeze
Canned fish	Refrigerate
Dried fish	Freeze
Other fish (paste, roe)	Freeze
Frozen shellfish	Freeze
Fresh shellfish	Freeze
Canned shellfish	Refrigerate
Dried shellfish	Freeze
Seafood products (crab cakes, cocktails)	Freeze
Frog legs	Freeze
Smoked fish	Freeze
Smoked shellfish	Freeze
Smoked crustaceans	Freeze

Food	Storage[a]
Flour and Flour Products	
Macaroni products	Room
Noodle products	Room
Pretzels, chips, and specialty foods	Room
Flour	Room
Corn meal	Room
Prepared mixes containing dried milk and dried eggs	Room
Fruits, Fruit Juices and Fruit Products	
Fresh fruits	Refrigerate
Frozen fruits	Freeze
Canned fruits	Refrigerate
Dried fruits	Refrigerate
Fruit juices	Refrigerate
Frozen fruit juices	Freeze
Jams, jellies, preserves, and butters	Refrigerate
Fig paste	Refrigerate
Olives	Refrigerate
Grain and Cereal Products	
Breakfast cereals	Room
Whole grains and beans	Refrigerate

Food	Storage[a]
Rice	Room
Oatmeal	Room
Infant Foods	
Infant cereals	Refrigerate
Milk bases, dehydrated infant food	Refrigerate
Milk bases, liquid	Refrigerate
Canned infant food	Refrigerate
Meats and Poultry	
Meat and meat products	Freeze
Poultry and poultry products	Freeze
Miscellaneous By-Products	
Oilseed by-products (cottonseed meal)	Refrigerate
Slaughtered animal by-products (bone meal)	Refrigerate
Fish and marine by-products (fish meal)	Refrigerate
Poultry and fowl by-products	Refrigerate
Fruit and vegetable by-products	Refrigerate
Dairy by-products	Refrigerate
Cereal by-products	Refrigerate

Food	Storage[a]
Nuts and Nut Products	
Nuts	Refrigerate
Nut products	Refrigerate
Pet Foods and Animal Feeds	
Animal feeds, dry	Refrigerate
Animal feeds, moist	Freeze
Animal feeds, canned	Refrigerate
Pet foods, dry	Refrigerate
Pet foods, moist	Freeze
Pet foods, canned	Refrigerate
Processed and Prepared Foods	
Desert mixes, dry	Room
Pudding mixes, dry	Room
Frozen dinners	Freeze
Canned dinners	Refrigerate
Prepared salads	Freeze
Canned soups	Refrigerate
Dehydrated dinners	Refrigerate
Gelatin (dry)	Room
Yeast (dry)	Room

Food	Storage[a]
Spices, Flavorings, and Condiments	
Whole spices	Room
Ground spices	Room
Mixed spices	Room
Extracts and flavors	Refrigerate
Essential oils	Refrigerate
Raw materials for extracts	Refrigerate
Dressing for salad	Refrigerate
Dry salad dressing mix	Refrigerate
Other condiments	Refrigerate
Vegetables and Vegetable Products	
Fresh vegetables	Freeze
Frozen vegetables	Freeze
Canned vegetables	Refrigerate
Dried vegetables	Room
Pickles	Refrigerate
Vegetable oil	Refrigerate

[a]Storage temperatures should be as follows: room temperature (21-23°); refrigeration (4°); freezing (-20°).

ANNEX 7

Calibration of Partial Immersion Thermometer

1. Cover a pipet tray, measuring approximately 13 x 23 x 46 cm, with a flexible plastic sheet. Tape the sheet to the tray to make it as nearly airtight as possible.

2. Make a resealable entry port to allow placement and removal of the reference thermometer calibrated by an appropriate standardizing organization.

3. Add 4 liters of distilled water to the tray.

4. Place the reference thermometer on supports in the tray so that the thermometer does not rest in contact with the tray.

5. Seal entry port to prevent evaporation of water.

6. Place bulb of thermometer to be calibrated as close as possible to bulb of reference thermometer.

7. Seal pipet tray and place in an incubator set at or very near the temperature at which thermometer will be used.

8. Let thermometers equilibrate for at least 3 hours.

9. Remove thermometer to be calibrated and read temperature with the aid of a magnifying glass. Estimate the reading to the nearest 0.1°.

10. Remove sealed tray containing the reference thermometer from the incubator.

11. As quickly as possible, open the port and read the reference thermometer, while it is still submerged, using a magnifying glass. Consult the correction certificate for the reference thermometer to determine the true temperature.

12. Determine the correction factor (the amount to be added to or subtracted from the reading of the thermometer being calibrated to obtain the correct or true temperature).

13. Write this correction factor and a number to singularly identify the thermometer on a piece of tape and attach it to the newly calibrated partial immersion thermometer. Record these calibration data in the hard-bound record book containing the temperature log.

ANNEX 8

Calibration of Microscope

1. Place an eyepiece micrometer grid, 1 mm grid, with lines 10 μm apart into one of the microscope oculars (10X magnification).

2. Place a stage micrometer grid, 2 mm grid, with lines 10 μm apart onto microscope stage.

3. Using lowest power microscope objective, focus ocular micrometer onto stage micrometer.

4. Align the two grids so that the far left lines of each are superimposed.

5. Count the number of stage micrometer lines that are covered by the 100 lines of the ocular micrometer.

6. If at 10X magnification, the 100 lines of the ocular micrometer cover 60 lines (600 μm) of the stage micrometer, the distance between each line of the ocular micrometer is 6 μm.

7. If at 40X magnification, the 100 lines of the ocular micrometer cover 15 lines (150 μm) of the stage micrometer, the distance between each line of the ocular micrometer is 1.5 μm.

8. If at 100X magnification, the 100 lines of the ocular micrometer cover 6.5 lines (65 μm) of the stage micrometer, the distance between each line of the ocular micrometer is 0.65 μm.

9. If a microbiological specimen is observed at 100X magnification to have a length spanning 4 ocular micrometer lines and a width covering 2.5 ocular micrometer lines, its length and width are 2.6 μm and 1.63 μm, respectively.

ANNEX 9

Determination of Bacterial Effectiveness of Ultraviolet Lamps

1. Dispense plate count agar (20 ml) into 15 x 100 mm petri plates.

2. Prepare a series of 10-fold dilutions of a culture of Enterobacter aerogenes so that 0.5 ml of inoculum will result in a count of 200-250 colonies per plate.

3. For each dilution, pipet a volume of 0.5 ml onto surfaces of four petri plates.

4. Use a sterile glass spreader rod to spread the inoculum evenly over entire surface of agar.

5. Repeat this procedure with other petri plates. Use separate glass spreader rod with each dilution of culture.

6. For each dilution, remove covers of four petri plates. Expose two of these plates to the ultraviolet lamp for 2 minutes at points where sterility is desired. In addition, expose two plates to ordinary lighting for 2 minutes.

7. Close plates and incubate at 35° for 24-48 hours.

8. Remove plates from incubator and count. Plates of appropriate dilution exposed to laboratory lighting should contain 200-250 colonies. Plates receiving this same dilution of inoculum exposed to ultraviolet light should show a 99% reduction in counts. If reduction is less than 80%, the lamp must be replaced. Record these data in hard- bound record books.

ANNEX 10

Determination of Bacteriostatic/Bactericidal Residue on Laboratory Glassware Surfaces

1. Wash six petri dishes according to the normal washing routine of the laboratory, and designate these dishes as Group A.

2. Wash six additional dishes as above, rinse 12 times with distilled water, and designate as Group B.

3. Wash another six dishes according to the laboratory's normal procedure, dry without further rinsing, and designate as Group C.

4. Sterilize petri dishes in Groups A, B, and C by the laboratory's normal procedure.

5. If testing of presterilized plastic petri dishes is desired, designate six sterile dishes as Group D.

6. To each petri dish add 1 ml of a pure culture dilution of Enterobacter aerogenes that will yield 50-150 colonies per plate.

7. To each plate, add 20 ml of plate count agar and mix thoroughly with inoculum.

8. After solidification, incubate plates at 35° for 48 $\pm$ 2 hours and then count.

9. Interpret counts as follows:

   a. Less than 15% difference in the average plate counts for plates of Groups A, B, C, and D indicates no detergent residual with bacteriostatic or bactericidal properties or that the presterilized plates are acceptable.

   b. A difference in colony counts of more than 15% between Groups A and B or D and B indicates the presence of an inhibitory detergent residue.

   c. A difference in counts of less than 15% between Groups A and B and more than 15% between Groups A and C indicates that the detergent has inhibitory properties that are removed during routine washing.

ANNEX 11

Determination of Suitability of Water for Preparing Microbiological Media and Reagents

Principle

This procedure is based on the growth of a 24-hour culture of Enterobacter aerogenes in a chemically defined, minimal growth medium. If the growth of this culture increases or decreases 20% or more than a control culture, it is concluded that a growth stimulatory or inhibitory agent, respectively, is present in the water.

Glassware

Only borosilicate glass that has been thoroughly rinsed with distilled water before dry heat sterilization should be used.

Reagents

Chemicals of the highest purity must be used. Reagents are prepared in water freshly distilled from a glass still. The following reagents are required:

(a) Sodium citrate solution. Dissolve 0.29 g sodium citrate, $Na_3C_6H_6O_7.2H_2O$, in 500 ml of water.

(b) Ammonium sulfate solution. Dissolve 0.60 g ammonium sulfate, $(NH_4)_2SO_4$, in 500 ml of water.

(c) Salts-mixture solution. Dissolve 0.26 g magnesium sulfate, $MgSO_4.7H_2O$, 0.17 g calcium chloride, $CaCl_2.2H_2O$, 0.23 g ferrous sulfate, $FeSO_4.7H_2O$, and 2.50 g sodium chloride, NaCl, in 500 ml of water.

(d) Stock phosphate buffer solution. Dissolve 34.0 g potassium dihydrogen phosphate, KH_2PO_4, in 500 ml of distilled water, adjust to pH 7.2 $\pm$ 0.5 with 1 N sodium hydroxide, NaOH, and dilute to 1 liter with distilled water.

(e) Phosphate buffer solution. Dilute stock phosphate buffer solution 1:25 in distilled water.

Boil stock solutions 1-2 minutes to kill vegetative bacterial cells; store in sterile glass-stoppered bottles in the dark at 5° up to 3 months. These stored solutions must be tested for sterility before use. The salts-mixture solution will become turbid 3-5 days after preparation as the ferrous salt is converted to the ferric state. If long-term storage is desired, prepare the salts-mixture solution without the ferrous sulfate. Just before use,

add the proper amount of ferrous sulfate to complete the formulation of the salts-mixture solution. Do not use any solution showing turbidity.

Samples

Collect approximately 200 ml of water of unknown quality and 200 ml of control water in borosilicate glass flasks and boil only for 1-2 minutes to prevent chemical changes in the water. Redistill control water to meet the following specifications: conductivity >0.5 megohms resistance or <2 μmhos/cm at 25°, pH 5.5-7.5, total organic carbon <1.0 mg/liter, single heavy metals (Cd, Cr, Cu, Ni, Pb, and Zn) <0.5 mg/liter, total heavy metals, ≤ 1.0 mg/liter, ammonia/organic nitrogen <0.1 mg/liter, total chlorine residual less than detection limit, and heterotrophic plate count $<1,000$ colonies/ml.

Preparation of flask components

Prepare five flasks containing components as shown in Table 1.

Preparation of E. aerogenes inoculum

On the day before initiation of suitability test, inoculate a strain of E. aerogenes onto a slant of nutrient agar having a slant of approximately 6.3 cm contained in a 16 x 125 mm screw- cap tube. Streak entire surface uniformly and incubate 18-24 hours at 35°. Pipet 1-2 ml sterile dilution water from a 99 ml water blank onto the culture and emulsify gently with a pipet. Pipet suspension back into the 99 ml water blank. Make a 1:100 dilution of the original bottle into a second water blank, a further 1:100 dilution of second bottle into a third water blank, and a 1:10 dilution of the third bottle into a fourth water blank. This procedure should result in a suspension containing 30-80 viable cells per ml. Pipet 1.0 ml of the fourth dilution ($1:10^5$) into each flask as indicated in Table 1.

Verify cell density by making a series of plate counts from the third dilution. Choose the proper volume from this third dilution, which, when diluted by the 30 ml in flasks A, B, C, D, and E, will contain 30-80 viable cells per ml.

Calculations

To determine the presence of growth-inhibiting substance, the following ratio is used:

$$\text{Ratio} = \frac{\text{colony count/ml, flask B}}{\text{colony count/ml, flask A}}$$

A ratio of 0.8-1.2 indicates there are no inhibitory substances present in the water. A ratio of less than 0.8 indicates the presence of growth-inhibiting substances, whereas a ratio above 1.2 indicates growth-promoting sources in the water.

To determine the presence of nitrogen and carbon sources that promote growth, the following ratio is used:

$$\text{Ratio} = \frac{\text{colony count/ml, flask C}}{\text{colony count/ml, flask A}}$$

To determine nitrogen sources that promote growth, the following ratio is used:

$$\text{Ratio} = \frac{\text{colony count/ml, flask D}}{\text{colony count/ml, flask A}}$$

To determine carbon sources that promote growth, the following ratio is used:

$$\text{Ratio} = \frac{\text{colony count/ml, flask E}}{\text{colony count/ml, flask A}}$$

Interpretation of results

When the ratio of colony counts from flask B/flask A exceeds 1.2, it may be assumed that growth-stimulatory substances are present. However, this is a very sensitive procedure and, in actual practice, ratios up to 3.0 have little significance. Thus, for ratios between 1.2 and 3.0, tests C, D, and E (Table 1) are not done.

When the ratio of colony counts from flask B/flask A is below 0.8, it may be assumed that there are toxic substances in the water. This test includes all allowable tolerances, and corrective action must be taken at once when this ratio is less than 0.8. This corrective action would involve an inspection of the distillation equipment and a careful review of the production and handling of the distilled water.

Table 1. Flask components for water suitability test*

Component	Control test (ml)		Optional tests (ml)		
	Control	Unknown distilled water	Food available	Nitrogen source	Carbon source
	A	B	C	D	E
Sodium citrate solution	2.5	2.5	-	2.5	-
Ammonium sulfate solution	2.5	2.5	-	-	2.5
Salts-mixture solution	2.5	2.5	2.5	2.5	2.5
Phosphate buffer (pH 7.3±0.1)	1.5	1.5	1.5	1.5	1.5
Unknown water sample	-	21.0	21.0	21.0	21.0
Redistilled water control	21.0	-	5.0	2.5	2.5
Total volume per flask	30.0	30.0	30.0	30.0	30.0

* From reference 3, Geldreich, E. E., and H. F. Clark. 1965. J. Milk Food Technol. 28:351-355. Reproduced with permission of the publisher.

ANNEX 12

Maintenance of Microbiological Stock Cultures

Bacillus cereus

A. Short term

1. Inoculate nutrient agar slant and incubate at 30-35° for 24 ± 2 hours.
2. Hold at room temperature (21-23°) for additional 1-2 days so that sporulation is complete.
3. Refrigerate broth culture at 4°.
4. Subculture every 6 months.

B. Long term

1. Inoculate nutrient agar slant and incubate at 30-35° for 24 ± 2 hours.
2. Hold at room temperature (21-23°) for additional 1-2 days so that sporulation is complete.
3. Suspend growth from agar slant with distilled water and mix 1:1 with 20% glycerin salt solution. Prepare this solution by dissolving 4.2 g NaCl and bringing volume to 800 ml with distilled water. Add 12.4 g K_2HPO_4 (anhydrous), 4.0 g KH_2PO_4 (anhydrous), and 200 ml glycerin (reagent grade). Adjust pH to 7.2 and autoclave 15 minutes at 121°.
4. Freeze 3 ml volumes of 1:1 mixture immediately in solid CO_2 or in ultra-low freezer and store at -55 to -90°.
5. Subculture every 2 years.

Campylobacter jejuni

A modified atmosphere of 85% N_2, 10% CO_2, and 5% O_2 must be used for all incubations.

A. Short term

1. Inoculate tube containing 10 ml casamino acids-yeast extract-salts broth. Prepare this broth by dissolving the following ingredients in 1 liter of distilled water: 20 g casamino acids, 6 g yeast extract, 2.5 g NaCl, and 8.71 g K_2HPO_4 (anhydrous). Adjust pH so that value after autoclaving is 8.5 ± 0.2. Autoclave 15 minutes at 121°.
2. Incubate inoculated medium at 41-44° for 48 ± 2 hours.
3. Streak broth culture to either chocolate agar or blood agar base slants, and incubate at 41-44° for 48 ± 2 hours.
4. Make subcultures weekly.

B. Long term

1. Inoculate tube containing 10 ml casamino acids-yeast extract-salts broth and incubate at 41-44° for 48 ± 2 hours.
2. To culture add glycerol to a final concentration of 10-20%.
3. Freeze 2-3 ml portions at -70° or below.
4. Subculture every 6 months.

Clostridium botulinum

A. Short term

1. Inoculate deaerated cooked meat medium or Trypticase-peptone-glucose-yeast extract broth with trypsin (TPGYT) and incubate at 26 or 35°, depending on type of strain cultured. Cooked meat medium, incubated at 35°, is preferable for proteolytic strains of types A, B, F, and G. TPGYT, incubated at 26°, is preferable for nonproteolytic types B, E, and F.

   For preparation of cooked meat medium, add 12.5 g of commercially available medium to 100 ml of cold distilled water. Mix and let stand 15 minutes to wet particles thoroughly. Alternatively, distribute 1.25 g of commercially available medium into 20 x 150 mm tubes, add 10 ml of cold distilled water, and mix thoroughly to wet all particles. Autoclave 20 minutes at 121°. Final pH should be 7.2 ± 0.2.

   TPGYT is not commercially available and must be made from individual ingredients. For preparation of base,dissolve the following ingredients in 1 liter of distilled water: 50 g Trypticase, 5 g peptone, 20 g yeast extract, 4 g glucose, and 1 g sodium thioglycollate. Dispense 15 ml portions into 20 x 150 mm tubes and autoclave 10 minutes at 121°. Final pH should be 7.0 ± 0.2. Refrigerate sterilized media and add trypsin immediately before use.

   For preparation of trypsin solution, add 1.5 g trypsin (1:250, Difco) to 100 ml of distilled water. Stir trypsin in water to suspend. Allow particles to settle and filter-sterilize supernatant through 0.45 μm membrane.

   Before use, steam or boil base for 10 minutes to expel dissolved oxygen. Add 1 ml trypsin solution to each 15 ml of base.

2. Incubate inoculated media for 5 days or longer at respective temperatures indicated.
3. Store at 4° up to 6 months.

B. Long term

1. Culture strain in broth as indicated above.
2. Centrifuge a portion of TPGYT culture in sterile centrifuge tubes to sediment spores.
3. Wash spore suspension twice with 10-20 ml volumes of sterile distilled water.
4. Resuspend washed spores in sterile distilled water and store at 4°.
5. Spore suspension should be stable for 10-20 years.

<u>Clostridium perfringens</u>

A. Short term

1. Inoculate tube containing 10 ml of freshly steamed (deaerated) fluid thioglycollate broth and incubate aerobically at 35-37° for 18-20 hours.
2. Subculture to deaerated cooked meat medium and incubate aerobically at 35-37° for 24 $\pm$ 2 hours.
3. Hold at room temperature (21-23°) for additional 1-2 days so that sporulation is complete.
4. Refrigerate broth culture at 4°.
5. Subculture every 30 days.

B. Long term

1. Inoculate tube containing Trypticase-peptone-glucose-yeast extract broth (buffered). Prepare this medium by dissolving the following ingredients in 1 liter of distilled water: 50.0 g Trypticase, 5.0 g peptone, 20.0 g yeast extract, 4.0 g glucose, 5.0 g Na_2HPO_4, and 1.0 g sodium thioglycollate. Adjust medium to pH 7.3 $\pm$ 0.2; dispense 15 ml portions into 20 x 150 mm tubes, and autoclave for 8 minutes at 121°. Refrigerate autoclaved medium until used.
2. Incubate inoculated medium for 18 hours at 35-37°.
3. Add 1 part broth culture to 1 part sterile skim milk and lyophilize 1-2 ml portions. Alternatively, add 1 part broth culture to 1 part 10% glycerin salt solution. Prepare solution by dissolving 4.2 g NaCl and bringing volume to 900 ml with distilled water. Add 12.2 g K_2HPO_4 (anhydrous), 4.0 g KH_2PO_4 (anhydrous), and 100 ml glycerin (reagent grade). Adjust pH to 7.2 $\pm$ 0.2 and autoclave 15 minutes at 121°.
4. Freeze 1-3 ml volumes of 1:1 mixture of culture and glycerin salt solution using solid CO_2 and ultra-low temperature freezer. Store at -55 to -90°.
5. Subculture every 2 years.

Escherichia coli

A. Short term

1. Inoculate brain heart infusion agar slant and incubate at 35° for 2 hours.
2. Refrigerate slant culture at 4°.
3. Subculture monthly.

B. Long term

1. Inoculate brain heart infusion agar slant and incubate at 35° for 24 ± 2 hours.
2. Subculture slant growth to tube containing 10 ml brain heart infusion broth.
3. Incubate at 35° for 24 ± 2 hours.
4. Centrifuge cell suspension and wash cells twice with 10 ml volumes of Butterfield's phosphate buffer.
5. Suspend washed cells in 10 ml Butterfield's phosphate buffer.
6. To 1 part washed cell suspension add 1 part double strength reconstituted nonfat dry milk, which is prepared by dissolving 200 g nonfat dry milk powder in 1 liter of distilled water and autoclaving for 15 minutes at 121°.
7. Shell-freeze and lyophilize 1- to 2-ml portions of this 1:1 mixture.
8. Culture is stable for 4-5 years at room temperature (21-23°).

Listeria monocytogenes

A. Short term

1. Inoculate tube containing 10 ml Trypticase soy broth with 0.6% yeast extract or 10 ml tryptose phosphate broth.
2. Incubate at 30-35° for 48 ± 2 hours.
3. Subculture broth to a slant of Trypticase soy agar with 0.6% yeast extract or a slant of nutrient agar.
4. Incubate at 30-35° for 48 ± 2 hours.
5. Refrigerate slant culture at 4°.
6. Subculture monthly.

B. Long term

1. Inoculate tube containing 10 ml Trypticase soy broth with 0.6% yeast extract or 10 ml tryptose phosphate broth.
2. Incubate at 30-35° for 48 ± 2 hours.
3. To broth culture add glycerol to final concentration of 10%.
4. Freeze 2- to 3-ml volumes at -70° or below.
5. Subculture every 6 months.

Salmonella

A. Short term

1. Inoculate brain heart infusion agar slant and incubate at 35° for 24 ± 2 hours.
2. Maintain culture at room temperature (21-23°) in the dark.
3. Subculture every 2 weeks.

B. Long term

Lyophilization is not recommended for long term storage of Salmonella cultures because flagellar and somatic antigens may be damaged.

1. Inoculate blood agar base slant to obtain growth over most of the surface and incubate at 35° for 24 ± 2 hours.
2. Collect growth from slant surface with sterile loop.
3. Use growth to inoculate tube containing semisolid Trypticase agar base (without phenol red). Prepare this medium by dissolving 20.0 g Trypticase and 3.5 g agar in 1 liter of water. Dispense 1- to 2-ml in 10 x 75 mm tubes with foil cover. Sterilize tubes at 116-118° (not over 12 pounds of pressure) for 15 minutes. When cool, aseptically seal the tubes with corks that have been in boiling paraffin for 5 minutes.
4. Incubate inoculated tubes at 35° for 24 ± 2 hours.
5. Store cultures in the dark at room temperature (21-23°).
6. Subculture every 3 years.

Shigella

A. Short term

Same as for E. coli.

B. Long term

Same as for E. coli.

Staphylococcus aureus

A. Short term

1. Inoculate tube containing 10 ml Trypticase soy broth and incubate at 35° for 24 ± 2 hours.
2. Streak broth culture to slant of Trypticase soy agar and incubate at 35° for 24 ± 2 hours.
3. Store at room temperature (21-23°).
4. Subculture weekly.

B. Long term

1. Inoculate tube containing 10 ml Trypticase soy broth and incubate at 35° for 24 ± 2 hours.
2. Add glycerol to final concentration of 20% and freeze 2- to 3-ml portions at -80°. Alternatively, add enough sterile mineral oil to submerge Trypticase soy agar slant culture.
3. For both types of long-term culture preservation, subculture annually.
4. Alternatively, add 2 ml of 6- to 12-hour Trypticase soy broth culture to 2 ml of sterile 80% glycerol in cryotube and freeze immediately at -70°. Subculture at 6-month intervals.

Vibrio cholerae

A. Short term

1. Inoculate tube of T_1N_1 medium by stabbing deeply into medium. Prepare this medium by dissolving the following ingredients per liter of distilled water: 10 g Trypticase, 10 g NaCl, and 20 g agar. Dispense 4 ml portions into 13 x 100 mm screw-cap tubes. Autoclave 15 minutes at 121°. No pH adjustment is necessary.
2. Loosen caps and incubate inoculated tubes at 35° for 24 ± 2 hours.
3. Tighten caps and store at 4°.
4. Subculture weekly.

B. Long term

1. Inoculate tube containing 10 ml T_1N_1 broth and incubate at 35° for 6-12 hours.
2. Add 2 ml of 6- to 12-hour T_1N_1 broth culture into 2 ml of sterile 80% glycerol in cryotube and freeze immediately at -70°.
3. Subculture at 6-month intervals.

Vibrio parahaemolyticus

A. Short term

1. Inoculate tube of preservation medium by stabbing semisolid medium deeply. Prepare this medium by dissolving the following ingredients per liter of distilled water: 3 g yeast extract, 10 g peptone, 30 g NaCl, and 3 g agar. Dispense 4 ml portions into 13 x 100 mm screw-cap tubes. Autoclave 15 minutes at 121°. No pH adjustment is necessary.
2. Loosen caps and incubate inoculated tubes at 35° for 24 ± 2 hours.
3. Tighten caps and store at room temperature (21-23°) only. Do not refrigerate.
4. Subculture weekly.

B. Long term storage

1. Inoculate tube containing 10 ml of Trypticase soy broth with 3% NaCl (final concentration) and incubate at 35° for 6-12 hours.
2. Mix 0.09 ml of sterile dimethyl sulfoxide with 1 ml of broth culture in sterile cryotubes and freeze immediately at -70°.
3. Subculture every 6 months.

Vibrio vulnificus

Same as for V. parahaemolyticus.

Yeasts and moulds

A. Short term

Before subculturing, examine cultures visually for presence of contamination by other yeasts and moulds. Use dissecting microscope to determine if mites are present.

1. Streak plate containing 30 ml of potato dextrose agar supplemented either with tartaric acid or antibiotics as described below. Antibiotics are preferred to tartaric acid because stock solutions are relatively easy to prepare and a low pH, inhibitory to some yeast and mould species, does not result.

   Use 10% solution of tartaric acid to adjust agar medium to pH 3.5 $\pm$ 0.1. Sterilize solution by filtering through 0.45 μm membrane. Titrate to determine amount of solution needed to adjust pH to 3.5. After adding solution to medium, verify pH by allowing a portion of the medium to solidify and checking with a pH meter.

   Chlortetracycline-HCl, at a concentration of 40 ppm, is the preferred antibiotic. Other antibiotics, e.g., chloramphenicol or streptomycin, may be used, but always at the same concentration as chlortetracycline-HCl and in addition to it. Prepare stock solution of antibiotic by dissolving 1 g of antibiotic in 100 ml of sterile distilled water and filtering through a 0.45 μm membrane. Store stock solutions in the dark at 4-8°. Shelf life does not exceed 1 month. Equilibrate stock solutions to room temperature (21-23°) immediately before use. If agar medium is in 250-ml aliquots, add 1 ml of 100-ml stock solution to obtain 40-ppm concentration. For other volumes of melted potato dextrose agar, add proportional volume of antibiotic solution.

2. Incubate agar plate at 22-25° until colonies of 3-5 cm have developed (about 1 week for moulds and 2 weeks for yeasts).
3. Pick well-isolated colony to slant of potato dextrose agar supplemented with either tartaric acid or antibiotics, as described above.
4. Incubate tubes at 22-25° for 1 (moulds) or 2 (yeasts) weeks.

5. Refrigerate cultures at 4°.
6. Subculture every 2 months.

B. Long term

1. Inoculate potato dextrose agar slant supplemented either with tartaric acid or antibiotics, as described above.
2. Incubate tubes at 22-25° for 1 (moulds) or 2 (yeasts) weeks.
3. Submerge agar slant culture with sterile mineral oil or glycerol.
4. Store at 4°.
5. Subculture every 6 months.
6. Alternatively, flood actively growing agar slant culture with sterile distilled water. Vortex slant to bring spores/cells into suspension.
7. Transfer 1 ml of suspension to test tube containing 5 g of sterile loam soil (autoclaved 1 hour at 121°).
8. Store at 4°.
9. Subculture annually.

<u>Yersinia enterocolitica</u>

Plasmids for pathogenicity may be lost if cultures are incubated above 30° or if they are subcultured too frequently.

A. Short term

1. Inoculate tube containing 10 ml of veal infusion broth or brain heart infusion broth and incubate at 26° for 48 $\pm$ 2 hours.
2. Streak broth culture to Trypticase soy agar slant and incubate at 26° for 48 $\pm$ 2 hours.
3. Store at room temperature in the dark.
4. Subculture monthly.

B. Long term

1. Inoculate tube containing 10 ml of veal infusion or brain heart infusion broth and incubate at 26° for 48 $\pm$ 2 hours.
2. Add glycerol to a final concentration of 10-20%.
3. Freeze 2- to 3-ml portions at -70° or below.
4. Subculture every 4-6 months.

ANNEX 13

Example Animal Health Certificate

ANIMAL HEALTH CERTIFICATE

SHIPPED:

DATE _____ VENDOR _____ PURCHASE ORDER NO. _____

RECEIVED:

DATE _____ TIME _____ ROOM NO. _____

BY WHOM _____

DESCRIPTION:

SPECIES _____ STRAIN _____ NUMBER _____

WEIGHT _____ S E X _____

CONDITION OF ANIMALS: _____

QUARANTINE:

DATE IN _____ DATE OUT _____ ROOM NO. _____

OBSERVATIONS _____

DIAGNOSIC TEST(S) PERFORMED: _____

DISPOSITION: _____

STUDY DIRECTOR: _____ PROJECT/NO. _____

_____
VETERINARY MEDICAL OFFICER

DATE _____

ANNEX 14

Procedure for Mouse Restraint and Intraperitoneal Injection

1. Fill syringe with appropriate volume of inoculum to be injected. Set aside.

2. Take hold of the mouse near the base of its tail. Do not hold mouse by its tail for a prolonged period as this will induce stress.

3. Remove the mouse from its cage and let it grasp a firm surface.

4. With one hand holding the base of the tail, use the other hand to grasp the nape of the neck.

5. With the same hand grasping the nape of the mouse's neck, place the mouse's tail between analyst's fingers to secure and control the animal.

6. Using one hand to fully restrain the animal, use the other hand to swab injection area with 70% ethanol.

7. Insert needle into lower left or lower right quadrant of abdomen at a 30° angle. Vital organs are not present in this area.

8. Gently aspirate the syringe to ensure proper placement. Any sign of blood or fluid indicates improper injection, and the needle must be withdrawn and repositioned.

9. Inject the inoculum in a steady, uninterrupted motion.

10. After injection, promptly place the syringe and needle in a puncture-resistant container, autoclave, and discard.

11. After inoculation, swab the injection site with 70% ethanol.

ANNEX 15

Procedures for Euthanizing Mice

A. Cervical dislocation

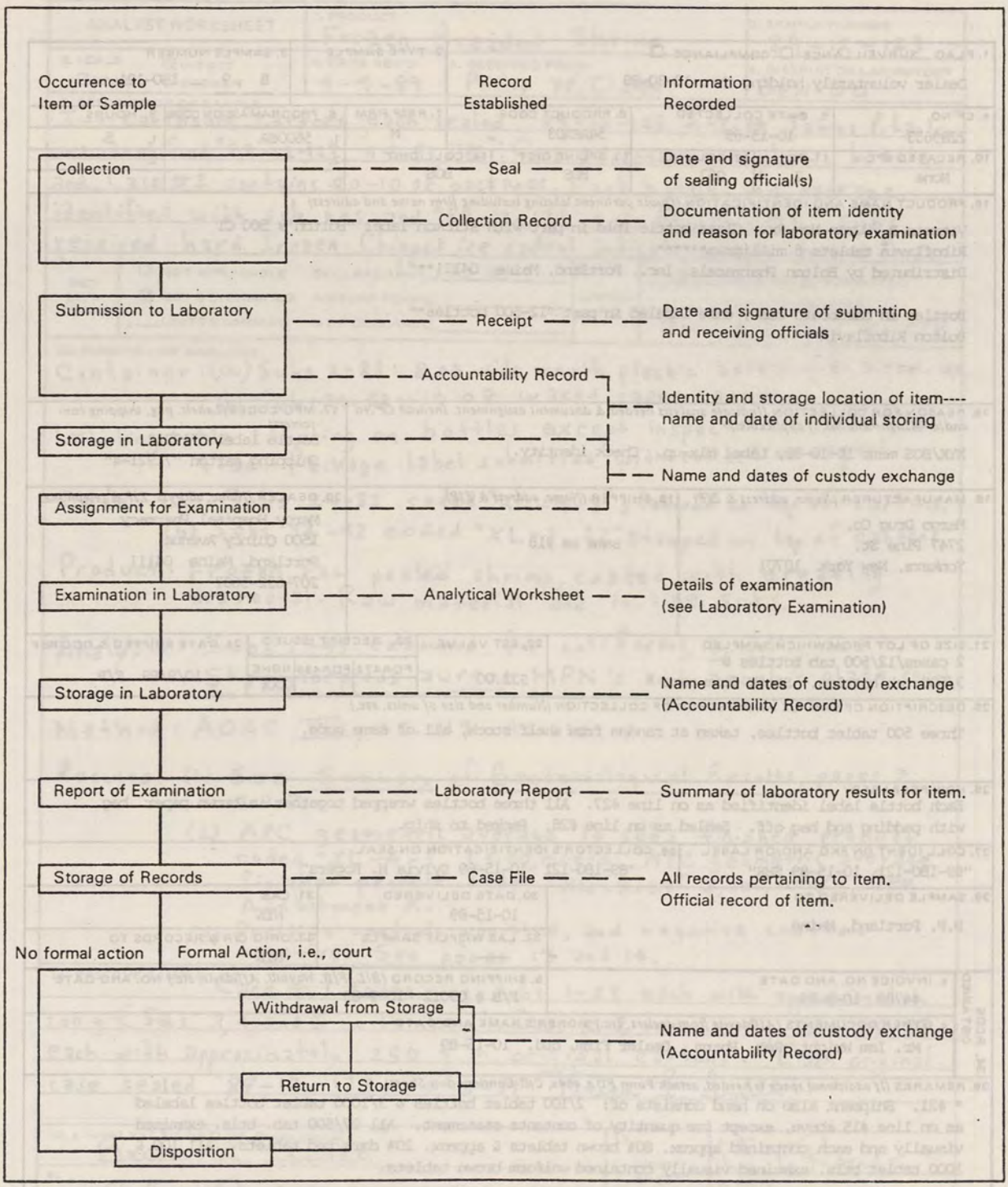
1. Take hold of the mouse near the base of its tail.
2. Remove the mouse from its cage and let it grasp a firm surface.
3. Pull the tail gently, but firmly, until the mouse stretches itself.
4. Place a metal bar, e.g., scissors or forceps, firmly across the nape of the neck.
5. Pull tail upward sharply to dislocate neck and to achieve an immediate death.

B. Carbon dioxide gassing

1. A laboratory desiccator may be used as the gassing container. Place Dry Ice below the supporting platform within the desiccator. Dry Ice must not come in direct contact with the mice.
2. Pour enough warm water over the Dry Ice to generate carbon dioxide gas.
3. Replace desiccator lid to form air-tight enclosure.
4. Let carbon dioxide gas saturate interior of desiccator.
5. Partially remove lid to place mice in the carbon dioxide-saturated chamber and immediately secure lid.

ANNEX 16

Example Flow Chart of Sample Submitted for Laboratory Examination



From Optimizing Chemical Laboratory Performance Through the Application of Quality Assurance Principles, Frederick M. Garfield, Nancy Palmer, and George Schwartzman, Eds., Proceedings of a Symposium, Association of Official Analytical Chemists, 94th Annual Meeting, Oct. 22-23, 1980, Washington, DC. Reproduced with permission of the publisher.

ANNEX 17

Example Collection Report

1. FLAG SURVEILLANCE ☐ COMPLIANCE ☐ Dealer voluntarily holding thru 10-30-89					2. TYPE SAMPLE	3. SAMPLE NUMBER 8 9 — 180-121		
4. CF NO. 2289653	5. DATE COLLECTED 10-15-89		6. PRODUCT CODE 54AFA03		7. RESP FIRM M	8. PROGRAM ASSIGN CODE 1. 56008A 2. 3.	9. HOURS 1. 5 2. 3.	
10. RELATED SPL None	11. PC 2	12. COLL NO. 057	13. SPLING DIST BOS	14. COLL DIST BOS				

15. PRODUCT NAME AND IDENTIFICATION *(Quote pertinent labeling including firm name and address)*

Vitamin B_2 Human Non-Rx. Glass bottle lbld in part with stickon label "Bolton's 500 CT Riboflavin tablets 5 milligram******
Distributed by Bolton Pharmacals, Inc., Portland, Maine 04111**"

Bottles in cardboard case, case labeled in part "12-500 bottles**
Bolton Riboflavin**"

16. REASON FOR COLLECTION *(Indicate analysis needed & document assignment. Include CP No. and/or Assignment No. if applicable.)*	17. MFG CODES *(Labels, pkg, shipping containers)*
NYK/BOS memo 10-10-89, Label mix-up. Check identity.	Bottle label "71921" Shipping carton "71921-4"

18 MANUFACTURER *(Name, address & ZIP)*	19. SHIPPER *(Name, address & ZIP)*	20. DEALER *(Name, address, ZIP & telephone)*
Margo Drug Co. 2747 Pine St. Yonkers, New York 10701	same as #18	Mercy Hospital Pharmacy 1500 Quincy Avenue Portland, Maine 04111 207-632-5687

21. SIZE OF LOT FROM WHICH SAMPLED	22. EST VALUE	23. RECEIPT ISSUED			24. DATE SHIPPED & DOC REF
		FDA472	FDA484	NONE	
2 cases/12/500 tab bottles & 3/500 tab bottles. Stock from same *	$32.00			XXX	10/9/89 F/B

25. DESCRIPTION OF SAMPLE AND METHOD OF COLLECTION *(Number and size of units, etc.)*

Three 500 tablet bottles, taken at random from shelf stock, all of same code.

26. HOW PREPARED

Each bottle label identified as on line #27. All three bottles wrapped together in brown paper bag with padding and bag off. Sealed as on line #28. Packed to ship.

27. COLL IDENT ON PKG AND/OR LABEL	28. COLLECTOR'S IDENTIFICATION ON SEAL
"89-180-121 10-15-89 SHR"	"89-180-121 10-15-89 Sylvia H. Rogers"

29. SAMPLE DELIVERED TO	30. DATE DELIVERED	31. LAB
P.P. Portland, Maine	10-15-89	NYK
	32. LAB W/SPLIT SAMPLE ——	33. ORIG C/R & RECORDS TO NYK

34. RCDS OBTAINED

a. INVOICE NO. AND DATE
#4789 10-8-89

b. SHIPPING RECORD *(B/L, F/B, Waybill, Affidavit, etc.)* NO. AND DATE
F/B # 09012 10-9-89

c. OTHER DOCUMENTS *(Affidavits from dealers, etc.)* SIGNER'S NAME AND DATE
Mr. Iam Wright, Reg. Pharm., Dealer Firm, dtd. 10-15-89

35. REMARKS *(If additional space is needed, attach Form FDA 464a, C/R Continuation Sheet)*

* #21. Shipment also on hand consists of: 2/100 tablet bottles & 3/1000 tablet bottles labeled as on line #15 above, except for quantity of contents statement. All 27/500 tab. btls. examined visually and each contained approx. 80% brown tablets & approx. 20% dark red tablets. All 100 & 1000 tablet btls. examined visually contained uniform brown tablets.

36. CONSUMER COMPLAINT NO. OR RECALL NO.	37. AMT. $ 4.25	38. 704(d) SPL	39. COLLECTOR *(Typed name and signature)*
N/A	CRED. CD. C ☐ V ☐ B ☐	YES ☐ NO ☒	Sylvia H. Rogers Sylvia H. Rogers

COLLECTION REPORT

ANNEX 18

Example Analyst Worksheet

FLAG

ANALYST WORKSHEET	1. PRODUCT: Frozen Breaded Shrimp		2. SAMPLE NUMBER: 89-12-123
3. SEALS: ☒ INTACT ☐ BROKEN ☐ NONE	4. DATE REC'D: 4-4-89	5. RECEIVED FROM: Philip W. Owen	6. DISTRICT OR LABORATORY: DEN-DO

7. DESCRIPTION OF SAMPLE

Two cardboard cartons each sealed "89-12-123 4-3-89 James P. Landry" and identified "89-12-123 4-3-89 JPL". Case #1 contains 23 plastic bottles and Case #2 contains 20-10 oz packages. Each bottle and package identified with sub nos and "89-12-123 4-3-89 JPL". All subs received hard frozen. Chipped ice control indicates sample received frozen.

8. NET CONTENTS		9. LABELING	
☐ NOT APPLICABLE	DECLARE/UNIT: 10 oz	3	ORIGINAL(S) SUBMITTED
☒ NOT DETERMINED	AMOUNT FOUND:		COPIES SUBMITTED
UNITS EXAMINED	% OF DECLARED:	☐ NONE	Attached to C/R

10. SUMMARY OF ANALYSIS

Container: (a) Subs 1-23: 8 oz wide mouth plastic bottle with screw-cap.
(b) Subs 24-43: 10 oz waxed paper carton.

Labeling: No labeling on bottles except inspector's identification. Package label submitted with collection report.

Code: (a) Subs 24-33 coded "XL-4-3" stamped on top of cartons.
(b) Subs 34-43 coded "XL-3-27" stamped on top of cartons.

Product: Frozen, raw peeled shrimp, coated with breading material. Raw material and in-line subs.

Analysis: Subs 1-43 examined for coliforms, E. coli, and Staphylococcus aureus MPN's and aerobic plate count.

Method: AOAC XIV, 46.013-46.016.

Results: (1) See Summary of Bacteriological Results, pages 2 and 3.
(2) APC geometric average 10 subs finished product coded "XL-4-3" 1,600,000/g. APC geometric average finished product coded "XL-3-27" 2,100,000/g. See Attachment A.
(3) Positive controls positive, and negative controls negative. See pages 13 and 14.

11. RESERVE SAMPLE: Case #1 containing subs 1-23 each with approximately 200 g (Subs 3, 4, and 5 with 300 g). Case #2 containing subs 24-33 each with approximately 250 g in original cartons. Each original case sealed "89-12-123 4-5-89 Brian L. Smith".

12. a. ANALYST SIGNATURE (Broke Seal ☒)	13. WORK-SHEET CHECK	a. BY
Brian L. Smith		C. T. Rouse
b. Alice E. Williams		b. DATE 4-14-89
c.	14. DATE REPORTED	4-14-89

PAGE 1 OF 4 PAGES

ANNEX 19

Summary of Bacteriological Results

SUMMARY OF BACTERIOLOGICAL RESULTS — SEE BACTERIOLOGICAL DEFINITIONS — Page 2 of 14 Pages

1. PRODUCT: Frozen Breaded Shrimp
2. SAMPLE NUMBER: 89-12-123
3. MANUFACTURER: Le Blanc Shrimp Co., Grand Isle, La.
4. DATE COLLECTED: 4-3-89

SUBSAMPLE A	DESCRIPTION B	COLIFORMS MPN PER GRAM C	E. COLI MPN PER GRAM D	COAGULASE POSITIVE STAPHYLOCOCCI MPN PER GRAM E	AEROBIC PLATE COUNT ORGANISMS PER GRAM F
1	Breading from previously unopened bag	15	<3	<3	8,000
2	Dry batter from previously unopened bag	7.3	<3	<3	12,000
3	Frozen, peeled shrimp from unopened bag	7.3	<3	<3	45,000
4	Frozen, peeled shrimp from 2nd unopened bag	23	3.6	3.6	60,000
5	Frozen, peeled shrimp from 3rd unopened bag	15	<3	<3	33,000
	1st In-line sampling				
6	Shrimp after thawing in pan overnight (55°F)	460	<3	<3	450,000
7	Liquid batter freshly mixed from pot (56°F) 8:18 A.M.	15	3.6	3.6	5,000
8	Breader from machine 8:22 AM	11	<3	<3	560
9	Shrimp from trays at table 8:25 A.M.	1,100	<3	<3	550,000
10	Shrimp after 1st batter 8:30 A.M.	460	3.6	3.6	700,000
11	Shrimp after 1st breading 8:34 A.M.	210	<3	3.6	790,000
12	Shrimp after 2nd batter 8:39 A.M.	240	7.3	7.3	850,000
13	Shrimp after 2nd breading 8:43 A.M.	240	9.3	15	600,000
14	Shrimp from packing table 8:48 A.M.	460	9.1	11	740,000
	2nd In-line sampling				
15	Shrimp from thawing bin (iced) 50°F 1:35 P.M.	1,100	<3	<3	950,000
16	Liquid batter from pot, 66°F 1:40 P.M.	>1,100	75	240	2,300,000
17	Breader from machine 1:44 P.M.	460	23	11	250,000
18	Shrimp from trays at table 1:50 P.M.	>1,100	<3	3.6	790,000
19	Shrimp after 1st batter 1:54 P.M.	>1,100	28	150	1,800,000
20	Shrimp after 1st breader 1:59 P.M.	>1,100	43	210	2,400,000
21	Shrimp after 2nd batter 2:04 P.M.	>1,100	93	93	1,900,000
22	Shrimp after 2nd breader 2:08 P.M.	>1,100	150	240	1,500,000
23	Shrimp from packing line 2:13 P.M.	>1,100	75	93	1,800,000

1"<3" means "not found in 1/10 gm. portion"

SUMMARY OF BACTERIOLOGICAL RESULTS — SEE BACTERIOLOGICAL DEFINITIONS — Page 3 of 14 Pages

1. PRODUCT: Frozen Breaded Shrimp
2. SAMPLE NUMBER: 89-12-123
3. MANUFACTURER: Le Blanc Shrimp Co., Grand Isle, La.
4. DATE COLLECTED: 4-3-89

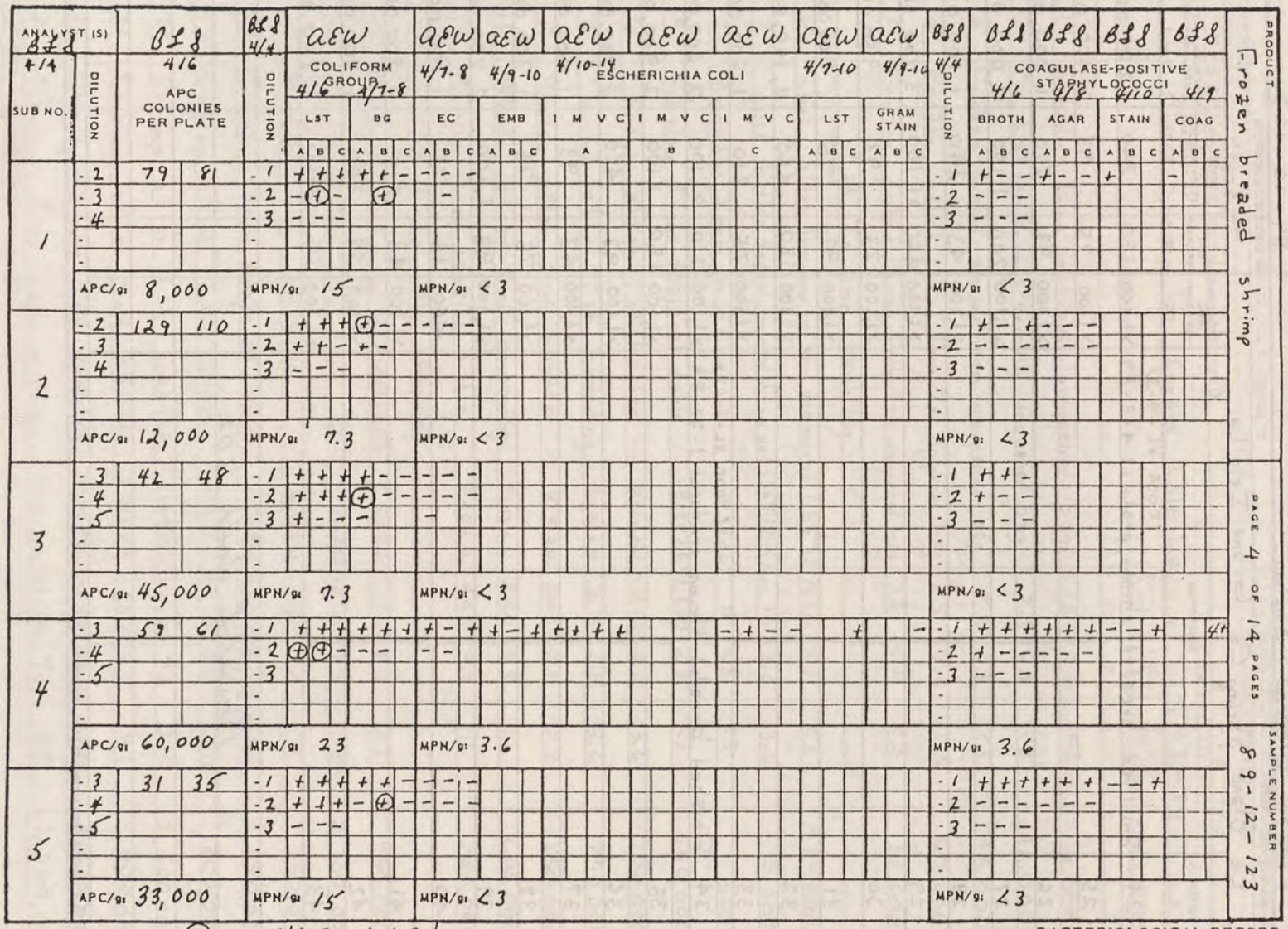
SUBSAMPLE A	DESCRIPTION B	COLIFORMS MPN PER GRAM C	E. COLI MPN PER GRAM D	COAGULASE POSITIVE STAPHYLOCOCCI MPN PER GRAM E	AEROBIC PLATE COUNT ORGANISMS PER GRAM F
24	(CODE XL-4-3) Finished breaded shrimp, produced 4-3-89	>1,100	150	240	1,200,000
25	↓	>1,100	75	93	1,400,000
26		>1,100	93	210	1,800,000
27		>1,100	240	1,100	2,600,000
28		>1,100	43	240	1,100,000
29		>1,100	460	>1,100	3,700,000
30		>1,100	23	93	950,000
31		>1,100	93	75	900,000
32		>1,100	150	460	2,100,000
33	↓	>1,100	75	210	1,600,000
34	(CODE XL-3-27) Finished breaded shrimp, produced 3-27-89	>1,100	210	>1,100	3,600,000
35	↓	>1,100	150	1,100	2,900,000
36		>1,100	93	460	2,700,000
37		>1,100	43	93	1,400,000
38		>1,100	75	75	1,300,000
39		>1,100	93	1,100	2,700,000
40		460	15	75	1,200,000
41		>1,100	43	210	2,800,000
42		>1,100	28	240	1,800,000
43	↓	>1,100	75	460	1,800,000

Brian L. Smith 102

1 "<3" means "not found in 1/10 gm. portion"

ANNEX 20

Bacteriological Record



ANALYST (S) BSS / BSS 4/4	BSS	BSS 4/4	aew	aew	aew	aew	aew	aew	aew	aew	BSS 4/4	BSS	BSS	BSS	BSS	PRODUCT: Frozen breaded shrimp
4/4	4/6		COLIFORM GROUP 4/6 4/7-8	4/7-8	4/9-10	4/10-14 ESCHERICHIA COLI			4/7-10	4/9-10		COAGULASE-POSITIVE STAPHYLOCOCCI 4/6 4/8 4/10 4/9				
SUB NO.	APC COLONIES PER PLATE		LST	BG	EC	EMB	I M V C	I M V C	I M V C	LST	GRAM STAIN		BROTH	AGAR	STAIN	COAG	
	DILUTION		A B C	A B C	A B C	A B C	A	B	C	A B C	A B C	DILUTION	A B C	A B C	A B C	A B C	PAGE 4 OF 14 PAGES
1	-2 79 81		- 1 + + + + + -	- - -	-							- 1 + - -	+ - -	+ - -	+	-	
	-3		- 2 - + -	+	-							- 2 - - -					
	-4		- 3 - - -									- 3 - - -					
	-		-									-					
	-		-									-					
	APC/g: 8,000		MPN/g: 15		MPN/g: < 3							MPN/g: < 3					
2	-2 129 110		- 1 + + + + - -		- - -							- 1 + - + - - -					
	-3		- 2 + + - + -									- 2 - - - - - -					
	-4		- 3 - - -									- 3 - - -					
	-		-									-					
	-		-									-					
	APC/g: 12,000		MPN/g: 7.3		MPN/g: < 3							MPN/g: < 3					
3	-3 42 48		- 1 + + + + - -		- - -							- 1 + + -					
	-4		- 2 + + + + - -		- - -							- 2 + - -					
	-5		- 3 + - - -		-							- 3 - - -					
	-		-									-					
	-		-									-					
	APC/g: 45,000		MPN/g: 7.3		MPN/g: < 3							MPN/g: < 3					
4	-3 59 61		- 1 + + + + + +		+ - + + - + + + +		- + - -			+	-	- 1 + + + + + - - +				4'	
	-4		- 2 + + - - -		- -							- 2 + - - - - -					
	-5		- 3									- 3 - - -					
	-		-									-					
	-		-									-					
	APC/g: 60,000		MPN/g: 23		MPN/g: 3.6							MPN/g: 3.6					SAMPLE NUMBER: 89-12-123
5	-3 31 35		- 1 + + + + + -		- - -							- 1 + + + + + - - +					
	-4		- 2 + + + - + -		- - -							- 2 - - - - - -					
	-5		- 3 - - -									- 3 - - -					
	-		-									-					
	-		-									-					
	APC/g: 33,000		MPN/g: 15		MPN/g: < 3							MPN/g: < 3					

(+) = positive at 48 hrs

BACTERIOLOGICAL RECORD

ANALYST (S)

SUB NO.	DILUTION	APC COLONIES PER PLATE	DILUTION	COLIFORM GROUP LST A	LST B	LST C	BG A	BG B	BG C	ESCHERICHIA COLI EC A	EC B	EC C	EMB A	EMB B	EMB C	I M V C A	I M V C B	I M V C C	LST A	LST B	LST C	GRAM STAIN A	GRAM STAIN B	GRAM STAIN C	DILUTION	COAGULASE-POSITIVE STAPHYLOCOCCI BROTH A	BROTH B	BROTH C	AGAR A	AGAR B	AGAR C	STAIN A	STAIN B	STAIN C	COAG A	COAG B	COAG C
Positive media control -	-	E. coli	-	+			+			+			+			+ + - -			+			-			-											
	-	E. aerogenes	-	+			+			+			+			- - + +			+			-			-											
	-	S. aureus	-																						-	+			+			+			++		
	-		-																						-												
	-		-																						-												
	APC/g:		MPN/g:							MPN/g:															MPN/g:												
Pipette	-	0	0	-																					-												
	-			-																					-												
	-			-																					-												
	-			-																					-												
	-			-																					-												
	APC/g:		MPN/g:							MPN/g:															MPN/g:												
Plate count agar and Petri dish	-	0	0	-																					-												
	-			-																					-												
	-			-																					-												
	-			-																					-												
	-			-																					-												
	APC/g:		MPN/g:							MPN/g:															MPN/g:												
Diluents (90 and 450 ml)	-90	0	0	-																					-												
	-450	0	0	-																					-												
	-			-																					-												
	-			-																					-												
	-			-																					-												
	APC/g:	90 ml - 0 450 ml - 0	MPN/g:							MPN/g:															MPN/g:												
Blender	-	0	0	-																					-												
	-			-																					-												
	-			-																					-												
	-			-																					-												
	-			-																					-												
	APC/g:	0	MPN/g:							MPN/g:															MPN/g:												

PRODUCT: Frozen breaded shrimp

PAGE 13 OF 14 PAGES

SAMPLE NUMBER: 89-12-123

BACTERIOLOGICAL RECORD

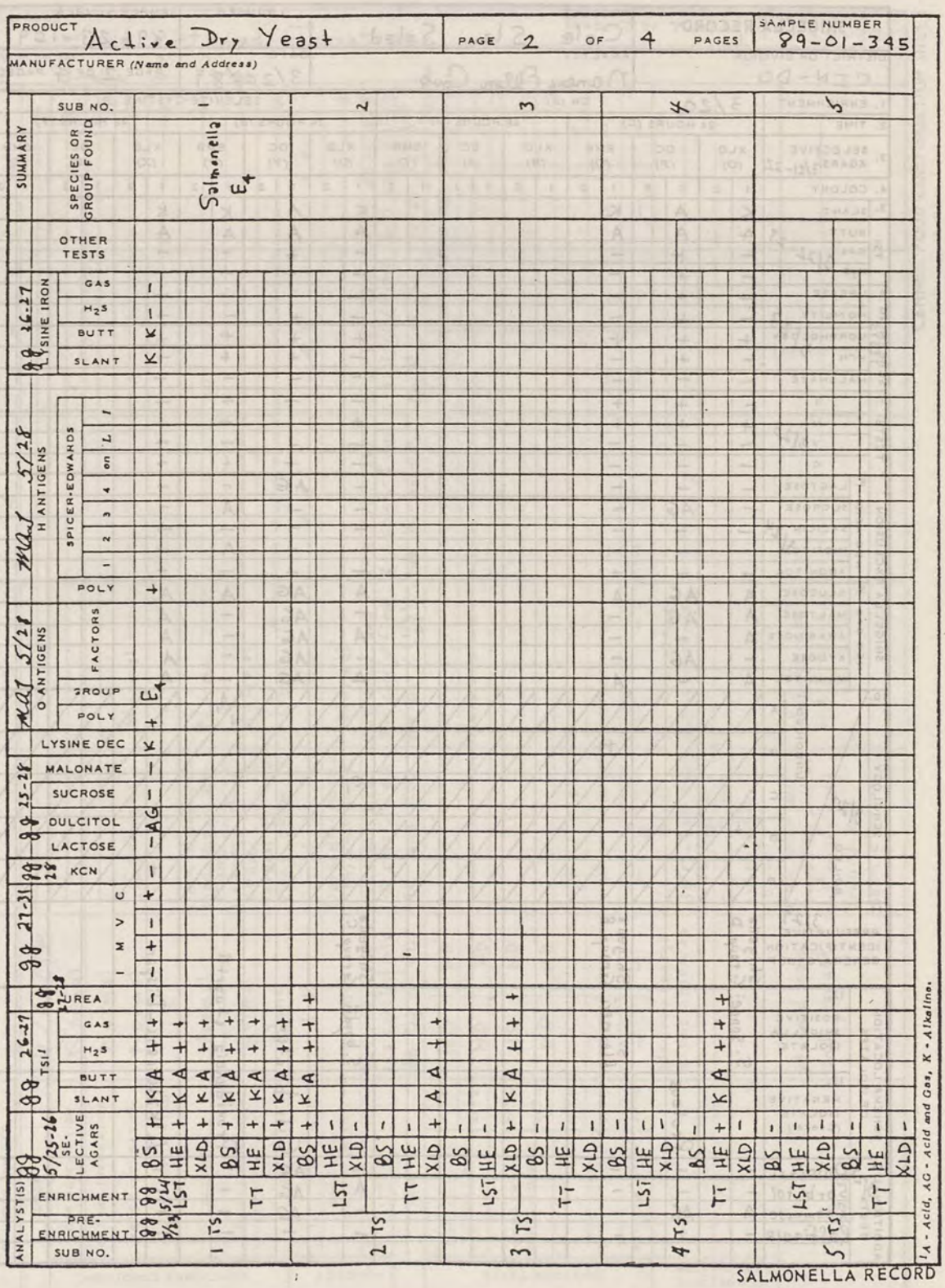
BACTERIOLOGICAL RECORD

PRODUCT	PAGE	SAMPLE NUMBER
Frozen breaded shrimp	PAGE 14 OF 14 PAGES	89-12-123

Group	Test	Tube/entry	Negative media control
COAGULASE-POSITIVE STAPHYLOCOCCI	COAG	A B C	
	STAIN	A B C	
	AGAR	A	1
	BROTH	A	1
DILUTION			MPN/g:
ESCHERICHIA COLI	GRAM STAIN	A B C	
	LST	A	1
	I M V C	C	
	I M V C	B	
	I M V C	A	1 1 1 1
	EMB	A	1
	EC	A	1
			MPN/g:
COLIFORM GROUP	BG	A	1
	LST	A	1
DILUTION			MPN/g:
APC COLONIES PER PLATE			0
			0
DILUTION			APC/g:
ANALYST (S)	SUB NO.		Negative media control

ANNEX 21

Salmonella Record



ANNEX 22

Shigella Record

SHIGELLA RECORD				PRODUCT: Cole Slaw Salad		I NUMBER		SAMPLE NUMBER: 89-23-169	
DISTRICT OR DIVISION: CIN-DO				ANALYST: Nancy Ellen Cook		DATE: 3/20/89		PAGE 2 OF 4 PAGES	
1. ENRICHMENT	3/20 GN (A)					SELENITE-CYSTINE (B)			
2. TIME	24 HOURS (C)			48 HOURS (D)		24 HOURS (E)		48 HOURS (F)	
3. SELECTIVE AGARS 3/21-22	XLD (O)	DC (P)	EMB (Q)	XLD (R)	DC (S)	EMB (T)	XLD (U)	DC (V)	EMB (W)	XLD (X)	DC (Y)	EMB (Z)
4. COLONY	1 2	1 2	1 2	1 2	1 2	1 2	1 2	1 2	1 2	1 2	1 2	1 2
5. TSI SLANT	K	A	K				K	A	K	K		
TSI BUTT	A	A	A				A	A	A	A		
TSI GAS 3/22-23	–	+	–				–	+	–	–		
TSI H_2S	–	+	–				–	+	–	–		
6. SCREENING TESTS UREASE	–	+	–				–	–	–	–		
SCREENING TESTS MOTILITY	–	+	–				–	+	–	–		
SCREENING TESTS MORPHOLOGY 3/23	+	+	+				+	+	+	+		
SCREENING TESTS KCN	–	+	–				–	+	+	–		
SCREENING TESTS MALONATE	–	–	–				–	–	–	–		
7. TESTS I	–	+	+				–	–	+	+		
TESTS M	+	+	+				+	+	+	+		
TESTS V 3/23	–	–	–				–	–	–	–		
TESTS C	–	–	–				–	+	+	–		
8. SHIGELLA EXCLUSION NEG ACID LACTOSE	–	–	–				–	AG	–	–		
NEG ACID SUCROSE	–	AG	–				–	–	A	–		
NEG ACID SALICIN	–	–	–				–	–	–	–		
NEG ACID INOSITOL 3/24	–	–	–				–	–	A	–		
NEG ACID ADONITOL	–	–	–				–	–	–	–		
9. NEG GAS GLUCOSE	A	AG	A				A	AG	A	A		
NEG GAS MALTOSE	A	AG	–				–	AG	–	A		
NEG GAS ARABINOSE	A	–	–				A	AG	–	A		
NEG GAS XYLOSE	–	AG	–				–	AG	–	A		
NEG GAS MANNITOL	A	–	A				A	AG	–	A		
10. SEROLOGY UNBOILED A									A_1 +			
SEROLOGY UNBOILED B			+									
SEROLOGY UNBOILED C							C_1 +					
SEROLOGY 3/27 BOILED D	+											
SEROLOGY BOILED AD										+		
11. 3/27 PRESUMPTIVE IDENTIFICATION GENERA/GROUP	Shigella Group D		Shigella Group B				Shigella Group C_1					
SCHEMA LOCATION OF ISOLATES 12. POSITIVE SHIGELLA ISOLATE	S. sonnei		S. flexneri				S. boydii	Citrobacter	Providencia stuartii	Alkalescens-Dispar		
SCHEMA LOCATION OF ISOLATES 13. NEGATIVE ISOLATE (Leaker)		Proteus										
14. ADDITIONAL TESTS 3/23 Dulcitol	–	–	–				–	AG	–	–		
ADDITIONAL TESTS Sorbitol	–	–	–				A	AG	–	–		
ADDITIONAL TESTS Rhamnose	A	AG	–				–	AG	–	–		
ADDITIONAL TESTS Raffinose	–	–	–				–	–	–	–		

Canned Food Continuation Sheet

PRODUCT: Canned Stewed Tomatoes | ANALYST(S): Leslie S. Paige | PAGE 3 OF 7 PAGES | SAMPLE NUMBER: 89-35-321

SUBSAMPLE NUMBER			1		2		3		4		5		6		
CODE			W1/60/TW		—		—		—		—		—		→
CONTAINER CONDITION	C/R DESCRIPTION		Normal		—		—		—		—		—		→
	BEFORE INCUBATION		Normal		—		—		—		—		—		→
	AFTER INCUBATION		Normal		Springer		Normal		—		—		—		→
	NO. DAYS INCUBATED		14		—		—		—		—		—		→
	INTERNAL		Normal		Dark discoloration around can seam		Normal		—		—		—		→
	SEAM EXAMINATION		Normal		Leakage along seam		Normal		—		—		—		→
	MICRO-LEAK		None		Several pin holes along seam		None		—		—		—		→
PRODUCT	pH		4.4		4.9		4.5		4.4		4.3		4.4		
	ODOR AND APPEARANCE		Normal		Dark discoloration, sour smell		Normal		—		—		—		→
	SMEAR		3-7 rods and 1-3 cocci/field		2-4 rods and 1-6 cocci/field		2-5 rods and 3-4 cocci/field		1-5 rods and 2-3 cocci/field		1-4 rods and 6-13 cocci/field		9-15 rods and 5-7 cocci/field		
GAS			No H_2 or CO_2		H_2 and CO_2		No H_2 / slight CO_2		No H_2 or CO_2		No H_2 or CO_2		No H_2 / slight CO_2		
CULTURE TESTS	COOKED LIVER (Cooked meat)	35°C	ND	ND	—	—	—	—	—	—	—	—	—	—	→
		55°C	ND	ND	—	—	—	—	—	—	—	—	—	—	→
	BROM-CRESOL PURPLE BROTH	35°C	ND	ND	—	—	—	—	—	—	—	—	—	—	→
		55°C	ND	ND	—	—	—	—	—	—	—	—	—	—	→
	ACID BROTH	30°C	—	—	+	+	—	—	—	—	—	—	—	—	
		55°C	—	—	+	+	—	—	—	—	—	—	—	—	
	MALT EXTRACT	30°C	—	—	—	—	—	—	—	—	—	—	—	—	
MICROSCOPIC EXAMINATION			No microorganisms observed		Acid broth, 30°C - both tubes with bacilli		No microorganisms observed		—		—		—		→
					55°C - both tube's with cocci										
SUMMARY			Normal can and product		Can with decomposing product and microleaks around can seam closure		Normal can and product		—		—		—		→

CANNED FOOD CONTINUATION SHEET

ANNEX 24

Botulinum Continuation Sheet

PRODUCT: Smoked Salmon | PAGE 2 OF 5 PAGES | SAMPLE NUMBER: 89-11-110

METHOD OF PREPARATION

200 gms fish + 200 ml sterile gel phosphate buffer + approx. 10 gms sterile sand ground in a sterile mortar with a sterile pestle. Mixture centrifuged and filtered through Millipore filter. Filtrate adjusted to pH 6.1. Half of the filtrate used for toxicity screening test; remainder stored at 0°C for confirmation test.

A sub = filtrate heated 10 min at 100°C (undiluted)
B sub = filtrate non-trypsinized
C sub = filtrate trypsinized. All injections 0.5 ml intraperitoneally.

ANALYST(S): Edward H. Lossinski | DATE: 5/8/89

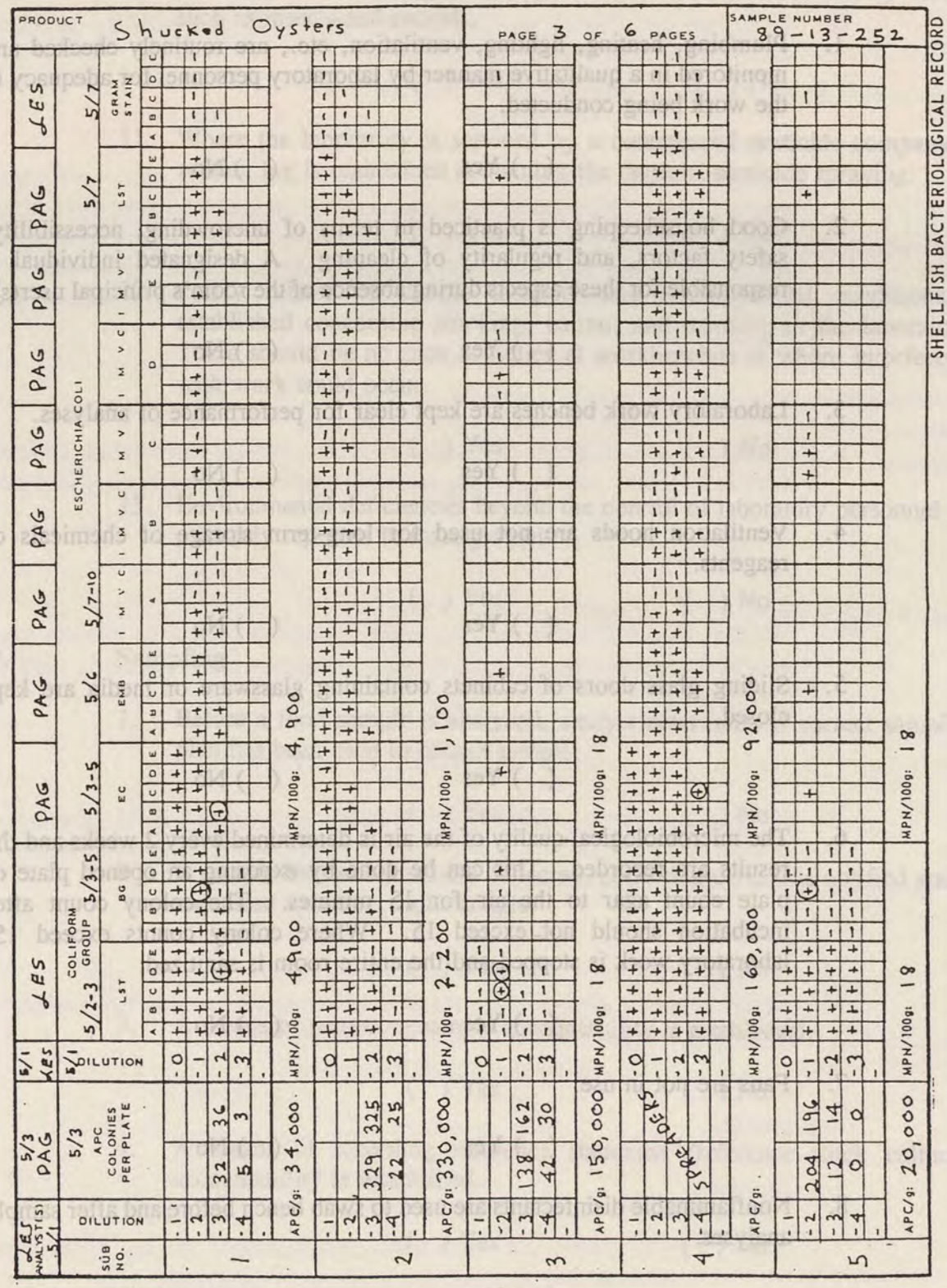
SUB NO.	TOXICITY SCREENING TEST	NEUTRALIZATION				SUMMARY
		UNPROTECTED	PROTECTED			
1A	*0					
1B	0-1-2: 0 0 0					
1C	0-1-2: 0 0 0					
2A	0					
2B	0-1-2: 2 1 1					
2C	0-1-2: 2 2 2	2 2 2	Protected A: 2 2 2	Protected B: 2 2 2	Protected E: 0 0 0	Mice protected by E antitoxin; not by A or B
3A	0-1-2: 0 2 0	0 0 0	0 1 0	0 0 0	0 0 0	See page 5 for repeat analysis on filtrate. No toxicity.
3B	0					
3C	0-1-2: 0 0 0					
4A	0					

* NUMBER OF DEAD MICE OUT OF 2 INOCULATED

BOTULISM CONTINUATION SHEET

ANNEX 25

Shellfish Bacteriological Record



ANNEX 26

Quality Assurance Review Check List

A. Environment

1. Plumbing, heating, lighting, ventilation, etc., are routinely checked and monitored in a qualitative manner by laboratory personnel for adequacy to the work being conducted.

( ) Yes ( ) No

2. Good housekeeping is practiced in terms of uncrowding, accessibility, safety factors, and regularity of cleaning. A designated individual is responsible for these aspects during absence of the room's principal user(s).

( ) Yes ( ) No

3. Laboratory work benches are kept clear for performance of analyses.

( ) Yes ( ) No

4. Ventilation hoods are not used for long-term storage of chemicals or reagents.

( ) Yes ( ) No

5. Sliding glass doors of cabinets containing glassware or media are kept closed.

( ) Yes ( ) No

6. The microbiological quality of the air is determined every 2 weeks and the results are recorded. This can be done by exposing an opened plate of plate count agar to the air for 15 minutes. The colony count after incubation should not exceed 15. Where colony counts exceed 15, laboratory work is stopped and the entire room is sanitized.

( ) Yes ( ) No

7. Fans are not in use.

( ) Yes ( ) No

8. Nonflammable disinfectants are used to swab bench before and after sample analyses.

( ) Yes ( ) No

9. System of disposing of pathogenic materials is adequate.

( ) Yes ( ) No

10. Measures are taken to prevent, eliminate, or reduce infestation by vermin such as insects and rodents.

( ) Yes ( ) No

11. Where the laboratory is serviced by a commercial pesticide company, a written log is maintained containing the dates of pesticide spraying.

( ) Yes ( ) No

12. Policy related to sample integrity and environmental conditions is established concerning smoking, eating, and drinking in the laboratory. There should be no such activities at workbenches or where interference with work could occur.

( ) Yes ( ) No

13. Environmental deficiencies beyond the control of laboratory personnel are promptly reported to management.

( ) Yes ( ) No

B. Sampling

1. Before a food sample is analyzed, analyst determines if correct sampling plan has been used to collect sample.

( ) Yes ( ) No

2. If sample has been invalidly collected, collecting official is notified and is requested to collect valid sample.

( ) Yes ( ) No

3. A means of recording sample accountability is established.

( ) Yes ( ) No

4. A means of recording reference standards (reference stock cultures) accountability is established.

( ) Yes ( ) No

5. If sample consists of several sample units, each unit must be labeled to maintain its individual integrity during analysis.

( ) Yes ( ) No

6. If sample consists of several sample units, each unit must be analyzed individually unless otherwise directed.

( ) Yes ( ) No

7. Analyst ensures that each sample is accompanied by a collection report containing the following information: food or sample type, sample number, date of collection, collecting official's name or identification number, reason for collection, name and address of manufacturer, manufacturer's code, size of lot sampled, description of sample, method of collection, and delivery destination of sample.

( ) Yes ( ) No

8. If samples are collected using aseptic technique, the appropriate controls (sterile container, sterile gloves, and sterile collecting utensils) are included with sample.

( ) Yes ( ) No

9. Analyst determines that microbiological integrity of sample has not been altered during shipment by determining temperature of maximum reading thermometer.

( ) Yes ( ) No

10. If sample cannot be analyzed immediately upon arrival at the laboratory, analyst ensures that it is stored under appropriate conditions to maintain microbiological integrity.

( ) Yes ( ) No

11. Before analysis, analyst examines physical condition of sampling containers to ensure absence of tears, pin holes, etc.

( ) Yes ( ) No

C. **Equipment**

1. General

a. All pieces of equipment are located in an appropriate environment that will assure proper functioning.

( ) Yes ( ) No

b. All pieces of equipment are properly operated in consultation with manufacturer's recommendations.

( ) Yes ( ) No

c. Instruments are calibrated/standardized before use, and as needed during use.

( ) Yes ( ) No

d. Instrument and equipment manuals are kept in a location readily accessible to laboratory personnel.

( ) Yes ( ) No

e. The laboratory has a program of preventive maintenance for instruments and equipment that can be maintained by laboratory personnel. Any irregularity in equipment function is immediately reported to supervisor.

( ) Yes ( ) No

f. All equipment not in regular use or not properly functioning is tagged immediately.

( ) Yes ( ) No

g. A written log is kept on maintenance of laboratory equipment.

( ) Yes ( ) No

h. Principal user(s) for delicate instruments and equipment are identified.

( ) Yes ( ) No

i. Personnel are familiar with procedures for obtaining instrument/equipment repair.

( ) Yes ( ) No

2. Specific

a. Incubators

(1) Interior is clean with no spillage areas.

( ) Yes ( ) No

(2) Internal temperature is monitored by one or more calibrated partial immersion thermometers, the actual number being dependent on size of incubator. These readings are made first thing in the morning and last thing in the afternoon and are recorded in temperature log.

( ) Yes ( ) No

(3) Air incubators, set at 35°, maintain this temperature within a tolerance of 2°.

( ) Yes ( ) No

(4) Partial immersion thermometers are calibrated annually and the results recorded.

( ) Yes ( ) No

(5) Graduations of partial immersion thermometers do not exceed 0.1°.

( ) Yes ( ) No

(6) Liquid mercury columns in partial immersion thermometers are inspected daily for separations.

( ) Yes ( ) No

(7) Temperature recording charts are signed and dated when inserted and removed.

( ) Yes ( ) No

(8) Temperature recording charts are changed before overprinting occurs.

( ) Yes ( ) No

(9) Temperature recording charts are maintained in a separate file for 3 years.

( ) Yes ( ) No

(10) The amount of humidity in the incubator is indirectly determined every 3 months by calculating the

percentage of weight loss of agar plates incubated at 35° for 48 ± 2 hours, and this information is maintained in the temperature log.

( ) Yes ( ) No

(11) Percentage of weight loss of agar plates incubated at 35° for 48 ± 2 hours does not exceed 15%.

( ) Yes ( ) No

b. Water baths

(1) Water baths are clean and free of rust.

( ) Yes ( ) No

(2) Cover fits securely on waterbath.

( ) Yes ( ) No

(3) Temperature of water bath is monitored by calibrated total immersion thermometer. These readings are taken first thing in the morning, last thing in the afternoon, and 3-4 times during a normal working hours. These readings are recorded in the temperature log.

( ) Yes ( ) No

(4) Total immersion thermometers are calibrated annually and the results recorded.

( ) Yes ( ) No

(5) Graduations of total immersion thermometers do not exceed 0.1°.

( ) Yes ( ) No

(6) Liquid mercury columns in total immersion thermometers are inspected daily for separations.

( ) Yes ( ) No

(7) Temperature recording charts are signed and dated when inserted and removed.

( ) Yes ( ) No

(8) Temperature recording charts are changed before overprinting occurs.

( ) Yes ( ) No

(9) Temperature recording charts are maintained in a separate file for 3 years.

( ) Yes ( ) No

(10) Where a temperature recorder is not used, cultures of Escherichia coli and Enterobacter aerogenes are used to ensure that the temperature did not extend beyond the accepted tolerance during nonworking hours.

( ) Yes ( ) No

c. Refrigerators and freezers

(1) Interior is clean with no spillage areas.

( ) Yes ( ) No

(2) Internal temperature of refrigerator (4°) and of freezer (-20°) is monitored by one or more calibrated partial immersion thermometers. Daily readings are recorded in temperature log.

( ) Yes ( ) No

(3) Partial immersion thermometers are calibrated annually and recorded.

( ) Yes ( ) No

(4) Graduations for partial immersion thermometers do not exceed 1°

( ) Yes ( ) No

(5) Liquid mercury columns in partial immersion thermometers are inspected daily for separations.

( ) Yes ( ) No

d. Autoclaves

(1) Autoclave is able to maintain an internal temperature of 121° under a pressure of 15 psi.

( ) Yes ( ) No

(2) No more than 45 minutes is required for a complete cycle.

( ) Yes ( ) No

(3) Autoclave contains pressure and temperature gauges on the exhaust side and an operating safety valve.

( ) Yes ( ) No

(4) Autoclave is equipped with a temperature recorder to provide a permanent record of sterilizing cycle.

( ) Yes ( ) No

(5) Autoclave temperature recorder charts are maintained for at least 3 years.

( ) Yes ( ) No

(6) Air bubbles are not produced in fermentation vials during depressurization.

( ) Yes ( ) No

(7) A microbiological indicator, such as a Bacillus stearothermophilus spore ampule, a maximum registering thermometer, or sterility indicator tape is used with each load.

( ) Yes ( ) No

(8) A time and temperature log is maintained for each load.

( ) Yes ( ) No

(9) Autoclave are serviced annually and a record is maintained.

( ) Yes ( ) No

e. Hot air ovens

(1) Oven is equipped with a calibrated thermometer capable of registering in the range of 160-180°.

( ) Yes ( ) No

(2) Thermometer is calibrated annually and results recorded.

( ) Yes ( ) No

(3) Graduations for this thermometer do not exceed 1°.

( ) Yes ( ) No

(4) A record of time and temperature is maintained for each sterilization cycle.

( ) Yes ( ) No

(5) Commercially available sterility indicator tape is used for each load.

( ) Yes ( ) No

f. Balances

(1) Balance pans are clean.

( ) Yes ( ) No

(2) All high-precision balances are cleaned and calibrated annually.

( ) Yes ( ) No

(3) Records of annual calibrations are maintained in bound book or in files.

( ) Yes ( ) No

g. pH meters

(1) pH meter casing is clean and dust-free.

( ) Yes ( ) No

(2) When not in use, glass electrodes are stored in a pH 7 buffer solution.

( ) Yes ( ) No

(3) When not in use, reference electrodes are stored in 0.1 M KCl solution.

( ) Yes ( ) No

(4) pH meters are standardized before each use.

( ) Yes ( ) No

(5) Only unexpired pH buffer standards are used.

( ) Yes ( ) No

(6) Aliquots of pH buffer standards are not reused.

( ) Yes ( ) No

h. Blenders

(1) Blender base is clean.

( ) Yes ( ) No

(2) Rotation speed of blender has been calibrated with a tachometer, and this information has been recorded.

( ) Yes ( ) No

i. Laminar flow hood

(1) All procedures involving pathogenic organisms, dispensing of sterile media, or the analysis of canned foods for sterility are conducted under a laminar flow hood.

( ) Yes ( ) No

(2) Hood interior is wiped before and after each use with a liquid disinfectant.

( ) Yes ( ) No

(3) Bunsen, Fisher, or other laboratory burners are used within the hood.

( ) Yes ( ) No

(4) Performance of hood is monitored monthly by exposing blood agar plates to the air flow for 1 hour. After incubation at 35° for 24 and 48 hours, no colonies are present on plates. These results are recorded.

( ) Yes ( ) No

(5) Hood is serviced annually by qualified technician and results are maintained in record book.

( ) Yes ( ) No

j. Microscopes

(1) Microscopes are not moved from location to location.

( ) Yes ( ) No

(2) When not in use, microscopes are protected by dust cover.

( ) Yes ( ) No

(3) Microscope stand is clean.

( ) Yes ( ) No

(4) Microscope objectives are clean.

( ) Yes ( ) No

(5) Microscopes are serviced annually and a record is maintained.

( ) Yes ( ) No

k. Glassware

(1) Laboratory glassware is made of borosilicate glass.

( ) Yes ( ) No

(2) Glassware items are free of cracks or etching.

( ) Yes ( ) No

(3) Glass pipet tips are not chipped.

( ) Yes ( ) No

(4) Mouths of dilution bottles do not have chips.

( ) Yes ( ) No

(5) Glassware items are clean and free of residues or dried medium.

( ) Yes ( ) No

(6) Screw-cap containers have leakproof, nontoxic liners that can withstand repeated autoclaving for 15 minutes at 121°.

( ) Yes ( ) No

(7) Calibrated glassware items have clearly visible graduations.

( ) Yes ( ) No

D. Chemicals, media, reagents

1. An inventory record is maintained for all chemicals and dehydrated media.

( ) Yes ( ) No

2. Only dyes certified for bacteriological use are ordered.

( ) Yes ( ) No

3. Bottles of media and chemicals are dated and initialed when received and opened.

( ) Yes ( ) No

4. Expired media, sera, and reagents are discarded.

( ) Yes ( ) No

5. Dehydrated media are not used if caked or discolored.

( ) Yes ( ) No

6. Dehydrated media and chemicals are kept in tightly closed bottles protected from dust, excessive humidity, and direct sunlight.

( ) Yes ( ) No

7. Laboratory pure water is used in the preparation of all media.

( ) Yes ( ) No

8. Dehydrated media are completely dissolved before dispensing.

( ) Yes ( ) No

9. A record is maintained for the preparation of all rehydrated media and chemical reagents.

( ) Yes ( ) No

10. The pH is checked on each batch of media after preparation and autoclaving.

( ) Yes ( ) No

11. Media are autoclaved according to the specific recommendations (time, temperature, and pressure) of the procedure being used.

( ) Yes ( ) No

12. Media with fermentation tubes are examined after autoclaving. Fermentation tubes with air bubbles are discarded.

( ) Yes ( ) No

13. An autoclaving log is maintained so that the sterilizing conditions of each batch of media may be traced.

( ) Yes ( ) No

E. Standards

1. Bacterial stock strains are checked for purity and authenticity before use. The results are entered into a bound notebook.

( ) Yes ( ) No

2. Reference cultures are maintained under long-term and short-term conditions to assure viability and stability of biochemical, serological, and pathogenic characteristics.

( ) Yes ( ) No

F. Methodology

1. The appropriate method of analysis is used. Whenever possible, this method has been officially validated by an organization such as the Association of Official Analytical Chemists, Nordic Committee of Food Analysis, International Commission on Microbiological Specifications for Foods, etc.

( ) Yes ( ) No

2. All methods used must be followed exactly as written.

( ) Yes ( ) No

3. If extenuating circumstances require one or more modifications in the official method, these deviations are noted on the analyst work sheet.

( ) Yes ( ) No

4. If an unofficial method must be used, this method is validated in the analyst's own laboratory.

( ) Yes ( ) No

5. Appropriate controls (see section 9.2) are included with each sample analysis.

( ) Yes ( ) No

G. Use of Animals

1. Mice are quarantined at least 1 week before use.

( ) Yes ( ) No

2. Floors of animal room are swept and mopped daily with disinfectant.

( ) Yes ( ) No

3. Walls of animal room are disinfected at least monthly.

( ) Yes ( ) No

4. Temperature and humidity of the animal room are monitored with a hygrothermograph.

( ) Yes ( ) No

5. Hygrothermograph charts are changed weekly before over-printing occurs.

( ) Yes ( ) No

6. Analyst who inserts and who removes hygrothermograph charts initials and dates these charts.

( ) Yes ( ) No

7. Used hygrothermograph charts are taped in record book.

( ) Yes ( ) No

8. Animal room is provided with timer to provide alternating 12-hour periods of light and darkness.

( ) Yes ( ) No

9. Cages are large enough to prevent crowding.

( ) Yes ( ) No

10. Cages are labeled or identified completely.

( ) Yes ( ) No

11. Animal feed is changed twice a week.

( ) Yes ( ) No

12. Water is changed 3 times a week.

( ) Yes ( ) No

13. A log is maintained documenting the frequency of feeding and watering the mice.

( ) Yes ( ) No

14. Appropriate procedures for restraining and injecting laboratory mice (see section 10.7) are followed.

( ) Yes ( ) No

15. Appropriate procedures for disposing of mice (see section 10.8) are followed.

( ) Yes ( ) No

H. Documentation

1. The information on the analyst worksheet is compatible with the information on the collection report.

( ) Yes ( ) No

2. The worksheet describes completely the appearance of the sample and its condition when received by the analyst.

( ) Yes ( ) No

3. The worksheet completely and accurately reflects the continuity and integrity of the sample.

( ) Yes ( ) No

4. The use of all types of media and chemicals with their lot numbers is indicated on the worksheet.

( ) Yes ( ) No

5. The use of all reference control cultures is indicated on the worksheet.

( ) Yes ( ) No

6. All calculations on the worksheet are checked for accuracy and are easy to follow.

( ) Yes ( ) No

7. If more than one person took part in the analysis, the worksheet clearly indicates who did what.

( ) Yes ( ) No

8. Sample reserve or disposition is clearly indicated on the worksheet.

( ) Yes ( ) No

9. All results on the worksheet are recorded in ink.

( ) Yes ( ) No

10. Any errors on the worksheet are "crossed through" with a single line. The correct entry above is initialed and dated.

( ) Yes ( ) No

11. Information on the sample accountability record is accurate, complete, and compatible with sample collection record and analyst worksheet.

( ) Yes ( ) No

12. The sample accountability record correctly reflects the handling and storage of sample.

( ) Yes ( ) No

13. The sample collection records are filed appropriately.

( ) Yes ( ) No

14. Samples are stored in a clean, secure room under appropriate conditions of temperature and humidity.

( ) Yes ( ) No

FIAT PANIS

M-82/T0451E/1/4.91/2400
ISBN 92-5-103053-7