Association of Public Analysts

Mastership in Chemical Analysis Examination

Training Guide

Microbiology

Date 2004

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FOREWORD

This training guide is one of a series produced by the Association of Public Analysts for use in the profession. It is particularly directed at candidates preparing for the Mastership in Chemical Analysis (MChemA) examination who have no background in microbiology.

The aim of this guide is to prepare candidates for the MChemA examination with sufficient guidance to enable them to deal with those aspects of the syllabus relating to the role of Food Examiner.

There are two objectives to this study guide:

i) To provide a basic minimum of background information as to the nature of micro-organisms, along with sources of information, which can provide specific detailed data if needed.

ii) To show links between the chemical, physical and microbiological aspects of foodstuffs. These will determine the shelf life of products, changes to taste or appearance due to inadequate control, and the possibility of illness in the consumer.

A knowledge of the types of environment favoured by particular organisms leads to a better understanding of the tests which should be applied, either as a regular check or in cases of investigation of food poisoning.

The MChemA is the qualification prescribed by the Food Safety (Sampling and Qualifications) Regulations 1990 for a person to be appointed as a public analyst in the United Kingdom. These regulations also provide that the MChemA is an authorised qualification for a person to practice as a Food Examiner. Responsibility for the examination and the award of a Mastership remain with the Royal Society of Chemistry, from which copies of current regulations and syllabus for the examination may be obtained.

Other training guides published by the Association of Public Analysts include the "Study Guide for the M.Chem.A.", "Candidate's Record of Professional Training and Experience", "Microscopy (2nd Edition)", "Certificate Writing", "Legislation" " Labelling of Food, Bottled Water and Agricultural Materials" and "Food Complaints". This guide on "Microbiology" complements the series.

The Training Committee would welcome corrections to the text and suggestions for improvement to future editions.

Charlie McDonald
Training Committee Chairman
Association of Public Analysts

Part 1

Glossary

Acidophile	grow best at pH below 5.5
Aerobic	requiring free oxygen for respiration
Alkaliphile	grow best at pH above 8
Anaerobe	not requiring free oxygen for respiration
Antibacterial	an agent that destroys bacteria
Background flora	micro-organisms that are naturally associated with a specific environment
Bactericide	same meaning as antibacterial
Bacteriophage	virus-like agents which destroy bacteria
Bacteriostat	an agent that stops bacterial growth but does not kill them
Binary fission	process by which bacterial cells replicate through an asexual process. During this process DNA replicates and attaches to the plasma membrane. The cell then divides and a bacterial cell wall forms.
cfu	colony forming unit (per gram or ml) -this is the standard form of measurement of bacteria level
Complex culture	ingredients and amounts not precisely known – general purpose supports growth of many common bacteria
Defined synthetic culture	all ingredients and exact amounts known, used to determine which nutrients a micro-organism requires for growth
Differential culture	growth of organism causes a visual change in the medium
Facultative anaerobe	grow aerobically when oxygen present, and anaerobically when it is absent
Gram stain	bacteriological staining procedure named after the discoverer Christian Gram. When bacteria are stained with certain basic dyes, the cells of some species can be easily decolourised with organic solvents (ethanol or acetone). These are Gram-negative bacteria. Species which resist decolourisation are Gram-positive bacteria.

Lenticule	plano-convex disc containing biologically active material in a solid water–soluble matrix. They can be used to reproduce countable numbers of cfu and hence useful for quality control
Mesophile	optimum growth between 25 and 40^{o}C
Microaerophiles	prefer low oxygen concentration
Obligate anearobe	killed by oxygen, only grows in its absence
Psychrophile	optimum growth at low temperatures (15 to 20^{o}C, some at 0^{o}C)
Psychrotroph	micro-organism which grows slowly at refrigeration temperature (less than 7^{o}C), but grow optimally at temperatures between 25 to 30^{o}C
Selective culture	supports a particular organism while inhibiting others
Thermophile	optimum growth at high temperatures (50 to 60^{o}C, some at 110^{o}C)
Total viable colony count	also known as “Aerobic Plate Count” and “Aerobic Colony Count”
Vacuoles	membrane bounded sacs that contain various substances important to the well being of the cell eg food, wastes, water. Some are temporary (food, waste), others are permanent (water)

Part 2

Micro-organisms

2.1 Nomenclature

Micro-organisms are named following the International Code of Nomenclature of Bacteria and Viruses. In this code a name consists of two parts, the first denotes the genera and the second denotes the species. The names are Latin/ Greek based and are written in Italics with the first word starting with a capital letter. The second word of the name is not capitalised and is usually descriptive. Through such a system therefore names such as *Bacillus albus* (white *Bacillus*) are formed. Where the genera are being referred to, the format is the genera name followed by "species" eg *Bacillus species*. "Species" can be shortened to "sp" in the singular and "spp" in the plural although common practice is to use "sp" for both singular and plural. It is not uncommon however for textbooks to omit the "sp" and refer only to the genera name eg "The *Campylobacter* are now a cause for concern……."

Another commonly used abbreviation is in reducing the genera name. However there are no rules applied. It can consist of the first letter followed by a full stop. *Bacillus albus* therefore becomes *B. albus*. Non-microbiologists need to take care since the same initial letter can used for different genera. For example, "*C.*" is used for "*Clostridium"*, "*Campylobacter"* and *"Cladosporium"* in some texts. Again however some texts refer to "*Cl.*" as the abbreviation for "*Clostridium"*.

Some other common usages may be encountered. For example, "S*taphylococcus"* may appear as "S*taph."* and "*Salmonella"* may appear as "*Salm."* Usually the meaning is clear, but non-microbiologists should take care.

To avoid confusion, full names will be used through out this study guide.

2.2 Categories of micro-organisms

2.2.1 Bacteria

Bacteria are unicellular organisms. They are prokaryotes, which means that the DNA is not held in a nucleus. They divide by simple binary fission and can occasionally exchange DNA, (the bacterial equivalent of sexual reproduction.)

The usual size range is 0.5 micron spheres to rods 10 microns long. Typical shapes are spheres (cocci), rods (bacilli) or spirals (spirilla). Individual bacteria can be viewed by staining and examination at x600 magnification. A common technique is to use a Gram stain; the resulting colour of the bacteria depending on the structure of the cell wall. Various other stains can be used to highlight characteristics of certain types of bacteria. Alternatively, live bacteria can be viewed using phase contrast microscopy, which allows observation of the characteristic patterns of motility.

Mature cultures of *Bacillus sp* and *Clostridium sp* will have spores visible under the microscope with the position of the spores being diagnostic. When viewed microscopically, certain bacteria appear to be evenly spaced. This is due to the production of a layer of gum surrounding each bacterium and is typical of bacteria, which adhere to surfaces.

Bacteria grow by absorbing simple nutrients from their surroundings. They are predominantly found in foods with a water activity (a_w) greater than 0.9. They normally grow in a pH range of 4 to 10. Non spore-forming bacteria are normally killed by desiccation, but some survival may occur in a high protein environment (eg on a dirty meat cutting board).

2.2.2 Moulds

Moulds characteristically have a filamentous form and can have a "furry" appearance when they develop into a visible colony. Individual strands of mould are called hyphae and the mass of interwoven hyphae, the mycelium. Individual hyphae consist of thick walled, branching tubes, which may be divided into sections by cross walls called septa. The size range of hyphae is 10 to 100 microns diameter.

During development, colonies of mould change colour due to the production of coloured spores e.g. white *Penicillium sp* mycelium develops a blue-green centre. Identification is normally based on the structure of the sporing heads of cultures growing on agar. If the mould is developing in liquid, such as in a carton of fruit juice, then the characteristic appearance may be modified. It is necessary to grow the mould in a standard culture environment to ensure accurate identification.

Moulds can grow in media with lower pH range and lower water activity than that suitable for bacteria.

2.2.3 Yeasts

Yeasts are characterised in appearance by regular oval shapes with smaller buds at the ends. Size can vary from 5 to 10 microns. In certain liquids the yeast colonies can appear almost spherical and can be confused with fat droplets. However, yeasts are much more regular in size than fat droplets, which show a range of sizes. With phase contrast microscopy, the vacuoles and other internal structures are visible within the yeasts, but not the fat droplets. Oil red O stain can also be used to distinguish fat droplets where the fat is stained red and the yeast is unaffected.

The ideal environment for yeast growth is a sugar solution of concentration 5% to 10% and a pH range of 2 to 8. The presence of yeast can also lead to alcohol production in food due to their action on carbohydrate.

2.2.4 Algae

Algae show a wide range in size and shape, ranging from the size of yeast to long strands of seaweed. They are characterised by always containing chlorophyll and usually have the characteristic green colour. Some small algae appear almost colourless, when viewed singly under the microscope and can resemble yeast. Typical algae have thick cell walls and a large central vacuole.

They normally only develop in liquids with low nutrient levels (not foodstuffs) and ample light supply. The dirt, which is found in milk bottles, often contains traces of algae, because the recycled bottles are washed out by users then left outside on the doorstep in the dust and rain for collection. Presumptive algae from these bottles can be encouraged to grow by inoculating into sterilised water with a trace of fertiliser (eg Growmore) and leaving the water on a bright windowsill for at least a week. This can be used to distinguish algae killed by the bottle washing process from unwashed algae.

The organisms referred to as "blue/green algae", which occur as toxic blooms in waters, are in fact more closely related to bacteria than true algae.

2.2.5 Viruses

Viruses consist of a piece of DNA or RNA in a protein coat. The size range is 20 to 300 nm, meaning that they can only be viewed with an electron microscope.

The presence of viruses can be determined by their effects on the host, for example mottling patterns on the leaves of plants. A large proportion of water and food borne human illness is probably due to viruses. They can be spread to new hosts by coughing and sneezing. The viruses do not multiply in the foodstuff, only in the contact human, animal or plant host.

2.2.6 Protozoa

These are unicellular organisms, which are normally larger than yeasts and often have cilia on the surface. They come in a wide variety of shapes and sizes from 1micron to 300 micron. High levels are present in polluted rivers and lakes. Some protozoa are pathogenic

2.2.7 Nematodes and other parasites

Nematodes are types of worm with round or cylindrical shape, some of which can have long term health implications.

Nematodes (round worms, *Trichinella sp*.) can be present in cysts in pork and beef. These can cause infection and long term illness in humans who eat inadequately cooked meat. Nematodes can also be found in large numbers in the guts of fish.

Tapeworms (*Taenia sp*.) are found in meat originating from countries with poor hygiene standards.

Liverflukes (*Fasciola hepatica*) are the cause of enlarged blood vessels in packs of liver. They are normally dead because most liver has been frozen at some time before sale. There have been cases of human infection through eating watercress, grown in water contaminated with cattle faeces.

2.3 Indicator Organisms

Indicator organisms are common, innocuous bacteria, which are normally present in high levels in situations where pathogenic organisms may be found. For example, waste sewage can contain in excess of 10^6 *Escherichia. coli* bacteria per ml and in the mixture there may be a low level of *Salmonella sp., Shigella sp.* or other pathogen (one per litre). In a situation where there is possible contamination of a

water supply by this sewage, it would be difficult and time consuming to test for every known pathogen. If the levels of pathogen are low it could be easily missed in the examination. It is more practical to examine for the more common associated indicator organisms and then to assume that if these are present then the pathogens may also be present.

It is necessary to choose the indicator organism with care, depending on the type of pathogens that one may expect to be present.

Ideally the indicator organism should have the following characteristics:

- have the same source as the pathogen
- be more common by a large factor
- have the same susceptibility to treatments such as pasteurisation
- survive for longer in the water or foodstuff
- be easy to detect
- be detected quickly

2.3.1 Indicator organisms for water

Water samples need to be checked for possible faecal waste contamination. The faecal waste indicator organisms are coliform sp, faecal *Enterococci sp (Streptococci sp*) and *Escherichia coli. Escherichia coli* are most closely linked to faecal material, but they rapidly die out in stored water and only last about one week. The coliform group of bacteria, which includes *Escherichia coli*, contains organisms that survive for longer in stored water. Some may even multiply in soil and dirty water and are therefore not true indicators of sewage. *Enterococci sp* survive for longer in stored water than *Escherichia coli*. Examination for these bacteria is relatively simple, but the initial concentration in faecal waste is lower.

There is still debate as to the ideal indicator organism. Coliphages are viruses, which infect and destroy *Escherichia coli* bacteria. The coliphages have a similar survival time to the most resistant common pathogens, which are probably the human pathogenic viruses. However, the tests for coliphages have yet to be simplified.

Methods for examination of the indicator bacteria for water are given in *Microbiology of Drinking Water 2002*.

2.3.2 Indicator organisms for food

Many materials will have an inhomogeneous distribution of micro-organisms. They tend to form at interfaces such as the outer surface of meat or the top and bottom surfaces of the cream filling of a cake. Estimation of the total number of micro-organisms has to take this into account to ensure that the portion sampled is representative.

Bacterial levels can rise rapidly if conditions are favourable. It is therefore necessary to be able to examine samples submitted to the laboratory within a few hours. It is also necessary to discover the history of the sample in terms of time spent at room temperature and time spent in a fridge or freezer. This information may assist in considering levels at the time of purchase, although great care is needed in extrapolating microbiological loading.

Public health examinations are based on levels of pathogenic bacteria and their associated food poisoning. A Food Examiner also has to consider the presence of innocuous organisms that may just make the food unpleasant to eat. The most commonly used tests are for *Enterobacteriaceae sp, Escherichia coli* and total viable count (TVC).

2.3.2.1 *Enterobacteriaceae sp*

The method of examination for this group of bacteria which includes the *coliform sp* with the addition of glucose fermenting bacteria is found in BS 5763 Part 10 1993 (1999).

The test method is simple; the main problem is adaptation of the method to suit the variety of different food products available. Products such as cream may increase the opacity of the plates so much that the bacterial colonies are not visible. This can be overcome by making multiple plates each containing a smaller portion of the sample. Products such as cakes tend to break up into tiny white fragments, which are identical to the colonies on the plates. This can be overcome by either using a lined stomacher bag which filters the suspension, or to examine the plates prior to incubation and mark any false colonies. Margarine and chocolate samples will require stomaching after warming to about 37^{o}C to ensure an adequate emulsion is formed, followed by chilling to re-separate the fat.

2.3.2.2 *Escherichia coli*

Although non-pathogenic strains of *Escherichia coli* can be isolated from the intestines of warm blooded animals, many strains are pathogenic to humans. These latter strains consist of four groups, enteropathogenic (EPEC), enterotoxigenic (ETEC) (also known as verotoxigenic (VTEC)), enteroinvasive (EIEC) and enterohaemorrhagic (EHEC). The commonest group ETEC is generally the cause of traveller's diarrhoea and has been implicated in several large outbreaks of food poisoning associated with various meats and poultry, mashed potatoes, milk and cheese.

Care is needed in using non-pathogenic *Escherichia coli* as an indicator organism. Even the presence in food in large numbers does not necessarily imply recent heavy faecal contamination. The level can be influenced by the use of unclean utensils and operators as well as truly faecal contact. A high level of organism may also be due to low level contamination with subsequent growth within the food. (However high levels of *Escherichia coli* in a food should be taken to suggest some sort of contact with faecal matter and places a question mark over the safety of the food.

2.3.2.3 Total Viable bacterial counts

An estimation of the total viable bacterial count of a product, combined with a knowledge of the expected bacterial levels can be very informative. In general, few foods become hazardous to health or unpleasant to taste until the bacterial count exceeds 10^{6}/g. However there are a few exceptions, such as products containing *Escherichia coli O157,* and *Campylobacter sp* which have low infectious doses.

There is some debate as to the most suitable incubation temperature for total viable counts. Most sources recommend 30^{o}C although some will use 35^{o}C or 37^{o}C.

Some foods however, have a naturally high total viable bacterial count. The normal count for yoghurt is about 10^8/g and reduction of this, combined with an increase in mould and yeast counts is an indicator that the yoghurt is of unsatisfactory quality. The presence of the correct species and levels of live lactic acid bacteria in yoghurt is essential for the maintenance of a low pH and development of bactericidal compounds. These in turn inhibit harmful bacteria and allow a fairly long shelf life. The addition of fruit and other ingredients such as nuts can also affect the natural balance.

Similarly, bacon normally has an unusually high count of innocuous bacteria as can wild game products. Cream, which is nearing its best before date, can have a bacterial count of 10^7/g and still be acceptable. One survey of creams found that the high count was predominantly *Pseudomonas sp* and was not a concern from a food poisoning perspective.

2.4 Pathogenic Organisms

The pathogenic organisms of interest to a public health microbiologist are the species of *Salmonella, Campylobacter, Listeria, Staphylococcus, Bacillus, Clostridia, Streptococcus, Escherichia coli O157, Shigella, Legionella, Rotoviruses, Giardia, Cryptosporidium* and *Entamoeba*. In addition bacterial toxins and those of moulds and yeast require attention.

The US Food and Drug Administration have produced an excellent web site called the Bad Bug Book, (http://www.cfsan.fda.gov/~mow/intro.html), which gives details for each of the common pathogenic foodborne bacteria as well as for dangerous protozoa and toxins such as scromboid and shellfish associated toxins. The site details the incubation times, affective dose, typical foodstuff where found and recent associated poisoning outbreaks

Illness can be caused by bacterial toxins or by bacterial infection.

An example of bacterial toxin is that produced by *Staphylococcus aureus*. If this is allowed to multiply in food to levels in excess of 10^6/g, certain strains of this bacterium will secrete toxins into the foodstuff. The effect on the consumer is a rapid attack of illness, 2-4 hours after consumption. Some toxins can be heat resistant. The food may have been heated sufficient to kill the bacteria, thus making it non-detectable, but the toxin will still be present and hazardous. Another common example of toxin production is in boiled rice. This high starch foodstuff is an ideal environment for the growth of *Bacillus sp* bacteria. Given a warm environment, the *Bacillus sp* have a relatively high growth rate and a toxin producing strain could result in contaminated rice in a period of hours, even if the rice is subsequently reheated.

Moulds can also produce toxins that remain in the foodstuff even if the mould is removed and can have long term health implications for liver damage and cancer development. For example, the presence of *aflatoxins* in milk is due to bovine consumption of *aflatoxin* contaminated feed. Similarly, *ochratoxin* is found in a range of commodities particularly dried fruit, and *patulin* can be found in apple juice.

Bacterial **i**nfection, on the other hand, can be caused by very low levels of the pathogen. For example, infection by *Campylobacter sp* could probably be caused by ingesting less than 10 bacteria. The organism multiplies in the gut and illness develops after several days.

The following is a summary of pathogenic organisms, symptoms and typical foods in which they are found:-

Organism	Incubation period	Symptoms				Duration	Typical foods
		Vomiting	Diarrhoea	Cramps	Fever		
Bacillus cereus (emetic)	30min to 4 hrs	+++	+ (possible)	+ (possible)	-	short	Rice
Bacillus cereus (diarrhoeal)	8 to 12 hrs	-	++	++	-	1 to 2 days	Soups Milk products
Staphylococcus aureus	4 to 6 hrs	++	+	++	-	6 to 12 hrs	Cold meats Dairy products Salami
Clostridium perfringens	8 to 22 hrs	-	++	++	-	12 to 24 hrs	Reheated meats
Salmonella sp	12 to 48 hrs	+	++	-	+	48 hrs to 7 days	Poultry Meats Eggs
Clostridium botulinum	24 to 72 hrs	-	-	-	-	3 days	Home bottled produce
Vibrio sp	8 to 48 hrs	-	+++	-	+	2 to 3 days	Seafoods Meat
Campylobacter sp	2 to 11 days	-	+++	++	++	3 days to 3 weeks	Milk Water Poultry Mushrooms
Escherichia coli O157	2 to 5 days	-	+++	+++	+ (possible)	5 to 10 days	Meat Milk

2.5 Spoilage Organisms

The average foodstuff carries a wide range of bacteria and moulds, most of which are completely innocuous. In time, these organisms will multiply to produce a putrid smell or taint or visible patches of mould, all of which deter the consumer from eating the product.

A problem area in food technology now is that modern processing techniques can inhibit the natural spoilage organisms while favouring the development of pathogens. For example, long term storage at low temperatures can result in high levels of *Listeria sp.* Similarly, *botulinum* toxins develop in products that are inadequately heat treated. This can occur where a minimal heat treatment designed to retain as much water as possible in a cooked ham is sufficient to kill the majority of bacteria. If a trace of the *Clostridium botulinum* spores remain then they could increase in the absence of competitors with serious consequences for the consumer.

Items stored in bags with modified atmospheres will have extended shelf life dates on the pack, but once the packaging is damaged or opened, this pack life becomes the same as its non-prepacked counterpart. Slight puncture holes in the packaging may not be easily visible, but can allow entry of contaminants (usually mould spores).

The type of spoilage that develops on a food depends on factors such as water activity, pH and packaging. The type of spoilage may also be affected by additives such as preservatives or through the presence of natural competing bacteria as are found in eg yoghurt.

The introduction of "healthy lifestyle" foods can result in reduced shelf life. An example is in production of low fat spreads with low salt content. The higher water activity in these products can make the conditions for bacterial or mould growth more favourable.

2.5.1 Examples of Spoilage Organisms

2.5.1.1 Raw Meat

Under chill conditions, mesophiles grow slowly and psychrotrophs such as *Pseudomonas sp*, *Acinetobacter sp* and *Psychrobacter immobilis* cause spoilage. Surface counts of 10^7/cm² will produce off taints while levels of 10^8/cm² causes slime formation. At ambient temperature, the predominant spoilage flora are the *Enterobacteriaceae sp* and *Acinetobacter sp*. If the surface is dry, colonies of yeasts and moulds may appear.

2.5.1.2 Comminuted Meat Products

Putrefactive spoilage is caused by *Pseudomonas sp* and is usually near the surface of comminuted meat and sausages. Inside the product, spoilage is due mainly to Gram positive bacteria, *Brocothrix sp* and lactic acid bacteria. Psychrotrophic *Enterobacteriaceae sp* also form part of the flora.

2.5.1.3 Cooked Perishable Uncured Meats

Spoilage of cook-in-bag 'sous-vide' products, kept cool, will be by psychrotropic, spore-forming bacilli, while meat pies will normally spoil due to the outgrowth of mould of the species *Mucor, Penicillium*, *Rhizopus* and *Aspergillus.*

2.5.1.4 Retorted Shelf-stable Uncured Meat Products

With these products, which include canned meat products and retorted pouches, spoilage due to underprocessing may result in survival of spore formers such as *Clostridium sporogenes.* while post-process contamination through seams, pinholes and rough handling usually gives rise to a mixed flora of organisms.

2.5.1.5 Cooked Perishable Cured Meats

These products include canned hams, pates and frankfurter style sausages that contain nitrite. They are heated to approx. 80ºC and are intended to be refrigerated after cooking. Insufficient heat treatment can result in souring, gas formation or greening due to survival of the relatively heat resistant psychrotroph *Lactobacillus viridescens*. If held at ambient temperature, thermotolerant mesophiles such as *Bacillus cereus* or *Bacillus licheniformis* can produce off-odours in the presence of oxygen. *Enterobacteriaceae sp* and halophilic *Vibrios* have caused hydrogen sulphide production in vacuum-packed bacon and ham, when muscle pH >6.0, salt content <4.0% and storage temp. >15ºC.

2.5.1.6 Fresh Fish

Fish caught at sea may be gutted, washed in sea-water and held in ice. Total viable counts can reach 10^8/g after 12-14 days storage at 0ºC. This number can be reached after only 5-6 days at 7ºC. Spoilage is mainly due to *Pseudomonas sp* producing mercaptans, sulphides and trimethylamine.

2.5.1.7 Crustaceans

Shrimps, prawns and lobster are either frozen on being caught or are kept alive until ready to be boiled prior to consumption. Spoilage can be rapid due to the presence of *Alteromonas sp* and the production of ammonia, trimethylamine, hypoxanthine and acetic acid.

2.5.1.8 Molluscs

Oysters, scallops and mussels contain approximately 3% carbohydrate and souring spoilage due to poor removal of *coliform sp* can result. The properly cleaned product can spoil due to the production of volatile bases with the microflora consisting mainly of *Acinetobacter sp* or *Moraxella sp*.

2.5.1.9 Milk

Raw milk is spoiled by lactic acid bacteria, growing mainly above 10ºC, producing souring and coagulation.

Pasteurised milk is usually treated at 72ºC for 15 seconds (known as High Temperature Short Time or HTST), then cooled rapidly to <10ºC. Thermoduric bacteria such as *Streptococcus thermophilus*, *Micrococcus luteus, Corynebacterium lacticum* and *Bacillus cereus* survive the HTST treatment. 'Sweet curdling' of pasteurised milk (curdling without acid formation) is due mainly to *Bacillus cereus*. HTST pasteurisation destroys psychrotrophs, so that milk that has been pasteurised and not subsequently contaminated in the plant, should have a shelf life of 7-10 days when stored at a temperature below 7ºC.

Ultra High Temperature (UHT) milk has been heated at >132ºC for at least 1 second. Spoilage can be due to survival and outgrowth of the spore formers *Bacillus subtilis* or *Bacillus stearothermophilus*. Gelation of stored UHT milk may be due to proteases that have been produced by psychrotrophs in the raw milk, prior to UHT treatment.

2.5.1.10 Butter

Bacteria such as *Pseudomonas sp* that may enter the product after pasteurisation can grow out at refrigeration temperatures and hydrolyse fat to fatty acids causing rancidity.

2.5.1.11 Cheese

Cheese is prepared from a mixed starter culture of *Streptococcus sp* and *Lactobacillus sp*. The *streptococci sp* then decline and the majority of organisms in the ripened cheese is *lactobacilli* sp. If the pH is too high during ripening, *Pseudomonas* s*p* can grow out causing sliminess. 'Gassy' cheese is caused by *Enterobacter sp* or *Clostridia sp* fermenting lactose and producing carbon dioxide.

2.5.1.12 Eggs

The chicken egg is virtually sterile after being laid but can become contaminated by Gram negative organisms through faults in the shell. The following are some of the commonly occurring types of bacterial spoilage:-

Type	Bacteria Involved	Type of Spoilage
Green rot	*Pseudomonas fluorescens*	Bright green fluorescent albumen under UV light. Yolk may disintegrate. Fruity odour.
Pink rot	*Pseudomonas sp*	Pink discolouration of albumen.
White rot	*Pseudomonas sp*	Watery albumen. Yolk may disintegrate.
Black rot	*Proteus vulgaris*) *Aeromonas sp*) *Pseudomonas sp*)	Watery albumen turning brown. Yolk disintegrates and turns black. Hydrogen sulphide odour.
Red rot	*Serratia marcescens*	Bright red albumen and yolk. No smell.

2.5.1.13 Fruit and Vegetables

Bacterial spoilage of fruit and vegetables is due mainly to *Erwinia sp* and *Pseudomonas sp* which affect most vegetables, but particularly tomatoes and cucumbers. *Erwinia carotovora* can infect damaged tissue at harvest, grow rapidly and produce pectic enzymes that break down the structure of the vegetable.

Mould spoilage of fruit and vegetables can take the following forms:-

Genus	Type of spoilage	Principle fruit and vegetables involved
Alternaria sp	Firm black rot	Cabbage, cauliflower, sprouts, potato, tomato. lemon, orange, apple, pear
Aspergillus sp	Black mould	Banana, peach, plum, grape
Botrytis sp	Grey mould	Most fruit and vegetables
Cladosporium sp	Green mould	Cucumber, peach, plum, grape
Fusarium sp	White/pink rot at stem end	Root vegetables, tomato, banana, orange
Geotrichium sp	Sour rot	Potato, tomato, citrus fruit
Mucor sp	Soft rot	Strawberry
Penicillium sp	Blue/green mould	Most fruits, some vegetables
Phytophthora sp	Downy mildew, brown rots (blight)	Potato, carrot, leafy vegetables, tomato, strawberry, lemon
Rhizopus sp	Soft rot	Many fruit and vegetables
Sclerotina sp	Soft rot (vegetables); Brown rot (fruits)	Many fruit and vegetables

2.5.1.14 Cereals and Bakery products

The low water activity of harvested grain prevents the growth of bacteria.

Bacterial spoilage of bread by *Bacillus sp*, 'rope' is now rare due to chill storage and the use of propionic acid preservative. Spoilage of bread is now normally due to mould growth, black *Rhizopus nigrificans*; green *Penicillium sp* and *Aspergillus sp*; and red *Neurospora sitophila.*

Mould spoilage is also associated with cakes having a high water activity.

2.5.1.15 Beer

Cloudiness in beer, flavour defects and pellicle formation can occur in cask beer due to the outgrowth of yeasts.

2.5.1.16 Frozen Foods

Microbial growth is inhibited at the commercial storage temperature of -18ºC. If frozen foods are allowed to thaw over a long period at, for example, 3-10ºC, psychrotrophs can become predominant and cause spoilage. Particular problems can arise with the thawing of large packs such as turkeys where high temperatures at the surface (>20-25ºC) can cause the rapid outgrowth of bacteria.

In the case of frozen peas, the normal microflora is replaced due to contamination on the production line by lactic acid bacteria such as *Leuconostocs sp* and *Streptococci sp*, The thawed product can spoil due to acid produced from natural sugars, and develop 'vinegary' and 'buttery' odours.

Part 3

Factors Affecting the Growth of Micro-organisms

3.1 Optimum Environments

Each organism has an ideal environment in which it will grow (a fact that is used in their identification and enumeration in the laboratory). Factors that influence the growth of bacteria include pH, water activity, temperature and nutrient levels. There are four phases in the growth cycle of a microbiological population, lag, growth, stationary and death. A useful summary is given on:
http://www.cfsan.fda.gov/~mow/app3.html.

3.2 Bactericidal Treatments of Food and Products

Although heat, cold, dehydration and chemicals all result in stress to bacteria, they can survive this stress by the development of spores or by alternative metabolic changes. Stressed bacteria will still be viable, but may not respond to standard tests, because the shock of treatment with selective media and/or temperature change is the final straw. A more gentle pre-enrichment stage may be necessary to revive them.

3.2.1 Physical

3.2.1.1 Temperature

Heat is an ideal way to kill micro-organisms; with wet heat being more effective for sterilisation than dry heat. Most bacteria begin to die at about 60^{o}C, however, it is necessary to maintain this temperature for a period of time to ensure the destruction of the entire population. In any culture of bacteria, some will die and some will survive, depending on their phase of development, on the surrounding bacteria and the food matrix. This makes the calculation of the ideal temperature to ensure adequate kill a complex matter and computer programs are now available to manufacturers for guidance.

Low temperatures on the other hand can have the opposite effect to that desired. Storage at low temperatures for example will favour psychrophiles such as *Listeria sp*.

Freezing will kill *Campylobacter sp*, but many other types of bacteria will be relatively unaffected. It is the process of freezing and the creation of ice crystals in the bacteria that damages them. Once hard frozen, particularly if surrounded by a suitable nutrient medium, the bacteria will survive indefinitely. For this reason, it is necessary to keep any freeze/thaw cycles of samples for microbiological examination to an absolute minimum.

3.2.1.2 Dehydration

Many products, such as cakes and jams, depend on low water activity (high sugar levels) to inhibit growth of bacteria and moulds. It is important to note, that the overall water activity level of a cake may be adequate, but localised areas under the plastic film wrapping can lead to condensation and patches of mould, unacceptable to consumers, developing in these droplets of water.

3.2.1.3 Irradiation

Ionising irradiation kills bacteria and other micro-organisms by causing DNA damage and production of toxic reactant products. It is permitted for use on foods within the UK through legislation and licence controls. One of the concerns with this technique is that the food, prior to irradiation, may have been of poor microbiological quality and their dead cells and toxins will still be present although the bacterial count is low.

3.2.1.4 Modified Atmospheres

Packaged fresh food is still metabolically active and continues to use up oxygen in the headspace. This increases the carbon dioxide and water levels in the headspace, which encourages the growth of spoilage micro-organisms particularly with fresh fruit. With some foods such as processed meats and cheeses, a high carbon dioxide level may have a beneficial antimicrobial effect. The composition of the atmosphere within retail packages is adjusted to give a gas mix as close as possible to that which will optimise the shelf-life and may consist of a mixture of oxygen, nitrogen, carbon dioxide, ethylene and water vapour.

In these products the composition of the gas is controlled by the rate at which it defuses (or not) through the packaging material. In active packaging systems, the packaging material interacts with the internal gas environment to continually modify the system by removing gases from, or adding gases to, the headspace.

3.2.2 Chemical Additives

Permitted additives can be added specifically for their preserving action and the choice will be related to the specific foodstuff and the target organism most likely to be of concern. For example the microbial activity of benzoic acid is highest at the low pH's of carbonated soft drinks while sorbic acid is more active against moulds up to pH's of 6.5. More information of this aspect can be found in *Handbook of Food Additives.* The permitted additives currently listed in the Miscellaneous Additives Regulations 1995 are:-

Acetic acid	Orthophenyl phenols
Benzoates	Natamycin
Biphenyl	Nitrates/ nitrites
Borax	Nisin
Dimethyl dicarbonate	Para –hydroxybenzoates
Hexamethylene tetramine	Propionates
Lactic acid.	Sorbates
Lysozyme	Sulphur dioxide

Trends towards “additive free” foods are reducing the maximum time between production and consumption of food and consumers need to be aware of the reduced shelf life of such products. The common example of this is preservative free bread, which cannot be stored at room temperature in bread bins in domestically heated kitchens for more than a few days.

3.2.3 Natural Bactericidal Agents

Some food ingredients have natural bactericidal or bacteriostatic properties in food eg salt and alcohol and may have both a flavouring and preserving action depending on the concentration present.

Herb and spice extracts have natural bacteriostatic activity due to the presence of terpenoids and phenolic compounds. These compounds will have a preserving action on the herbs and spices themselves, but will not be present in compound foods at a sufficient level to have a technological function overall.

Traditional methods used to restrict the growth of pathogens include the use of high levels of salt, smoking and dehydration. These techniques are not compatible with the modern trends to low salt and moist foods. Modern equivalents are not so robust and require temperature controlled storage or shorter shelf life.

Similarly the level of other natural bacteriostatic agents in food is reducing due to lifestyle changes. The lowering of acetic acid levels in pickled foods and sugar levels in jam all result in reductions in keeping quality and increase the potential for the growth of pathogenic organisms. Although these products have a long shelf life till opened, they will rapidly deteriorate once micro-organisms enter the container. The after opening shelf life of these products will be much shorter than their predecessors, but this message is not necessarily getting to the consumer.

Bacteriocins are small proteins produced by bacteria mainly lactic acid bacteria, and which kill competing closely related bacteria by attacking cell walls and allowing contents to leach out. The damaged cells are then incapable of producing the energy needed for survival and die. By using these systems, the growth of spoilage and pathogenic micro-organisms in food can be controlled. Nisin, produced by *Lactococcus lactis*, is an example and is active against *Clostridium botulinum* and *Bacillus cereus*. A second example, Piscicolin 126 is produced by the lactic acid bacterium C*arnobacterium piscicola* JG126 and is active against *Listeria monocytogenes*. Only Nisin is permitted in the UK.

3.2.4 Disinfectants

These products are not usually used directly on or in food, although some prepared vegetables can be washed in solutions containing free chlorine to reduce the bacterial load. This can lead to consumer complaints of “chemical taint” in the final product.

Disinfectants are now also being incorporated into products that come into contact with foods, such as chopping boards and plastic bags. This raises two questions:

- whether the disinfectant has any noticeable bacteriocidal effect on the chopping board.

- whether migration of the bactericide into the food takes place.

Part 4

Examination for Micro-organisms

4.1 Sampling Issues

Unlike sampling for chemical analysis, microbiological sampling can carry a high risk of cross contamination. Similarly the potential for sample deterioration between sampling and examination is also higher than with samples for chemical analysis. Practical sampling details for food are given in *Code of Practice No 7* and the LACORS document *Guidance on Food Sampling* for Microbiological Examination. Sampling details for waters can be found in *Microbiology of Drinking Water (2002) – Part 2 – Practices and procedures for sampling,* published by the Environment Agency.

4.2 Basic Techniques

A working knowledge of the following procedures and terms is essential for an understanding of the microbiological examination of foods and waters.

- Most Probable Number (MPN), pour plates, spread plates, spiral plating, membrane filtration, dip slides
- Diluents
- Surface hygiene tests (eg adenosine triphosphate luminesence (ATL), protein tests, swab tests, contact plates
- Stains (eg Gram, spore, capsule) and other microscopical techniques
- Motility tests
- Standard chemical tests (oxidase, Kovacs reagent, catalase)
- Serotyping (immunoassay, latex attached antibodies, magnetic separation)
- Polymerised Chain Reaction (PCR)
- Types of media – enrichment, selective, chromogenic, rapid methods
- Type culture storage and source (cultiloops, frozen bead storage, lenticules, National Collection of Type Culture vials)
- Direct Epifluorescent Filter Technique (DEFT)
- Dye reduction
- Electrical conductance methods

4.3 Methods for Bacterial Identification

A number of hygiene related statutory instruments specify the methods to be used for the microbiological examination of the foods covered by the regulation eg milk and dairy products. When a method is not specified it is preferred to use an internationally accepted validated method published by CEN or ISO. These methods are usually adopted as British Standards and are referenced in the BSI catalogue. For drinking waters the methods published by the Environment Agency with respect to private water supplies can be considered to be the standard against which any alternative method would need to validated for performance.
The following sources are given in alphabetical order. The most appropriate method to use will depend on the circumstances of the investigation being undertaken.

- American Public Health Association
- Association of Agricultural Chemists
- Bhavan's Association of Microbiologists
- British Standards Institute
- Chorleywood and Campden Research Association methods
- Health Canada Compendium of Microbiological Methods
- International Commission on Microbiological Standards for Foods
- Microbiology of Drinking Water (2002) Parts 4 –10 (Environment Agency)
- Ministry of Agriculture Fisheries and Food Methods Manual
- Oxoid or Difco manual
- HPA Standard Operating Procedures
- Statutory Instruments

Part 5

Managing the Microbiology Laboratory

5.1 Setting up a Microbiology Laboratory

Microbiological laboratories investigate and identify micro-organisms that can cause disease in man, animals and plants. Measures are therefore necessary to protect workers from infection and to prevent the escape of infectious material into the community.

There is general agreement that micro-organisms should be classified into hazard groups according to their pathogenicity i.e. ability to cause disease. These groups numbered 1 – 4 in increasing order of hazard are shown in the table below:

Hazard Group / Class	**Definition**	**e.g. test / organism**	**Containment level**
HG1 : harmless	An organism that is most unlikely to cause human disease	total viable counts	1
HG2 : low risk	An organism that may cause human disease and which might be a hazard to laboratory workers but is unlikely to spread to the community. Laboratory exposure rarely produces infection and effective prophylaxis or effective treatment is usually available.	*Clostridium botulinum, Legionella pneumophila, Staphylococcus aureus*	2
HG3 : medium risk	An organism that may cause severe human disease and presents a serious hazard to laboratory workers. It may present a risk of spread to the community but there is usually effective prophylaxis or treatment available.	*Escherichia coli O157:H7, Salmonella typhi, Hepatitis B*	3
HG4: high risk	An organism that causes severe human disease and is a serious hazard to laboratory workers. It may present a high risk of spread to the community and there is usually no effective prophylaxis or treatment	*Lassa fever, Ebola zaire*	4

The hazard group rating for a particular micro-organism can be determined by reference to the Second Supplement to: *Categorisation of biological agents according to hazard and categories of containment (Fourth Edition, 1995)* available from HSE website, Health Directorate, reference C40 02/00 MISC208.

It follows that different levels of "containment" – the total sum of precautions necessary for safe handling - will vary according to the hazard group of the organisms being handled. A summary of containment level requirements is given below:

Containment requirements	Containment levels			
	1	**2**	**3**	**4**
Laboratory site : isolation	No	No	Partial	Yes
Laboratory : sealable for fumigation	No	No	Yes	Yes
Ventilation : inward airflow / negative pressure	Optional	Optional	Yes	Yes
through safety cabinet	No	Optional	Optional	No
mechanical: direct	No	Optional	Optional	No
mechanical: independent ducting	No	No	Optional	Yes
Airlock	No	no	Optional	Yes
Airlock with shower	No	No	No	Yes
Wash basin	Yes	Yes	Yes	Yes
Effluent treatment	No	No	No	Yes
Autoclave site: on site	No	No	No	No
in suite	No	Yes	Yes	No
in lab – free standing	No	No	Optional	No
in lab – double ended	No	No	No	Yes
Microbiological safety cabinet / enclosure	No	Optional	Yes	Yes
Class of cabinet / enclosure	-	Class I	Class I/III	Class III

This scheme was set out by the Advisory Committee on Dangerous Pathogens in their report *Categorisation of pathogens according to hazard and categories of containment (1990) HMSO.*

5.2 Other Design Considerations

Unless the laboratory is self contained and free standing it should be placed on the top floor of buildings.

Ancillary areas such as media preparation and sterilisation, incubation and refrigeration need to be in close proximity to avoid moving infectious or hazardous materials further than is necessary.

Handwashing basins should be available with elbow, wrist or knee operated taps in each room and placed near to the exits.

Although there is no official requirement for air flows in Level 2 laboratories it is highly desirable that it should be from “clean” to “dirty” areas i.e. from corridors and hallways into the laboratory and not in the reverse direction.

A microbiological safety cabinet should be available for physical containment. Most laboratory techniques are known to produce inadvertent aerosols. The cabinets are used as barriers to minimise the hazard of airborne infections by preventing the escape of these aerosols into the laboratory environment. Cabinets are classified as Class I, II or III according to the hazard level of organism to be handled. All involve the use of a High Efficiency Particulate Air (HEPA) filter.

Walls and floors should be smooth and well sealed so as to be easy to clean. Flat surfaces apart from benches and shelves should be avoided as they gather dust and micro-organisms. Benches and floors should be impervious to water, easy to clean and resistant to acids, alkalis, solvents and disinfectants.

One service room should be dedicated to the decontamination of equipment and of infected waste before it is finally disposed of as required by the Department of the Environment and the HSE.

At least one autoclave will be required for sterilisation purposes and this will require regular inspection as a pressure vessel as well as servicing and 12-point calibration (UKAS quality system requirement) to ensure that sterilisation temperatures are achieved throughout the various load types.

Entry, especially to containment level 2 laboratories should be restricted to trained personnel by means of locks or signs. Particular attention should be paid to the training of non-technical cleaning staff.

5.3 Quality Issues

5.3.1 UKAS

The requirements for UKAS laboratory accreditation for microbiological examination are detailed in UKAS document L38 and BS EN ISO 17025:2000. Particular attention usually needs to be given to the quality control aspects of microbiology work in a public analyst laboratory due to the negative nature of most of the results.

5.3.2 Quality Control Schemes

5.3.2.1 Proficiency Test Schemes

Participation in external quality control schemes, where they exist, is an UKAS requirement. Regular trials for a variety of organisms are organised by the following organisations.

- Don Whitley Scientific Ltd
- Food Examination Performance Assessment Scheme (FEPAS)
- Health Protection Agency (Food and Waters)
- Water Authorities

5.3.2.2 Internal

Microbiological quality control rests very strongly on comparison of observed and expected or “acceptable” variability. Microbiological viable count methods measure living organisms. The analyte (the microbial population) takes an active part in the quantitative procedure. The examiner, the bacterial population and the culture medium interact in many ways in the process. As a consequence the results may display variability that is unpredictable but can be understood as a biological phenomenon.

The microbiological laboratory is no different to a chemical laboratory with respect to the requirements for the calibration and monitoring of measuring equipment and temperature measurement. It is particularly important that incubators are temperature profiled to ensure that the tight temperature tolerances required e.g. 37°C ± 0.5°C can be achieved throughout the internal space, especially when the incubator is full of plates. Similarly autoclaves need 12 point calibrations of the load chamber to be established for each of the different sterilisation cycles used.

A number of quality control checks should be carried out with each batch of tests required. These include:

Check	Definition
Parallel plating	a volume of the final test suspension is examined in duplicate
Blank control	a sterile liquid e.g. water or Ringers, is treated to all steps of the examination process
Positive controls	a suspension of the target organism (or a closely related species if target is pathogenic) with an average count of the right order of magnitude is examined. It is important that neither an excessive number nor too small a number of organisms is present
Negative controls	a suspension of a selected non-target organism (usually detailed in the method) that may be present in the sample but which should be successfully distinguished from the target organism by the examination
Standard addition	a positive or negative control organism suspension is added to a sample under investigation and the recovery is evaluated. This procedure is valuable for presence/absence tests where interference is suspected. The introduction of lenticules containing known quantities of micro-organisms is making such recovery exercises more feasible for quantitative procedures
Colony counts	on different volumes e.g. 1ml, 0.1ml, 0.01ml.

The results of internal quality controls should be recorded for each batch and the data plotted on to graphs to provide a visual assessment of any data trends. It is generally recommended that results are converted to the $\log_{10}$ scale before plotting on control charts as the results can range over several orders of magnitude. Constructing the chart on graph paper with a logarithmic scale on the ordinate is a workable procedure for on-going daily recording in the laboratory without recourse to log tables.

One of the major problems for quantitative QC in microbiological analysis is the fact that some methods are highly sensitive to different interpretation e.g. plate counting, colony growth characteristics. It is therefore important that all examiners are involved in the examination of external proficiency testing samples and that regular repeat examinations involving two examiners are carried out.

Part 6

Food Production and Control

6.1 Hazard Analysis Critical Control Point (HACCP)

HACCP is an approach to controlling the areas of food production and storage that present the greatest hazard in terms of potential for microbiological contamination. In basic terms, the process is assessed to establish the points of production where contamination is most likely to occur, and how this contamination can be controlled. Measures are then put in place to ensure elimination of the hazard, and records kept to demonstrate that these controls are being adhered to. Procedures for carrying out an assessment are given in *Hazard Analysis Critical Control Point Evaluations* (WHO). There is also useful information given on the FSA web site.

The Meat (Hazard Analysis Critical Control Point) Regulations 2002 require that meat plant establishments have a hygiene system based on the HACCP principles in place as part of their quality systems. Butcher shops similarly are required to have a HACCP system in place as part of their licensing provision (*Food Safety (General Food Hygiene) Regulations 1995*, Regulation 4A). Other establishments are covered by the general provisions of this latter set of Regulations (Regulation 4(3)).

6.2 Food Complaints

6.2.1 Food Complaints

Many problems that give rise to food complaints, are caused by micro-organisms. Such complaints can be divided into two categories; those that arise from food spoilage and those alleged to have caused food poisoning.

6.2.1.1 Food Spoilage

Food spoilage can be defined as food that is damaged or injured so that it is made undesirable for human consumption and spoilage organisms can bring this about. However, undesirable for human consumption is not necessarily "unfit" for human consumption and "unpalatable" does not necessarily mean "unsafe".

Microbial food spoilage results in deterioration in quality that can be detected visually, and/or organoleptically by the consumer. Visual spoilage can be due to the effect of mould colonisation, slime development or rotting, while organoleptic spoilage can be due to conditions such as souring, taint development or rancidity. Most problems are the result of poor storage conditions, i.e. inadequate temperature control, pest control or atmosphere control (humidity).

The symptoms of microbial food spoilage include:-

- **Mouldiness:** Surface growth of moulds.
- **Rots:** Black rot in eggs, soft rots of fruit and vegetables.
- **Sliminess:** Bacterial slimes on vegetables, meat, fish.

- **Taints and odours:** Products of proteolysis and lipolysis associated with slimes.

- **Abnormal Colours due to Moulds or Bacterial Colonisation:** e.g. *Serratia sp* (red), *Sarcina sp* (yellow), *Pseudomonas sp* (green fluorescence).

- **Ropiness:** Capsulated *Leuconostoc sp* in sugar; rope from starch or protein in bread or milk.

- **Fermentative spoilage:** Souring, acids and alcohols from sugars, gas production. Bone taint by *Clostridia sp* in meat. Fat sours by *Bacillus sp* in canned foods, blown cans by gas producers. 'Wild' fermenters in fermented foods, cheese, beer, etc.

- **Putrefaction:** Anaerobic decomposition of proteins causing foul odours in under-processed, anaerobic packaged canned meat and vegetables.

- **Aerobic hydrolysis:** Bitter flavours from proteins; lipid oxidation and lipid hydrolysis flavour defects, e.g UHT milk.

The rate of deterioration of any product depends on a number of factors, the important ones being the chemical structure, physical characteristics, the type of micro-organisms present, the number of micro-organisms present, temperature, pH and the water activity.

The success of micro-organisms as a whole is due to their ability to utilise a vast range of nutrient sources and to colonise and tolerate even the most extreme of environmental conditions. Individual species of micro-organisms have different growth characteristics; typically Psychotrophs double every 1-2 hours at their optimum temperature, whilst mesophiles and thermophiles double every 15-20 minutes at their optima. Changes of temperature not only affect the rate of growth, but will also affect the balance of microbial flora. Even a small temperature change may encourage the growth of different organisms and hence cause different spoilage characteristics.

6.2.1.2 Food Poisoning

Many consumer complaints relate to food that the consumer alleges to have caused illness and it is often the case that such complaints cannot be substantiated because:

- The original foodstuff is not available, and the control sample provided is satisfactory.

- The consumer has mis-identified the foodstuff that caused them to become ill.

- The illness that the consumer has experienced was not microbiological in nature.

- Spoilage of the food has led the consumer to believe they have suffered from food poisoning symptoms

Organisms that cause food poisoning are called pathogens. These are agents that are capable of producing disease although their ability to produce disease varies. The degree of pathogenicity, or ability to cause infection is termed “virulence”.

The majority of illness complaints are associated with symptoms of vomiting or diarrhoea hence the assumption of microbial pathogen poisoning. However, consumption of spoiled food (rancid) or food containing high numbers of spoilage organisms can also lead to gastric disturbances. Food poisoning can occur as a consequence of the presence of harmful chemical substances and/or micro-organisms. Microbiological food poisoning occurs when food which contains either pathogenic micro-organisms and/or their toxins is consumed. The gastro-intestinal symptoms result from ingestion of a preformed toxin, from a toxin produced within the intestinal tract, or from infection by the pathogen. The onset of symptoms therefore ranges from as little as 2-3 hours in the case of a preformed toxin, to days in the case of microbial infection. The difficulty in identifying the source of infection is therefore self evident.

It should be noted that food poisoning can also occur through non-microbiological activity. For example Scromboid poisoning is caused by high levels of histamine in this family of fish. The source of the poison however is a mute point since the histamine level rises when enzymes produced by bacteria break down the amino acid histadine. The purist may therefore argue that this is also microbiological food poisoning. Symptoms including vomiting, stomach pains and diarrhoea can occur immediately, to several hours after consumption where high levels of histamine are involved. They usually last for a few hours but in some cases can last several days.

6.2.2 Investigation of Complaints

When investigating a complaint it is important that consideration is given as to whether or not the complaint could be due to the action of micro-organisms.

Some forms of microbial spoilage are more easily assessed by chemical analysis, eg souring of milk by measurement of acidity, putrefaction of meat by TVN. In such cases, any microbiological testing is not particularly meaningful, other than to confirm that micro-organisms are present in high numbers. In the case of spoiled milk, a gram stain can indicate whether spoilage is due to a monoculture, suggesting survival of thermophiles through pasteurisation, or a mixed culture, suggesting post pasteurisation contamination.

Techniques that may be used include both quantitative and qualitative methods. These may involve the use of non-selective or selective media. For some qualitative examinations, a selective enrichment process may be used to increase the number of the target organism, before a selective media is used to demonstrate whether the organism is present.

When investigating food complaints, consideration should be given to carrying out both chemical analysis and microbiological examination as one set of results can compliment or reinforce the other.

6.3 Food Poisoning Outbreaks

Food poisoning incidents may affect any number of individuals. An Outbreak is defined either as “two or more linked cases of the same illness” or “as a situation when the observed number of cases of an illness exceeds the expected number”.

NHS Boards are responsible for investigating food poisoning outbreaks and usually initialise the setting up of an Outbreak Control Team (OCT), the role of which is to :-

- Reduce the number of cases by promptly recognising the incident, defining how cases have been exposed to the hazard, identifying and controlling the source of that exposure.

- Minimise the mortality and morbidity by arranging care of those affected.

- Keep the public/media informed of risks and how to minimise these risks.

- Collect information to prevent and manage future incidents.

The team usually comprises a consultant in public health medicine, a clinical microbiologist and local authority chief environmental health officer. Other EHO’s and medical personnel may also join the team depending on the circumstances. Also whilst fulfilling this remit, the team may liase with other agencies, including public analysts laboratories in circumstances where food samples are available for chemical testing and/or microbiological examination.

Information obtained from analysis and/or examination of food samples is not essential to the success of the OCT in managing the outbreak. In many cases, appropriate food samples cannot be obtained. The OCT operates by forming a hypothesis based on all the information available including interviews with the victims and their relations. Results of clinical samples examined in medical laboratories would add to the weight of evidence supporting a given hypothesis. The results of examination of food samples can be the “icing on the cake” which completes the investigation.

Microbiological examination of food samples is normally carried out based on the known facts relating to the incident. This may include:

- Information relating to the foodstuffs considered to be implicated. Many pathogenic organisms are known to be associated with particular foods, eg *Bacillus cereus* is often associated with cooked rice dishes.

- Information relating to the symptoms displayed, eg bloody diarrhoea is associated with *Campylobacter sp* and with *Escherichia coli O157.*

- Information relating to the onset of symptoms, eg where onset of symptoms occurs within a few hours of consumption, organisms such as *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* are often implicated, whilst organisms such as *Campylobacter jejuni* can take up to 10 days for symptoms to develop.

Examination techniques used may range from enumeration on an appropriate selective agar to detection using a combination of enrichment, selective enrichment and selective agars as appropriate.

The routine testing for pathogens is usually confined to organisms presenting a moderate risk, eg *Salmonella typhimurium*, *Bacillus cereus*, *Staphylococcus aureus*, *Campylobacter sp*, *Clostridium perfringens* and *Vibrio parahaemolyticus*. The following bacteria are Category 3 pathogens and as such should only be handled by experienced microbiologists in a laboratory operating at Containment Level 3: *Bacillus anthracis, Salmonella typhi, Shigella sp, Vibrio cholerae* and *Brucella melitensis.* If these organisms are found, the sample should be sealed immediately, all plates containing the sample should be destroyed by autoclaving, and the original material and details of its source referred to a public health laboratory immediately. (*Escherichia* coli *O157* is also a Category 3 organism, but a derogation exists which allows laboratories with Category 2 facilities to perform routine screening for the organism.)

Indicator organisms include species such as *Bacillus cereus* and *Staphylococcus aureus* which occur more or less ubiquitously. In small numbers these organisms are not a problem, although they may proliferate under conditions of poor storage. In large numbers they suggest that the food was prepared under conditions of poor hygiene, and that spoilage or food poisoning problems will occur sooner rather than later.

Staphylococcus aureus may occur as an indicator of generally unhygienic conditions, or it may produce Staphylococcal enterotoxin causing very rapid symptoms (two hours) of gastro-enteritis. Similarly *Escherichia coli* can cause an infection, be toxigenic, or be an indicator of faecal pollution while *Bacillus cereus* is a pathogen causing food poisoning when found in large numbers in rice. This latter organism however is not generally associated with gastric problems when found on other foods, e.g. cream.

6.4 Shelf Life Determination

Shelf life represents the useful storage life of food. At the end of its shelf life, food is developing characteristics eg changes in taste, aroma, texture or appearance that are deemed unacceptable or undesirable. The underlying cause for the change may be chemical eg rancidity, physical eg freezer burn or microbiological.

Establishing the microbiological shelf life for many foods is important at all stages in their history including product design, formulation change, packaging or storage change and changes in microbiological criteria. Regardless of the when or why, numerous variables must be considered in the experimental design of the shelf-life study to approach a useful result.

Accurate prediction of shelf life necessitates a carefully planned and executed series of experimental studies. Shelf life should be re-evaluated in the event ingredient, formulation, processing, packaging or storage changes are anticipated. The knowledge gained from these studies reliably promotes confidence that the product delivered to the customer is safe and of high quality and that the product remains consistent over time with respect to spoilage rate.
For the delivery of a product with maximum quality, the shelf life of a product should be determined by organoleptic evaluation and microbiological examination.

6.4.1 Organoleptic Evaluation

The taste, odour and appearance of a food are the ultimate criteria used by consumers to judge a food's acceptability. In the laboratory, organoleptic evaluation of a food is also used as a direct method for determining shelf life. The food is prepared and periodically examined for changes in appearance, aroma, texture and taste until it becomes unacceptable. The organoleptic determination is easily accomplished by those familiar with the product's desired characteristics. Shelf life based on organoleptic analysis, however, may vary significantly from consumer to consumer, since tastes, expectations and ability to detect changes differ greatly.

The organoleptic quality of food changes as its micro-flora grow and metabolise available nutrients. The sensory changes at first might be subtle, but they eventually make the food unacceptable. Generally, sensory changes are not detectable until the microbial population is high. The number of organisms required to cause spoilage varies with the food item and the type(s) of micro-organisms growing in it.

Shelf life may be estimated on the basis of microbial density. As a rule of thumb, 10 million bacteria per gram, 100,000 yeast per gram, or visible mould, signal the end of microbiological shelf life. Noticeable degradation of the product is likely at these levels. Whether the changes are acceptable is determined by the organoleptic evaluation.

6.4.2 Microbiological Examination

High numbers of micro-organisms are normal in certain foods, but indicate deterioration in other foods. Therefore, it is desirable to know, even in the absence of objectionable organoleptic changes, the microbiological state of food as it nears the end of its shelf life.

Microbial growth in foods for estimation of shelf life is most commonly monitored using agar plating procedures. The procedures are quantitative for the number of viable organisms present at the time of examination. Because of differences in growth requirements among the different types of micro-organisms that may be found in food, no single procedure is available to enumerate all micro-organisms. Although a simple, useful procedure is the aerobic plate count, usually over an incubation temperature of 30°C for 72 hours, many organisms are not detected using this incubation profile. These include organisms that grow only at low or high temperatures, most lactic acid bacteria, strict anaerobes and yeast and mould. Thus, the plating procedures are usually selected on the basis of the type or types of organisms anticipated or known to be present in the food. If the "right" procedures are not selected, it is very possible to have obvious microbiological spoilage, but no experimental data to support the organoleptic observations.

During the time the micro-organisms in food are in the lag phase, the food appears to be microbiologically stable. Once the cells enter into the growth phase and begin to multiply, the product begins to change and is considered unstable. At some point along the microbial growth curve, the food usually will spoil. Therefore, for shelf life, the significant points concerning the microbial growth cycle are the duration of the lag phase, the growth rate and the microbial count at the end of the growth phase. The end of shelf life usually occurs near the end of the growth phase.

How often a food is examined for micro-organisms during the shelf-life study must be decided with care in order to detect significant microbiological events. To identify the

different transition points along the growth path, the food is sampled periodically to quantify the number of organisms present. An excessive period between samplings increases the risk of under- or over-estimating shelf life. The more examinations that are completed, the more accurate will be the shelf-life determination. For most foods, the anticipated shelf-life time is divided into 5 to 12 intervals for sample collection and examination. The number of intervals chosen is generally an estimation based on experience with similar foods.

The distribution of micro-organisms in a sample of food, or even between samples of food from the same production lot, is not necessarily uniform. For example, if 1 in 5 bottles of food solution contains a spoilage organism, then only 1 of 5 may display evidence of spoilage. Similarly, a spoilage organism present in a solid or viscous food may exhibit localised spoilage while another area without the organism is free of spoilage. Sampling plans must take into account the possible distribution of micro-organisms within the lot, with types with even microbiological distributions requiring fewer samples. Generally, at least three carefully selected samples of a heterogeneous product are needed to obtain an acceptable representation of microbiological activity in the product. Sample numbers are especially critical when the initial levels are lower than 10 cells per gram, which is the normal sensitivity of the agar plating procedures used for examinations. Single packages represent the most easily distinguishable analytical unit.

A shelf-life study conducted on a single batch of food is valid for that food and any other production lot that is identical. If the micro-organism type or number differs significantly among batches, shelf-life duration may differ. Replication of the study will enhance the accuracy of the prediction.

6.4.3 Designing a shelf-life study

Shelf-life studies for each product should be designed specifically for that product because of the number of variables that must be considered. These include temperature, water content, time, types of micro-organisms, suitability of analyses, sampling and replication.

6.4.3.1 Temperature

Of the factors influencing microbial growth eg water, acidity, temperature, nutrients, preservatives and atmosphere all but temperature become essentially fixed at the time of product formulation, processing and packaging. Normally, these factors are not intentionally altered in a shelf-life study. Storage temperature usually determines the length of microbiological shelf life of perishable foods.

Each species of organism has a different minimum, optimum and maximum growth temperature range. Moreover, differences may be observed among isolates of the same species. The important point about temperature and growth is that when the storage temperature of a product changes, not only does the shelf life change, but the types of spoilage flora also will likely change. Small changes in storage temperature may have a significant effect on shelf life. A few degrees may determine the difference between good shelf life and premature spoilage.

The most useful shelf-life information is obtained for a product kept at its intended storage temperature. However refrigerator and room temperatures are not standardised (eg refrigeration can mean anything from -3° to 10°C). Shelf life at -3°C may be very different from that at 10°C, and to produce a meaningful study and to compare different studies, temperatures used must be known. Temperatures of 4°C and 24°C are therefore commonly used for refrigerator and room temperature storage, respectively.

Although food is subjected to temperature cycles during distribution and retail presentation, temperature cycling in laboratory studies makes data interpretation difficult, and is not recommended. A significantly better understanding of shelf life can be obtained when several storage temperatures are used.

6.4.3.2 Water

Water level determines the characteristics of many foods. Some foods are expected to be dry, some appear moist, and others obviously contain water. Water is essential for microbial growth, and if the amount of free water changes, a food's susceptibility to spoilage may change. For example, if a dry product that is resistant to spoilage becomes damp, it will likely spoil. In contrast, a moist food will not spoil if it dries.

Food packaging plays an essential role in the control of moisture, and has a significant effect on shelf life. There is exchange of moisture between the atmosphere and the food. This exchange continues until the food reaches equilibrium with the atmosphere. Hermetically sealed packages contain a limited amount of air, and the smaller the headspace, the quicker equilibrium is attained between food and air. For hermetically sealed samples, humidity control need not be considered as a study variable as long as the package remains intact.

Most foods are packaged so that little moisture exchange occurs during the life of the product. Thus, humidity control and/or monitoring is required mainly for foods that are subjected to temperature extremes, exposed to the atmosphere (such as cakes, pies and pastries), or packaged in air-permeable containers. Relative humidities of 40%, 60% and 80% represent a practical range for experimentation.

6.4.3.3 Duration

A study's duration should at least match the target shelf life for the food being considered. Conversely, a study may be designed to exceed the shelf-life goal if expectations are met and the point of spoilage needs to be determined.

If a product fails halfway through a study, there is little point in continuing with the trial. On the other hand, if the product is stable during one segment of a study eg no microbial activity is observed, the trial should be continued to the next segment. Sterile products do not require repeated testing beyond the time expected for outgrowth of any contaminating micro-organisms. It is not unusual for microbial levels to stay constant, and even decrease, over a period of hours, days or weeks before beginning to increase.

The microbiological shelf life of a food to be stored at one temperature cannot be used to extrapolate the shelf life at other storage temperatures. Each storage temperature must be trialled separately.

An organism that grows at one temperature may not grow, or may not be the main spoilage organism, at another. Accelerated microbiological shelf-life predictions are therefore not useful and full term studies are the only reliable predictors of shelf life.

6.4.3.4 Challenge Testing

A food may exhibit an exceptionally long shelf life even though conditions permit microbial growth. This may result from the absence of micro-organisms in the samples tested, or because the contaminating organisms will not grow in the particular product formulation. Understanding the stability of these foods in the event of a chance contamination requires a microbiological shelf-life study in which a product is challenged by inoculating it with appropriate spoilage organisms, thus adding several more variables to the study.

The product is inoculated with known spoilage micro-organisms and treated and stored in accordance with the shelf-life study guidelines. The types of organisms and the number of strains of each type, as well as inoculation levels need to be decided. The spoilage organism used is usually one that has been isolated previously from similar foods that have spoiled eg lactobacilli and yeast are the most common spoilage organisms of salad dressings and sauces. The more isolates included in the challenge study, the greater will be the confidence in the accuracy of the shelf-life assessment. In practice, 5 isolates of *lactobacillus sp.*, 5 of yeast, and 5 of mould represent a reasonable selection for a salad-dressing challenge study. Such is the sensitivity of micro-organisms to their environment that the organisms' preparation conditions must be carefully chosen to account for a study's specific needs.

Contamination of product by both un-adapted and adapted organisms can occur. The use of organisms that are not specifically adapted for growth in the product simulates organisms originating in the environment and entering the food through contact. Adaptation simulates product-to-product contamination.

The number of organisms added to the food is generally significantly higher than what would normally be found as a result of contamination during processing and are generally greater than 10 per gram, offering easy observation of the presence of the challenge organisms. If the sample has a low initial inoculation level and die-off occurs, one might incorrectly conclude that the product is stable. Using high inoculation levels will prevent this error. A level of about 10,000 cells per gram is useful for observing either decreases or increases in levels, even if an initial 100-fold die-off is observed. Lower levels can be detected, but usually at significantly greater expense and with lower accuracy.

When the level of the challenge organisms does not increase during shelf-life storage, the product formulation is resistant to that microbial growth. However, if the organisms are present in sufficient numbers, it is still possible that the metabolic activity of the non-growing cells will cause undesirable changes in the product.

Challenge studies using pathogens are conducted to measure the behaviour of those micro-organisms in foods. If the pathogens do not grow, the food is considered stable with respect to the ability of the food system to inhibit their growth. Commonly used pathogens are *Salmonella sp, Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Clostridium perfringens, Yersinia enterocolitica, Clostridium botulinum* and *Escherichia coli.*

6.4.4 Mathematical Modelling

The various factors influencing microbial growth are easily measured and by collecting sufficient data it is possible to derive a mathematical equation of growth. This could allow a quick estimation of shelf life by entering the required variables for the food into the equation. Mathematical models that include some of the growth-controlling variables for bacterial pathogens are available, but are not necessarily accurate when applied to foods. They are useful for “what if" scenarios, such as: "How much longer is the growth of *Salmonella sp* delayed by decreasing the pH of the food from 5.6 to 5.4?" Future refinements will most certainly make models more useful but much further work is necessary before they can reliably replace experimental shelf-life studies.

Part 7

Report Writing

7.1 Microbiological Standards and Guidelines

7.1.1 Food

7.1.1.1 Food Safety Act

This Act defines food as including:-

- Drink
- Articles and substances of no nutritional value which are used for human consumption
- Chewing gum and other products of a like nature and use
- Articles and substances used as ingredients in the preparation of food

In general terms the Food Safety Act 1990 provides as follows:

Section 8

- Any person who sells for human consumption …….any food which fails to comply with food safety requirements, shall be guilty of an offence.

- Food fails to comply with food safety requirements if …..(b) it is unfit for human consumption; (c) it is so contaminated (whether by extraneous matter or otherwise) that it would not be reasonable to expect it to be used for human consumption in that state.

- Where any food which fails to comply with food safety requirements is part of a batch, lot or consignment of food of the same class or description, it shall be presumed …….until the contrary is proved, that all the food in that batch, lot or consignment fails to comply with those requirements.

Section 14

- Any person who sells to the purchaser's prejudice any food which is not of the nature or substance or quality demanded by the purchaser shall be guilty of an offence.

7.1.1.2 Regulations

There are several sets of Legislation which set out microbiological standards for certain types of food:-

- Minced Meat and Meat Products Preparation (Hygiene) Regulations 1995 SI 1995/3205

- Egg Products Regulations 1993 SI 1993/1520

- Food Safety (Fisheries Products and live Shellfish) (Hygiene) Regulations 1998 SI 1998/994

- Dairy Products (Hygiene) Regulations 1995 SI 1995/1086

For some organisms eg *Salmonella sp* the standard is "absent" and no enumeration is needed. If the organism is present on the culture medium then the sample has failed the standard. Some organisms have a less than figure such as *Escherichia coli* in shellfish where the standard is <230 colonies/100g. Some standards eg *Staphylococcus aureus* in raw goats' milk are set out in threshold fashion with a number of units in a sample being above a limit and the sample still being acceptable. The legislation is formatted through n, c, m and M where:-

n number of sample units comprising the sample
c number of sample units where the bacteria count may be between m and M, the sample being considered acceptable if the bacterial count of other sample units is m or less
m threshold value for the number of bacteria, the sample is considered satisfactory if the number of bacteria in all sample units does not exceed m
M maximum value for the number of bacteria, the sample is considered unsatisfactory if the number of bacteria in one or more sample units is at or exceeds M

In the raw goats' milk example n = 5, c = 2, m = 500 and M = 2000. A sample therefore consists of 5 units (eg cartons) of milk and the sample examination can have three possible outcomes:-

i) If all five cartons have a bacterial count of 500/ml or less then the sample is satisfactory.

ii) If 1 or 2 units have a bacterial count between 501/ml and 2000/ml, and the other units have counts of 500/ml or less, then the sample is acceptable.

iii) If one unit has a count of 2001/ml or more, then the sample fails, no matter how low the counts are in the other cartons of the sample.

A list of legislation dealing with food hygiene issues is given in Appendix 1.

7.1.1.3 Health Protection Agency

The HPA has published guidance in the form of *Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale – 2000***.** These list a range of foodstuffs in 5 categories and set out the Aerobic Colony Count (ACC) for each category.

Ready-to-eat foods are categorised according to their susceptibility to microbial growth:

1 Beefburgers, meat pies, sausage rolls, scotch egg, raw pickled fish, mousse/dessert, bhaji.

2 Faggots, kebabs, meat meals (shepherds pie, casseroles), unsliced poultry, sausages, cakes, pastries, desserts (no dairy cream), tarts, flans, pies, cheese based bakery products, flan/quiche, mayonnaise/dressings, samosa, vegetables and vegetable meals, ice cream, non dairy milk shakes, pasta/pizza, ready-to-eat meals in general.

3 Sliced meat (beef, pork, poultry), crustaceans, cooked fish, seafood meals, cakes, pastries, desserts (with dairy cream), trifle, pate, satay, spring rolls, coleslaw, dried fruit and vegetables, rice.

4 Brawn, sliced meat (ham, tongue), tripe and other offal, molluscs, smoked fish, taramosalata, houmous and dips, prepared salads, sandwiches (no salad).

5 Raw ham (Parma), salami and fermented meats, smoked sausages, cheesecake, bean curd, fresh fruit and vegetables, cheese, yoghurt, sandwiches (with cheese/salad).

The guidelines are given in the following table:-

Food Category	Parameter	Microbiological quality (cfu/g unless stated)			
		Satisfactory	Acceptable	Unsatisfactory	Unacceptable/ Potentially hazardous
Aerobic Colony Count 30ºC/48hrs					
1		$<10^3$	10^3 - 10^4	$>10^4$	N/A
2		$<10^4$	10^4 - 10^5	$>10^5$	N/A
3		$<10^5$	10^5 - 10^6	$>10^6$	N/A
4		$<10^6$	10^6 - 10^7	$>10^7$	N/A
5		N/A	N/A	N/A	N/A
Indicator organisms					
1 - 5	*Enterobact-eriaceae sp*	$<10^2$	10^2 - 10^4	10^4	N/A
1 – 5	*Escherichia coli*	<20	20 - 10^2	$>10^2$	N/A
1 – 5	*Listeria sp*	<20	20 - 10^2	$>10^2$	N/A
Pathogens					
1 - 5	*Salmonella sp*	ND in 25g			Present in 25g
1 - 5	*Campylobacter sp*	ND in 25g			Present in 25g
1 - 5	*Escherichia coli O157* and other *VTEC sp*	ND in 25g			Present in 25g
1 - 5	*Vibrio cholera*	ND in 25g			Present in 25g
1 - 5	*Vibrio parahaemo-lyticus*	<20	20 - 10^2	10^2 - 10^3	$>10^3$
1 - 5	*Listeria mono-cyogenes*	<20	20 - 10^2	N/A	$>10^2$
1 - 5	*Staphylococcus aureus*	<20	20 - 10^2	10^2 - 10^4	$>10^4$
1 - 5	*Clostridium perfringens*	<20	20 - 10^2	10^2 - 10^4	$>10^4$
1 - 5	*Bacillus cereus* and other pathogenic *Bacillus sp*	$<10^3$	10^3 - 10^4	10^4 - 10^5	$>10^5$

The guidelines recognise that foods in category 5, by their nature, cannot have upper limits for ACC.

High ACC, in the absence of other quality criteria such as chemical indicators of spoilage, cannot be used as the basis for a prosecution.

All these guidelines must be interpreted with great care. For example, *Staphylococcus aureus* levels in excess of 10^4 cfu/g are stated to be unacceptable/potentially hazardous, but levels of 10^6 cfu/g are needed to produce Staphylococcal toxin. There is no indication in the guidelines of the equivalence to 'safety' or 'quality', as required by the Food Safety Act 1990.

7.1.1.4 Institute of Food Science and Technology (IFST)

The Institute of Food Science and Technology has issued guidance in their publication *Development and Use of Microbiological Criteria for Foods*.

The monograph has chapters which include:

- Factors affecting the microbiology of foods
- Sampling for microbiological testing
- Considerations in choosing microbiological methods
- Food categories (frozen, dried/ambient stable, fresh/chilled, heat treated, fermented foods)
- Microbiological limits

The criteria in this monograph differ from the HPA guidelines in that they approach the subject from the point of view of the practical food microbiologist rather than the enforcement scientist. They are based on Good Manufacturing Practice (GMP). To that end, the criteria for individual foods or groups of foods relate to pathogens and to indicator and spoilage organisms and provide levels for good manufacturing practice and an upper limit. Where appropriate, the criteria also include limits for mycotoxins, histamine and amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP) and paralytic shellfish poisoning (PSP).

Some examples of the IFST criteria are (all figures per g or ml, unless otherwise stated):-

Raw Meat

Examples of products in this category are, meat joints, mince, diced meats, offal, burgers, sausages, bacon, marinaded products, and cured products.

In terms of pathogenic organisms, criteria for absence is not generally applicable. *Salmonella sp, Campylobacter sp, Escherichia coli O157* and parasites may be present, while *Yersinia enterocolitica* may be important in pork; and *Staphylococcus aureus* in bacon. Monitoring the incidence of bacterial pathogens may be useful for trend analysis.

Indicators and spoilage organisms of interest are as follows:-

	GMP	Maximum
Aerobic Plate Count (APC) (indicator of quality)	$<10^4$	10^7
Escherichia coli (indicator of hygienic slaughter practice)	$<10^2$	10^4
Yeasts (sausages and Marinated products)	$<10^4$	10^6

Fruit and Fruit Juices

Examples of products in this category are, whole, sliced or chopped fruits, freshly squeezed juices, pasteurised juices and fruit cocktail.

In terms of pathogenic organisms, the presence and growth in acidic environments is unlikely, although *Salmonella sp* and *Escherichia coli O157* may survive. *Escherichia coli* may grow on apple substrates and mycotoxin monitoring (patulin) may be appropriate. Organisms/ toxins of interest are:

	GMP	Maximum
Criteria for absence of pathogens in products not generally applicable.		
Salmonella sp – chilled desserts (non heat processed fruit)	ND in 25g	ND in 25g
Listeria monocytogenes – chilled desserts (non heat processed fruit)	ND in 25g	10^3
Patulin - apple juice	ND in 25g	50ppb

Indicator and spoilage organisms of interest are:

Yeasts	GMP	Maximum
Chilled desserts unpasteurised	$<10^3$	10^6
Fruit juice unpasteurised	$<10^3$	10^6
Fruit juice pasteurised	10	10^3

Processed Foods

Examples of products in this category are, Ready meals, cooked meats and fish products, pies, pasties, quiches, flans, sandwiches, sous vide products, fermented meats, cured meats, desserts and moist bakery products.

Pathogens and toxins of interest are:

	GMP	Maximum
Salmonella sp	ND in 25g	ND in 25g
Listeria monocytogenes	ND in 25g	10^3
Clostridium perfringens – cooked meat, vegetables and pulses –	$<10^2$	10^3
Bacillus cereus – rice products, spiced products and bakery products	$<10^2$	10^4
Staphylococcus aureus	20	10^3
Vibrio parahaemolyticus – warm water fish	ND in 25g	10^2
Escherichia coli O157 – raw fermented meats	ND in 25g	ND in 25g
Histamine - scromboid fish	<5mg / 100g	5mg / 100g
PSP – bivalve mollusc flesh	ND / 100g	<80g / 100g
DSP– bivalve mollusc flesh	ND in bioassay	ND in bioassay
ASP – edible parts of molluscs	Must not exceed 20 ug domoic acid/ g by HPLC	

Indicators and spoilage organisms of interest are:

APC- fermented products	No criteria for live fermented products	
APC – non-fermented products	$<10^4$	Variable dependant on product
APC – cooked sliced meats	Possibly up to 10^8 on storage due to lactic acid bacteria	
Enterobacteriaceae sp (hygienic practice)	$<10^2$	10^4
Escherichia coli sp	<10	10^3
Gram negative *Bacillus sp.* (spoilage)		10^7

7.1.2 Waters

Standards for potable waters are contained in three sets of legislation:-

- The Water Supply (Water Quality) Regulations 2000, which deal with mains supply
- The Private Waters Supply Regulations 1991, which deal with water supplied to domestic or business premises other than by a mains supply or in a container
- The Natural Mineral Water, Spring Water and Bottled Drinking Water Regulations 1999, which deal with water supplied in containers for human consumption

The Food Safety Act 1990 amended the Water Act 1989 to include "food production purposes" in terms of the quality of water used at such premises, through either a mains or private supply. Food production purposes are defined as water use in the manufacture, processing, preserving or marketing of the food for consumption off the premises. There is an exemption contained in the second set of Regulations above for water used solely for crop washing after harvesting as long as the quality of the final food or drink product is not compromised.

7.1.3 Agriculture

The Feeding Stuffs Regulations 2000 set limits on the level of rye ergot (a hardened mass of mould mycelium) in certain feeds and also for Aflatoxin B_1 in nut based feeds. Other than these, there are no microbiological standards set for animal feeding stuffs and it is left to the Agricultural Analyst to assess results on an individual basis, having regard to the debilitating effect on the animal in question. The specifics of each case require attention. For example would the presence of *Salmonella* in a chicken feed be reported against? The reporter has to rely on the generality of Section 73A of the Agricultural Act, which creates the offence of "unwholesome" of a feeding stuff. This can be taken to mean not favourable to, or promoting good health in animals.

7.2 Reporting Comments

7.2.1 Food

The following are the types of comments that would be used when reporting formally on a food failing to meet guidelines or standards.

7.2.1.1 Ready to Eat Food

Results of examination of a sample of cooked beefburger :-

Total Viable Colony Count (30^0C)	2.3×10^6 colony forming units per gram
Escherichia coli	50 colony forming units per gram

Comments :

In my opinion, having considered the Health Protection Agency Guidelines for Ready to Eat Foods (September 2000), a ready to eat beefburger must have a total viable colony count at 30^0C of not more than 1×10^4 colony forming units per gram.

The sample total viable colony count at 30^0C exceeded this level and therefore was of unsatisfactory microbiological quality.

I am further of the opinion having considered the Health Protection Agency Guidelines for Ready to Eat Foods (September 2000), that a ready to eat beefburger must have an *Escherichia coli* count of less that 20 colony forming units per gram to be of satisfactory microbiological quality, but that counts of not greater than 100 colony forming units per gram are of acceptable microbiological quality.

The sample *Escherichia coli* count exceeded 20 colony forming units per gram but was less than 100 colony forming units per gram. In my opinion this indicates that there may have been a problem associated with hygiene in the production and/or at retail sale of the food.

I am therefore of the opinion that the sample was not of the quality demanded by the purchaser within the meaning of Section 14 of the Act.

7.2.1.2 Shellfish

Results of examination of a sample of live muscles :

Salmonella 5 colony forming units per 25 grams flesh

Comments:

Under the terms of the Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations 1998, live muscles for human consumption must not contain the *Salmonella* organism.

The sample contained 5 *Salmonella* colony forming units per 25 grams of flesh.

I am therefore of the opinion that the sample was of unsatisfactory microbiological quality and unfit for human consumption within the meaning of Section 8 of the Act.

7.2.1.3 Milk

Results of examination of a formal pasteurised milk sample submitted as five units:-

Coliforms	Unit 1	0	colony forming units per millilitre
	Unit 2	2	colony forming units per millilitre
	Unit 3	0	colony forming units per millilitre
	Unit 4	1	colony forming units per millilitre
	Unit 5	0	colony forming units per millilitre

Comments:

Under the terms of the Dairy Products (Hygiene) Regulations 1995, a sample of pasteurised milk taken for examination must comprise of 5 units which are examined individually.

Also under the terms of these Regulations, when examined individually, a maximum of 1 unit is permitted to have a coliform count of greater than 0 colony forming units per millilitre but must not be greater than 5 colony forming units per millilitre.

The sample contained 2 units with a coliform count of greater than 0 colony forming units per millilitre and not greater than 5 colony forming units per millilitre.

I my opinion therefore, the sample was of unsatisfactory microbiological quality with respect to the stated Regulations.

7.2.1.4 Complaint of Mould on Food

Results of examination of a specimen of liver sausage with a complaint of the presence of mould.

Comments:

The sausage was, as received, contained in a piece of plastic film, in a clear plastic bag closed with a security tie marked “FDS3398”.

On the end face of the sausage was an area of approximately 1.7cm x 0.9cm which consisted of material of different colour from the rest of the sausage. The area consisted of three interlinked circles with blue centres and white surrounds. This material was identified as viable *Penicillium* mould.

I am therefore of the opinion that the liver sausage, as received, was so contaminated that it would not be reasonable to expect it to be consumed in that state, within the meaning of Section 8 of the Food Safety Act 1990.

7.2.2 Water

7.2.2.1 Drinking Water

Results of examination of a sample of tap water:-

Escherichia coli	10	colony forming units per 100 millilitres.

Comments:

Under the terms of the Water Supply (Water Quality) Regulations 2000, water supplied for human consumption, by a water authority, must not contain any *Escherichia coli* colony forming units per 100 millilitres at the consumers' tap.

The sample contained 10 *Escherichia coli* colony forming units per 100 millilitres of water and therefore in my opinion was of unsatisfactory microbiological quality with respect to the stated standard.

7.2.3 Agriculture

7.2.3.1 Feedingstuff

The results of examination of a sample of compound feeding stuff for feed to cows :-

Total coliforms	200 colony forming units per gram
Escherichia coli	50 colony forming units per gram
Faecal Streptococci	20 colony forming units per gram

Comments:

The above Act requires an animal feeding stuff to be wholesome such that it is not unfavourable to good health in animals.

In my opinion, the results of examination indicate the presence of faecal matter in the sample, this being detrimental to the health of the animal if consumed.

I am further of the opinion therefore that the sample is not wholesome within the meaning of Section 73A of the Act.

Part 8

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Appendix 1

Legislation Dealing with Hygiene Issues in Food

1959	No	277	Milk and Dairies (General) Regulations
1959	No	734	Ice-Cream (Heat Treatment etc) Regulations
1982	No	1727	Food (Revision of Penalties) Regulations
1984	No	1918	Imported Food Regulations
1985	No	67	Food (Revision of Penalties) Regulations
1990	No	1323	Ungraded Eggs (Hygiene) Regulations
1993	No	1520	Egg Products Regulations
1994	No	3082	Meat Products (Hygiene) Regulations
1995	No	539	Fresh Meat (Hygiene and Inspection) Regulations
1995	No	540	Poultry Meat, Farmed Game Bird Meat and Rabbit Meat (Hygiene and Inspection) Regulations
1995	No	1086	Dairy Products (Hygiene) Regulations
1995	No	1763	Food Safety (General Food Hygiene)
1995	No	2148	Wild Game Meat (Hygiene and Inspection) Regulations
1995	No	3205	Minced Meat and Meat Preparations (Hygiene) Regulations
1998	No	994	Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations
1998	No	2095	Meat (Hygiene and Inspection) (Charges) Regulations
2002	No	889	Meat Hazard Analysis Critical Control Point Regulations

Appendix 2

British Standards Containing Methods of Examination

BS 4285	Microbiological examination for dairy purposes.
BS 4833	Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of micro-organisms. Colony-count technique at 30°C.
BS 5763	Methods for microbiological examination of food and animal feeding stuffs.
BS 6579	Microbiology of food and animal feeding stuffs. Horizontal method for the determination of salmonella spp.
BS 6887	Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.
BS 6888	Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase positive staphylococci.
BS 7850	Methods for microbiological examination of meat and meat products
BS 7932	Microbiology. General guidance for the enumeration of Bacillus cereus. Colony count technique at 30°C.
BS 8261	Milk and milk products. General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination.
BS 11290	Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of Listeria monocytogenes.
BS 11866	Milk and milk products. Enumeration and presumptive Escherichia coli.
BS 15214	Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of mesophilic lactic acid bacteria. Colony count technique at 30°C.
BS 16649	Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of β-glucuronidase- positive Escherichia coli.
BS 16654	Microbiology of food and animal feeding stuffs. Horizontal method for the detection of Escherichia coli O157.
BS 17410	Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of psychrotrophic micro-organisms.