Part I

General issues

1

Microbiological analysis and food safety management: GMP and HACCP systems

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1.1 Introduction

There are two different approaches to deliver food safety, Quality Control (QC) and Quality Assurance (QA). Both systems share tools, but the emphasis is very different. Both approaches are legitimate, but they need totally different organisations, structures, skills, resource and ways of working (Kilsby, 2001). QC is a reactive approach influenced by the pressures in the external world. In a QC organisation the emphasis is on measurement, which needs to be robust and statistically relevant, and the focus is on legal and commercial issues. In contrast, QA is a preventative approach driven by the company's internal standards. The emphasis is on operational procedures, which must be robust and regularly reviewed, and the focus is on the consumer.

There are several problems associated with relying on testing for product safety assurance (van Schothorst and Jongeneel, 1994). In order to apply any statistical interpretation to the results, the contaminant should be distributed homogeneously through the batch. As microbiological hazards are usually heterogeneously distributed, this means that there is often a major discrepancy between the microbiological status of the batch and the microbial test results (ICMSF, 1986). Even if the microbial distribution is homogeneous, it may still be prohibitive to test a sufficient number of sample units for all the relevant hazards to obtain meaningful information. Perhaps most significantly, microbiological testing detects only the effects and neither identifies nor controls the causes. As a consequence there has been an inexorable move from QC to QA in the management of microbiological hazards in food, with the focus on preventative control measures rather than finished product testing. Although microbiological analysis has subsequently borne the brunt of much denigrating,

it still has a vital role to play as part of a QA framework, albeit with a shift in application and emphasis.

1.2 Food safety management systems

Food safety management relies on the interplay of a number of fundamental elements, including:

- knowledge
- tools (e.g. risk assessment)
- mechanisms (e.g. HACCP) (van Schothorst, 1998; Ross and McMeekin, 2002).

At the centre lies the provision of safe food defined by a food safety objective (FSO), which is a statement of the frequency or maximum concentration of a microbiological hazard in a food considered acceptable for consumer protection. The mechanism by which the FSO is achieved is by application of a number of systems, which have been adopted by the food industry and are used in an integrated fashion. These include good manufacturing practice (GMP), good hygiene practice (GHP) and the hazard analysis critical control point (HACCP) system.

HACCP is a food safety management system that uses the approach of identifying and evaluating hazards and controlling their fate at critical control points (CCPs) in the supply chain. The widespread introduction of HACCP has promoted a shift in emphasis from end-product inspection and testing to the preventative control of hazards during production, especially at the CCPs. It is generally agreed that the most successful implementation of HACCP is done within an environment of well-managed prerequisite programmes (PRPs) (Mortimore and Mayes, 2002). Although definitions vary, the concept of PRPs does not differ significantly from what may be termed GMP. GMP is concerned with the general (i.e. non-product specific) policies, practices, procedures, processes, and other precautions that are required to consistently yield safe, suitable foods of uniform quality. GHP is the part of GMP that is concerned with the precautions needed to ensure appropriate hygiene and as such tends to focus on the prerequisites required for HACCP.

Generally, GMP/GHP requirements include the following:

- the hygienic design and construction of food manufacturing premises
- the hygienic design, construction, proper use and maintenance of machinery
- cleaning and disinfection procedures for plant and equipment
- general hygienic and safety practices in food processing, including:
  - microbial quality of raw materials and supplier quality assurance
  - hygienic operation of each process step
  - hygiene of personnel and their training in hygiene and the safety of food
  - pest control
  - water and air control

– product rework and recall procedures
– waste management
– labelling and traceability systems
– transportation (Brown, 2002; Mortimore and Mayes, 2002).

For steps in the manufacturing process that are not recognised as CCPs, the use of GMP is essential to provide assurance that suitable controls and standards are present. In turn, the identification and analysis of hazards within the HACCP programme will provide information to interpret GMP requirements and indicate staff training needs for specific products or processes (Brown, 2002).

Although GMP cannot substitute for a CCP, collectively it can minimise the potential for hazards to occur, thus eliminating the need for a CCP. The implementation of effective GMP will control 'general' or 'establishment' hazards that would otherwise have to be controlled by a CCP. Failure to have GMP in place will inevitably lead to a large number of CCPs in the HACCP plan covering both 'general/establishment' hazards and product specific ones.

Food safety management is required from 'farm to fork' and systems analogous to GMP have been developed throughout the food supply chain. These include systems targeted at food production: good agricultural practice; good working practices of animal husbandry (Johnston, 2002); and good aquacultural practice; as well as those targeted at food handlers and consumers: good catering practice; and good domestic kitchen practice (Griffith, 2002).

1.3 Types of testing used in GMP and HACCP systems

The types of tests that have a role in GMP and HACCP systems depend on the specific application and range from standard detection and enumeration methods through to the most sophisticated finger printing techniques. Although full details of these methods are covered elsewhere in this book, it is worth taking time to briefly consider the importance of tests for indicator organisms and the application of challenge tests and predictive microbiology models.

1.3.1 Pathogen vs. indicator testing

The numbers of pathogenic microorganisms in most raw materials and food products are usually low and so pathogen tests may provide little information of use for the implementation and maintenance of GMP and HACCP systems. Instead, the enumeration of so-called 'indicator organisms' has an important role. Indicator organisms are groups of microorganisms that are indicative for the possible presence of pathogens. Although there is not necessarily a relationship between indicator and pathogen numbers, it can be generally assumed that the possible numbers of a pathogen are less than the numbers of the organisms indicative for it. It can also be assumed that reduction in the numbers of the indicator organisms will produce a similar reduction in the numbers of

any corresponding pathogen (Brown *et al*., 2000). For the same reasons indicator organisms can also provide a measure of post-pasteurisation contamination that might lead to pathogen contamination. As different indicator organisms imply the possible presence of different pathogens, there are several groups of tests that may be appropriate, e.g. total aerobic counts, coliforms, *Enterobacteriaceae*, *E. coli*, faecal streptococci and aeromonads (Brown *et al*., 2000).

1.3.2 Microbiological challenge testing and predictive microbiology

When assessing the safety of a product and/or process the use of microbiological challenge testing may be required. This type of test can be helpful in determining the ability of a food to support the growth of pathogens and in the validation of processes that are intended to deliver a defined degree of lethality against a target organism (IFT, 2001). In essence microbiological challenge testing involves the inoculation of a food with specific microbial hazards and monitoring their growth, survival or death during storage and/or after specific process steps. However, there are a number of important factors that must be considered when designing and implementing a challenge test, including:

- selection of appropriate challenge organisms
- inoculum level
- duration and number of analyses
- storage conditions and packaging
- methodology
- interpretation (Vestergaard, 2001).

This type of microbiological testing is expensive, time-consuming and is very product/process specific and therefore may have to be repeated if the product and/or process is modified. These factors have been some of the main drivers for developments in the field of predictive microbiology, the concept and history of which have been reviewed in detail by McMeekin *et al*. (2002). Mathematical microbiology models can help describe the growth, survival and death of microorganisms in food as affected by the intrinsic factors (characteristics of the food, e.g. pH, a_W, preservatives) and extrinsic factors (characteristics of the environment, e.g. temperature). In addition to the numerous predictive microbiology models that have been published, several software systems incorporating microbiology models have been produced, some of which are commercially or freely available (Blackburn, 2000). Information about this software is provided at the end of this chapter.

Predictive models have the potential for a range of safety and spoilage applications including shelf-life determination and extension, distribution and storage condition assessment, product formulation and reformulation, process design, risk assessment, GMP, HACCP, and as an alternative or adjunct to challenge testing (Vestergaard, 2001). However, the extent to which the application of predictive models can be relied upon has been the subject of considerable debate. The US Department of Agriculture Food Safety and Inspection Service

(USDA FSIS) has gone so far as to say that 'it is not possible or appropriate to rely solely upon a predictive modelling program to determine the safety of foods and the effectiveness of processing systems' (FSIS, 2002). The FSIS also state that predictive models do not replace the need for challenge testing. The Institute of Food Technologists (IFT) take a more balanced view, highlighting the value of combining predictive models with challenge tests and the potential for using challenge test data for model development and validation (IFT, 2001). Taking a pragmatic approach it is clear that predictive models can provide a powerful source of information and a tool for its practical application, whilst not completely negating the need for microbiological testing. Utilising predictions requires a considerable amount of knowledge of the food, the process, the microorganism of concern and the model itself, and therefore models do not replace the judgement of a trained and experienced microbiologist.

Developments in information technology have also led to the construction of microbiological expert systems. Originating from the artificial intelligence field of research, expert systems are essentially computer programs that attempt to emulate the performance of human experts. As an example of what can be achieved, an expert system for ready-to-eat meals has been described (Adair and Briggs, 1993). The system contained databases on product design, manufacturing and microorganisms, and several predictive bacterial growth models. In response to user inputs a rule base was applied and the output comprised the required assembly and packaging conditions, the minimum thermal process and the maximum shelf-life to ensure a microbiologically safe product.

1.4 Microbiological analysis and GMP systems

GMP/GHP systems have been found to be effective provided that they are well documented with standard operating procedures (SOPs), are fully implemented, and include monitoring records and verification procedures (Kvenberg and Schwalm, 2000). There are several principal sources of microbial contamination of a product that require control: raw materials, equipment, process/production environment and people. The extent to which microbial testing plays a role, and the degree of sampling required, should reflect the category of risk associated with the particular raw material, area or operation. For example, a 'high-risk' raw material that is added to a product post-pasteurisation may require more testing to verify compliance with a specification than one added before pasteurisation. Also the food contact surfaces and air quality in a 'high care/hygiene' area may require a higher level of sampling.

1.4.1 Determining the source and significance of hazards

Whether GMP/GHP or HACCP eventually controls the hazards, hazard identification is an important first step to ensure safe food products. Microbiological testing can play an important role in identifying potential hazards as well as

linking them to a source, assessing their significance for the final product, and verifying that controls are effective and successfully implemented. For example, in a meat processing plant microbial testing demonstrated that the most important factor contributing to the microbial contamination of ground beef and retail cuts was from incoming raw materials obtained from different suppliers (Eisel *et al*., 1997). Environmental sources of contamination were shown not to be a significant source of overall microbial contamination, although it was highlighted that cleaning and sanitation programmes and safe handling were still important. In a similar way, predictive microbiology models have helped determine the significance of different microbiology hazards in establishing the shelf-life of pasteurised milk (Griffiths and Phillips, 1988). This exercise highlighted the importance of good hygienic processing to reduce the post-pasteurisation contamination. The use of molecular characterisation techniques has further increased the microbiologist's armoury and epidemiological tracking of strains can provide a more in-depth knowledge of the food process. This may enable the determination of sites of contamination helping to highlight where controls are required, whether they be through GHP or CCPs (Dodd, 1994).

1.4.2 Raw materials

The quality of raw materials can affect the overall quality of the finished product. Microbiological testing may often be required to verify that raw materials are delivered to the agreed specification and as a means of monitoring or selecting suppliers. Although frequently covered as part of a HACCP study, raw material specifications may not be identified as a CCP, in which case they are usually covered by GMP/GHP. Testing may then involve confirming the absence of specific pathogens or that indicator organisms are within defined limits.

Raw materials may also be the means of introducing contamination into the food processing or production environment. This is particularly important from the point of view of controlling contamination in animal husbandry. Although control is particularly difficult in a farm environment, prevention of feed contaminated with pathogens being introduced into e.g. broiler flocks, is an important control point. Here microbial testing can be an important means of verifying that pathogens are absent and that the batch conforms to agreed specifications.

1.4.3 Equipment

Food contact surfaces are a particularly important potential source of contamination, and sanitation (cleaning and disinfection) is the major day-to-day control. When undertaken correctly, sanitation programmes have been shown to be cost effective and easy to manage, and, if diligently applied, can significantly reduce the risk of microbial contamination (Holah and Thorpe, 2002). In this regard microbial testing is useful in the validation of standard

sanitary operating procedures (SSOPs) and the verification that they have been carried out effectively.

In general, the microbial tests used are indicator organisms such as TVC, coliforms, *Enterobacteriaceae* or yeasts and moulds. However, it is very easy to forget that visual examination can provide a valuable first step in validating and/or verifying an SSOP and can save a lot of wasted effort and expense. Conventional methods for monitoring surface contamination include:

- contact plates/dipslides (for flat surfaces)
- swabbing and plating (ideal for more inaccessible areas)
- sponges (for sampling larger areas) (Fraser, 2002).

An estimate of the microbial load per unit of plant is obtained and can be compared with predetermined specifications. An aerobic colony count of 100 cfu/cm^2 is often used as a standard and counts below that level are indicative of clean surfaces (Griffiths, 1997). In closed systems that are sanitised by cleaning in place (CIP) procedures, cleanliness is usually determined by analysing rinse water samples for microbial load.

Although of value in validating and/or optimising an SSOP, verification of sanitation based on colony counting techniques can generally only be of use for trend analysis. However, the application of the ATP bioluminescence technique for hygiene monitoring now provides the advantages of immediate corrective action, which fits with the QA approach. There are now a plethora of different types of systems for measuring ATP based on the analysis of swabs, rinse waters, and determining ATP *in situ*, which is only really applicable to flat surfaces. ATP bioluminescence can be useful to help refine and improve SSOPs and optimise the use of chemicals and water and in some cases can lead to a reduction in sanitation costs. Although of great potential benefit the technique does have its limitations. In particular, some food products have naturally low levels of ATP. In these cases hygiene monitoring using ATP bioluminescence would not be an appropriate method. However, rapid tests for measuring other hygiene indicators, e.g. protein and catalase activity, have also been developed and may be more applicable where ATP analysis is not. Although a microbial surface may not be a source of contamination after sanitation, food residue on that surface during production can provide the opportunity for microbial growth, which could then be a source of recontamination to the product. With the production pressures to keep lines running as long as possible between SSOPs, microbial testing can provide valuable information to maximise line efficiency without compromising the microbial safety or quality of the product.

1.4.4 Environment

The food production/process environment can be a source of general contamination. Many surfaces not directly in contact with food may harbour microorganisms, e.g. non-food contact equipment surfaces, walls, floors, drains,

overhead structures. These microorganisms can then be transferred to the food in the air via water droplets and dust. Sampling of this environment can provide information on the likely presence and incidence of pathogens, their distribution in relation to processing lines and thus the risk of product contamination (Cordier, 2002). This allows preventative measures to be established in the framework of GHP, such as layout of processing lines and zoning within the factory.

Sampling the cleaning equipment is a very useful index of what is actually present in a production environment, because cleaning 'collects' dirt and bacteria from all parts of the factory, e.g. floor mops, brushes, vacuums (Fraser, 2002). In a similar way sampling of drains also gives a better chance of determining whether a particular pathogen is present in the production environment, e.g. *Listeria*. This can often be a better approach than sampling end products. In addition, other wet areas such as sinks, taps, cleaning cloths and brushes, and boot-washing baths should also be checked routinely. Aerosols can be created from such areas and contamination can find its way into products on the manufacturing line. Testing for indicator organisms generally gives the most useful information on the environmental hygiene, an exception to this being the testing for *Listeria* in high-hygiene environments.

Air quality can be a good index of the overall sanitary condition of a production environment. Air can contain microbial contamination from both external and internal sources, depending on the set-up of the factory. For example, if filters and air-conditioning units are not properly maintained, microorganisms can enter the plant from outside. Internal contamination can occur from skin particles shed from factory personnel, dust particles from packaging materials, and aerosols created during either production or on-going cooling or cleaning processes. The records from the routing monitoring of air quality can build a picture of the general standard of air hygiene in a plant (and identify the areas/sources of highest contamination). Generally, the methods for measuring air quality are either settle plates or the use of a portable, battery-operated, air sampler. Because airborne counts can fluctuate widely depending on activities around the area, it is important to note what is happening (e.g. cleaning, shift-change) in order to correlate data with events (Fraser, 2002).

The temperature of the production environment can obviously affect microbial proliferation and here predictive microbiology can play a role in GMP/GHP. For example, a dynamic Temperature Function Integration (TFI) model was used jointly by regulators and processors to develop justifiable criteria for the management of refrigeration during the production of hot and warm-boned meat, the post-slaughter handling of ovine carcasses and the handling of offals (Armitage, 1997). Similarly, the use of predicted lag times and growth rates of coliform bacteria have been used to support a proposal to alter the temperature of cutting rooms for chilled meat carcasses as stipulated by public health authorities in several countries (Baker, 1995).

1.4.5 People/training

Food production staff and food handlers are a potential source of contamination of food products. For this reason, it is important that adequate training is given, and that proper supervision ensures adherence to all hygiene measures, particularly hand washing. The use of microbiological testing should not be underestimated as a part of hygiene training. The impact of seeing agar plates covered in colonies that have been isolated from swabs taken from hands pre-washing or surfaces pre-cleaning and the reduction achieved following washing or sanitation can be significant. The rapid results achievable by ATP bioluminescence can be particularly useful for the motivation and training of sanitation and production staff by providing a means by which they can access their own performance and by demonstrating the importance of their work. Regular swabbing of hands can also help to reinforce hygiene procedures.

1.5 Microbiological analysis and HACCP systems

Successful implementation of a fully validated HACCP study means that the supposed reliance on microbiological testing, with all its sampling limitations, is relinquished and this should enable a significant reduction in the volume of testing. Some in the food industry went so far as to surmise that microbiological testing would become obsolete (Struijk, 1996). In reality, however, microbiology testing has continued albeit with a shift in application and emphasis and accompanying changes in the role of the microbiologist (Kvenberg and Schwalm, 2000).

The HACCP process comprises seven principles, which are further broken down into stages, and microbiological analysis has an important role to play in several of them (Table 1.1), including:

- hazard analysis
- determination of CCPs
- defining target values and critical limits
- verification.

Although, not defined as a separate stage, validation of the HACCP study is an essential verification activity and can benefit from microbiological test data. Validation is concerned with obtaining evidence that the elements of the HACCP plan are effective, i.e. ‘doing the right things’. This contrasts with other verification activities, which determine the effectiveness of the HACCP system once defined and implemented, i.e. ‘doing things right’.

The extent and scope of microbial testing is likely to vary with differences in facilities and equipment, the scales of processes, and the types of products involved (Brown *et al*., 2000). In reality the HACCP process often starts with a product/process concept where design control points (DCPs) rather than CCPs are the output. At this point predictive models can be of particular value as the

Table 1.1 The seven principles of the HACCP system

1	Conduct a hazard analysis
2	Determine the critical control points (CCPs)
3	Establish limits for each CCP
4	Establish a system to monitor control of each CCP
5	Establish the corrective action to be taken when a critical limit is exceeded
6	Establish procedures to verify that the HACCP system is working effectively
7	Establish documentation for the HACCP system

product and/or process goes through a number of iterations for reasons of product development, marketing, production or safety.

1.5.1 Hazard analysis

The first principle of the HACCP process is to conduct a hazard analysis. Hazard analysis is the process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore which should be addressed in the HACCP plan. For each raw material and process step this includes consideration of the likely occurrence of the hazards, qualitative/quantitative evaluation of the hazards, survival or multiplication of the hazards and identification of appropriate preventative measures. Published sources of microbiological data, including epidemiological and surveillance data, together with knowledge gained through commercial experience, can provide the HACCP team with relevant information on the likely hazards associated with the product and process. However, when existing data is lacking microbiological testing is often needed (Kvenberg and Schwalm, 2000). This may involve determining the incidence of pathogens or indicator organisms in raw materials, the presence of pathogens (e.g. *Listeria*) in the environment, and microbial loads in foods and on equipment (Stier, 1993). Here the links with GHP are important.

Validation of the technical accuracy of the hazard analysis and effectiveness of the preventative measures is important before the HACCP study is finalised and implemented. Examples where microbiological methods may be used for validation include pre-operation checks of cleaning and sanitising, screening of sensitive raw materials, challenge testing, and monitoring of critical sites for microbiological build-up during processing (Hall, 1994). Predictive models can be used to help assess the risk and determine the consequence of a microbiological hazard in food during the different process steps (Elliott, 1996). The advantage of using predictive models is that the effect of adjusting the product formulation and/or processing parameters can be rapidly assessed. To obtain predictions from most models a starting concentration of microorganisms is required and here information from microbial testing can be of value. Where predictive models are appropriate this may allow a reduction in, or negate the need for, challenge testing, which might otherwise be required

to provide this information. Predictive models have a particularly important role to play in obtaining information about microorganisms that require specialist facilities for data generation. For example, models for *Clostridium botulinum* have been used to predict the safe refrigerated shelf-life of *sous vide*-type food products (Baker and Genigeorgis, 1993) and vacuum-packed fish (Hyytiä *et al.*, 1999). Even though the FSIS (2002) has stated that 'generally, a microbial pathogen computer model (MPCM) would not be the only documentation relied upon to support an element of a HACCP plan' it is conceded that 'in certain circumstances, a microbiologist … may determine the MPCM program is the most appropriate (and sole) source of data' and *Cl. botulinum* is used as an example.

1.5.2 CCPs

A CCP is a step at which control can be applied (and is essential) to prevent or eliminate a food safety hazard or reduce it to an acceptable level. The second, third and fourth principles of HACCP involve determining CCPs, setting critical limits and establishing a system to monitor control of each CCP. Challenge testing and predictive models can provide useful information for both the determination of CCPs and the setting of critical limits (Baker, 1995; Elliott, 1996; Griffiths, 1997; Fujikawa and Kokubo, 2001; IFT, 2001; FSIS, 2002). This information is often required to set maximum times and temperatures for storage conditions and minimum times and temperatures for heat processes. Microbial testing can play a major role in the validation of CCPs to demonstrate their effectiveness (van Schothorst, 1998; Blackburn, 2000; Kvenberg and Schwalm, 2000). For safe product design a defined reduction of target microorganisms may be required, either in one CCP or over a series of process steps. Quantitative data may be required to demonstrate that the process can deliver the defined level of microbial kill or that the end product meets the specification for safety and/or stability. This is particularly true if unconventional or unique control measures and/or critical limits are used.

Predictive microbiology models can be used for 'what if' scenarios to provide an indication of the severity of problems caused by process deviations or the complete breakdown of any of the CCPs (FSIS, 2002). They can also be used to provide useful information on the assessment of equivalence of HACCP plans (Fujikawa and Kokubo, 2001). In many cases it is still necessary to conduct challenge tests to validate CCPs as current models will not be appropriate for all situations that may be encountered in food production. Microbial methods, particularly molecular characterisation ones, can be useful in answering questions that may arise as part of the HACCP validation exercise. For example, if a hazardous organism appears in a product at a point in the production line beyond the CCP designed to control it, does this mean failure of the CCP, or does it indicate post-process contamination (Dodd, 1994).

The HACCP process requires the establishment of systems to monitor all identified CCPs. In most cases it is not feasible to use microbial testing to

monitor CCPs due to the long analysis time, low method sensitivity and heterogeneous nature of pathogen contamination. However, there are some notable exceptions. The receipt of raw materials within defined microbiological specification is often identified as a CCP. As a consequence, preventative measures may include a Certificate of Analysis for selected contaminants and in-house laboratory tests to confirm acceptability and when screening new suppliers. Here again, the use of indicator organisms testing is often used. ATP bioluminescence kits are widely used for checking the sanitation of equipment. As results from these methods can be obtained in only a few minutes, it allows sufficient time for equipment to be resanitised before production begins thus preventing contamination. Consequently, sanitation of equipment and monitoring using ATP bioluminescence may be identified as a CCP, although this is frequently covered as part of a GHP programme. Although limited in their availability, other 'real-time' methods such as flow cytometry have been proposed for CCP monitoring (Griffiths, 1997).

1.5.3 Verification

The sixth HACCP principle involves the establishment of verification procedures to confirm that the HACCP system is working effectively. The traditional view is that verification does not need to include microbial testing because, in large part, it is accomplished by reviewing HACCP monitoring records (Kvenberg and Schwalm, 2000). However, it is clear that many companies use microbial testing in verification programmes for both incoming ingredients and finished products. This may involve pathogen testing, although quantitative indicators can provide a much more effective tool for verifying that HACCP is properly implemented (Swanson and Anderson, 2000). The choice of appropriate indicators is product and process specific. For example, testing for coliforms provides an effective verification technique for the pasteurisation of milk and water potability. However, in certain applications finished product testing for even indicator organisms provides no meaningful data (e.g. canned products). In theory, a well-functioning HACCP plan should only require occasional testing as part of the verification process. However, sometimes local legislation, customer requirements or the company's own standards demand a higher level of testing (Stier, 1993).

Microbiological data can provide valuable sources of information for trend analysis and statistical process control and for this purpose they are generally under-utilised. In this regard, quantitative tests are more informative to a processor than negative pathogen tests as trends can be examined and early warnings of problems or loss of control can be obtained. Loss of operation control may give rise to dramatic changes to microbiological test results, however it may manifest itself in much more subtle gradual changes in microbial counts only detectable via trend analysis. If microbiological data are examined proactively it is then conceivable for microbiological problems to be prevented making the exercise compatible with the QA approach to food safety.

The goal should be for data to be directed towards process improvement and microbiological analyses should not be done solely for the sake of generating data. Many organisations have test results and baseline data for indicator organisms collected over many years. New criteria that replace historic baselines must be carefully reviewed to ensure that the processor retains a solid understanding of the microbial profiles of their processes and products. For example, the implementation of a new test method can make previously developed baseline data worthless if the new testing protocol does not provide equivalent results.

HACCP is a living system and therefore review of the HACCP plan is an important aspect to ensure that it remains fully valid and implemented. A formal review should be triggered if there is a change to the product or process, but if this is not the case then it should be reviewed at regular intervals, e.g. annually. In these reviews it may be decided that microbiological data are required to assess the significance of a new hazard or to ensure that the CCPs can still control the existing hazards in light of any proposed changes to the product or process.

1.5.4 Troubleshooting and forensic investigation

It has been pointed out that in spite of meticulous adherence to HACCP-based good practices occasional human, instrumental or operational hiatuses can and will occur (Struijk, 1996). Microbiological methods are still required for troubleshooting and forensic investigation in order to identify the cause of the problem and rectify it. Usually the first action required is to identify and control the affected product, which may or may not be identified as having deviated from HACCP critical limits. Microbiological testing may be appropriate to determine, or confirm, whether there is a microbiological problem and, if so, whether it is a safety or spoilage incident. In combination with a review of the process records, particularly at CCPs, and any historical microbiological test data, it may be necessary to instigate a microbiological sampling and testing plan to determine how much product is affected. As speed is often critical, rapid microbiology methods can play an important role (Stier, 1993). In addition, predictions from microbiology models may help to determine the extent of the microbiological problem (Fujikawa and Kokubo, 2001). Once this information has been obtained, decisions can be made regarding segregation, blocking, recall and salvaging of affected batches and the status of further production.

Microbiological analysis is often required to determine the cause or source of the problem and the type and extent of testing required will vary enormously depending on the situation. Rapid techniques like ATP bioluminescence can be useful troubleshooting tools to identify problem areas quickly. Tests ranging from indicator organisms, through specific pathogen detection methods to the genetic fingerprinting of strains may also be appropriate. Following this immediate action an assessment of the integrity of the HACCP plan is required. It has to be determined whether the HACCP has failed due to its validity or its

implementation. Here again, microbiological analysis may have a role to play in any subsequent review and revalidation.

1.6 Future trends

The food industry has responsibilities to produce safe and wholesome food and providing this assurance is ultimately the microbiological goal. A microbiology test that could analyse a batch of food non-destructively, on-line and with the required accuracy, sensitivity and specificity is the 'Holy Grail' and would provide this assurance. Our current technical capabilities, and even the likely developments in the foreseeable future, fall well short of this ideal situation and so food safety management using the QA approach is here to stay.

As can be seen throughout this chapter the full range of existing methods have an important place in our armoury against the threats posed by micro-organisms in food. Due to the diversity of applications and user requirements new method developments still have the potential to bring benefits. Methods that are faster, cheaper, easier to use, more accurate and/or more sensitive are likely to find welcome recipients. There is also a push to standardise methods and demonstrate equivalence to address the increasingly global market for food.

The rapid monitoring of hygiene using ATP bioluminescence is probably the best example of a 'microbiological' test applied in a GHP/HACCP environment. It is likely that the range of other compounds that could be used to monitor hygiene will extend further. Increased use of genetic fingerprinting methods to better understand the microbial ecology of the factory, manufacturing line, and production process may also bring benefits in targeting better control of the hazards. Biosensor development for very rapid pathogen detection and indicator organism enumeration could also be of benefit for application within GHP and HACCP (Fung, 2002). A variety of biosensors are now commercially available to monitor microorganisms, but they are not yet suitable for routine testing in the food industry. It has been proposed that rapid alert kits for food spoilage and detection of food pathogens will be developed for catering and home use and that more developments in this area are needed (Fung, 2002). However, there is a danger that if these kits are marketed at the expense of hygiene training and education then we may fall into the trap of imposing a QC rather than a QA mentality on the food handler and consumer.

The use of predictive models, particularly those based on probabilities, for GMP and HACCP has yet to be fully realised. It has been stated that their utility will be further enhanced when predictive microbiology is recognised as a rapid method (McMeekin *et al.*, 2002). This will require an increased availability and applicability of models and improvements to the accuracy of predictions as well as greater understanding of the benefits and limitations by the user. The deviations between predictions from current models and observed data in foods that are seen are often due to a factor not included in the model (e.g. a preservative) or differences in the factors used (e.g. type of acid or humectant).

The physiological state of microorganisms in food, particularly if injured or preconditioned, can have a dramatic effect on their fate and growth or survival kinetics (Blackburn and Davies, 1994; McMeekin *et al.*, 2002). Combining knowledge of microbial kinetics in food with an understanding of the underlying physiological processes offers great benefits for the management of food safety in the future. Ultimately, the combining of predictive models with rule bases in expert systems offers the potential for greater assurance for food safety, while still providing scope for innovation by food developers and producers.

1.7 Sources of further information and advice

Food MicroModel software and enquiry service are currently available from Leatherhead Food International, Randalls Road, Leatherhead, Surrey KT22 7RY, UK. Tel. +44 (0)1372 376761; Fax. +44 (0)1372 386228. http://www.leatherheadfood.com

Forecast service and ERH CALC software are available from Campden & Chorleywood Food Research Association, Chipping Campden, Gloucestershire GL55 6LD, UK. Tel. +44 (0)1386 842000; Fax. +44 (0)1386 842100. http://www.campden.co.uk

Food Safety and Inspection Service (FSIS) website. http://www.fsis.usda.gov

Institute of Food Technologists website. http://www.ift.org

MicroFit and DMfit are available from the Institute of Food Research Reading and can be downloaded from the Internet. http://www.ifr.bbsrc.ac.uk

Pathogen Modeling Program is available from the USDA and can be downloaded from the Internet. http://www.arserrc.gov/mfs/pathogen.htm

Seafood Spoilage Predictor is available from the Danish Institute of Fisheries Research and can be downloaded from the Internet. http://www.dfu.min.dk/micro/ssp

1.8 References

ADAIR C and BRIGGS P A (1993) The concept and application of expert-systems in the field of microbiological safety, *Journal of Industrial Microbiology*, **12** 263–7.

ARMITAGE N H (1997) Use of predictive microbiology in meat hygiene regulatory activity, *International Journal of Food Microbiology*, **36** 103–9.

BAKER D A (1995) Application of modelling in HACCP plan development, *International Journal of Food Microbiology*, **25** 251–61.

BAKER D A and GENIGEORGIS C (1993) Predictive modeling, in Hauschild A H W and Dodds K L (eds) Clostridium botulinum: *Ecology and Control in Foods*, Marcel Dekker Inc., New York, pp. 343–406.

BLACKBURN C DE W (2000) Modelling shelf-life, in Kilcast D and Subramaniam P (eds) *The Stability and Shelf-Life of Food,* Woodhead Publishing Limited, Cambridge, pp. 55–78.

BLACKBURN C DE W and DAVIES A R (1994) Effects of preconditioning on pathogenic bacteria in foods, *Food Technology International Europe 1994*, 37–40.

BROWN M (2002) Safe process design and operation, in Blackburn C de W and McClure P J (eds) *Foodborne Pathogens Hazards, Risk Analysis and Control*, Woodhead Publishing Limited, Cambridge, pp. 197–228.

BROWN M H, GILL C O, HOLLINGSWORTH J, NICKELSON II R, SEWARD S, SHERIDAN J J, STEVENSON T, SUMNER J L, THENO D M, USBORNE W R and ZINK D (2000) The role of microbiological testing in systems for assuring the safety of beef, *International Journal of Food Microbiology*, **62** 7–16.

CORDIER J-L (2002) Sampling and testing for pathogens essential in safe food manufacture, *New Food*, **2** 37–40.

DODD C E R (1994) The application of molecular typing techniques to HACCP, *Trends in Food Science and Technology*, **5** 160–4.

ELLIOTT P H (1996) Predictive microbiology and HACCP, *Journal of Food Protection*, Suppl. 48–53.

EISEL W G, LINTON R H and MURIANA P M (1997) A survey of microbial levels for incoming raw beef, environmental sources, and ground beef in a red meat processing plant, *Food Microbiology*, **14** 273–82.

FRASER E (2002) Environmental monitoring in the food industry, *New Food,* **5** 9–14.

FSIS (2002) Use of microbial pathogen computer modelling in HACCP plans, USDA Food Safety and Inspection Service, Washington, DC, FSIS Notice 55-02.

FUJIKAWA H and KOKUBO Y (2001) Practical applications of predictive microbiology software programs to HACCP plans, *Journal of the Food Hygienic Society of Japan*, **42** 252–6.

FUNG D Y C (2002) Predictions for rapid methods and automation in food microbiology, *Journal of AOAC International*, **85** 1000–2.

GRIFFITH C (2002) Good practices for food handlers and consumers, in Blackburn C de W and McClure P J (eds) *Foodborne Pathogens Hazards, Risk Analysis and Control*, Woodhead Publishing Limited, Cambridge, pp. 257–76.

GRIFFITHS M W (1997) Rapid microbiological methods with hazard analysis critical control point, *Journal of AOAC International*, **80** 1143–50.

GRIFFITHS M W and PHILLIPS J D (1988) Modeling the relation between bacterial growth and storage temperature in pasteurised milks of varying hygienic quality, *Journal of the Society of Dairy Technology*, **41** 96–102.

HALL P A (1994) Scope of rapid microbiological methods in modern food production, in Patel P D (ed.) *Rapid Analysis Techniques in Food Microbiology*, Blackie Academic, Glasgow, pp. 255–67.

HOLAH J and THORPE R (2002) Hygienic plant design and sanitation, in Blackburn C de W and McClure P J (eds) *Foodborne Pathogens Hazards, Risk Analysis and Control*, Woodhead Publishing Limited, Cambridge, pp. 151–95.

HYYTIÄ E, HIELM S, MOKKILA M, KINNUNEN A and KORKEALA H (1999) Predicted and observed growth and toxigenesis by *Clostridium botulinum* type E in vacuum-packaged fishery product challenge tests, *International Journal of Food Microbiology*, **47** 161–9.

ICMSF (1986) Micro-organisms in food-sampling for microbiological analysis Principle and specific applications, Blackwell, Oxford.

IFT (2001) Microbiological challenge testing, in *Evaluation and Definition of Potentially Hazardous Foods*, A Report of the Institute of Food Technologists for the Food and Drug Administration of the US Department of Health and Human Services, IFT/FDA Contract No. 223-98-2333.

JOHNSTON M (2002) HACCP in farm production, in Blackburn C de W and McClure P J (eds) *Foodborne Pathogens – Hazards, Risk Analysis and Control*, Woodhead Publishing Limited, Cambridge, pp. 127–50.

KILSBY D (2001) Personal communication.

KVENBERG J E and SCHWALM D J (2000) Use of microbial data for hazard analysis and critical control point verification Food and Drug Administration perspective, *Journal of Food Protection*, **63** 810–14.

MCMEEKIN T A, OLLEY J, RATKOWSKY D A and ROSS T (2002) Predictive microbiology: towards the interface and beyond, *International Journal of Food Microbiology*, **73** 395–407.

MORTIMORE S and MAYES T (2002) The effective implementation of HACCP systems in food processing, in Blackburn C de W and McClure P J (eds) *Foodborne Pathogens – Hazards, Risk Analysis and Control*, Woodhead Publishing Limited, Cambridge, pp. 229–56.

ROSS T and MCMEEKIN T (2002) Risk assessment and pathogen management, in Blackburn C de W and McClure P J (eds) *Foodborne Pathogens Hazards, Risk Analysis and Control*, Woodhead Publishing Limited, Cambridge, pp. 97–126.

STIER R F (1993) Development and confirmation of CCP's using rapid microbiological tests, *Journal of Rapid Methods and Automation in Microbiology*, **2** 17–26.

STRUIJK C B (1996) The Hamlet option in food microbiology: to analyze or not to analyze food specimens as marketed once HACCP implemented, *Acta Alimentaria*, **25** 57–72.

SWANSON K M J and ANDERSON J E (2000) Industry perspectives on the use of microbial data for hazard analysis and critical control point validation and verification, *Journal of Food Protection*, **63** 815–18.

VAN SCHOTHORST M (1998) Principles for the establishment of microbiology food safety objectives and related control measures, *Food Control,* **9** 379–84.

VAN SCHOTHORST M and JONGENEEL S (1994) Line monitoring, HACCP and food safety, *Food Control*, **5** 107–10.

VESTERGAARD E M (2001) Building product confidence with challenge studies, *Dairy, Food and Environmental Sanitation*, **21** 206–9.