Predictive Microbiology: Quality Assurance Tool or Computer Game?

R.G. Bell, D.M. Phillips and R.J. Jones
Meat Industry Research Institute of New Zealand (Inc.)
PO Box 617, Hamilton, New Zealand

ABSTRACT

Predictive microbiology describes the mathematical, largely computerised, procedures used to predict the most probable developmen microorganisms under a particular set of environmental conditions. Predictions are effected by equations that integrate growth over time in presence of one or more environmental variables such as temperature, water activity, or substrate pH. The foundation premise of predic microbiology is that the responses of microbial populations to particular environmental conditions are reproducible.

Predictive microbiology has, to date, failed to realise the practical position in Quality Assurance that its early proponents envisaged: poss reasons for these are discussed. The four major obstacles to wider acceptance of predictive microbiology appear to relate to datum points (in assumptions), monitoring procedures, validation, and regulatory auditability. Regulatory approval for the use of predictive systems will be ga only after their performance has been satisfactorily validated in extensive field trials and the type and nature of predictive microbiological rec are established, to provide trace-back to, and assurance of, the circumstances under which they were obtained; that is, the predictive systems r be fully auditable. The future of predictive microbiology as a quality assurance tool will remain uncertain until all parties concerned accept the data it generates are equivalent in all respects to those obtained by current cultural methods.

INTRODUCTION

The traditional cultural methods used in food microbiology are diagnostic; that is, they confirm that particular microbiological events h occurred. The information they provide is both historical and temporally frozen. Dynamic events, such as microbial development during storage, can be determined retrospectively as a series of "frozen frame pictures". Future events can, however, be anticipated from such histo snap shots using the "*déjà vue* principle"; that is, predictions can be made based on the analyst's prior experience. The fundamental basis allo experience to be used as a predictive tool is the belief that microbial responses to a set of environmental conditions are reproducible. confidence placed on such assertions depends on the analyst's microbiological expertise and the degree to which the conditions causing past ev have been duplicated. This type of predictive microbiology is more an art than an exact science and in the 1990s like common sense, is b challenged by the power of the silicon chip and its entourage of computer "wizards".

"Predictive Microbiology" and "Microbiological Modelling" are generally considered to be synonyms for the mathematical, largely computer procedures used to predict the probable development of microbiological population on or in a given substrate subjected to a particular s environmental conditions. Predictions are effected by the adding together of growth predicted by equations that relate growth over time in presence of one or more other environmental variables; for example, Temperature Function Integration relates microbial growth to the ti temperature history experienced. Consideration of further variables such as acidity (pH) or water activity (a_w) adds to the complexity of equations on which the models operate.

As with old fashioned experience, the foundation premise of predictive microbiology is that the responses of microbial populations to partic environmental conditions are reproducible. Provided this is the case, it should be possible from past quantitative observations to quantitati predict the most probable response of microorganisms contained within a particular environment. The difference between this type of predic and that of the expert analyst is that the computer, not its operator, is the repository of the past observations, converted into the forma mathematical equations.

Meat and meat products provide amiable habitats for a growth of the diverse range of contaminating microorganisms introduced during produc and processing. Which of those contaminating microorganisms will develop to cause product spoilage or to compromise the produ microbiological safety is determined by the very complex processes of microbial selection and succession. These processes, of which relat microbial ecology, are influenced by the quantitative and qualitative composition of the initial contaminating microflora and the effects of vari intrinsic and extrinsic factors, including microbial interactions, on the proliferation, mere survival or demise of components of that microfl The utility and acceptance, indeed the very quality of predictive models can be gauged by how well they accommodate both population environmental variables. There is, as might be expected, a great diversity of opinion among modellers concerning mathematical mecha required to produce more accurate predictions of microbial growth. Elegant as many of these procedures may be, they will all be evaluate the real world by their practical applications. Thus, practical usefulness will, in the end, be the judge and jury that determines whether predic microbiology remains an academic experience or realises its potential as a welcome addition to the armoury of Quality Assurance.

It is not the intent of this review to preempt the ultimate decision on the future of predictive microbiology. Instead, it comments on why methodology has, to date, failed to realise the practical potential anticipated so eagerly by its early proponents. The authors have chosen no cite any sources of illustrative data they have used; thus, the reader may regard all experimental data presented to be either real or fictitious as t feel is appropriate.

PREDICTIVE MICROBIOLOGY IN QUALITY ASSURANCE: PLEA AND REPLICATION

Case for Predictive Microbiology

The case for predictive microbiology opens, as it has for the past twenty odd years, by castigating cultural methods on the indisputable grou that they are labourious, time consuming, expensive and, as is often the situation with meat, may be compromised by non-representative sampli The brief presented by learned counsel then challenges the interpretation placed on counts by questioning whether the organisms that enumerated indeed reflect the microbial risk or condition being assessed. At this point, the surrealistic world of the microchip casually enters

argument with the confident announcement that predictive microbiology will soon relegate classical cultural techniques of microbial enumeration to a verification role in Quality Assurance and eventually may eliminate the need for cultural methods completely.

In the aghast silence that follows this iconoclastic statement, learned counsel proclaims that predictive microbiology will enable rational decisions to be made concerning the microbiological quality and safety of products at any time after some predetermined datum, (starting) point; for example in the case of primal meat cuts the microbial population on completion of vacuum packaging. Without giving the pragmatists time to consider the significance of the datum points on which the question of validity undoubtedly rests, subjective support for the predictive approach in the form of its increasing application is presented. This support is couched in comfortable terms that are familiar to those employed in Quality Assurance. For example the concept of *Time-Temperature Tolerance* is espoused with comments made about:

(i) Improved accuracy of estimations of microbial product quality, storage-life and safety.
(ii) Ease of identifying the consequences of changes, both deliberate and otherwise, within processing and distribution systems.

or the subject of *Product Processing and Packaging Effects* is included with mention made of:

(i) Rational development of products through easy, low-cost quantification of the consequences of changes in their formulation.
(ii) Determination of processing specifications suitable for use in HACCP-type Quality Assurance systems
(iii) Determination of the influence of packaging on product performance during storage.

The question that does, however, remain unaddressed is, if predictive microbiology can produce all these important benefits, why is it virtually absent from national and international food legislation and regulations.

Case against Predictive Microbiology

The case against predictive microbiology is built not on criticism of the concept of predictive microbiology, which would indicate a dated mind set, but rather on a myriad of reservations associated with the validity of its practical application. Among the most important of these are:

(i) Definition of the initial datum point; that is, the qualitative and quantitative characterization of the initial microbiological status of the product.
(ii) Differences between the lag resolution times for the various elements of that datum population.
(iii) Possible interactions between members of the developing microflora.
(iv) Product heterogenicity in respect to chemical composition, extrinsic factors and the distribution of the microflora.
(v) Interference by non-microbial causes of product deterioration.

The next set of concerns relates to the validity and limitations of predictive models, particularly:

(i) Relationships between laboratory-derived kinetic data and those measured in practical situations.
(ii) The ability of models to accommodate biological variation in general and the aberrant behaviour of microorganisms following their exposure to stress conditions in particular.
(iii) The effect of changing extrinsic factors.
(iv) The technical ability to adequately monitor extrinsic effects and to relate those effects to the development of microflora.

Notwithstanding this formidable list of arguably important concerns about the credibility and capability of microbiological modelling, it is true to say that as the knowledge base expands, confidence in its use is growing.

PREDICTIVE MICROBIOLOGY IN QUALITY ASSURANCE: THE PRESENT SITUATION

Today, the benefits of using predictive microbiology are being promoted in various sectors of the food industry, including meat processing. These promotions are made by those involved in modelling who, perhaps faced with the new funding criterion of relevance, feel they have an orphan technology that deserves a good home. For a variety of reasons, not the least of which has been the availability of research funding, predictive microbiology has tended to focus on individual pathogens rather than or the more complex issue of microbial spoilage. So far, because of the statistical nature of predictive microbiology, fledgling applications relate primarily to process Quality Assurance — an approach that has proved very confusing to those concerned with product Quality Assurance, for whom "quality control" means end product testing. Unfortunately, few individual sample analyses produce results that can be described as being comfortably close to the predicted values. Arguments that models provide a "Fail Safe" feature, in that the predicted values are greater than the observed values is, to many traditionalists, simply an expedient apology for a poor model. Similar variation between individual plate counts would, on the other hand, simply be accepted as an expected consequence of natural variation.

Commercial under-use of such systems has been variously justified, including:

(i) incompatibility with current practices
(ii) unreliability of equipment
(iii) cost

Each of these issues can, be addressed, either by training, to educate people with dated mind sets and ensure that appropriate techniques are used or through recent advances in computer and monitoring equipment technology.

Regulatory authorities, on the other hand, have been reluctant to embrace predictive microbiology because of a lack of confidence in predictive data exacerbated by the general inadequacy of documentation procedures for capturing, treating and analysing the results. In other words, it is believed that such systems will be difficult to audit and this, in turn, throws further doubt on the validity of predictive results. Validity of prediction is also highly dependent on the correct placement and functioning of environmental sensory, particularly those recording temperature. Careful, well-documented research establishing sensor design and positioning, both on/in product and within a process, is essential, as is operator

training, to ensure that the appropriate parameter is indeed being measured. Without such assurance, acceptable predictive numbers co erroneously indicate that a process is under control while in fact the acceptable results are due to probe misplacement. Therefore there is so justification for the current caution on the part of regulatory authorities in respect to the acceptance of predictive microbiological methods for demonstrating regulatory compliance.

The three major obstacles to the wider acceptance of predictive microbiology appear to be acceptance of datum points, the adequacy of monitor procedures and problems of verification and validity.

Datum points

A "datum" is a "thing known or granted, assumption or premise from which inferences may be drawn". In the case of predictive microbiolo a datum is the starting point or more particularly the initial number of organisms present at time zero. The importance of a known, or logica derived, datum point cannot be over emphasized if the validity of predictions is not to be in doubt. For example the microbial quality of prod exiting from a chilling procedure is influenced not only by the conditions experienced during that procedure, but also by the product's microl status when it entered that procedure. In other words, the past history of product must be recognised as being relevant, and consequently m be communicated from one procedure to the next.

To predict the microbial quality of product that has been subjected to a chilling or a storage/transport process, for example, it is crucial to kn or to predict the microbial loading on the product before it entered that process. Two datum points are available - one when no microbial grow has occurred, the zero point or start of the process, and the other when the product is spoiled, the end point. Use of the zero point requires t assumptions be made about the levels of contamination acquired during the various earlier stages of processing. This is especially true in the c of fresh meat, as slaughter and dressing hygiene as well as pre-packaging handling conditions can markedly influence initial post-packag microbial numbers. For processes that are under control, the zero point can be reasonably established from historical data. The predictive mo can then be used to move forward to determine an end point.

An end point datum can also be used, but the concept is more difficult to understand than the zero point datum. Such a system may work, example, by using a temperature function integration algorithm that inter-converts time and microbial numbers as is illustrated in Figure 1. assured storage-life (a realistic minimum) is assumed unless an actual figure determined in trials performed at the production plant is availab This storage-life is expressed in time units and also in microbial numbers. The assured storage life (time at a given temperature or temperat "history") is first translated by the program [(1) to (2) in Figure 1] into a notional initial contamination, the zero point, from which an accumulat of growth due to the actual recorded product temperature history is then made [(3) to (4) in Figure 1]. The remaining storage life is the differe between the final growth accumulation number calculated for the actual recorded product history and the number accepted as the spoilage po converted to time at an appropriate storage temperature [(5) to (6) in Figure 1].

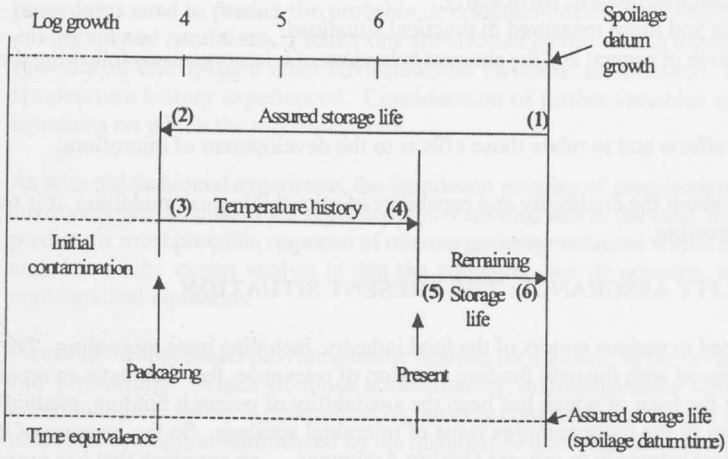

Figure 1.
Illustration of the use of a spoilage or end point-datum program.

In practice, confidence in the use of this or similar systems requ that a realistic assured storage life figure is set for economic sensible shipment sizes. If the assured storage life is unrealistic long, product may spoil earlier than the system anticipates, and is unrealistically short, large shipments may be precluded, ther increasing freight costs.

Assured storage life, the end point datum, would most likely determined by a storage trial under a given set of conditi Alternatively, a predictive approach could be taken, but the vali of that result would require that the organism modelled was likel dominate the spoilage flora developing on the product concern Consequently, there appears to be an ongoing requirement classical microbiology to verify that the model used is appropriat the product concerned. This raises the interesting question as whether one assumes a "worst case" and models organisms of h spoilage potential, for example *Brochothrix thermosphacta* vacuum packed lamb, or aims for an "average case" and mode more benign organism such as a *Lactobacillus* sp.

It is the validity of the datum point for the number of bacteria initi present at the start of a process that determines whether predictive microbiological methods can be confidently used for product as well as proc assurance. With pathogen food safety models, initial cell numbers must be entered, otherwise the program will run on predetermined assum numbers. Unless the process being considered is under control, i.e. subject to a HACCP or other effective quality management system, start numbers in individual units of product may be highly variable. This situation will compromise and consequently invalidate the use of predict microbiology as a product assurance tool but not as a process assurance tool. In fact, the ongoing conflict between process and product assura confounds or confuses the role that predictive microbiology does, or could, play in commercial Quality Assurance, Total Quality Managem and HACCP systems.

An alternative to the use of a datum point, but one amenable only to process assurance, is the use of unitless "Process Indices". These indic such as the Process Hygiene Index (PHI) and the Process Storage Index (PSI) developed by MIRINZ, provide an estimate of the potential growth of an appropriate organism under the monitored conditions. As a predictive model, the *E. coli*-based PHI can be faulted becaus considers temperature, measured at the slowest cooling site, as the only factor limiting growth. Consequently, the index it produces, expres as potential generations of growth, is not representative of, for example, other parts of carcass and generally exceeds the observed increase by m than can be reasonably "excused" as providing a "fail safe" prediction. Its limitations as a predictive model do not, however, detract from

application as a Quality Assurance tool for monitoring and comparing cooling processes. The companion *Lactobacillus delbrueckii*-based PSI produces a potential generations index for a given transport and storage scenario, but unlike a datum point system does not provide either an indication of microbial numbers or remaining storage life. Provided the entire process is under control the latter can, however, be estimated from historical data obtained from previous shipments.

Monitoring Procedures

As the name Temperature Function Integration suggests measurement of temperature is fundamental to the process by which microbial growth is predicted. The credibility that can be placed on the predicted results is largely determined by the accuracy and consistency of the measurements on which they are based. The accuracy of predictions will be strongly influenced by errors in the measuring instruments and the measurement techniques used, and this influence will be cumulative. In the case of cooling carcasses, that means both how and where the temperature is measured. For accurate prediction, temperature must be measured at a site of microbial growth, that is, at the meat surface, and not, as conventional time-temperature hygiene regulations require, at a deep, unexposed tissue site, which is effectively sterile and consequently is rarely, if ever a site of microbial growth.

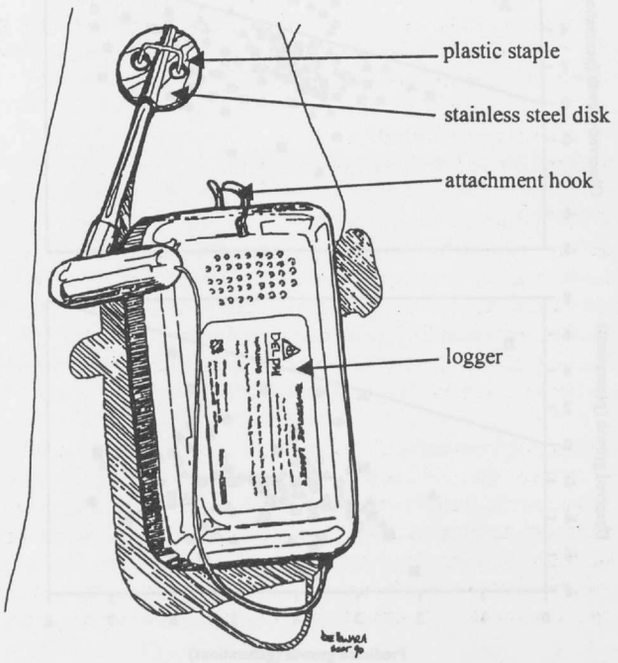

Figure 2.
Logger assembly for meat surface temperature measurements.

Meat surface temperature measurements are notoriously difficult to perform in a consistent manner unless the equipment employed forces a standard approach that reduces the difficulties experienced by those who are unfamiliar with the techniques. For use with the PHI systems for assessing the hygienic adequacy of cooling processes a carcass surface temperature monitoring procedure using a relatively large disc attachment to hold a temperature probe in place has been developed, Figure 2. The disc is attached to the carcass by a small plastic staple (non heat conducting) and the tapered temperature probe is then inserted into the disc. It is recognized, however, that the assembly may, initially distort the actual surface temperature reading a little because of its bulk and subsequently by preventing evaporation from the carcass surface it covers.

The site of temperature measurement remains a matter of contention, with selection largely determined by the purpose of the monitoring procedure. For the purposes of process control, the slowest cooling site is appropriate as this site theoretically affords the greatest potential for growth, that is, the worst-case scenario. Slowest cooling sites tend to be species specific and may even vary with the class of stock within a species. For product assurance purposes a site representing average cooling may be more appropriate.

Verification and Validity

"Verification" is the establishment of truth or correctness by examination or demonstration". Verification in respect to mathematical growth models is accomplished by experimental demonstration that predicted values are similar to observed values. An example of an experimental verification is shown in Figure 3. However, the validity of verification must be questioned in respect the proposed application of the model to predict the growth of *E. coli* on hot de-boned beef.

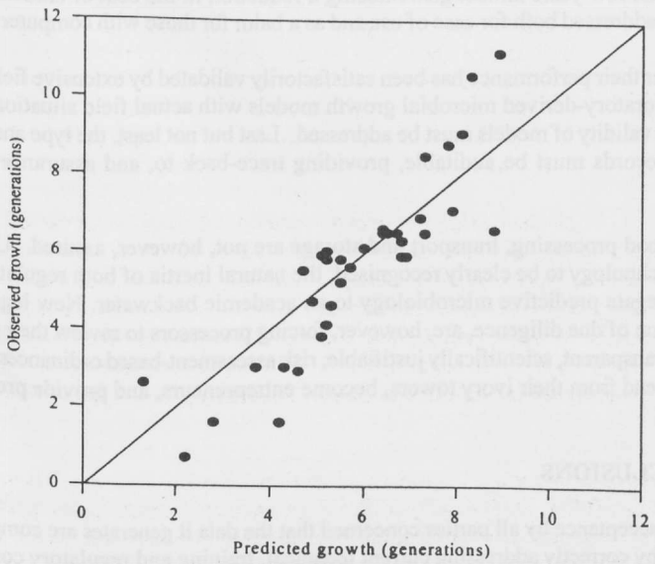

Figure 3.
Comparison of predicted and observed *E. coli* growth on agar squares in cartoned hot-deboned beef during cooling.

"Validity" derived from the Latin *validus* (strong) in the case of an argument, which most certainly is the case with predictive microbiology, implies soundness, defensibility and being well founded.

Continuing with the verification example illustrated in Figure 3. Although great care was taken to ensure that the temperature was measured at the site of microbial growth. The growth measured was that of *Escherichia coli* growing in a half strength Brain Heart Infusion 2.5% agar square sandwiched between two high pH post rigor chilled beef slices equilibrated to 25-30°C, wrapped in plastic, and then cooled within a carton of hot deboned beef. While the predictive accuracy of this model was demonstrated for the circumstances of the trial, the "validity" (the property being well founded and applicable), of the result in respect to *E. coli* growing directly on cooling pre-rigor cartoned beef is open to challenge. Verification of predicted growth by observed values in meat products such as paté, being less contrived are perhaps more valid and consequently less open to challenge.

On a cooling lamb carcass, where variables other than temperature, such as relative humidity and air velocity are not stringently controlled predicted and *E. coli* growth observed

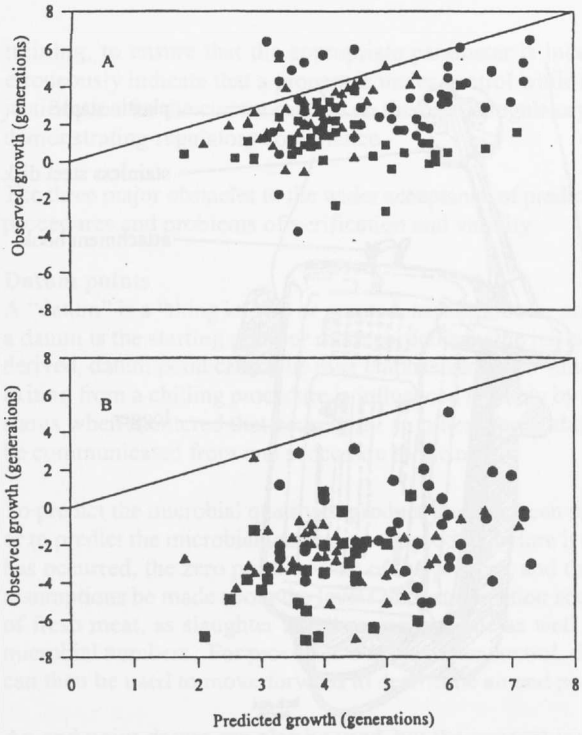

Figure 4.
Comparison of predicted and actual E. coli growth adjacent to the fifth lumbar vertebra (●), and on the flap (▲) and thigh (■), observed in plastic wrapped BHI agar squares placed on a lamb carcass panel A) and painted directly on the carcass surface (Panel B).

in plastic wrapped half strength Brain Heart Infusion agar squares, is generally less than satisfactory (Figure 4A). The correlation is even worse when *E. coli* growth is measured on the carcass surface rather than within a protected agar square, Fig. 4B. However, even under the latter conditions, a number of observed results do approach the predicted values indicating that on occasion temperature and time may be the only variables influencing growth. Irrespective of temperature monitoring site, predictions tend to exceed observed values.

In other words, for unwrapped product, humidity, or perhaps more correctly surface water activity, plays a part in determining actual growth and so ought to be included in predictive equations. However, the effect of humidity can be excluded for the specific purpose of predicting the hygienic efficacy of carcass cooling processes, as there may be sites on a carcass where humidity does not restrict growth. Predictive results in three circumstances, although lacking general accuracy, do provide a valid basis on which to compare the hygienic adequacy of cooling processes.

Preliminary results obtained using the *Lactobacillus delbrueckii* based PSI system applied to transport and storage of vacuum-packed lamb, show a much closer relationship between predicted and observed populations. This observation suggests that within a vacuum-pack, time and temperature are indeed the only variables that need to be considered in respect to predicting growth of spoilage microflora.

PREDICTIVE MICROBIOLOGY IN QUALITY ASSURANCE: THE FUTURE PROSPECTS

The key to the wider adoption of predictive microbiology as a Quality Assurance tool lies in its acceptance by regulatory authorities. Howe it would be naïve to believe that predictive microbiology will ever completely replace diagnostic microbiology. Even the most zealous devot of predictive microbiology crave the reassurance of real world data over the uncertainty associated with that generated in cyber space by an ov heated silicon chip. Similarly, only the foolhardy would change a commercial formulation or process as the result of a computer prediction with first conducting a confirmatory trial.

Regulators cannot, however, be expected to force the use of predictive microbiology for Quality Assurance purposes. New systems must st on their merit as acceptable alternatives to those in current use. Both the establishment of the systems and the training in their correct use m rely heavily on the expertise of microbiologists working in conjunction with the other disciplines involved. Any shortcomings, including th of cost, must be rectified in respect both to computer hardware and software and to monitoring equipment and its application. The technol of field measurement equipment has advanced significantly in the last few years almost guaranteeing a reduction in the cost of hardware. T requirements for software - in particular its user friendliness - must be addressed both for ease of use and as a balm for those with computer phob

Regulatory approval for the use of new systems can be gained only after their performance has been satisfactorily validated by extensive field tri Where appropriate, more field work may be required to compare laboratory-derived microbial growth models with actual field situations. T need for, and frequency of, periodic reassessment or verification of the validity of models must be addressed. Last but not least, the type and nat of predictive microbiological records must be established. Such records must be auditable, providing trace-back to, and assurance of, circumstances under which they were obtained.

The prospects for wider application of predictive microbiology in food processing, transport and storage are not, however, assured. Unles fundamental change in attitudes occurs, allowing the benefits of the technology to be clearly recognised, the natural inertia of both regulators a processors alike seems destined, as it has for the past decade, to relegate predictive microbiology to an academic backwater. New legislati introducing mandatory HACCP systems and demands for demonstration of due diligence, are, however, forcing processors to review their Qual Assurance practices, while regulatory authorities are moving towards transparent, scientifically justifiable, risk assessment-based ordinances. Th the window of opportunity is now wide open for the modellers to descend from their ivory towers, become entrepreneurs, and provide predict microbiology with a practical future as well as an academic past.

CONCLUSIONS

The future of predictive microbiology in Quality Assurance lies in the acceptance by all parties concerned that the data it generates are compara to those obtained by current cultural methods. When this is achieved, by correctly addressing current technical, training and regulatory concer predictive microbiology will, through use, become its own best advocate. Predictive microbiology will not, however, completely repl diagnostic microbiology, as there will be an ongoing requirement for occasional reality checks.

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