VALIDATION OF THE CHILLING OF HOT BONED MANUFACTURING MEAT AND PRIMALS

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Prepared by:

Hot Boning Expert Panel

Meat & Livestock Australia
Locked Bag 991
North Sydney NSW 2059

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Preface

This publication is the result of the work of an expert panel convened by Meat & Livestock Australia (MLA) in 2000. The Hot Boning Expert Panel had representatives of regulators, meat processors and research organisations, chaired by MLA. A large number of people were involved in the work of the panel over its life.

Those involved most directly in the work described here were:

Marc Chambers- E.G. Green & Sons
Peter Greenham- H.W. Greenham & Sons
Ian Jenson- Meat & Livestock Australia
Noel Kelson- Midfield Meats
Karen Krist- Australian Meat Industry Council
Tom McMeekin- University of Tasmania and Australian Food Safety Centre of Excellence
Tom Maguire- Australian Meat Industry Council
Jim Murray- Australian Quarantine and Inspection Service
John Sumner- Meat & Livestock Australia
Paul Vanderlinde- Food Science Australia

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Executive summary

Chilling requirements for hot boned meat in Australia have been established based on predictive microbiology precepts.

Hot boning is regulated via AQIS Notice 2001/20 using a Hot Boning Index (HBI) to describe predicted growth of *E. coli*.

Company chilling regimes are assessed against the following criteria at the site of microbiological concern (the slowest cooling point):

- Average Hot Boning Index (HBI) of no more than 1.5
- 80% of the HBIs must be no more than 2.0
- Upper target HBI of no more than 2.5

A predictive microbiology model has been developed with parameters of pH 6.5, water activity 0.993 and lactate 51.7 mM (Ross *et al.* 2003).

The model has been evaluated and found to predict growth more accurately than existing predictive models (Mellefont *et al.* 2003).

Validation of the model has been carried out by inoculating hot boned meat in cartons with faeces or *E. coli* cultures with acceptable correlation between observed and predicted growth (R^2=0.89).

Validation of the model has been carried out by inoculating lean or fatty surfaces of primals with faeces or *E. coli* cultures with a reasonable correlation between observed and predicted growth (R^2=0.56). It is expected that the use of different model parameters to account for fatty or lean surfaces would improve the correlation and lead to a more realistic estimate of the HBI.

Simulated boxed product modelling suggests that the average HBI for a whole box of product would be almost 1 less than the HBI at the slowest cooling point. Similarly, the average HBI for the whole mass of a slow-cooling rump (not just the surface) was determined to be almost 1 less than the HBI at the slowest cooling point.

Microbiological and chilling data from in-plant monitoring demonstrate that the HBI is achievable and that the microbiological quality of the chilled product is excellent.

In the next revision of the Export Meat Orders, the term “HBI” will become subsumed within the term “Refrigeration Index” (RI) which will be used to regulate chilling of meat throughout the Australian industry. The criteria used to assess whether a meat processor is conforming with the RI are identical with those now used for the HBI.

Introduction

The hot boning process is regulated by AQIS Notice 2001/20, in which the temperature control of the chilling phase is based on predictive microbiology principles. Specifically, each company is required to establish that each chilling regime meets performance criteria for predicted growth of *E. coli* at the site of microbiological concern (the slowest cooling point) in which potential $\log_{10}$ increase is a mean of 1.5; a target upper limit of 2.5; and an 80th percentile of 2.0.

The purpose of the present document is to supply scientific information supporting the formulation of AQIS Notice 2001/20 and validation of the model used to measure predicted growth of *E. coli* by individual companies.

1 Background to regulation of hot boning in Australia

The concept of deboning meat from "hot" carcases was first mooted in the early-1970s with advantages cited such as reduced chilling space, labour costs from avoiding double handling, and better yields (Visser 1977; Cuthbertson, 1979). The main disadvantage was considered microbiological, because meat remains at a temperature suitable for growth of any contaminating microorganisms for some hours. An early code of practice in Australia stipulated chilling meat to 7°C or colder within 3 hours of slaughter (2 hours from commencement of boning) and then freezing within a further 10 hours, a regime which, unfortunately, could not be achieved by normal cooling practices (Grau and Herbert, 1974).

By 1980, CSIRO researchers had developed an equation which predicted growth of *E. coli* on beef trim in cartons during chilling. Predicted growth had also been compared with actual growth of *E. coli* in blended meat at temperatures between 10 and 40°C (Table 1) confirming the opportunity for 2-3 log growth on meat packed "hot" (Herbert and Smith, 1980).

Table 1: Growth of *E. coli* on fresh meat

Approximate initial temperature (°C)	Calculated *E. coli* increase ($\log_{10}$)	Actual *E. coli* increase ($\log_{10}$)	Difference ($\log_{10}$)
35	2.36	2.29	+0.07
25	2.35	2.43	-0.08
15	1.21	1.16	+0.05

Herbert and Smith (1980) also calculated cooling rates required to cool hot boned meats so that, on average, through a carton, *E. coli* grew no more than one generation (i.e. doubled). The calculation took into account that meat on the outside of the carton would cool more quickly (with no, or little increase in *E. coli*) while meat at the thermal centre might increase 2-3 log. Finally, the authors stipulated cooling regimes which would satisfy the requirement of an average *E. coli* increase of one doubling. For 27.2kg cartons cooled in air blast freezers (the industry "norm") meat could be no warmer than 25°C if the freezer air temperature was –35°C.

This requirement made boning directly off the slaughter floor ("true hot boning") impossible and companies resorted to preliminary cooling of carcases in a chiller, followed by boning – a process variously termed "warm boning", "boning-on-the-curve" or "same-day boning".

As the responsible regulatory authority, the Commonwealth Department of Primary Industry was concerned about ensuring meat safety when faced with the proposal that meat could be boned without first being reduced in temperature. Consideration was given to the potential for bacteria on the meat to proliferate when the warm meat was placed in a carton before refrigeration occurred. The carton and the air within would act as insulation and would also reduce drying of the surfaces thus negating one of the primary antimicrobial effects of conventional chilling. Intuitively, the product was considered to represent a considerable hazard unless procedures were put in place to minimise that hazard.

The first mention of hot boning in export legislation is a 1981 amendment to the Bureau of Animal Health Manual of Instruction for Meat Inspection and Meat Handling Procedures. The amendment contains detailed instructions relating to the agreements to be entered into and assurances to be made in order to permit hot boning to occur.

When the Export Meat Orders (EMOs) were published in 1985 hot boning was addressed by EMOs 282 to 284 and associated references in the Australian Export Meat Manual (Volume I). The EMOs required an approved program to be in place before hot boning could commence and Volume I

contained the requirements to be addressed, including *"documentary evidence from a competent expert that the facilities can, in practice, consistently meet the temperature and time combinations proposed".*

In 1988, hot boning requirements were introduced to Volume III of the Meat Manual and the table of time/temperature combinations, in 1°C increments, appeared. The table was based upon the premise that no more than a potential tenfold (1 $\log_{10}$) increase in mesophilic bacteria should be permitted at any point in a carton during the cooling process to 8°C. The maximum times allowed to cool meat to 8°C for meat packed between 35-20°C (the range of hot and warm boning) are summarised in Spooncer (1993) and range from 417 minutes for meat packed at 35°C to 793 minutes if meat were packed at 20°C. Spooncer concluded with *"For meat boned straight off the slaughter floor, the cooling rates that apply to hot-boned meat cannot be achieved with conventional packing and freezing techniques"* but suggested satisfactory cooling could be achieved if cartons were reduced in cross sectional area and cooled either in a plate freezer or in an air blast at unusually low temperature (colder than –35°C) and high air velocity (>3m/s).

During the 1990s true hot boning became more attractive because of advantages such as:

- Reduced processing time from slaughter to load-out
- Lower chilling space and other capital cost requirements
- Reduced energy consumption and other chiller costs
- Increased boning yield
- Improved productivity
- Elimination of occupational health and safety problems associated with hard fat

In 1994 an AQIS Meat Notice (94/7) was issued that introduced some flexibility in the arrangements provided the final product remained microbiologically equivalent to conventionally boned meat. This became known as the alternate protocol. The Notice superseded the entries in Volume I and Volume III and retained the table of times but changed the target temperature from 8^0C to 7^0C. It also introduced the possibility of saving chilled cuts. The Notice allowed companies to operate under a Hot Boning Approved Program. Each company was required to provide microbiological data to support their application for an approved program obtained by an "expert authority" approved by the Secretary of the Australian Quarantine and Inspection Service (AQIS).

During this period several new plants were constructed in Australia specifically for hot boning. These plants were mostly equipped with plate freezers and had slaughter and dressing rates which were in equilibrium with the time required for carcases to be hot boned i.e. boning and packing rates dictated slaughter chain speed. However, even with the efficiency of plate freezing, it became apparent that primal cuts (vacuum-packed) could not be chilled to meet the requirements of AQIS Notice 94/2. Thus, hot boning plants were required to operate under an approved program, with attendant monitoring requirements which were considered onerous by the industry.

A number of plants constructed in Australia were of similar design with those operated in New Zealand, where hot boning of beef had become a major component of the industry. These plants were able to operate in accordance with NZ regulations and since product from that country was exported to many countries (including Australia) it was questioned whether AQIS Notice 94/2 should be reconsidered.

In 1999 a scientific panel was convened by Meat and Livestock Australia (MLA) to consider alternative approaches to chilling and freezing of hot boned meat. The panel was satisfied that the hot boning industry was producing bulk-packed frozen meat equivalent to that produced as a result of conventional boning and proposed a revised approach. Vanderlinde and Murray (1995) were able to segment data from the first national baseline study (Vanderlinde *et al.* 1999) and conclude that there was little difference in the microbiological profile of manufacturing meat from hot- and cold-boned carcases. The second national baseline study of 1998 provided confirmation (Table 2) with little difference between hot boned and conventionally boned meat for several microbiological parameters (Phillips *et al.* 2001).

Table 2: Microbiological profile of hot boned and cold boned meat (Phillips 1999)

	Hot boned meat (n=131)	Cold boned meat (n=857)
Mean log Total Viable Count per gram (25°C)	1.92	2.09
E. coli (% detected)	0.8	5.7
S. aureus (% detected)	17.9	15.3

AQIS Notice 2000/06 provided details of a year-long study where companies could operate under trial conditions while their processes were assessed under new predictive microbiological criteria that took into account variability in the production process. The Notice also defined the term Hot Boning Index (HBI).

During the trial, time/temperature recordings were collected from the time of carton closure until the product temperature fell below 7^{o}C. It is generally accepted that freezing and frozen storage reduces the number of *E. coli* present, therefore, the HBI should overestimate the potential growth at the thermal centre of the carton. Cartons freeze from the outside in, hence bacterial growth is inhibited most quickly in the outer layers. The slowest cooling point in the carton (the thermal centre) represents only a small proportion of the total amount of meat in the carton. Therefore, the HBI for each carton represents a *worst-case* i.e. potential growth at the slowest-cooling portion of the carton. It provides a more stringent assessment than would be obtained by normal sampling procedures e.g. microbiological testing of samples drilled aseptically from frozen cartons.

Using the data, a Hot Boning Index (HBI) was calculated for each carton of logged product based on log increase of *E. coli*. The HBI is obtained using a predictive microbiology model that estimates the number of generations of *E. coli* at the centre of the carton from time/temperature recordings collected during the refrigeration process (see section 2 below). The number of generations can then easily be converted into logarithms of growth. The HBI, similar to the Process *Hygiene* Index used in New Zealand, is used to assess the refrigeration processes of hot-boning plants.

When each company had surveyed all chilling/freezing regimes the data (HBIs) were assessed against the following criteria:

- Average Hot Boning Index (HBI) of no more than 1.5
- 80% of the HBIs must be no more than 2.0
- Upper target HBI of no more than 2.5

The three criteria selected were similar to those which comprised the PHI used in New Zealand, reflecting the fact that this country had satisfied controlling authorities regarding the efficacy of the predictive microbiology approach.

An expert panel of scientists and industry representatives reviewed the results of the trial and recommended retaining the interim criteria as criteria for approval and on-going compliance by plants conducting boning operations prior to completion of carcase chilling in accordance with Export Meat Order 250.1.

Following completion of the trial, AQIS Notice 2001/20 was published advising that the panel had decided to adopt the HBI criteria used during the trial. It advised that all previous approved programs were superseded. Establishments that had submitted data demonstrating their processes met the criteria received on-going approval of their programs. Where the criteria had not been met or where plants had failed to submit data, program approvals lapsed and new validations were required.

Currently, hot boning is regulated via AQIS Notice 2001/20, which essentially confirms the interim criteria specified in AQIS Notice 2000/06.

It should be noted that, in Australia's next issue of the Export Meat Orders, the term "HBI" will become subsumed within the term "Refrigeration Index" (RI) which will be used to regulate chilling of meat throughout the Australian industry. The criteria used to assess whether a meat processor is conforming with the RI are identical with those now used for the HBI.

2 Predictive microbiology model used for regulation of hot boning

The model, developed by University of Tasmania, is based on 236 growth rate data sets and includes factors additional to those of Herbert and Smith (1980), specifically, pH, lactate and water activity. Further details are given in Ross *et al.* (2003). The equation (shown in Section 3) provides the growth rate in generations per hour (Table 3).

Table 3: Growth rates at various temperatures using the equation of Ross *et al*. (2003)

Temperature (°C)	Generations/h
35	2.79
30	2.02
25	1.33
20	0.77
10	0.105

The model parameters for pH, a_W and lactate were set based on data for bulk-packaged meat. It is impractical for processors to measure these values routinely; therefore average values were used that gave good agreement with observed growth rates. The parameters used were pH 6.5, a_W 0.993 and lactate 51.7 mM. Five generations (1.5 log) are deducted from the predicted potential increase on the basis of evidence from CSIRO regarding the effect of lag phase (Smith, 1985) and from Ross (1999).

The latter author reviewed the uncertainty and unpredictability of bacterial lag times and the limitations this imposes on the application of predictive microbiology. He analysed lag time information obtained from the literature as well as information from novel experiments. The information obtained suggested that:

- While lag times are highly variable, apparent variability can be reduced by using the concept of relative lag times or 'generation time equivalents' i.e. the ratio of lag time to generation time (LGR).
- Although lag times may take almost any value, there is a common pattern of distribution of relative lag times for a wide range of species across a wide range of conditions.
- That common distribution of relative lag times has a sharp peak in the range 4-6 generation time equivalents.

These results have significance for the application of predictive microbiology to interpret the hygienic adequacy of carcase chilling and other meat processing and handling operations.
From a practical perspective, a lag time of 3 generation time equivalents reduced the expected growth without lag by 0.9 $\log_{10}$ cfu, i.e. almost a factor of ten. Four or 5 generation times of lag equate to a reduction in the expected growth of 1.2 and 1.5 log cfu respectively.
The practical significance of these results to the meat industry is that there is now a substantial body of information to justify the inclusion of lag times in calculations of the effects of different meat processing and handling procedures. Ross (1999) observed that there was a substantial lag phase equivalent to 4-5 generation times when *Klebsiella oxytoca*, an enteric surrogate for *E. coli*, was inoculated on to meat carcasses during in-plant studies.

Similar observations were noted during the development of the US and UK predictive models databases (respectively, Pathogen Modelling Program and FoodMicroModel). For the former, Buchanan (pers. comm.) indicated a lag phase duration equivalent to approximately 4 generation times and observed that this was consistent with pathogen behaviour on foods. For the latter, Robinson *et al.* (1998) reported that the lag time of *L. monocytogenes* was, on average, 8 times that of the mean generation time. The universality of such observations is further suggested by the results of Widders, Coates and Ross (unpublished) for spoilage pseudomonads growing on pork meat in retail trade.

The model developed for predicting growth of *E. coli* on meat takes into account temperature, pH, water activity and lactate. The parameters used were pH 6.5, a_W 0.993 and lactate 51.7 mM. The lag phase is considered to account for 5 generations of *E. coli.*

3 Model development for chilling of hot boned meat

The model currently used has been developed by scientists at University of Tasmania. Previously, models for the combined effects of temperature and water activity on *E. coli* growth rate (Salter *et al.*, 1998) and for the effects of temperature, pH and lactic acid (Presser *et al.*, 1997) were developed. The current model combines terms for high and low temperature, high and low water activity, high and low pH and dissociated and undissociated lactic acid to yield a new general model form (Ross *et al.,* 2003).

All data used in generation of the model are shown in Appendix 1 which comprises the full text of Ross *et al.*, 2003. Data were fitted to a reduced form of the general model as shown

$$\begin{aligned}\sqrt{r} = \; & c \bullet (T-T_{min}) \bullet (1-\exp(d\bullet(T-T_{max}))) \\ & \bullet \sqrt{(a_w-a_{wmin})} \\ & \bullet \sqrt{(1-10^{(pHmin-pH)})} \bullet \sqrt{(1-10^{(pH-pHmax)})} \\ & \bullet \sqrt{(1-[LAC]/(U_{min} \bullet (1+10^{(pH-pKa)})))} \\ & \bullet \sqrt{(1-[LAC]/(D_{min} \bullet (1+10^{(pKa-pH)})))} \\ & \pm e\end{aligned}$$

Equation 1 (Budavari, 1989)

where:
r = relative growth rate or specific growth rate (time^{-1}), c, d and g = fitted parameters
a_w = water activity
a_{wmin} = theoretical minimum water activity below which growth is not possible
T = temperature, T_{min} = theoretical minimum temperature below which growth is not possible
T_{max} = theoretical maximum temperature beyond which growth is not possible
pH has its usual meaning
pH_{min} = theoretical minimum pH below which growth is not possible
pH_{max} = theoretical maximum pH beyond which growth is not possible
[LAC] = lactic acid concentration (mM)
U_{min} = minimum concentration (mM) of undissociated lactic acid which prevents growth when all other factors are optimal
D_{min} = minimum concentration (mM) of dissociated lactic acid which prevents growth when all other factors are optimal
pK_a is the pH for which concentrations of undissociated and dissociated lactic acid are equal, reported to be 3.86
e=error

Fitted parameter values and asymptotic standard errors on those estimates are shown in Table 4. Observed and predicted generation times (GT) are compared in Fig. 1, as residuals normalised to the observed generation time i.e. $\frac{(ObservedGT - PredictedGT)}{ObservedGT}$, as a function of generation time. To illustrate the validity of the use of the square root transformation to homogenise variance in the relative growth rate data, residuals in this transformation are presented as a function of generation time in Fig. 2.

The accuracy and bias factors (Ross, 1996) for the model predictions of generation time compared with the original data were 1.21 and 0.97, respectively. These will be discussed below in relation to model performance.

Table 4: Parameter values for the growth rate model for *E. coli*

Parameter	Estimate	Asymptotic Standard Error
c	0.2345	0.0083
T_{min}	4.14	0.63
T_{max}	49.55	0.42
pH_{min}	3.909	0.031
pH_{max}	8.860	0.19
U_{min}	10.43	0.52
D_{min}	995.5	106
$a_{w\ min}$	0.9508	0.0004
d	0.2636	0.038
Root Mean Square Error (RMSE) in √(1/(GT [h]))	0.0054	

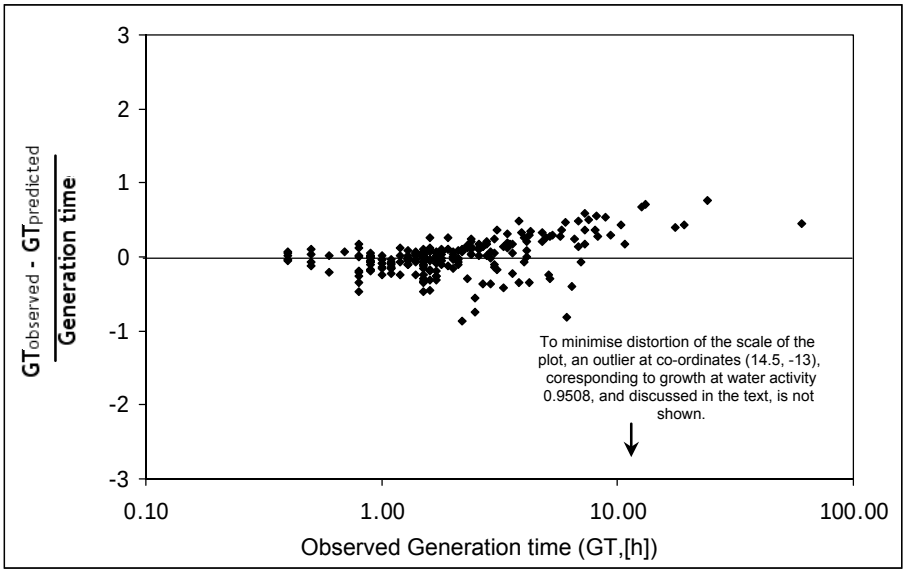


Fig. 1. Residuals plot of predictions of Equation 1 with the parameter values shown in Table 1 to the observations on which the model is based. The residual was divided by the corresponding observed generation time to normalise the deviation for the magnitude of the response

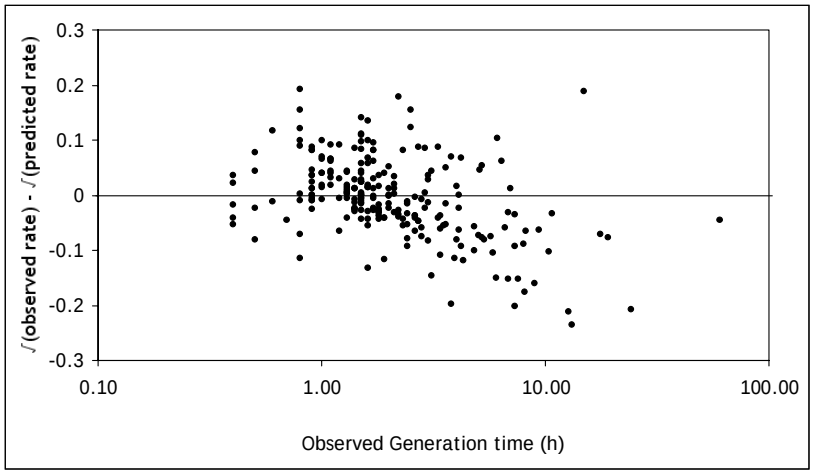


Fig. 2. Residuals plot for data used to derive the parameter values shown in Table 1 for Equation 1b. The observations and predictions are expressed as square root of relative rate to test the assumption that the square root prediction homogenises the variance in the data

The current model combines terms for high and low temperature, high and low water activity, high and low pH and dissociated and undissociated lactic acid to yield a new general model form.
Details of the model are published in the International Journal of Food Microbiology (Ross *et al.* 2003).

4 Performance of the model

The successful application of predictive modelling is dependent on the development of appropriate models but, prior to their application in industry, a performance evaluation of predictive models under conditions that were not used to derive the models is required. There are several ways in which model performance can be assessed. These include using sub-sets of the data set from which the model is derived, generating new data by laboratory experiments in liquid growth media or direct inoculation onto product, comparison to other data in the literature and trials in industry. It is generally accepted that comparing predicted responses to observed responses can assess the usefulness of a predictive model. Traditionally predictive models have been assessed statistically by the 'goodness of fit' of the data used to generate them and pictorial comparisons of observed and predicted data. Residual plots are also used to identify any non-linearity or non-constant variance in a model. Two indices of performance, the bias and accuracy factors (Ross, 1996, Baranyi *et al.*, 1999) are objective and quantitative measures that provide a simple means of reporting a readily interpretable assessment of model performance.

The bias factor (Bf) indicates systematic over- or under-prediction by a model. Perfect agreement between predictions and observations will give a bias factor of 1. Bias factors >1 predict generation time longer than observed and thus "fail dangerous" behaviour in the model. Conversely, bias factors <1 predict generation time less than observed and thus lead to "fail safe" predictions. As in many situations under- and over-predictions will tend to cancel out and the bias factor does not provide an indication of the average accuracy of estimates. This is provided by the accuracy factor which averages the deviation of each data point from the model's line of equivalence to measure the average 'distance' of the predictions from observations. Again a value of 1 indicates perfect agreement, whereas a factor of 2 indicates that the prediction is half or twice as large as the observation.

The following interpretation of the bias factor to assess model performance for pathogens was proposed by Ross, 1999:

Good:	0.90-1.05
Acceptable	0.70-0.89 or 1.06-1.15
Unacceptable:	<0.70->1.15

For the accuracy factor (Af), acceptable performance will depend on the number of parameters in the model as the error in growth rate estimates under controlled laboratory conditions is ~10% per independent variable (Ross *et al.,* 2000). Thus a model with 3 variables (temperature + pH + water activity) might be expected to have an error of ~30% or an accuracy factor of 1.3.

When model performance was evaluated for 1025 growth rate estimates in food, the bias and accuracy factors respectively were 0.92 and 1.29 indicating good performance. The UTAS model has generally outperformed the Pathogen Modelling Program (PMP) and Food MicroModel (FMM) and was particularly good when evaluated for 130 estimates of growth rate in meat with bias and accuracy factors of 0.97 and 1.26, respectively. That this was attributed to the inclusion of a term for lactic acid concentration in the UTAS model was demonstrated by poorer performance when the lactate term was removed i.e. bias factor 0.78 and accuracy factor 1.39.

Predictive model development and evaluation is a process involving continual improvement in model performance as more data describing the effects of additional environmental factors are included in the model. To illustrate this point, the performance of the new UTAS model was compared with the temperature only model of Salter *et al.* (1998) to describe independent data sets of Gill and Newton (1980), Grau (1983) and Smith (1985). The respective improvement in Bf and Af were as follows:

Data sets	Bf/Af using temperature-only model	Bf/Af using UTas model
Gill and Newton (1980)	0.43/2.31	0.70/1.43
Grau (1983)	0.53/1.90	0.72/1.40
Smith (1985)	0.90/1.18	1.04/1.09

A full description of how the performance of the new model was assessed is provided in Appendix 2, which comprises the manuscript of Mellefont *et al.* (2003).

> The performance of the model has been evaluated both by traditional criteria such as the 'goodness of fit' of the data and residual plots used to identify any non-linearity or non-constant variance in a model; in addition, two indices of performance, the bias and accuracy factors have been used. Full details of performance of the model have been published in the International Journal of Food Microbiology (Mellefont *et al.* 2003).

5 Validation of the model for predicting growth of *E. coli* during chilling and freezing of hot boned meat

A number of studies have been carried out in which hot boned meat has been inoculated with faeces or *E. coli* broth cultures and cooling followed under commercial conditions. These experiments have been done on meat trim in cartons and on chilled primals. The following sections detail the results of these trials.

5.1 Meat trim in cartons

Both sheep and cattle operations were assessed as part of studies carried out at CSIRO Meat Research Laboratory by Grau, Shay, Vanderlinde and others. Meat was inoculated with a suspension of faeces taken from similar animals slaughtered on the day of the trial. Faeces were diluted in water and filtered through cheesecloth before application. Meat was inoculated by brushing the inocula on to the surface of meat prior to packing. In all experiments coliform counts only were obtained, it is assumed that the majority of the coliform bacteria present were *E. coli* and in fact when measured *E. coli* usually accounted for >90% of the coliform count.

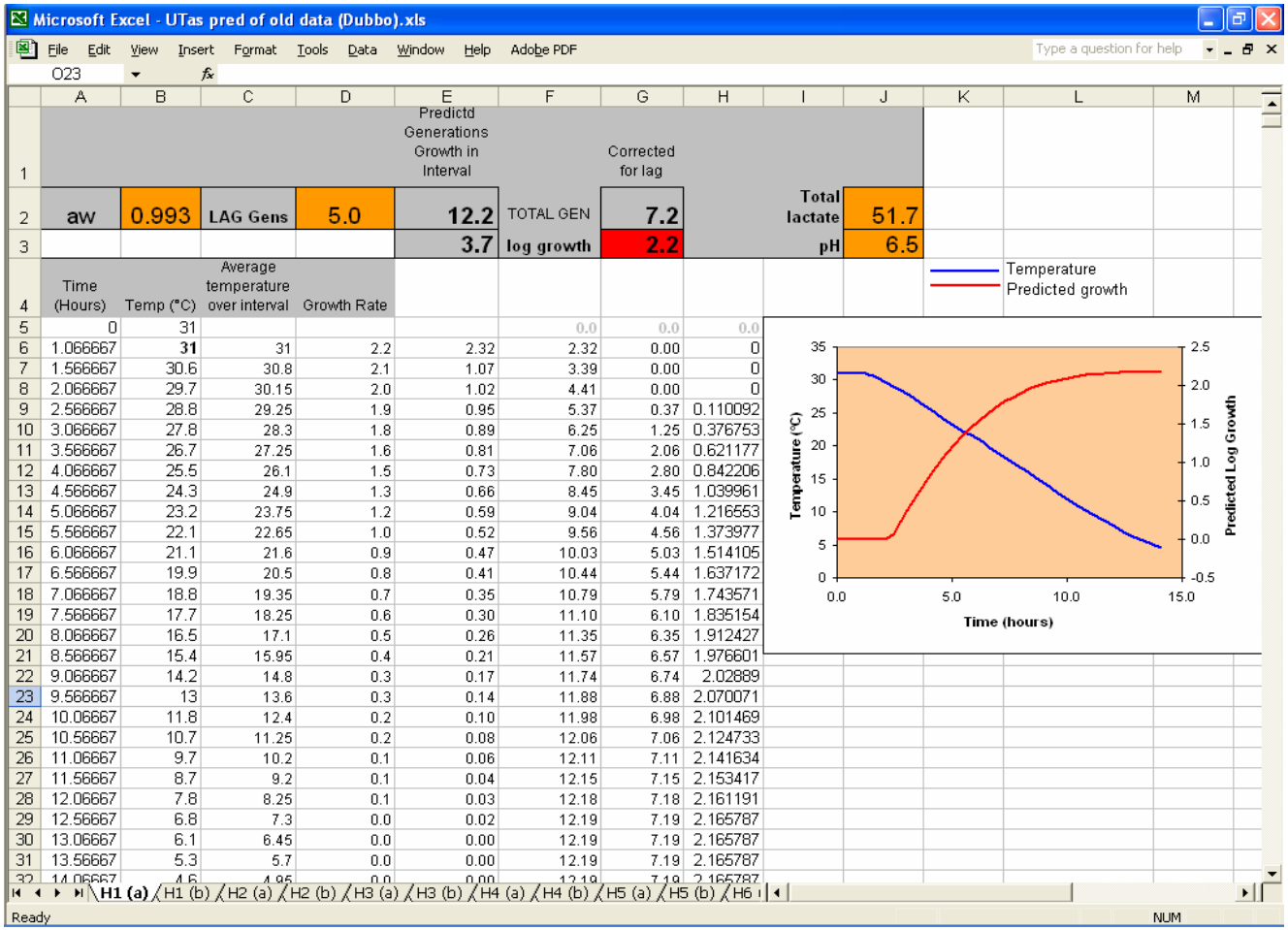
Samples for bacterial analysis were collected from the inoculated surfaces of meat placed as near to the thermal centre of cartons as possible. A sheet of plastic film was placed at the centre of each carton (between layers of meat) to allow the inoculated surface to be easily identified after freezing. Copper/constantan thermocouples were placed on either side of the plastic over the inoculated sites. Temperatures were monitored during cooling and freezing. Room temperatures were also recorded.

E. coli growth was modelled using an equation developed by the University of Tasmania. The equation requires input of the temperature history during cooling/freezing, the pH of the growth medium, the water activity and the lactate concentration. For meat in this trial the water activity was assumed to be 0.993. The lactate concentration in meat is related to the ultimate pH, for consistency the pH and lactate concentration were set for all calculations to 6.5 and 51.7 mM, respectively. A 5-generation lag was assumed before growth occurred.

Temperature histories from each trial were entered into an Excel (Microsoft) spreadsheet incorporating the predictive equation developed by the University of Tasmania and the predicted increase in *E. coli* estimated. An example of one of the calculations is given in Fig. 3 (a new calculation program has been developed that has a different appearance to figure 3 but makes exactly the same calculations). No allowance has been made for bacterial death as a result of freezing or of any reduction in *E. coli* numbers over time during frozen storage. Other models have been used to predict the growth of *E. coli* in meat during freezing; these models give similar predicted increases to those obtained using the University of Tasmania model once allowances are made for reductions that might occur during freezing. The predicted reductions obtained using the University of Tasmania model are compared to those found on inoculated sites in Fig 4. There was good agreement between the predicted increases and those found with 84% of the variability accounted for by the model (R^2=0.836).

On average, the model over predicts by 0.07 Log_{10} CFU/g, with the average difference between predicted and observed growth being 0.54 Log_{10} CFU/g (accepted by many as the limit of accuracy of the plate count technique see Jarvis, 1989). The 95% of the predicted values are <1.5 Log_{10} CFU/g different from the observed values. The largest deviation observed was 1.72 Log_{10} CFU/g.

Fig 3: Calculation of predicted growth in *E. coli* using an Excel spreadsheet model incorporating the predictive equation.



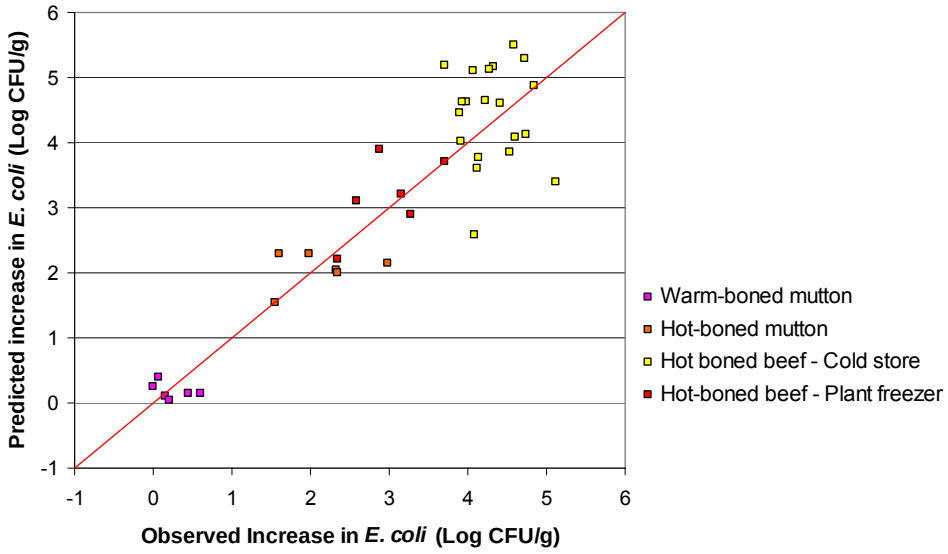


Fig 4: Observed increases (Log_{10} CFU/g) in coliform bacteria on inoculated surface (faecal suspension) of meat in cartons of boxed beef and sheep meat frozen under commercial conditions plotted against the predicted increase calculated using University of Tasmania model

A regression plot of the data showing the 95% confidence interval for the line and the 95% predictive interval is given in Fig. 5. When the two observation with large standard residuals were removed from the calculation a better fit (R^2 =0.89) was obtained (Fig. 6).

Data generated in these trials has some underlying variability. This may be due to the nature of the inoculum and the proportion of coliforms present in the faeces used in each experiment. The model developed by the University of Tasmania predicts the growth of *E. coli* and the growth of coliform bacteria may not be as well predicted by the model. A better fit can be obtained by using average values for the beef trial where zero time data were not collected (R^2 =0.9).

When the model was validated by trials involving inoculation of hot boned meat, there was good agreement between observed and predicted growth (R^2=0.89)

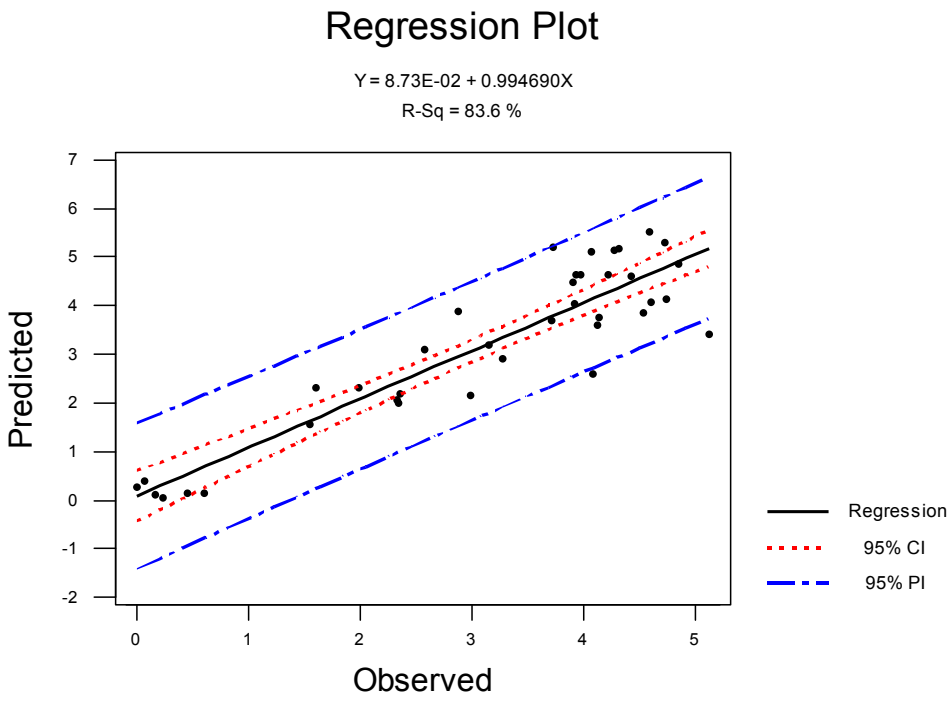


Fig 5: Regression plot of all data showing the line of best fit and its 95% confidence interval. The 95% prediction interval is also given

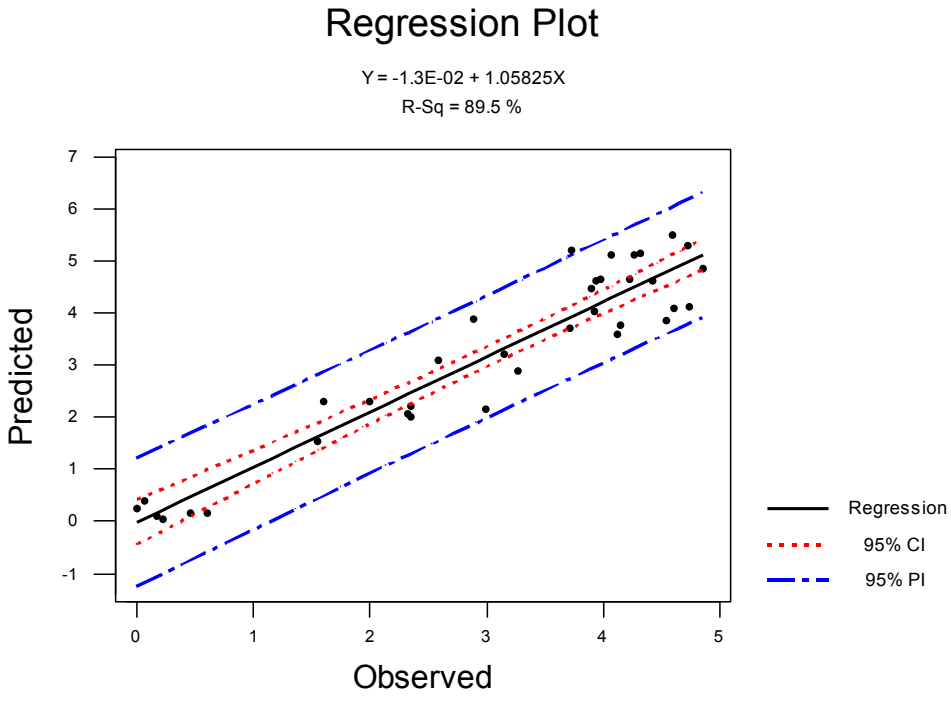


Fig 6: Regression plot of data without two unusual observations with large residues, showing the line of best fit and its 95% confidence interval. The 95% prediction interval is also given

5.2 Chilled primals

Growth of mesophilic bacteria on meat is dependent on the temperature, pH and tissue type. *Salmonella* and *E. coli* grew after only a short lag period, both aerobically and anaerobically, on beef fatty tissue and on high pH muscle (pH > 6) at 25 ^{0}C (Grau, 1983). During cooling of hot boned meat growth of *E. coli* and *Salmonella* is more likely on fatty tissue or muscles of high pH than on lean tissue of low pH. This could have a large impact on the predicted growth of *E. coli* on hot boned primals during cooling. Growth would be greater when fatty tissue is present at the surface of microbiological concern (i.e. the slowest cooling point in the carton) or when the meat has a high pH.

A number of experiments have been done over the past decade in which growth of *E. coli* was measured on lean and fat surfaces of primals (Grau, Shay, Vanderlinde and others, CSIRO Meat Research Laboratory). Organisms were inoculated either as broth culture or faecal slurry and the progress of cooling monitored by data logging. Bacterial counts were obtained from fatty tissue and lean surfaces before and after cooling to <7 ^{0}C. Predicted increases in *E. coli* were calculated using Equation 1, with parameters adjusted for low pH lean and high pH fat surfaces. The parameter values used in the model are given in Table 5. While these values do not necessarily represent the actual values of pH, a_W and lactate of the lean and fatty tissue used in the experiments, they are within the expected range of these parameters for these tissues and give the best overall fit to the observed data and therefore are average values industry can use for evaluating the effect of cooling on *E. coli* growth. Lactate concentrations were estimated based on published data (Grau, 1980; Bendall, 1979; Newbold and Scopes, 1967; and Puolanne and Kivikari, 2000).

Table 5: Parameter values used for estimating the growth of *E. coli* on lean and fatty surfaces of vacuum packaged primals during cooling.

	Low pH lean	Fat
pH	5.4	6.8
Lactate (mM)	86.5	0
a_W	0.993	0.990

Data are presented in Fig 7 for both faecal and broth inocula (with a 5-generation lag). The model accounts for 56% of the observed variability in the data. While not as good a fit as obtained with data for manufacturing meat it is still a reasonable fit given the underlying variability in the data. From experimental work conducted at CSIRO it is known that the pH of meat can vary both from muscle to muscle and at different locations within a muscle e.g. a striploin may have pH variability of 0.5 along the muscle. It is not practical to account for this variation in pH when applying the model on a day-to-day basis.

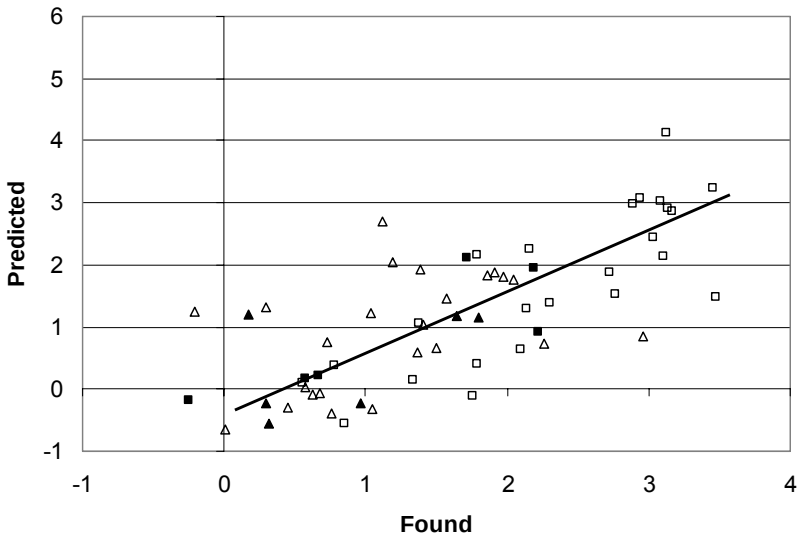


Fig 7: Observed increases (log_{10} CFU/cm^2) in E. coli on inoculated fat and lean surfaces (broth and faecal inocula) of hot boned primals during cooling. Meat was cooled in an experimental chiller. Predicted increase calculated using University of Tasmania model. Solid line is the regression line for all points. Open symbols broth culture, closed symbols faecal inocula. Squares are results from fatty tissue and triangles are from lean surfaces.

When the model was validated by trials involving inoculation of hot boned boxed meat, there was good agreement between observed and predicted growth (R^2=0.89). When the model was validated by trials involving the inoculation of primals, there was reasonable agreement between observed and predicted growth (R^2= 0.56).

6 Predicting meat temperature and the potential for pathogen growth on hot boned boxed trim during chilling

The risk posed by frozen boxed trim will depend, in part, on the amount of growth that occurs during chilling. The centre of a carton is the area of microbiological concern. This area may be small in relation to the overall size of the carton and therefore may not contribute significantly to the overall contamination of comminuted product derived from the trim after cooling.

An investigation was made to estimate the magnitude of growth occurring on trim during a typical cooling scenario and the subsequent bacterial load in minced meat

Meat temperatures throughout a carton of boxed beef were estimated using Food Product Modeller (FPM)[1]. Parameters in the model were set to represent a typical carton used by Australian manufacturers. The initial meat temperature was set at 31 ^{0}C and the cooling conditions simulated were -25 ^{0}C at an air speed of 3 ms^{-1}. To reduce the complexity of the spreadsheet model, *E. coli* growth was estimated using the CSIRO model. No allowance was made for a lag period prior to growth. The temperature history of the thermal centre of the meat is shown in Figure 8.

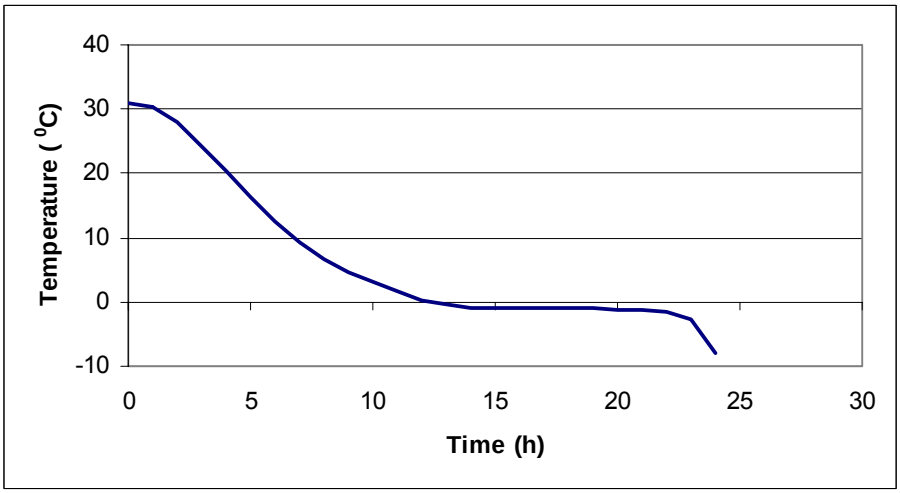


Fig 8: Predicted temperature history for boxed beef during cooling in a freezer operating at -25 ^{0}C with an air velocity of 3ms^{-1}

Temperature histories at 935 locations throughout the carton were modelled and the possible proliferation of *E. coli* estimated (5 x 11 x 17). An example of the calculated *E. coli* growth in the centre portion of the carton is shown in Figure 9.

[1] Version 2.00.1204. Copyright 1993-2000, MIRINZ Food Technology New Zealand and Meat & Livestock Australia

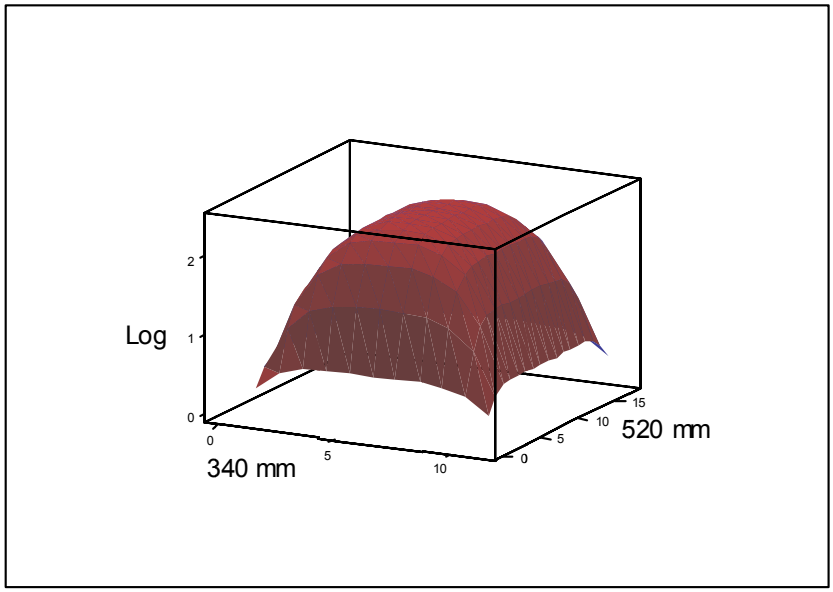


Fig 9: Estimated proliferation of E. coli in the central portion of a carton of meat during cooling at –25°C

The predicted proliferation was less at the surface (Figure 10).

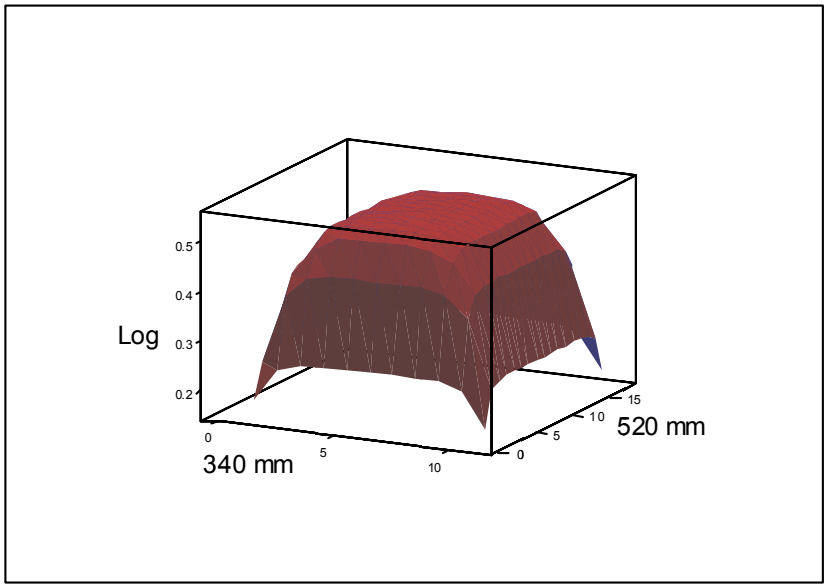


Fig 10: Estimated proliferation of E. coli near the surface of a carton of meat during cooling at -25 ^{0}C

It is not possible to get an estimation of the surface temperature using Food Product Modeller. This is due to the way that FPM proportionally sets temperature modelling points (or nodes) throughout the carton.

By adding the growth predicted at each temperature node and equating this to the weight of meat represented by a node and the total weight of met in a carton, it is possible to estimate the overall growth of *E. coli* during cooling. No allowance has been made for any death of *E. coli* as a result of chilling or freezing. In order to estimate the total number of *E. coli* after cooling it was assumed that the starting count was one *E. coli* per node i.e. 935 *E. coli* per carton (or 0.03 *E. coli* per g, since each node represents 29g). The estimated number of *E. coli* in the carton after cooling was 0.87 per g, an average increase of 1.4 logs. The maximum increase predicted at any site was 2.3 logs i.e. at the

thermal centre. The model cannot estimate surface temperatures due to the proportional allocation of nodes throughout the carton. The surface temperature was estimated for the top portion of the carton i.e. approximately 3 cm from the surface. The number of nodes that can be assigned is limited by computing power.

Modelling of temperatures throughout a carton of meat during cooling demonstrates that the overall increase in *E. coli* is lower than that calculated at the thermal centre
For a typical carton, growth at the thermal centre of a carton was estimated at 2.3 logs while the overall growth was only 1.4 logs, nearly one-log lower.

7 Predicting *E. coli* growth in chilled primals

There are a number of factors that influence the growth of bacteria on fresh meat. While temperature is the dominant controlling factor, intrinsic properties of the meat i.e. pH and water activity are also important. The general form of the predictive equation uses average values for pH and water activity for bulk packed meat to predict the growth of *E. coli* at various temperatures. This makes the models more 'user friendly' but also its applicability to all situations. The temperature used to calculate the growth of bacteria is also not representative of the whole product, being the temperature at the slowest cooling part of the carton. In effect these factors contribute to the inaccuracy of the model. The more a particular product differs from the general model the poorer the prediction obtained using the model.

Vacuum packaged primals are an example of a product that does not necessarily conform to the parameters used in the general model. Also, this type of product is usually consumed without comminuting; therefore contaminating bacteria will be destroyed on cooking. However, in some cases whole primals are ground before consumption and this needs to be considered when determining an acceptable hot boning index for this type of product. If an entire primal were ground and used for the purpose of making patties, the site of microbiological concern is shifted to the centre of the patty, with the possibility that pathogens introduced to the centre of the patty from the surface of the primal could introduce risk, typically if the patty were undercooked. If it is assumed that the entire carton is ground an estimate can be made of the overall increase in *E. coli* from the contact areas. There are other considerations as well. Muscle pH in primals is generally lower than that observed in bulk packed meat; therefore growth on exposed lean surfaces will be slower than that predicted using the general form of the model.

The following section details a practical example of how the potential risk of *E. coli* growth on vacuum packaged primals can be estimated. The example does not consider the effect of lean surfaces at the contact area and this may be something individual plants might like to consider when determining the refrigeration index for other products, such as heavily trimmed primals for specific markets. The example highlights the key data required for calculating the hot boning index for specific products i.e. the contact surface area in relation to the whole surface area and the weight of the primal

The following section details the findings from an investigation of:

- contact areas of large cuts such as rumps
- growth of *E. coli* on contact and non-contact surfaces of primals during cooling
- the effect of grinding on the overall increase of *E. coli*

Methodology

Rumps were selected because they represent large, slow-cooling primal cuts. Before the trial the total surface area of a number of rumps was estimated by removing the heat shrink vacuum bags and measuring the surface area of the bags. The surface area was then graphed against the weight of the primal (Fig 11). The relationship between primal weight and total surface area was used in the trial to estimate the surface area of each of the primals.

The cooling of vacuum packaged rumps was followed by locating thermocouples (copper/constantan) over contact surfaces and non-contact surfaces of selected cuts (Figs. 12-13). The contact area for each cut was estimated visually by sketching onto a piece of paper and then estimating the area with the aid of graph paper. Temperature histories were analysed using the model developed by researchers at the University of Tasmania. No allowance was made for differences between the pH of lean and fat surfaces at the contact area.

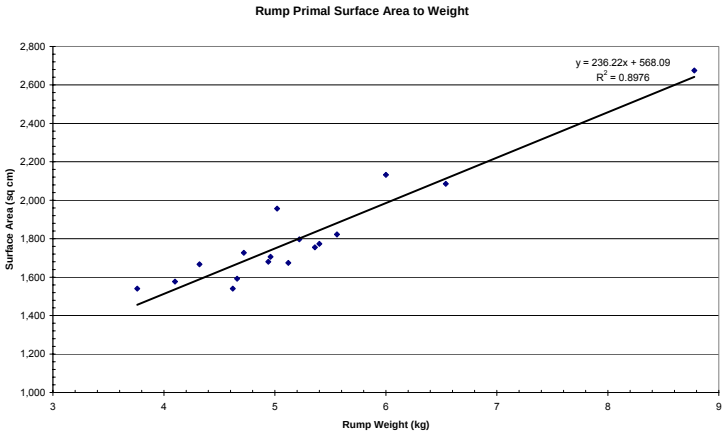


Fig 11: Relationship between the surface area and weight of rumps

Estimates were made of the predicted growth on all primals within each carton and of the count/g if the entire carton were ground. A correlation was made between the maximum HBI (located at the slowest cooling point) and the estimated log increase/g in ground primal.

Fig 12: Individually wrapped rumps showing placement of thermocouples and contact areas

Fig 13: Vacuum packaged rumps showing placement of thermocouples and contact areas

Results

Estimation of HBI across the primal surface

The Hot Boning Index (HBI) for sites across each primal monitored is given in Table 6. Only thermocouples located at the top and bottom of the cuts were outside the contact area. HBIs were calculated using the general form of the predictive equation (pH 6.5, a_W 0.993 and lactate 51.7 mM). HBIs at the centre of each contact area were higher than those towards the edge of the contact area. At the slowest cooling point (centre) HBIs ranged from 1.5-4.7. At contact points intermediate between the centre and periphery HBIs ranged from 1.3-4.3. At the edge of contact HBIs ranged from 0.1-2.7 and at the bottom (in contact with the carton floor) from 0-1.3. No growth in *E. coli* was predicted at the top of any of the rumps monitored during the trial.

Calculation of *E. coli* growth at the surface

The area was estimated of each of the zones listed in Table 6 and multiplied by the *E. coli* count/cm^2 prior to chilling, assumed to be 1CFU/cm^2 (this is a gross overestimation of the contamination but as we are only interested in the potential increase the starting count is irrelevant) The total surface area, calculated from the weight of each rump was used to estimate the total *E. coli* loading/carton before chilling. Bacterial loadings on each of the five zones listed in Table 6 were added to calculate the loading of *E. coli*/carton after chilling.

Table 6: Hot boning index calculated for surfaces on rumps during cooling

	Predicted log growth at each contact and non-contact point				
Carton	Centre	Intermediate	Edge of contact	Bottom and end	Top
1	2.9	2.4	1.4	1.2	0
2	3.7	3.1	1.1	1.3	0
3	3.8	3.0	0.6	0.8	0
4	2.8	2.0	0.2	0	0
5	4	3.6	0.9	0.5	0
6	4.7	4.0	1.4	0.5	0
7	3.7	2.9	0.4	0.3	0
8	3.3	2.9	1.0	0.5	0
9	2.6	2.8	2.1	0.5	0
10	2.6	2.6	2.2	0.5	0
11	2.7	2.6	2.0	0.2	0
12	2.3	2.1	1.5	0.4	0
13	1.5	1.3	0.1	0.1	0
14	1.9	1.6	0.2	0	0
15	3.1	3.1	0.9	0	0
16	3.6	3.0	2.1	0.6	0
17	4.5	3.2	0.8	0.4	0
18	4.6	4.3	2.7	0	0
19	4.4	4.1	2.4	0	0
20	2.7	2.0	0.4	0.5	0

Calculation of *E. coli* in ground primal

The total loading of *E. coli*/carton was converted to a count/g by dividing the total *E. coli*/carton by the weight of product in each carton. This was done both before and after chilling and, when the former was subtracted from the latter, it was possible to estimate the predicted increase in *E. coli*/g during chilling (Table 7). Since the initial count was assumed to be 1 CFU/g ($\log_{10}1=0$), then the $\log_{10}$ of the final count is equal to the HBI.

Table 7: Estimated *E. coli* counts (CFU/g) in ground rumps before and after chilling

	Log_{10} *E. coli*/g		
Carton	Before	After chilling	Increase
1	-0.45	0.97	1.42
2	-0.44	1.75	2.19
3	-0.48	1.54	2.02
4	-0.47	0.69	1.16
5	-0.48	2.16	2.64
6	-0.48	2.53	3.01
7	-0.47	1.62	2.09
8	-0.46	1.45	1.90
9	-0.46	1.21	1.68
10	-0.45	1.03	1.48
11	-0.46	1.18	1.63
12	-0.46	0.65	1.10
13	-0.47	0.01	0.47
14	-0.46	0.23	0.69
15	-0.46	1.39	1.85
16	-0.43	1.58	2.02
17	-0.46	2.18	2.65
18	-0.45	2.86	3.31
19	-0.48	2.55	3.03
20	-0.46	0.68	1.14

Correlation of maximum HBI and estimated increase in ground primals
It was possible to draw a correlation between the HBI predicted at the centre of the contact area for each carton of rumps and the estimated increase in *E. coli* count/g if the primals in the carton were ground (Figure 12). This correlation only applies to rumps packed and chilled under the conditions of the trial.

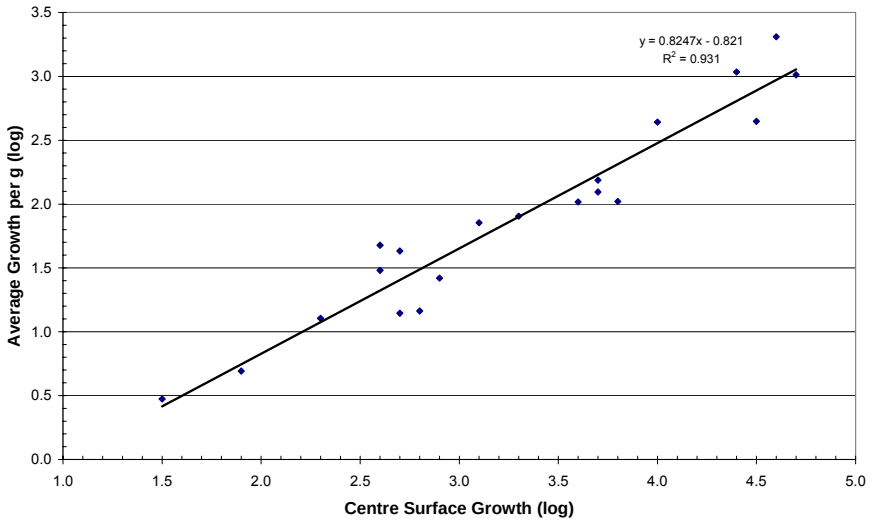


Fig 12: Relationship between centre HBI and estimated *E. coli*/g if all primals were ground

Discussion
There was a good relationship between the HBI and the predicted increase in *E. coli* should the entire carton be used for the manufacture of ground beef. Allowing for some variability, and the fact that

slower growth rates on lean surfaces were ignored, it appears that the HBI calculated at the slowest cooling point, overestimates the total growth per g by about 1-log or 10-fold.

The actual growth of *E. coli* on primals is not only a factor of the temperature but also the pH of the tissue and the surface area at slow cooling points.
The model indicates that growth will be slower on lean than on fatty surfaces. The area of the primal that cools slowly is relatively small.
In a trial on rumps, the average growth of *E. coli* was 1 $\log_{10}$ less than predicted by cooling at the slowest cooling site.

8 In-plant microbiological data

As part of the trial period during which HBIs were calculated for all chillers, plants undertook parallel microbiological analysis at the slowest cooling points. For manufacturing meat, this was the surface of a piece of trim place deliberately located at the centre of the carton. For primal cuts counts were carried out on large primals such as rumps at the slowest cooling point i.e. the centre of the contact area between cuts.

In Table 8 are presented summary data for plants A and B which monitored microbiological counts by drilling samples from defined sites of frozen manufacturing meat. Product means were log 1.90 and 2.5/g and prevalence of E. coli 2.0% and 11.5% at plants B and A, respectively.

Table 8: Total viable counts (TVC/g) from drilled samples of frozen manufacturing meat

	Log TVC/g			E. coli (%)
	Mean	SD	Maximum	
Plant A (n=113)	2.50	0.62	4.48	11.5
Plant B (n=737)	1.90	0.9	4.8	2.0

In Table 9 are presented TVCs taken from contact areas of primals after chilling in the carton at Plants A and C. Mean log TVC/cm^2 was 2.69 at Plant A and 1.47 at Plant C.

Plant C also monitored microbiology of product before and after chilling by sponging contact surfaces of large primals (rumps). As indicated in Table 9, for the 100 samples tested, the mean log TVC/cm^2 was 1.08 before chilling and 1.47 after.

Table 9: Total viable counts at contact surfaces of primals sampled at Plants A and C

	Log TVC/cm^2			E. coli (%)
	Mean	SD	Maximum	
Plant A (n=238)	2.69	0.68	5.0	12.6
Plant C unchilled (n=100)	1.08	0.55	3.23	Not done
Plant C chilled (n=100)	1.47	0.63	3.32	Not done

At Plant C data loggers placed on the area where counts were taken established a mean HBI of 1.1 with a maximum of 3.8 and minimum of zero. The mean increase in TVC (log 0.39) was less than that predicted by the HBI (1.1) which would be due to measuring TVC when the HBI predicts *E. coli* growth and also may be due to the testing of naturally-contaminated sites and that different sites were sampled pre- and post-cooling; the main criterion was that samples were taken from the centre of the contact area between cuts.

In Plant D, all cuts which had "high" HBIs (>3) were sampled. In Table 10 it can be seen that "worst case" counts (large, slow-cooling cuts) had a mean log TVC/cm^2 of 2.54, with counts ranging from log 1.44/cm^2 to log 3.40/cm^2. E. coli were detected on 2/18 samples (limit of detection 0.25/cm^2) with both counts being 0.5 cfu/cm^2.

Table 10: Total viable counts at contact surfaces of large primals which had high HBIs sampled at Plant D

	Mean	SD	Minimum	Maximum
Log TVC/cm^2 (n=18)	2.54	0.66	1.44	3.40

At Plant A, microbiological counts were obtained at the thermal centre of naturally-contaminated trim located at the slowest cooling point of the carton. After freezing a sample approximately 25cm^2 was chipped with a sterile chisel and the TVC and E. coli count obtained. As indicated from Table 11, the mean TVC was log 2.75/cm^2 at the slowest cooling point. *E. coli* was detected on 19/170 samples, 18 of which were <50/cm^2 and one was 810/cm^2.

Table 11: Total viable counts at contact surfaces of large primals which had high HBIs sampled at Plant A

	Mean	Minimum	Maximum
Log TVC/cm^2 (n=170)	2.75	1.44	3.40

In-plant chilling data (HBIs)

Plants routinely monitor rates of chilling by inserting data loggers at the slowest cooling points of manufacturing (bulk) meat and primals. In Table 12 is presented summary chilling data for manufacturing meat from Plants A and B where the former was able to conform with all three performance criteria (mean = 1.5; 80th percentile 2.0; maximum 2.5) while the latter (Plant B)conformed except for one logger which exceeded the target maximum of 2.5.

Table 12: HBIs for manufacturing meat from Plants A and B

Plant	Samples (n)	Mean	80th percentile	Maximum
A	308	0.5	1.0	2.5
B	39	0.4	0.7	3.0

In Table 13 is presented summary chilling data for primals at Plants A-D. HBIs were higher for primals than for manufacturing meat, particularly for large primals (e.g. rumps with mass greater than 6kg). Plant A conformed with all three performance criteria. Plants B-D were able to conform with the mean performance criterion (HBI=1.5) but were unable to meet the target maximum (HBI=2.5) or, in the case of large primals, with the 80th percentile (HBI=2.0).

Table13: HBIs for primals from Plants A-D

Plant	Samples (n)	Mean	80th percentile	Maximum
A	29	0.9	1.5	2.4
B	111	1.0	1.62	3.5
C*	81	1.4	2.6	5.1
C*	100	1.5	2.6	3.9
C	100	1.1	1.9	3.8
D	37	1.1	2.0	2.9

* Only large primals (rumps) surveyed

Plants are able to comply with the HBI, though problems may be encountered with large primals. Taken as a whole, these "worst case" data reflect low levels of post-chilling contamination, even when high HBIs have occurred.

9 On-plant operation of the Hot Boning Index

All hot boning plants undertake data logging of manufacturing meat and of primals in order to validate that chilling/freezing regimes can conform with the hot boning criteria.

For ongoing verification that the criteria are being met, plants will undertake data logging that reflects the range and volume of hot boned products manufactured. That is, both boxed beef and primals will be monitored in approximate proportion to volumes manufactured. A calculation tool (based on this publication) has been developed that allows selection of products according to whether they are trim or primals and, in the latter case, whether they are predominantly lean or have significant fat cover e.g. rumps. In this way, over time, continued conformance with the criteria will be verified.

While some products, such as large rumps, may have HBI > 2.5 at the contact area with the next primal in the carton, non-contact areas of the cut have HBI=0 (see Table 6).

Plants may apply a risk-based approach to deal with high HBIs. Assessment of risk takes account of the fact that:

- The site of microbiological concern is only a relatively small proportion of the surface area of the primal.
- Primals generally are consumed after a cooking process which destroys pathogens at the surface. In such cases the risk of consuming a pathogenic organism is extremely low.
- Occasionally, large cuts or portions of large cuts may be ground. The site of microbiological concern is now altered from surface contamination of the primal to the entire mass of the ground product. Should the product be undercooked, the risk is increased, relative to that of the risk associated with the intact primal.

A calculation tool (based on this publication) has been developed that may be used for rumps. It takes account of the risk associated with ground product by integrating the contact and non-contact areas and their respective HBIs to give a modified HBI. This approach may be used with all primals and modified to various levels of complexity by dividing primals into areas each of which has an HBI. This is the approach taken in Chapter 7.

The risk-based approach may be augmented by microbiological monitoring which will provide further evidence of satisfactory control of the process.

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Appendix 1: *E. coli* Growth Model

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Modelling the effects of temperature, water activity, pH and lactic acid concentration on the growth rate of *Escherichia coli*

T. Ross*, D.A. Ratkowsky, L.A. Mellefont, T.A. McMeekin

Centre for Food Safety and Quality, School of Agricultural Science, University of Tasmania, GPO Box 252-54, Hobart 7001, Tasmania, Australia

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Abstract

An extended square root-type model describing *Escherichia coli* growth rate was developed as a function of temperature (7.63–47.43 °C), water activity (0.951–0.999, adjusted with NaCl), pH (4.02–8.28) and lactic acid concentration (0–500 mM).

The new model, based on 236 growth rate data, combines and extends previously published square root-type models and incorporates terms for upper and lower limiting temperatures, upper and lower limiting pH, minimum inhibitory concentrations of dissociated and undissociated lactic acid and lower limiting water activity. A term to describe upper limiting water activity was developed but could not be fitted to the *E. coli* data set because of the difficulty of generating data in the super-optimal water activity range (i.e. >0.998). All data used to generate the model are presented.

The model provides an excellent description of the experimental data.

Keywords: *Escherichia coli*; Predictive model; Growth rate; Temperature; pH; Lactic acid; Water activity

1. Introduction

The provision of a safe and wholesome food supply is considered a basic tenet of a developed nation (McMeekin and Olley, 1995), yet the incidence of food-borne disease in the developed world is increasing and new pathogens continue to emerge (Maurice, 1994). Verocytotoxigenic *Escherichia coli* (VTEC) emerged as a serious food-borne threat to public health in the latter part of the 20th century (Johnson et al., 1996; Jaeger and Acheson, 2000). Pathogenic *E. coli* have been implicated in outbreaks involving meat products, contaminated recreational and drinking waters, contaminated vegetables and fruit juices and visits to farms or fields that have previously been grazed by cattle. In the majority of cases, the ultimate sources of the contamination or infections are believed to be faecal contamination from ruminants, particularly from cattle or sheep (Desmarchelier and Grau, 1997; Thorns, 2000).

During commercial slaughter of domestic animals, it is nearly impossible to guarantee that the carcass surface is not contaminated with faecal matter. Visual inspection and removal and carcass washing can reduce, but not reliably eliminate, contamination.

* Corresponding author. Tel.: +61-3-62-26-1831; fax: +61-3-62-26-2642.
E-mail address: Tom.Ross@utas.edu.au (T. Ross).

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Similarly, while decontamination strategies including acid washes (Smulders and Greer, 1998) and other methods (Sofos and Smith, 1998) can further reduce contamination, carcasses free from *E. coli* contamination cannot be guaranteed. The probability of human infection is generally considered to increase with the dose ingested (Coleman and Marks, 1998; Holcomb et al., 1999). Thus, to develop strategies to minimise public health risk, it is important to understand the potential for growth of *E. coli* on carcasses during processing, chilling, storage and distribution.

Temperature strongly influences the potential for, and rate of, growth of bacteria on carcass surfaces. In Australia, carcasses are chilled by circulation of cold air which introduces a second constraint, i.e. water activity, due to surface drying of the carcass which can decline to 0.95 and remain at levels below 0.97 for 20–30 h during and after chilling (Lovett, 1978; Salter, 1998). The pH, and in particular the level of organic acids (predominantly lactic acid), can also affect growth potential (Presser et al., 1997, 1998) with levels in red meat being of the order of 100 mM (Grau, 1981).

The long history of the use of *E. coli* as an experimental organism, its importance as an indicator organism, and latterly, its emergence as a serious foodborne pathogen, is reflected in the amount of data available describing its growth responses to different environmental conditions. Published kinetic models for *E. coli* growth include those of Gill and Phillips (1990), Heitzer et al. (1991), Buchanan et al. (1993), Buchanan and Bagi (1994), Rosso et al. (1995), Sutherland et al. (1995), Kovárová et al. (1996), Presser et al. (1997), Sutherland et al. (1997) and Salter et al. (1998). Those models were constructed using a variety of strains, substrate and environmental parameters (both constant and varying) but none consider the combined effects of temperature, water activity, pH and lactic acid concentration, variables that may be relevant to the prediction of *E. coli* growth in meat and on carcasses, particularly under Australian processing conditions. We describe here the development of a new model for the combined effects of temperature, water activity, pH and lactic acid concentration on the growth rate of *E. coli* that encompasses and extends the results and models of Presser et al. (1997) and Salter et al. (1998).

2. Materials and methods

2.1. Model development

Previously, square root-type models for the combined effects of temperature and water activity on *E. coli* growth rate (Salter et al., 1998) and for the effects of temperature, pH and lactic acid (Presser et al., 1997) were presented. We combine here the terms for high and low temperature, high and low water activity, high and low pH and dissociated and undissociated lactic acid to yield a new general model form, viz:

$$\begin{aligned}\sqrt{r} = {} & c(T - T_{\min})(1 - \exp(d(T - T_{\max}))) \\ & \times \sqrt{(a_w - a_{wmin})} \\ & \times \sqrt{(1 - \exp(g(a_w - a_{wmax})))} \\ & \times \sqrt{(1 - 10^{(pHmin - pH)})} \\ & \times \sqrt{(1 - 10^{(pH - pHmax)})} \\ & \times \sqrt{(1 - [LAC]/(U_{\min}(1 + 10^{(pH - pK_a)})))} \\ & \times \sqrt{(1 - [LAC]/(D_{\min}(1 + 10^{(pK_a - pH)})))} \pm e\end{aligned} \tag{1a}$$

where r = relative growth rate or specific growth rate (time^{-1}), c, d and g = fitted parameters, a_w = water activity, a_{wmin} = theoretical minimum water activity below which growth is not possible, a_{wmax} = theoretical maximum water activity above which growth is not possible, T = temperature, $T_{\min}$ = theoretical minimum temperature below which growth is not possible, $T_{\max}$ = theoretical maximum temperature beyond which growth is not possible, pH has its usual meaning, $pH_{\min}$ = theoretical minimum pH below which growth is not possible, $pH_{\max}$ = theoretical maximum pH beyond which growth is not possible, [LAC] = lactic acid concentration (mM), $U_{\min}$ = minimum concentration (mM) of undissociated lactic acid which prevents growth when all other factors are optimal, $D_{\min}$ = minimum concentration (mM) of dissociated lactic acid which prevents growth when all other factors are optimal, pK_a is the pH for which concen-

Table 1
Parameter values for the growth rate model for *E. coli*

Parameter	Estimate	Asymptotic standard error
c	0.2345	0.0083
T_{min}	4.14	0.63
T_{max}	49.55	0.42
pH_{min}	3.909	0.031
pH_{max}	8.860	0.19
U_{min}	10.43	0.52
D_{min}	995.5	106
a_{wmin}	0.9508	0.0004
D	0.2636	0.038
Root mean square error (RMSE) in $\sqrt{(1/(GT\ [h]))}$	0.0054	

trations of undissociated and dissociated lactic acid are equal, reported to be 3.86 (Budavari, 1989).

2.2. Data generation

Relative growth rate data (i.e. the reciprocal of generation time [h]), calculated from percent transmittance measurements and fitted to a modified-Gompertz model as previously described (McMeekin et al., 1993; Dalgaard et al., 1994), were collated from several studies including data previously reported by Salter (1998), Mellefont (2000) and Presser (2001). Some of these data were modelled by Presser et al. (1997) and Salter et al. (1998). Presser (2001) presented 96 data for the effects of temperature (20.88–22.76 °C), pH (4.02–8.28), water activity (0.986–0.998) and lactic acid concentration (0–500 mM) on the relative growth rate of *E. coli* M23. Similarly, Salter (1998) presented 96 data for the effect of temperature (7.63–47.43 °C) and water activity (0.966–0.997) on relative growth rate of *E. coli* M23 at pH 7.4. Relative growth rate data ($n=71$) for the effect of water activity (0.951–0.990) at temperature 25.2 ± 0.2 °C and pH 7.4 on *E. coli* SB1 was presented by Mellefont (2000). In the latter study, reported relative growth rates were estimated from $\log_{10}$(absorbance) data by linear regression of the transformed data representing the exponential growth phase. For consistency in this study, data from Mellefont (2000) were recalculated using the methods described above after converting the raw data to percent transmittance (%T) values using the relationship $\%T = 10^{(2\ -\ \text{absorbance})}$. All data used in generation of the model are shown in Appendix A.

To homogenise variance in the response variable (Ratkowsky et al., 1996), the growth rate model (Eq. (1a)) was fitted to the square root of *E. coli* relative

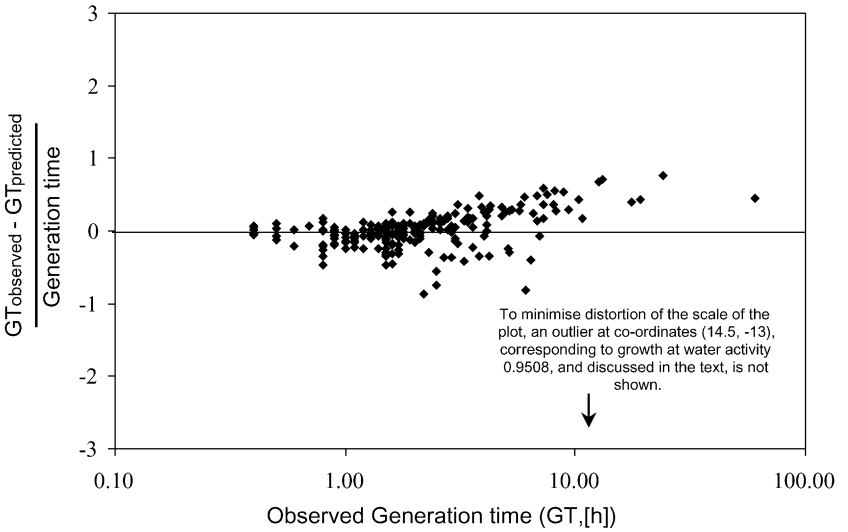

Fig. 1. Residuals plot of predictions of Eq. (1b) with the parameter values shown in Table 1 to the observations on which the model is based. The observations and predictions are expressed as generation times to aid understanding. The residual was divided by the corresponding observed generation time to normalise the deviation for the magnitude of the response.

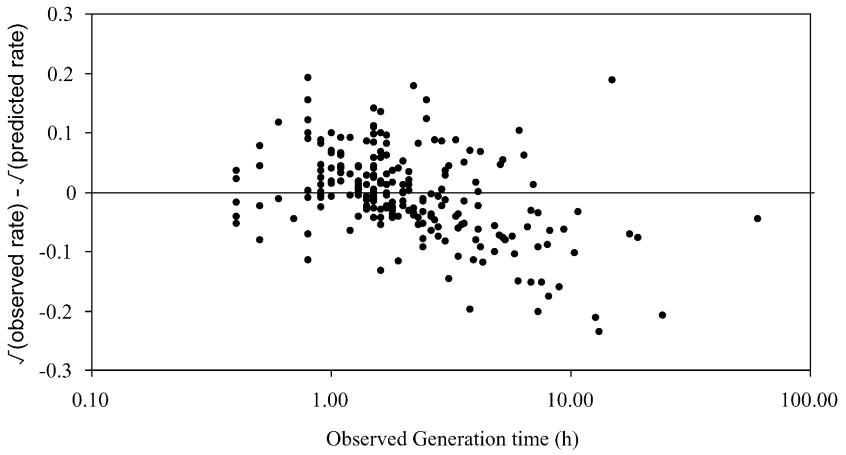

Fig. 2. Residuals plot for data used to derive the parameter values shown in Table 1 for Eq. (1b). The observations and predictions are expressed as square root of relative rate to test the assumption that the square root prediction homogenises the variance in the data.

growth rate data (Appendix A) using the SAS non-linear regression procedure PROC NLIN (SAS Institute, Cary, NC).

3. Results

For some parameters, convergence could not be achieved, due to insufficient data describing growth rates in the regions of interest. Specifically, relative growth rate data in the super-optimal a_w region, i.e. >0.998, were sparse and precluded reliable estimates of the parameter a_{wmax}. Consequently, data were fitted to a reduced form of the general model as shown, below:

$$\sqrt{r} = c(T - T_{min})(1 - \exp(d(T - T_{max}))) \times \sqrt{(a_w - a_{wmin})}\sqrt{(1 - 10^{(pHmin - pH)})} \times \sqrt{(1 - 10^{(pH - pHmax)})} \times \sqrt{(1 - [LAC]/(U_{min}(1 + 10^{(pH - pK_a)})))} \times \sqrt{(1 - [LAC]/(D_{min}(1 + 10^{(pK_a - pH)})))} \pm e \quad (1b)$$

where all terms are as previously described.

Fitted parameter values and asymptotic standard errors on those estimates are shown in Table 1. Observed and predicted generation times (GT) are compared in Fig. 1, as residuals normalised to the observed generation time, i.e. (Observed GT – Pre-Predicted GT)/(Observed GT), as a function of generation time. To illustrate the validity of the use of the square root transformation to homogenise variance in the relative growth rate data, residuals in this transformation are presented as a function of generation time in Fig. 2.

The accuracy and bias factors (Ross, 1996) for the model predictions of generation time compared to the original data were 1.21 and 0.97, respectively.

4. Discussion

The models of Presser et al. (1997) and Salter et al. (1998) were originally developed for the application to prediction of *E. coli* growth in unprocessed red meats. The model of Salter et al. (1998) describes the effect of water activity and temperature on *E. coli* growth rate, but does not consider the effect of pH or acidulant. The model of Presser et al. (1997) has five terms describing the inhibition of *E. coli* growth due to temperature, water activity, pH, the dissociated form of lactic acid and the undissociated form of lactic acid. That equation was developed primarily

for use with foods that are acidic to neutral and for which temperatures are suboptimal for *E. coli* growth rate. In both of those models, the amount and range of water activity data were limited.

Unlike many nations, Australian meat chilling is based on air cooling, resulting in water activity decreases at the carcass surface (Lovett, 1978; Salter, 1998) that are sufficient to severely inhibit *E. coli* growth rate. To create a model applicable to meat under Australian processing conditions, data from these studies were combined and supplemented with new data (Mellefont, 2000) that describe in detail the effect of water activity on *E. coli* growth rate. While the pH on carcasses during and after chilling is usually in the pH range 5.4–7.0, the existence of data for conditions beyond these ranges enabled inclusion in the model of terms for the super-optimal pH and temperature ranges resulting in a more complete, and thus more versatile, model potentially suitable for application to a wider range of foods.

The accuracy factor (Ross, 1996) for the model predictions compared to the original data was 1.21. Ross et al. (2000a,b) proposed that, as a "rule of thumb", the relative error in generation time or growth rate estimates under controlled laboratory conditions is around 10% per independent variable. The bias factor for a model compared to the data used to generate it would be expected to be 1.00 (Ross, 1996) unless the model was fitted to a transformation of the data other than the logarithm of the growth rate or generation time. In this study, the model was fitted to square root of rate data and may account for the bias factor of 0.97. These measures, together with the small standard errors of parameter estimates and the low fitting error (see Table 1), indicate that fitted model describes the data very well. Fig. 1 enables the fit of the model to the data to be visualised, and reveals slight, but systematic, lack of fit to the data for longer generation times, that would lead to the prediction of faster growth than what is observed. The same pattern of residuals was seen when non-normalised residuals were plotted (data not shown). This deviation is possibly due to the influence of the single, outlying, point observed at a water activity of 0.951, marginally above the estimated a_{wmin} of 0.9508. Due to the form of the model, at conditions very near to the notional minimal conditions for growth, predicted generation times are very long, as in this case where the predicted generation time (209 h) is vastly in excess of the observed generation time of 14.9 h. It is possible that other forms of predictive microbiology model could better describe the data set but, given the overall quality of fit of the model to the data, we did not explore this. The full data set used in the model is, however, presented in Appendix A enabling others to evaluate the merits of alternative model forms.

The utility of the model can be reliably assessed, however, only by comparison to independent data not used to generate the model. Before predictions of the new model can be compared to the growth rates reported by other workers, however, it is important to recognise that systematic differences between growth rate estimates derived from turbidimetric data have been reported (Dalgaard et al., 1994). Similarly, while growth data in predictive microbiology studies have often been fitted to the modification of the Gompertz model introduced by Gibson et al. (1987), microbial growth rate traditionally is determined from viable count assays by fitting, or estimating the line of best fit, to the exponential phase of growth (Koch, 1981). It is well documented (Whiting and Cygnarowicz-Provost, 1992; Baranyi, 1992; Ross, 1993; Dalgaard et al., 1994; Membré et al., 1999) that growth rate estimates derived from fitted Gompertz functions typically overestimate the calculated exponential growth rate by 10–15%. We consider that predictive models should provide estimates consistent with the traditional methods of growth rate determination and the assumption of exponential growth.

These systematic differences can be accommodated, however, by the inclusion in the fitted model of simple correction factors. The combined effect of the above systematic errors on growth rate estimates is such that the fitted model predicts growth rates that are ~71% of those that would be estimated from viable counts by the method of Koch (1981). To make predictions of the model consistent with estimates by those methods, the constant c in Table 1 should become 0.2790 (i.e. correction for $\mu_{\mathrm{VC}}/\mu_{\%T}=1.57$ (Dalgaard et al., 1994), correction for $\mu_{\mathrm{Gompertz}}=0.9$ (see above), $\sqrt{(1.57\times 0.9)}=1.19$, correction to b (Table 1) is $1.19\times 0.2345=0.279$). The fitted model, after inclusion of this correction factor, can be considered to be 'calibrated' to traditional methods of growth rate estimation by colony counts.

The over-prediction of slow growth rates as mentioned above was also noted when comparing the model predictions to independent data (Mellefont et al., in press). This resulted in the model having less accurate, but 'fail-safe', predictions for generation times longer than 5 h with the exception of meats for which bias and accuracy factors were 'good' by the criteria of Ross et al. (2000a,b) for all generation times. Interestingly, the same over-prediction of slow growth rates was also noted by Mellefont et al. (in press) for two other widely used predictive models for *E. coli* growth.

The evaluation of performance of the new, calibrated, model is described in detail by Mellefont et al. (in press) who reported that the model predicted well 1025 growth rate estimates reported in the literature after poor quality or unrepresentative data were excluded, with a bias value of 0.92, and an accuracy factor of 1.29.

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The authors would like to thank their colleagues Drs. Mark Salter and Kirsty Presser for providing their data. This study was funded by Meat and Livestock Australia and their support is gratefully acknowledged.

Appendix A. Data set used to create the model

Data source	Temperature (°C)	a_w	pH	LAC (mM)	GT (h)	sqrt(1/GT)
Presser (2001)	20.88	0.998	5.00	100	7.3	0.370
	20.88	0.997	4.42	25	4.0	0.500
	20.92	0.998	5.20	100	3.4	0.542
	20.94	0.991	5.57	100	2.1	0.690
	20.94	0.991	5.85	100	2.0	0.707
	20.94	0.991	7.25	100	2.4	0.645
	20.94	0.998	5.41	100	2.1	0.690
	20.94	0.998	5.74	100	1.7	0.767
	20.94	0.997	4.52	25	3.0	0.577
	20.94	0.997	4.57	25	2.3	0.659
	20.96	0.998	6.16	100	1.7	0.767
	20.96	0.997	6.02	25	1.5	0.816
	20.98	0.989	7.53	200	2.8	0.598
	20.98	0.989	8.14	200	3.9	0.506
	20.98	0.997	5.16	25	1.9	0.725
	21.00	0.997	6.73	25	1.6	0.791
	21.02	0.991	6.32	100	1.8	0.745
	21.08	0.997	4.02	0	7.3	0.370
	21.10	0.989	7.10	200	2.6	0.620
	21.12	0.991	5.58	100	2.4	0.645
	21.12	0.997	4.07	0	3.6	0.527
	21.14	0.989	7.86	200	3.0	0.577
	21.14	0.997	4.13	0	3.0	0.577
	21.18	0.997	4.39	0	2.3	0.659
	21.20	0.997	4.56	50	6.4	0.395
	21.20	0.997	4.60	0	1.9	0.725
	21.22	0.997	4.60	50	5.2	0.439
	21.23	0.989	5.77	200	2.9	0.587
	21.26	0.997	4.71	50	4.1	0.494
	21.26	0.997	5.27	0	1.5	0.816
	21.26	0.997	6.88	0	1.4	0.845
	21.28	0.986	8.14	500	2.5	0.632
	21.30	0.986	7.78	500	2.5	0.632

Appendix A (*continued*)

Data source	Temperature (°C)	a_w	pH	LAC (mM)	GT (h)	sqrt(1/GT)
Presser (2001)	21.30	0.997	5.08	50	2.0	0.707
	21.32	0.989	5.58	200	6.0	0.408
	21.32	0.989	6.32	200	2.1	0.690
	21.32	0.989	6.72	200	1.6	0.791
	21.32	0.989	8.28	200	2.8	0.598
	21.38	0.986	6.76	500	7.5	0.365
	21.38	0.997	6.13	50	1.4	0.845
	21.42	0.989	6.01	200	1.6	0.791
	21.56	0.989	7.28	200	2.2	0.674
	21.60	0.986	6.86	500	8.1	0.351
	21.70	0.989	6.69	200	1.5	0.816
	21.70	0.994	5.2	200	6.1	0.405
	21.76	0.986	7.75	500	3.1	0.568
	21.76	0.994	5.31	200	8.0	0.354
	21.82	0.994	5.84	200	1.7	0.767
	21.84	0.989	5.65	200	4.3	0.482
	21.88	0.986	8.14	500	2.2	0.674
	21.94	0.989	6	200	1.5	0.816
	21.96	0.991	7.51	100	1.8	0.745
	21.96	0.992	5.02	50	2.6	0.620
	21.98	0.989	7.45	200	2.3	0.659
	22.00	0.994	7.14	0	1.3	0.877
	22.02	0.992	6.68	50	1.5	0.816
	22.02	0.992	7.21	50	1.6	0.791
	22.02	0.994	5.14	0	1.6	0.791
	22.04	0.991	5.10	100	6.8	0.383
	22.04	0.991	7.10	100	1.5	0.816
	22.04	0.994	6.58	0	1.4	0.845
	22.06	0.989	6.96	200	1.6	0.791
	22.06	0.994	4.27	0	2.7	0.609
	22.06	0.994	6.14	0	1.4	0.845
	22.08	0.989	5.40	200	5.7	0.419
	22.08	0.992	5.56	50	1.8	0.745
	22.10	0.989	7.28	200	2.0	0.707
	22.10	0.991	6.04	100	1.6	0.791
	22.10	0.991	6.94	100	1.5	0.816
	22.10	0.992	6.01	50	1.5	0.816
	22.10	0.992	7.56	50	1.5	0.816
	22.10	0.994	5.86	0	1.4	0.845
	22.12	0.989	5.93	200	1.7	0.767
	22.12	0.992	4.78	50	3.6	0.527
	22.12	0.994	6.15	0	1.3	0.877
	22.12	0.994	6.94	0	1.3	0.877
	22.16	0.991	6.52	100	1.5	0.816
	22.16	0.991	7.19	100	1.5	0.816
	22.18	0.992	7.58	50	1.5	0.816
	22.18	0.994	4.88	0	1.5	0.816
	22.26	0.989	7.78	200	3.8	0.513
	22.26	0.992	7.49	50	1.5	0.816
	22.26	0.994	7.44	0	1.3	0.877
	22.26	0.994	7.60	0	1.3	0.877
	22.36	0.991	7.80	100	1.8	0.745
	22.38	0.991	7.61	100	1.8	0.745

(continued on next page)

Appendix A (*continued*)

Data source	Temperature (°C)	a_w	pH	LAC (mM)	GT (h)	sqrt(1/GT)
Presser (2001)	22.38	0.992	6.10	50	1.5	0.816
	22.40	0.989	7.65	200	2.4	0.645
	22.44	0.992	5.39	50	1.6	0.791
	22.46	0.989	6.25	200	1.5	0.816
	22.48	0.994	5.53	0	1.3	0.877
	22.50	0.991	5.59	100	1.7	0.767
	22.52	0.992	7.78	50	1.5	0.816
	22.60	0.989	7.88	200	3.1	0.568
	22.62	0.994	7.88	0	1.4	0.845
	22.76	0.991	8.00	100	1.8	0.745
Salter (1998)	7.63	0.997	7.40	0	60.5	0.129
	10.30	0.997	7.40	0	19.1	0.229
	12.03	0.997	7.40	0	9.3	0.328
	13.20	0.997	7.40	0	6.6	0.389
	14.48	0.997	7.40	0	4.8	0.456
	15.00	0.966	7.40	0	17.6	0.238
	15.00	0.969	7.40	0	10.7	0.306
	15.00	0.972	7.40	0	7.0	0.378
	15.00	0.976	7.40	0	5.1	0.443
	15.00	0.979	7.40	0	4.2	0.488
	15.00	0.982	7.40	0	3.8	0.513
	15.00	0.985	7.40	0	3.3	0.550
	15.00	0.991	7.40	0	2.9	0.587
	15.00	0.994	7.40	0	2.7	0.609
	16.03	0.997	7.40	0	3.5	0.535
	17.38	0.997	7.40	0	2.7	0.609
	18.53	0.997	7.40	0	2.2	0.674
	20.00	0.971	7.40	0	5.0	0.447
	20.00	0.971	7.40	0	5.8	0.415
	20.00	0.974	7.40	0	4.1	0.494
	20.00	0.974	7.40	0	4.8	0.456
	20.00	0.977	7.40	0	3.0	0.577
	20.00	0.977	7.40	0	3.3	0.550
	20.00	0.980	7.40	0	2.6	0.620
	20.00	0.983	7.40	0	2.0	0.707
	20.00	0.983	7.40	0	2.1	0.690
	20.00	0.986	7.40	0	1.7	0.767
	20.00	0.986	7.40	0	1.9	0.725
	20.00	0.988	7.40	0	1.5	0.816
	20.00	0.988	7.40	0	1.7	0.767
	20.00	0.991	7.40	0	1.5	0.816
	20.00	0.991	7.40	0	1.6	0.791
	20.00	0.994	7.40	0	1.4	0.845
	20.00	0.994	7.40	0	1.5	0.816
	20.18	0.997	7.40	0	1.7	0.767
	21.50	0.997	7.40	0	1.5	0.816
	22.68	0.997	7.40	0	1.2	0.913
	24.05	0.997	7.40	0	1.0	1.000
	25.00	0.974	7.40	0	2.0	0.707
	25.00	0.977	7.40	0	1.6	0.791
	25.00	0.980	7.40	0	1.2	0.913
	25.00	0.983	7.40	0	1.1	0.953
	25.00	0.986	7.40	0	1.0	1.000
	25.00	0.988	7.40	0	0.8	1.118

Appendix A (*continued*)

Data source	Temperature (°C)	a_w	pH	LAC (mM)	GT (h)	sqrt(1/GT)
Salter (1998)	25.00	0.991	7.40	0	0.8	1.118
	25.00	0.994	7.40	0	0.8	1.118
	25.43	0.997	7.40	0	0.9	1.054
	26.70	0.997	7.40	0	0.8	1.118
	27.90	0.997	7.40	0	0.6	1.291
	29.30	0.997	7.40	0	0.7	1.195
	30.00	0.971	7.40	0	1.9	0.725
	30.00	0.975	7.40	0	1.6	0.791
	30.00	0.978	7.40	0	1.2	0.913
	30.00	0.981	7.40	0	0.9	1.054
	30.00	0.984	7.40	0	0.9	1.054
	30.00	0.987	7.40	0	0.8	1.118
	30.00	0.991	7.40	0	0.8	1.118
	30.00	0.994	7.40	0	0.8	1.118
	30.60	0.997	7.40	0	0.6	1.291
	32.08	0.997	7.40	0	0.5	1.414
	33.60	0.997	7.40	0	0.5	1.414
	34.98	0.997	7.40	0	0.5	1.414
	36.70	0.997	7.40	0	0.4	1.581
	38.03	0.997	7.40	0	0.4	1.581
	40.08	0.997	7.40	0	0.4	1.581
	41.85	0.997	7.40	0	0.4	1.581
	43.63	0.997	7.40	0	0.4	1.581
	45.55	0.997	7.40	0	0.5	1.414
	47.43	0.997	7.40	0	1.3	0.877
Mellefont (2000)	25.20	0.999	7.40	0	0.9	1.054
	25.20	0.995	7.40	0	0.8	1.118
	25.20	0.991	7.40	0	0.9	1.054
	25.20	0.988	7.40	0	1.0	1.000
	25.30	0.984	7.40	0	1.1	0.953
	25.30	0.980	7.40	0	1.3	0.877
	25.30	0.976	7.40	0	1.5	0.816
	25.30	0.972	7.40	0	1.8	0.745
	25.30	0.969	7.40	0	2.4	0.645
	25.30	0.965	7.40	0	4.0	0.500
	25.40	0.961	7.40	0	8.9	0.335
	25.40	0.998	7.40	0	0.9	1.054
	25.40	0.995	7.40	0	0.8	1.118
	25.40	0.991	7.40	0	1.0	1.000
	25.40	0.987	7.40	0	1.0	1.000
	25.40	0.984	7.40	0	1.1	0.953
	25.40	0.980	7.40	0	1.3	0.877
	25.40	0.976	7.40	0	1.5	0.816
	25.40	0.973	7.40	0	1.8	0.745
	25.40	0.969	7.40	0	2.4	0.645
	25.40	0.965	7.40	0	2.9	0.587
	25.40	0.962	7.40	0	4.1	0.494
	25.40	0.958	7.40	0	6.8	0.383
	25.20	0.997	7.40	0	0.9	1.054
	25.20	0.994	7.40	0	1.0	1.000
	25.20	0.990	7.40	0	1.0	1.000
	25.20	0.986	7.40	0	1.1	0.953
	25.30	0.983	7.40	0	1.4	0.845

(continued on next page)

Appendix A (*continued*)

Data source	Temperature (°C)	a_w	pH	LAC (mM)	GT (h)	sqrt(1/GT)
Mellefont (2000)	25.30	0.979	7.40	0	1.4	0.845
	25.30	0.976	7.40	0	1.8	0.745
	25.30	0.972	7.40	0	2.2	0.674
	25.30	0.969	7.40	0	2.6	0.620
	25.30	0.965	7.40	0	3.4	0.542
	25.30	0.962	7.40	0	5.3	0.434
	25.30	0.958	7.40	0	10.4	0.310
	25.10	0.999	7.40	0	0.9	1.054
	25.20	0.995	7.40	0	0.9	1.054
	25.10	0.991	7.40	0	0.9	1.054
	25.20	0.988	7.40	0	1.1	0.953
	25.30	0.984	7.40	0	1.1	0.953
	25.30	0.980	7.40	0	1.4	0.845
	25.20	0.976	7.40	0	1.7	0.767
	25.20	0.972	7.40	0	2.0	0.707
	25.30	0.969	7.40	0	2.8	0.598
	25.30	0.965	7.40	0	4.2	0.488
	25.30	0.961	7.40	0	12.7	0.281
	25.20	0.998	7.40	0	0.9	1.054
	25.20	0.995	7.40	0	0.9	1.054
	25.20	0.991	7.40	0	0.9	1.054
	25.20	0.987	7.40	0	1.1	0.953
	25.20	0.984	7.40	0	1.2	0.913
	25.30	0.980	7.40	0	1.4	0.845
	25.30	0.976	7.40	0	1.7	0.767
	25.20	0.973	7.40	0	2.1	0.690
	25.30	0.969	7.40	0	2.6	0.620
	25.30	0.965	7.40	0	3.6	0.527
	25.30	0.962	7.40	0	5.2	0.439
	25.30	0.958	7.40	0	8.2	0.349
	25.40	0.951	7.40	0	14.9	0.259
	25.20	0.997	7.40	0	0.9	1.054
	25.20	0.994	7.40	0	0.9	1.054
	25.20	0.990	7.40	0	1.0	1.000
	25.30	0.986	7.40	0	1.1	0.953
	25.30	0.983	7.40	0	1.3	0.877
	25.30	0.979	7.40	0	1.5	0.816
	25.00	0.976	7.40	0	1.8	0.745
	25.00	0.972	7.40	0	2.4	0.645
	25.10	0.969	7.40	0	3.4	0.542
	25.00	0.965	7.40	0	7.3	0.370
	25.00	0.962	7.40	0	13.1	0.276
	25.20	0.958	7.40	0	24.2	0.203

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Appendix 2: Model Evaluation

Mellefont, L.A., McMeekin, T.A. and Ross, T. (2003) Performance evaluation of a model describing the effects of temperature, water activity, pH and lactic acid concentration on the growth of *Escherichia coli*. Reprinted from *Int. J. Food Microbiol*. 82: 45-58, copyright 2003, reproduced with permission from Elsevier.

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Performance evaluation of a model describing the effects of temperature, water activity, pH and lactic acid concentration on the growth of *Escherichia coli*

L.A. Mellefont *, T.A. McMeekin, T. Ross

Centre for Food Safety and Quality, School of Agricultural Science, University of Tasmania, GPO Box 252-54, Hobart 7001, Tasmania, Australia

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Abstract

A square root-type model for *Escherichia coli* growth in response to temperature, water activity, pH and lactic acid was developed by Ross et al. [Int. J. Food Microbiol. (submitted for publication).]. Predicted generation times from the model were compared to the literature data using bias and accuracy factors, graphical comparisons and plots of residuals for data obtained from both liquid growth media and foods. The model predicted well for 1025 growth rate estimates reported in the literature after poor quality or unrepresentative data ($n = 215$) was excluded, with a bias factor of 0.92, and an accuracy factor of 1.29. In a detailed comparison to two other predictive modes for *E. coli* growth, Pathogen Modeling Program (PMP) and Food MicroModel (FMM), the new model generally performed better. The new model consistently gave better predictions than the other models at generation times ≤ 5 h. Inclusion of the lactic acid term in the model is proposed to account for the consistently good performance of the model for comparisons to growth in meat, a parameter that is not explicitly included in the other models considered in the comparisons.

Keywords: *Escherichia coli*; Growth model; Performance evaluation; Validation; Predictive microbiology; Bias; Accuracy; Literature data; Meat

1. Introduction

Despite the advent of rapid genetic and immunological techniques for detecting foodborne pathogens such as *Escherichia coli* O157:H7, assessment of the microbiological quality and safety of foods is usually a retrospective process and is therefore only partially effective in protecting consumers from foodborne hazards. An approach with *predictive* value is required. Thus, predictive modelling has developed as an adjunct to traditional microbiological techniques. Essentially, the survival and/or growth of an organism of concern may be predicted on the basis of a mathematical relationship between microbial growth rate and environmental conditions (McMeekin et al., 1993). A number of environmental factors influence

* Corresponding author. Tel.: +61-3-62-261831; fax: +61-3-62-262642.
E-mail address: Lyndal.Mellefont@utas.edu.au (L.A. Mellefont).

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the growth, death and survival of microorganisms. While it is desirable to include in predictive models all factors that influence bacterial responses, in many situations, this is not practical. To develop an *appropriate* predictive model, it is necessary to include, as independent variables, those factors which are measurable and are relevant to the food and the conditions it is likely to encounter from the place of manufacture through to its final preparation and consumption. Temperature, water activity (a_w) and pH, modified primarily by lactic acid (Grau, 1981), are considered to have the predominant influence on microbial growth in fresh meats (Nottingham, 1982).

The successful application of predictive modelling is dependent on the development of appropriate models but, prior to their application in industry, a performance evaluation of predictive models under novel conditions that were not used to derive the models is required. There are several ways in which model performance can be assessed. These include using sub-sets of the data set from which the model is derived, generating new data by either laboratory experiments in liquid growth media or direct inoculation into product, comparison to other data in the literature and trials in industry. It is generally accepted that comparing predicted responses to observed responses can assess the usefulness of a predictive model. Traditionally predictive models have been assessed statistically by the 'goodness of fit' of the data used to generate them and pictorial comparisons of observed and predicted data. Residual plots are also used to identify any non-linearity or non-constant variance in a model. Two indices of performance, the bias and accuracy factors (Ross, 1996; Baranyi et al., 1999), are objective and quantitative measures that provide a simple means of reporting a readily interpretable assessment of model performance.

It is interesting to note the interpretation of the term 'validation' in the literature by authors reporting on model performance. The term evaluation, i.e. to assess, is often more appropriate than validation, i.e. to make valid or confirm, as many predictive models are never investigated in the production environment. Neumeyer et al. (1997) referred to their work on pseudomonad growth as a validation; industry trials were performed in addition to laboratory and literature performance evaluations. In comparison, Dalgaard and Jørgensen (1998) conducted seafood challenge tests only and referred to their comparisons as evaluations. Similarly, Miles et al. (1997) referred to their literature comparison as an evaluation. Despite that they applied no objective criteria, Walls and Scott (1996) entitled their work on challenge tests in raw ground beef as a validation. As a comparison to the literature data forms the basis of the assessment of the model of Ross et al. (submitted for publication) in this study, the use of the term 'performance evaluation' is preferred.

A complete performance evaluation of a model encompassing all combinations of factors affecting microbial growth is an enormous undertaking and, for models including many variables, an impractical one. The work required to test all variable combinations is immense, even if it entailed the examination of only one or two variables (e.g. pH and temperature). Extending the performance evaluation process to food matrices magnifies the task further, as well as providing potential complications in application of the test organism, adjusting independent variables in non-homogeneous matrices, the presence of other spoilage microbiota as well as logistical problems with enumeration techniques. An approach that allows an efficient examination of many variables without unreasonable demands on time and resources is required. Collation of literature data permits specific research questions to be answered without recourse to novel experiments, a strategy significantly more efficient than generating data de novo. Evaluating model performance by comparison to published data is an easier, and perhaps more robust, approach and also identifies ''gaps'' in the literature.

In this study, the performance of a new square root-type model for *E. coli* growth (Ross et al., submitted for publication) which incorporates parameters relevant to unprocessed meat products is assessed by a comparison of model predictions to published data for growth of *E. coli* in liquid growth media and foods. The performance of the model is also compared with two *E. coli* growth models included in Food MicroModel (FMM) and Pathogen Modeling Program Version 5.0 (PMP). FMM and PMP are software packages containing a range of predictive models that can estimate the effects of multiple variables on the growth and survival of foodborne pathogens. PMP is provided free of charge by the Microbiological Food Safety Research Unit of the United States Department

of Agriculture (USDA) and can be downloaded from: http://www.arserrc.gov/mfs/pathogen.htm. FMM is available for an annual fee from the Leatherhead Food Research Association, Surrey, England.

2. Materials and methods

2.1. Growth model

The square root-type model presented in Ross et al. (submitted for publication) contains terms describing the inhibition of *E. coli* growth due to temperature, water activity, pH, the dissociated form of lactic acid and the undissociated form of lactic acid. The fitted model is:

$$\begin{aligned}\sqrt{r} = {} & 0.2790 \times ((T - 4.14) \times (1 - \exp(0.2636 \\ & \times (T - 49.55))) \times \sqrt{(a_w - 0.9508)} \\ & \times \sqrt{(1 - 10^{(3.909-\mathrm{pH})})} \times \sqrt{(1 - 10^{(\mathrm{pH}-8.860)})} \\ & \times \sqrt{(1 - [\mathrm{LAC}]/10.433)} \times (1 + 10^{(\mathrm{pH}-3.86)}) \\ & \times \sqrt{(1 - ([\mathrm{LAC}]/(995.509 \\ & \times (1 + 10^{(3.86-\mathrm{pH})})))))} \pm 0.0054 \end{aligned} \quad (1)$$

where: r = relative growth rate (1/generation time (h)), T = temperature (°C), a_w = water activity, [LAC] = lactic acid concentration (mM).

2.2. Novel data collation

A comprehensive search of the literature for growth rate and generation or doubling time data for *E. coli* was performed and the data collated in computer spreadsheets. A total of 39 independent sources (35 publications, 2 personal communications and 2 novel data sets (Mellefont, 2000)) were identified. The number of growth rate data from each source ranged from 1 to 281. In sources where growth rates/generation times were not tabulated or specified, values were estimated from enlarged copies of graphs by linear regression of data derived from the graphs. If the report did not specify values for lactic acid concentration, pH or a_w, they were obtained from the other literature sources or estimated by comparison with analogous foods. Lactic acid concentrations were estimated for meat data from the values of Grau (1981). The a_w and pH values of laboratory media were derived from Chirife et al. (1982) and Atlas (1993). Percent NaCl values in Atlas (1993) were converted to a_w using the tables of Chirife and Resnik (1984). Details of the source, growth conditions and strains used for each of the published data sets are presented in Tables 1 and 2.

To prevent misleading assessment of model performance by comparison to poor quality or unrepresentative data, the collated literature data were subdivided into an edited data set. Data were excluded if:

- the growth media used could retard growth, e.g. minimal media or media containing antibiotics;
- in the case of complex laboratory media, if anaerobic conditions for growth were deliberately employed (data from potentially anaerobic foods were included);
- in the absence of tabulated data, there were insufficient data points for linear regression analysis for generation time estimation;
- in the case of foods, there were parameters not included in the predictive models that may exert an effect, e.g. modified atmosphere packaging, and growth data were generated under conditions beyond the range of variables used to create the model.

Eq. (1) is based on data in the ranges: 7.6–47.4 °C, a_w 0.951–0.999, pH 4.02–8.28 and lactic acid in the range 0–500 mM. PMP model limits are 8.9–42 °C, a_w 0.970–0.997 and pH 4.5–8.5. For FMM, the model limits are 10–30 °C, a_w 0.960–1.000 and pH 4.5–7.5.

2.3. Observed vs. predicted comparisons

The bias factor (B_f) is a measure of the relative average deviation of predicted and observed generation times and is expressed as the antilogarithm of the average of the logarithm of the ratio between the predicted and observed generation times:

$$B_f = 10^{\left(\sum \log(\mathrm{GT}_{\mathrm{predicted}}/\mathrm{GT}_{\mathrm{observed}})/n\right)} \quad (2)$$

where $\mathrm{GT}_{\mathrm{predicted}}$ = predicted generation time, $\mathrm{GT}_{\mathrm{observed}}$ = observed generation time and n is the

Table 1
Bias and accuracy factors for comparison of predicted generation times for *E. coli* (Eq. (1)) to published growth data in liquid growth media

Source: liquid media	*n*	Temperature	pH	[LAC]	a_w	Bias	Accuracy	*E. coli* strain	Environment
Barber (1908)	218	10 to 46.8	7.4	0	0.997	0.86	1.17	Bacillus coli	Beef peptone broth
Barber (1908)	24	30.1 to 43.8	7.4	0	0.997	0.83	1.21	Bacillus coli	Beef peptone broth
Barber (1908)	32	30 to 37.5	7.4	0	0.997	0.87	1.15	Bacillus coli	Beef peptone broth
Bernaerts et al. (2000)	8	15 and 35	7.4	0	0.993	0.71	1.52	MG1655 (wild type KIZ strain)	BHI
Buchanan and Bagi (1997)	4	12 to 28	5.5 to 7.5	0	0.987	1.55	1.55	O157:H7 933	BHI (adjusted with NaCl,10N HCl or 50% KOH)
Buchanan and Klawitter (1992)	58	10 to 42	4.5 to 8.5	0	0.957 to 0.987	1.38	1.68	O157:H7 cocktail	BHI (adjusted with NaCl, 10N HCl or 50% KOH)
Buchanan et al. (1993)	1	12	6.5	0	0.987	1.22	1.22	O157:H7 cocktail	BHI (adjusted with NaCl, 10N HCl or 50% KOH)
Demetz and Dantigny (2000)	14	14 to 39	7.3	0	0.995	1.01	1.22	TGI	TSB
Doyle and Schoeni (1984)	8	25 to 44.5	7.3	0	0.995	0.43	2.33	O157:H7-932	TSB
Eustace (personal communication, 1998)	5	8 to 37	7.3	0	0.995	0.92	1.16	NCTC 9001-non-pathogenic	TSB
Fratamico et al. (1997)	6	37	7	0	0.997	0.82	1.23	O157 cocktail	LB
Gill and Phillips (1985)	11	7.7 to 46	7.4	0	0.993	0.65	1.53	K12 ATCC 23716	BHI
Gill and Phillips (1985)	8	10 to 42.3	7.4	0	0.964	0.81	1.25	K12 ATCC 23716	BHI + 5% NaCl
Glass et al. (1992)	5	37	7.3	0	0.954 to 0.991	0.54	1.85	O157:H7 cocktail	TSB
Glass et al. (1992)	7	37	4.5 to 7.3	0	0.995	0.66	1.52	O157:H7 cocktail	TSB (adjusted with HCl)
Ingraham (1958)	20	8 to 46	7.3	0	0.995	0.62	1.63	K12	TSB
Jason (1983)	2	37	6.5	0	0.996	1.00	1.02	NCIB 9132	Broth′
Jennison (1935)	10	22 to 42	7.4	0	0.997	0.75	1.34	Not stated	NB
Kauppi et al. (1996)	20	8.5 to 12	7.4	0	0.995	1.06	1.21	O157:H7	BHI
Kauppi et al. (1996)	16	8.5 to 12	7.3	0	0.993	0.72	1.52	O157:H7, 0104:H21, O22:H8, 0111:NM	TSB
Lowry et al. (1989)	7	8.19 to 40	5.5	130	0.993	1.41	1.41	from chilled sheep livers	Synthetic Meat Medium
Maxcy and Liewen (1989)	5	10 to 30	7.2	0	0.998	0.76	1.32	Not stated	m-Plate Count Broth
Mellefont (2000)	46	9.9 to 45.5	7.47	0	0.995	0.89	1.15	SB1-non-pathogenic	NB
Palumbo et al. (1995)	3	10 to 37	7.4	0	0.993	0.95	1.23	O157:H7-A9124-1	BHI
Rajkowski and Marmer (1995)	46	8 to 28	5 to 7	0	0.977 to 0.991	0.99	1.46	O157:H7 cocktail	BHI
Salter (1998)	256	7.7 to 47.2	7.4	0	0.997	1.02	1.22	9 STEC strains	NB
Smith (1995)	1	25	7.4	0	0.997	0.89	1.13	SF (sheep feces)	NB

Table 1 (*continued*)

Source: liquid media	*n*	Temperature	pH	[LAC]	a_w	Bias	Accuracy	*E. coli* strain	Environment
Smith (1995)	1	25	7.4	0	0.997	0.89	1.13	SF (sheep feces)	NB Mg++ (NB + 5×10^3 M MgSO4)
Sutherland et al. (1995)	5	10 to 30	4.49 to 6.97	0	0.954 to 0.991	1.13	1.52	O157:H7 cocktail	TSB
Total	847								

number of observations used in the calculation (Ross, 1996). Careful interpretation of the bias factor is required. A bias factor of 1 indicates perfect agreement between observed and predicted generation times, however, under- and over-prediction will tend to 'cancel out' in this measure because the logarithm of the ratios will have opposite signs. Therefore, a bias factor of 1 is interpreted as indicating no *systematic* over- or under-prediction. A bias factor <1 indicates the model usually predicts generation times shorter than observed and a bias factor >1 that the model predicts generation times longer than observed. Under-prediction, i.e. $B_f<1$, may be regarded as 'fail-safe' and an over-prediction, i.e. $B_f>1$, as 'fail-dangerous'. Judgment must be exercised when assessing a model by this index because a low bias factor (i.e. <1), while indicating the model is 'fail-safe', also suggests a level of conservatism that may render the model not practically useful.

The accuracy factor (A_f):

$$A_f = 10^{\left(\sum |\log(GT_{predicted}/GT_{observed})|/n\right)} \quad (3)$$

where the terms are as previously defined, provides an indication of the spread of the results about the predicted value (Ross, 1996). An accuracy factor of 1 represents perfect agreement between observed and predicted values. The larger than 1 the value is, the less accurate the average estimate is between observed and predicted generation times.

Generation times from the collated literature data were compared to generation times predicted by Eq. (1), FMM and PMP using:

i) the bias and accuracy factors described by Eqs. (2) and (3);
ii) graphing $\log(GT_{observed})$ vs. $\log(GT_{predicted})$, and
iii) plotting the residuals $(\sqrt{r}_{observed} - \sqrt{r}_{predicted})$ against $\sqrt{r}_{predicted}$.

3. Results

Generation time values of 1240 for growth of pathogenic and non-pathogenic *E. coli* in liquid media and foods were collated. These data were edited according to the criteria described above to a data set comprising 1025 growth rate estimates. The edited data were separated into categories of data from studies in complex laboratory media (Table 1, $n=847$) and food (Table 2, $n=178$). The food category was further separated to meat only ($n=130$) and 'other foods' ($n=48$). Despite that Eq. (1) was generated in an aerobic broth system, anaerobic data were included in the analyses of the food category because of the relative paucity of food data and because it was not possible to quantify the degree of oxygen limitation. Anaerobic conditions would reduce the growth rate, likely leading to conservative or 'fail-safe' predictions of the models. In evaluating the model's performance, this was considered to be an acceptable compromise.

3.1. Graphical comparisons

The predicted values from Eq. (1) are compared to the corresponding ''observed'' literature values in Fig. 1 for 'media', and Fig. 2 for 'meat' and 'other foods'. Boundaries encompassing 90% of the data have been denoted with dotted lines. Much of the literature data for 'media' falls near the line of equivalence, i.e. where observed and predicted values are in perfect agreement. There are fewer points in the 'fail-dangerous' area, i.e. above the line of equivalence, and 26 data fall outside the 90th per-

Table 2
Bias and accuracy factors for comparison of predicted generation times for *E coli* (Eq. (1)) to published growth data in foods

Source: food	*n*	Temperature	pH	[LAC]	a_w	Bias	Accuracy	*E. coli* strain	Environment
Buchanan et al. (1993)	1	42	5.9	0	0.98	0.92	1.08	O157:H7 933	Canned tuna
Buchanan et al. (1993)	1	12	6.6	0	0.992	0.52	1.93	O157:H7 933	Canned dogfood
Buchanan et al. (1993)	1	28	6	0	0.976	1.34	1.34	O157:H7 933	Chicken broth
Buchanan et al. (1993)	1	19	6.5	0	0.985	0.80	1.25	O157:H7 933	UHT milk
Gill and de Lacy (1991)[a]	6	8 to 30	6.5	85	0.997	1.07	1.48	E10 from offal	High pH beef (striploin steaks)
Gill and Newton (1980)	2	20 and 30	5.5	130	0.997	0.76	1.32	Not stated	Meat slices
Gill and Newton (1980)	2	20 and 30	5.5	130	0.997	0.65	1.53	Not stated	Meat slices
Gill and Newton (1980)	9	20 and 30	5.5	130	0.997	0.78	1.43	Not stated	Meat slices + non-pathogenic psychrotrophs
Gill and Newton (1980)[a]	4	30	5.5	130	0.997	0.65	1.53	Not stated	Meat slices + non-pathogenic psychrotrophs
Grau (1983)	10	25	5.6 to 6.91	80	0.99	0.72	1.40	from sheep feces	Beef-thin layers of lean mince
Grau (1983)	1	25	5.6	130	0.997	1.35	1.35	from sheep feces	Fatty tissue
Grau (1983)[a]	8	25	5.5 to 6.79	80	0.99	0.93	1.33	from sheep feces	Beef-lean pieces
Grau (1983)[a]	1	25	5.6	130	0.997	1.35	1.35	from sheep feces	Fatty tissue
Kauppi et al. (1996)	14	8.5 to 12	7.2	0	0.986	0.91	1.18	O157:H7, 0104:H21, O22:H8, 0111:NM	Autoclaved whole milk
Mellefont (2000)	4	10 to 20	6	100	0.997	1.20	1.27	SR M23 (streptomycin resistant)	Sterile raw ground beef
Mellefont (2000)	3	10 to 20	6	100	0.997	0.87	1.15	SR M23 (streptomycin resistant)	Low background flora ground beef
Palumbo et al. (1997)	13	8 to 37	7.2	0	0.997	0.54	1.92	O157 cocktail	UHT-pasteurized milk
Palumbo et al. (1997)	4	12 and 15	7.2	0	0.997	0.53	1.90	O157 cocktail	Low background flora pasteurized milk
Palumbo et al. (1997)	3	12	7.2	0	0.997	0.64	1.57	O157 cocktail	High background flora pasteurized milk
Palumbo et al. (1997)	6	12 and 15	5.8	100	0.997	0.60	1.68	O157 cocktail	Irradiated ground beef (Initial 10^3 TVC)
Smith (1985)	1	8.2	6	85	0.997	1.15	1.15	SF	Raw blended mutton
Smith (personal communication)	67	10 to 40	6	85	0.997	1.10	1.16	SF	Raw blended mutton
Walls and Scott (1996)	6	12 to 35	5.7 to 6.4	85 to 130	0.997	1.05	1.11	O157:H7 cocktail	Raw ground beef (pH adjusted with 3N NaOH)
Wang et al. (1997)	10	8 to 22	6.9 to 7.1	0	0.997	0.65	1.54	O157:H7 cocktail	Unpasteurised milk
Total	178								

[a] Anaerobic growth conditions applied.

centile. In the most extreme case, the difference between the observed and predicted generation time is a factor of 6.9 (Fig. 1). At longer generation times, the model predictions, while 'fail-safe' on average, appear to be too conservative. For 'meat', much of the literature data falls near the line of equivalence (as is reflected in the lower A_f value), and most of the points are closer than that observed for growth in

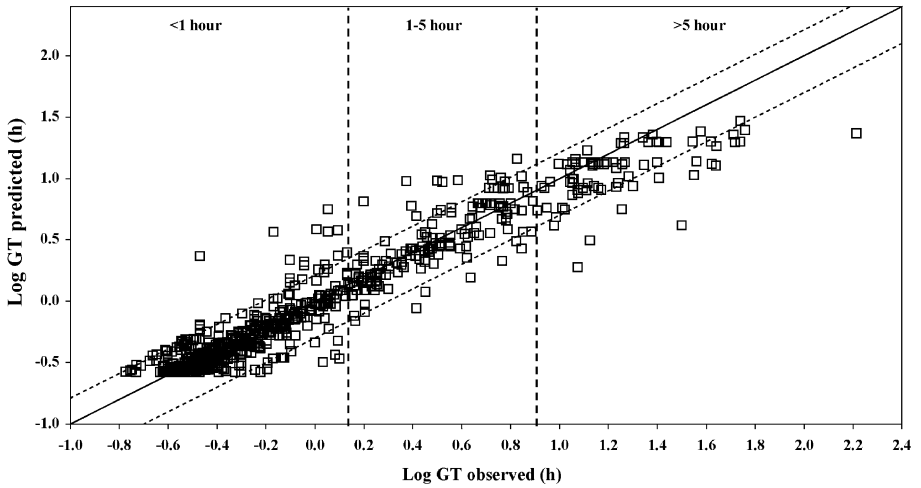

Fig. 1. Log($GT_{observed}$) vs. log($GT_{predicted}$) for the growth responses of *E. coli* reported in the published literature for 'media' and predictions from Eq. (1).

'media'. In comparison, the points for 'other foods' generally fall below the line of equivalence. For outliers in the food categories, the difference between observed and predicted generation times is smaller than those for 'media', with a maximum difference of 1.4 (Fig. 2). The residuals plot (Fig. 3) for all the 'edited' literature data compared to predictions from Eq. (1) shows little systematic deviation between observations and predictions, although the degree of scatter is slightly wider at faster generation times (Fig. 2).

3.2. Bias and accuracy

Eq. (1) performed better, as assessed by the bias and accuracy indices, than PMP and FMM for

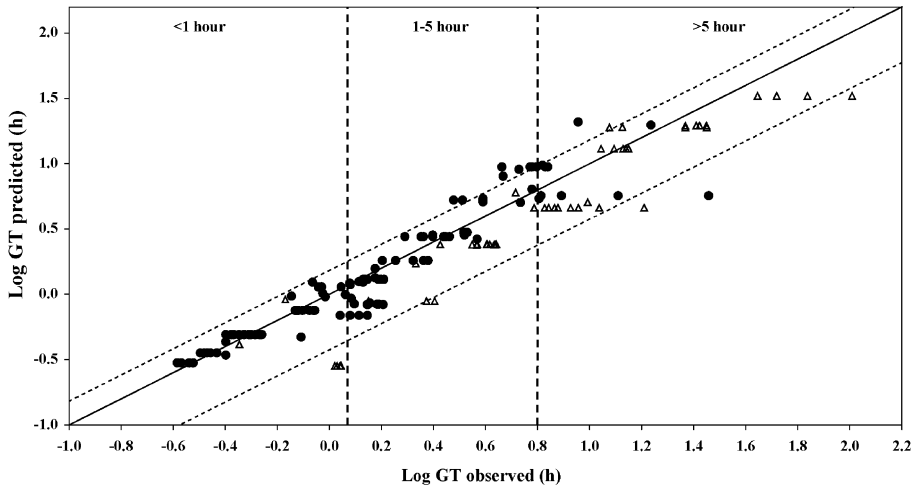

Fig. 2. Log($GT_{observed}$) vs. log($GT_{predicted}$) for the growth responses of *E. coli* reported in the published literature and predictions from Eq. (1) where: ● = 'meat' and △ = 'other foods'.

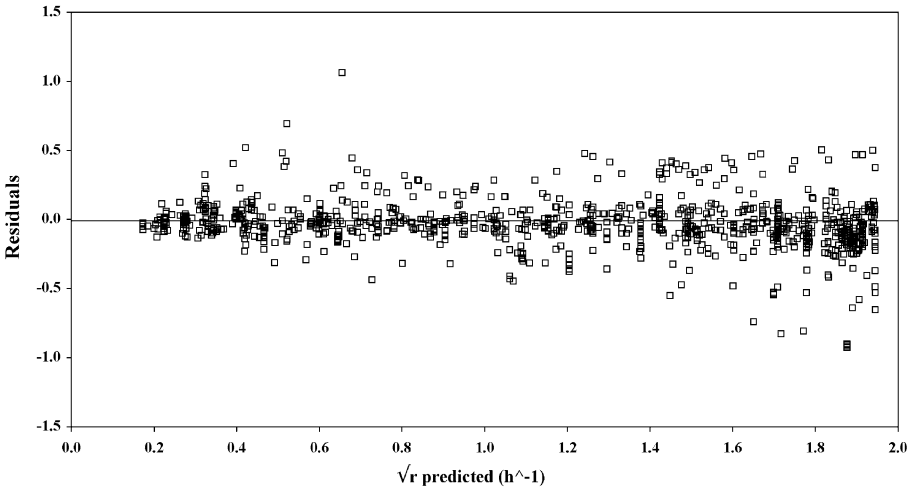

Fig. 3. The residual plot of ($\sqrt{r_{observed}} - \sqrt{r_{predicted}}$) against $\sqrt{r_{predicted}}$ for comparison of the growth responses of *E. coli* reported in the published literature (edited data set, $n = 1025$) and predictions from Eq. (1).

most of the data groups (Table 3). However, when the food category was divided into 'meat' and 'other foods', Eq. (1) performed best for 'meat' and FMM for 'other foods'. Foods other than meat consisted primarily of milk products. For this category only, FMM out-performed Eq. (1) for each of the performance indices. For the unedited data set, FMM had a lower bias factor than Eq. (1). The bias and accuracy indices for PMP were close to those of Eq. (1) for most data sets except 'other foods'.

To investigate whether the models' performance is better under some sets of growth conditions than others (e.g. 'harsh' vs. near optimal), generation time data were grouped into categories of:

- $GT_{observed} < 1$ h,
- $GT_{observed}$ 1–5 h, and
- $GT_{observed} > 5$ h,

based on the assumption that harsh conditions are associated with longer generation times. Bias and accuracy factors were calculated (Table 4).

Table 3
Bias and accuracy factors for growth of *E. coli* with data collated from the literature compared to Eq. (1), Pathogen Modeling Program and Food MicroModel (best bias and accuracy marked in bold)

Category	Eq. (1)			PMP			FMM		
	n	Bf	Af	*n*	Bf	Af	*n*	Bf	Af
All-unedited	1240	0.85	1.42	901	0.81	**1.39**	631	**1.07**	1.55
All-edited	1025	**0.92**	**1.29**	824	0.87	1.30	523	1.16	1.47
Media	847	**0.93**	**1.28**	664	0.91	**1.28**	387	1.34	1.47
All food	178	**0.86**	**1.36**	160	0.73	1.42	136	0.77	1.47
Meat	130	**0.97**	**1.26**	128	0.80	1.30	110	0.75	1.52
Other foods	48	0.63	1.68	32	0.50	2.01	26	**0.84**	**1.29**

Table 4
Bias and accuracy factors for generation time sub-sets of edited data for the growth of *E. coli* obtained from the literature compared to Eq. (1), Pathogen Modeling Program and Food MicroModel (best bias and accuracy marked in bold)

Category	GT (h)	Eq. (1)			PMP			FMM		
		n	Bf	Af	*n*	Bf	Af	*n*	Bf	Af
All-edited	<1	612	**0.97**	**1.20**	503	0.95	**1.20**	204	1.21	1.41
	1–5	254	**0.92**	**1.38**	217	0.85	1.39	228	1.17	1.57
	>5	159	0.76	1.56	104	0.61	1.70	91	**1.03**	**1.39**
Media	<1	561	**0.96**	**1.20**	454	0.95	**1.20**	172	1.33	1.41
	1–5	171	**0.99**	1.35	135	0.97	**1.32**	149	1.48	1.51
	>5	115	0.74	1.57	75	0.61	1.70	66	**1.09**	**1.39**
All food	<1	51	**1.05**	**1.11**	49	0.93	1.15	32	0.74	1.38
	1–5	83	**0.78**	**1.45**	82	0.68	1.52	79	0.75	1.46
	>5	44	0.82	1.54	29	0.60	1.69	25	**0.89**	**1.39**
Meat	<1	49	**1.05**	**1.11**	49	0.93	1.15	31	0.72	1.38
	1–5	64	**0.88**	**1.31**	64	0.74	1.39	64	0.72	1.60
	>5	17	1.09	1.57	15	0.68	1.50	15	**0.98**	**1.46**
Other foods	<1	2	**1.11**	**1.01**	1	1.36	**1.01**	0		
	1–5	18	0.49	2.03	17	0.46	2.18	14	**0.83**	**1.26**
	>5	28	0.71	1.52	15	0.55	1.84	11	**0.82**	**1.32**

Eq. (1) consistently performed best for generation times in the <1- and 1- to 5-h categories. The 'other foods' category was the exception, with poorer bias and accuracy factors for the 1- to 5-h category. For 'meat', Eq. (1) had a similar bias factor for each generation time category, although the accuracy factor deteriorated with increasing generation time. The best predictions for PMP were for the <1-h category. For 'media' data, PMP had similar bias and accuracy factors to Eq. (1) for the <1- and 1- to 5-h categories. Bias and accuracy factors deteriorated with increasing generation time for all categories. For FMM, the best bias factors were generally in the >5-h group with the exception of the 'other foods' category for which the best bias factor was observed in the 1–5-h group. Except for the <1-h category for 'other foods' (in which there was only one observation), accuracy factors were all higher than 1.27. The poorest accuracy factors for each category were found in the 1- to 5-h group. These outcomes are summarised in Table 3.

Overall, Eq. (1) appeared to predict better at generation times <5 h. PMP predicted well for the <1-h category, with bias and accuracy factors often similar to those for Eq. (1). FMM was consistently more accurate for generation times >5 h.

4. Discussion

Although graphical methods were used in this study to assess model performance, the basis of the evaluation of Eq. (1) was calculation of the bias and accuracy factors. While there are no agreed criteria by which a model can be said to have acceptable performance, i.e. to be 'validated', since the introduction of the bias and accuracy factors, several authors have suggested critical values of those indices that allow their interpretation for model validation, as discussed below.

The significance of the magnitude of the bias factor depends on the amount of growth that occurs, and is therefore dependent on whether the model being assessed is a model for spoilage or growth of pathogenic organisms. For example, if the bias factor were 0.9 and considering that the error in any viable count method is approximately ± 0.3 log cfu (Jarvis, 1989), differences between observed and predicted growth would not be measurable unless more than three log cycles of growth had occurred (i.e. 10% of $3 = 0.3$). From a shelf-life perspective, such an increase may be acceptable. However, for proliferation of pathogens with low infective doses, such an increase would be unacceptable. Thus, the bias factor must be interpreted in a manner consistent with the situation in which the predictive model is likely to be utilised. Dalgaard (2000) suggested that a B_f in the range 0.75–1.25 indicates a successful evaluation of seafood spoilage models. Ross (1999) considered that for pathogens less tolerance should be allowed for bias factors >1 as they would lead to under-predictions of growth, and are thus 'fail-dangerous' predictions. He proposed the following interpretation of the bias factor when used for model performance evaluations involving pathogens:

- 0.90–1.05 can be considered good;
- 0.70–0.90 or 1.06–1.15 can be considered acceptable;
- <0.70 or >1.15 should be considered unacceptable.

The error in growth rate estimates under controlled laboratory conditions is estimated to be around 10% per independent variable (Ross et al., 2000). Those authors proposed that as a 'rule of thumb', each environmental variable in a model is expected to add a similar amount of relative error. Thus, an acceptable accuracy factor can be determined by considering the effect of the *number* of environmental parameters in a kinetic model. For example, the best performance that might be expected from a kinetic model encompassing the effect of temperature, pH and a_w on growth rate, is $\sim 30\%$, or an accuracy factor of 1.3.

Normal experimental error and biological variability contribute to variation in results derived from experiments conducted by the researcher undertaking the evaluation, and are reflected in the accuracy factor. It is likely that this variability will be significantly larger when using data other than that used to generate the model and, in particular, data independent of the investigator's laboratory, methods and experimental strains. Ross (1996) observed that model accuracy decreased as the degree of experimental control is reduced.

Model performance reported in this paper and in Neumeyer et al. (1997) support the observations of Ross (1996). For Eq. (1) model, accuracy was 1.15 in growth experiments conducted by Mellefont (2000) in complex laboratory media and in inoculated, non-sterile ground beef (see Tables 1 and 2). Model accuracy for the 'unedited' literature data set, i.e. all the literature data, was poorer, $A_f = 1.42$. Similarly, in an evaluation of a growth model for *Pseudomonas putida* 1442, Neumeyer et al. (1997) reported model accuracy of $A_f = 1.23$ in laboratory media and 1.10 in homogenous liquid foods (milk and milk products), and 1.30 for a comparison to an unedited literature data set (the poorer model performance for the liquid media was attributed by Neumeyer et al. (1997) to inter-strain differences).

The critical values described above for the bias and accuracy factors enable objective performance evaluation of predictive models. However, they are imperfect because systematic deviations in predictions between observed and predicted responses may be obscured (Ross, 1996). Thus, graphical methods for comparison of observed vs. predicted growth are also important. Figs. 1 and 2 and the residuals plot (Fig. 3) reveal that, overall, Eq. (1) describes the data well. For the 'edited' literature data set, there is little evidence of any *systematic* deviation in predictions. It was noted, however, that some points in the 'media' category deviated markedly from the line of equivalence (i.e. outside the 90% boundary). Most outliers occurred at generation times ≤ 5 h and the difference between observed and predicted growth was several-fold away (Fig. 1). Many of those data, however, were generated with one or more environmental factors approaching growth limiting conditions. Additionally, 18 of the 26 outliers were derived from one source (Buchanan and Klawitter, 1992) and the bias factor, $B_f = 1.38$, reflects the over-prediction of growth by Eq. (1) for this data (Table 1). It is likely that data set is not representative of the response of most *E. coli*. The reasons for this are unknown but may be due to differences in strain types and/or experimental methodology. The model appears to be too conservative under conditions that lead to slow growth (i.e. the greater number of points below the line of equivalence in Fig. 1). This is reflected in the bias factor for the data set ($B_f = 0.92$). These points are usually generated under unfavourable conditions for growth. The reason for this behaviour is unknown, but is consistent with other reports for a variety of model types (McClure et al., 1994; Sutherland et al., 1995; Miles et al., 1997; Neumeyer et al., 1997; Salter, 1998; Augustin and Carlier, 2000).

For the 'meat' data set, which includes results from laboratory inoculation studies in ground beef (Mellefont, 2000), Eq. (1) describes the data well with most of the points lying close to the line of equivalence (Fig. 2), reflected in the bias factor ($B_f = 0.97$). However, for the 'other foods' category, Eq. (1) describes the data 'less well'. The majority of predictions are much faster than the observed growth, thus, the model is too conservative. This is reflected in the poor bias factor ($B_f = 0.63$).

The results from the graphing methods support the validity of the calculated bias factors. The limits proposed by Ross (1999) will now be used to evaluate the performance of Eq. (1).

Using the data from the literature, Eq. (1) performs well in almost all of the categories considered (Table 3). From the literature performance evaluation, the bias factors for the 'edited' ($B_f = 0.92$), 'media', ($B_f = 0.93$) and 'meat' ($B_f = 0.97$) data sets all fall into the best level proposed by Ross (1999), i.e. 0.90 to 1.05. Additionally, as the bias factors are < 1, the model can be considered 'fail-safe' on average. Eq. (1) has four independent variables. Therefore, according to Ross et al. (2000), the best performance that should be expected from Eq. (1) is an accuracy factor of ≤ 1.4. The accuracy factors of the 'edited' ($A_f = 1.29$), 'media' ($A_f = 1.28$) and 'meat' ($A_f = 1.26$) data sets are all < 1.4, indicating that Eq. (1) has an acceptable level of accuracy. Although Eq. (1) performed less well for the 'food' data set, $B_f = 0.86$ and $A_f = 1.36$, both of the performance indices are close to the levels considered to indicate a 'good' level of performance. This was also true for the unedited literature data set ($B_f = 0.85$ and $A_f = 1.42$).

The poorest level of performance for Eq. (1) occurred for the 'other foods' category ($B_f = 0.63$ and $A_f = 1.68$). It was noted previously that the majority of foods in this category are milk products, which account for 45 out of the 48 growth observations. Closer scrutiny of the individual data sets (see Table 2), and the results presented in Figs. 2 and 3, indicate that the low bias and high accuracy factors are

observed consistently, as opposed to over- and under-predictions cancelling each other out.

Eq. (1) performed better than PMP or FMM for the majority of the data sets considered (Table 3). Despite that PMP predictions were often close to those of Eq. (1), Eq. (1) outperformed this model for meat. It should be noted that due to differences in the model limits, Eq. (1) was compared to an average of 24% more data than PMP. While bias factors were 'good' for some data categories, accuracy was poor, suggesting predictions of FMM are potentially unreliable. FMM predicts the effects of three variables. The model is expected, thus, to have an accuracy of around 1.3. In only one category, 'other foods' was the accuracy factor within the expected limits ($A_f = 1.29$). The accuracy factors for the remaining categories were higher than 1.3, but covered a narrow range ($A_f = 1.47$ to 1.55). This suggests that no matter how the data is subdivided or regrouped, the accuracy factor will not improve, possibly because the model has poor B_f values.

The exceptions to the good performance of Eq. (1) were the 'unedited' and 'other foods' data sets. FMM appeared to perform well for the unedited data set, with a 'good' B_f of 1.07. However, the unedited data set contained many predictions outside the model limits for FMM. Due to its wider range of applicability, Eq. (1) was compared to approximately twice as much data. Closer scrutiny of the data reveals that FMM performs poorly in the 'media' and 'food' data sets. As these two categories comprise the bulk of the unedited data set, it is likely that the over-prediction in media is "cancelled out" by the under-prediction in the foods. Thus, for comparative purposes, the 'edited' data set is more appropriate, and although FMM had an acceptable bias factor, Eq. (1) performed better. For the 'other foods' category, although FMM had better bias and accuracy factors than Eq. (1), its performance was still considered 'poor' by the proposed critical limits. As no model performed well for this category, it would appear that none are applicable, possibly because important factors affecting *E. coli* growth in these products are not included in the models. This is termed 'completeness error' and arises because only a limited number of environmental factors can be included in the model in practice (Ross et al., 2000).

The consistently good performance of Eq. (1) for the 'meat' data may arise from the inclusion of the lactic acid term in the model, a parameter that is not explicitly included in the other models considered in the comparisons. This hypothesis was tested by removing the lactic acid terms from Eq. (1) and re-calculating the bias and accuracy factors for all the meat data. With the lactic acid terms included, bias and accuracy factors for all the meat data from the literature were 0.97 and 1.26, respectively (see Table 3). When the lactic acid terms were removed from Eq. (1), the bias and accuracy factors were 0.78 and 1.39. Thus, the hypothesis that inclusion of the lactic acid terms in Eq. (1) is responsible for the improved predictions for growth of *E. coli* in meat products is supported. The effect of additional variables on the performance of a model can be illustrated by comparing the performance of Eq. (1) to that of the model of Salter et al. (1998). The model of Salter et al. (1998) is for temperature only. Its performance, compared to that of Eq. (1), is summarised in Table 5. Eq. (1), which includes terms for pH, water activity and lactic acid, describes the data of Gill and Newton (1980) and Grau (1983) much better than the model of Salter et al. (1998). This highlights the requirement for appropriate predictive models relevant to the product and process.

The performance of the models was also assessed against sub-sets of the data divided on the basis of generation time (Table 4). Close-to-optimal environmental conditions are characterised by short generation times and conditions closer to the limits for growth are characterised by long generation times. The 1- to 5-h category is intermediate. Eq. (1) consistently performed best at the fast and intermediate

Table 5
Bias and accuracy factors for growth of non-pathogenic *E. coli* on meat presented in Salter et al. (1998) compared to Eq. (1) and the temperature model of Salter et al. (1998) (best bias and accuracy marked in bold)

Reference	*n*	Model			
		Eq. (1)		Salter et al. (1998)	
		Bias	Accuracy	Bias	Accuracy
Gill and Newton (1980)	4	**0.70**	**1.43**	0.43	2.31
Grau (1983)	9	**0.72**	**1.40**	0.53	1.90
Smith, 1985	8	**1.04**	**1.09**	0.90	1.18

generation times. PMP did not perform best for any of the generation time categories, although predictions were often similar between the two models. As for Eq. (1), the predictions by PMP were better than those of FMM. The best performance for PMP was observed at rapid generation times, i.e. < 1 h. However, as generation times increase, predictions become consistently less reliable, suggesting this model can only be used with confidence under conditions where rapid bacterial growth is expected.

Comparison of the performance of Eq. (1) against the other models also highlighted the influence of fidelity of the data used in the comparison. Under circumstances where a number of models predict poorly, it may indicate a deficiency in the literature data itself. Literature data sometimes provides incomplete information on experimental design and methods, or insufficient data may have been generated for growth calculations. Consistently poor model predictions may also reveal inter-strain differences. For example, Eq. (1) predicted poorly for the data of Ingraham (1958) in nutrient broth using *E. coli* K12 with $B_f = 0.62$ and $A_f = 1.63$ (Table 1). Other data for *E. coli* K12 are presented by Gill and Phillips (1985) and Bernaerts et al. (2000). When compared to those data for studies in unmodified BHI broth, Eq. (1) has performance indices of $B_f = 0.65$, $A_f = 1.53$ and $B_f = 0.71$, $A_f = 1.52$, respectively (Table 1). Given the similarity in the values of the performance indices for the data, it is likely that the poor performance of Eq. (1) is due to differences in growth characteristics between *E. coli* K12 and the strains used to develop Eq. (1). PMP and FMM also predicted poorly for this data (FMM $B_f = 0.68$, $A_f = 1.46$ and PMP $B_f = 0.57$, $A_f = 1.73$ for comparisons to Ingraham (1958); FMM $B_f = 0.74$, $A_f = 1.33$ and PMP $B_f = 0.65$, $A_f = 1.55$ for comparisons to Bernaerts et al. (2000)). It is probable that strains other than *E. coli* K12 were used to produce FMM, and PMP predictions are for *E. coli* O157:H7. Poor model performance due to inter-strain differences is expected because "predictive models are generally produced using fast growing strains so that predictions are guaranteed to be 'fail-safe'" (McMeekin et al., 1993).

McClure et al. (1994), Ross (1996), Miles et al. (1997), Neumeyer et al. (1997) and Salter et al. (1998) considered that there are to be many problems inherent in using literature data in model performance evaluations including deficiencies in the literature data and decreased experimental control which is reflected in a higher A_f. While our results from the literature performance evaluation reinforce those authors' observations, the role of a literature performance evaluation should not be undervalued. A search of the literature yielded 1240 growth rate data, which was edited to 1025, in a period of approximately 8 weeks. A similar time period was required to perform a laboratory inoculation study in ground beef which yielded seven growth rate data (Mellefont, 2000). The role of literature data in predictive model evaluations can be optimised by the implementation of assessment criteria, such as those described above, to improve the quality of the literature data used in evaluations. Editing of the literature data set allows model performance to be assessed more objectively, because the model's performance is not prejudiced by unrepresentative data.

4.1. Summary

The performance of three models for *E. coli* growth was rigorously evaluated against a large set of published and novel data. Eq. (1) performed well against food-based and media-based data. In a detailed comparison to other predictive models for *E. coli* growth, Eq. (1) consistently predicted more accurately at faster generation times, i.e. GT ≤ 5 h. The model was less accurate at generation times >5 h, and this is an important observation because most users of predictive models will be interested in predicting the consequences of conditions that lead to slower growth rates. However, it should be noted that Eq. (1) always encompassed the largest number of data, and therefore, the widest range of conditions, including those at which growth rates, are often more variable and more difficult to predict. In general, Eq. (1) predicted more accurately even with this larger domain. A notable exception to the good performance of Eq. (1) was for data from the 'other foods' category, largely data from milk and milk-based products. The other models also showed poor performance for this category, indicating that the poor predictive ability of Eq. (1) for these foods is not a problem unique to this model.

This performance evaluation has highlighted some of the difficulties and subtleties of the model evaluation process. Unless data is collected under well-

controlled conditions, variability in data can be expected due to normal experimental error and biological variability, and this variability is likely to be larger for data obtained from the literature. The bias and accuracy factors are useful tools in the model evaluation process, however, they do not generate absolute measures of performance: the values of the performance indices will be specific to the data sets used in the evaluation and care should be taken in their interpretation. The value of the data presented in the literature should not be underestimated, however, an awareness of its limitations is essential.

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