



# final report

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## Microbiological specifications for retail meat products

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# 1 Background

Meat and Livestock Australia (MLA) has facilitated consultations on microbiological criteria for raw meat in the retail supply chain. The team included microbiologists, processors, retailers and marketers which met to consider microbiological guidelines suitable for meat processors. The purpose of this document is to record the principles used in the guideline-setting process.

The document includes sections on:

- The process by which guidelines can be set
- Principles for setting microbiological criteria for meat safety and shelf-life
- Setting guidelines for red meat products at entry to the retail system
- Linkage between guidelines and shelf-life requirements
- Shelf-life testing

Two approaches were taken to the setting of criteria and assessment of whether raw meats could conform to the shelf-life requirements of a retail supply chain. One approach used initial levels of contamination frequently achieved by abattoirs and judged product as acceptable or not at the end of shelf-life. The second approach used several different initial levels of contamination and then calculated the likely remaining shelf-life past the end of that expected. Both approaches used predictive microbiology and general 'rules of thumb' that anyone setting specifications and shelf-life periods, will find useful.

The document also examines the microbiological quality of meat from Australian abattoirs against international standards and guidelines and suggests that, if the current commercial shelf-life is satisfactory, then the standards being achieved by the industry are satisfactory.

It is believed that the process followed here can be used on an industry-wide basis, providing that the analysis is tailored to a specific supply chain.

## **2 INTRODUCTION Shelf-life testing: the claimable life of raw meat**

*This chapter contains excerpts from a publication in the Meat Technology Update series, (2/06, April 2006) and is reproduced with permission of Food Science Australia. The full text of the update can be found in Appendix 1.*

Generally a food is considered to be past its shelf-life when it is no longer acceptable to the consumer. It can be that the colour, flavour, texture, aroma or nutrient content have deteriorated to the point that it is no longer acceptable. It can also be when it becomes a food safety issue, where the food product may make consumers ill.

Whilst shelf-life is usually equated with spoilage, for fresh meat particularly, the end of shelf-life might be reached before spoilage is evident. For example, the loss of bloom of mince or steaks, or reaching a microbial count specified as an acceptable maximum by a retailer, may be the determinant of retail shelf-life whereas spoilage as defined by off-odour and slime would be the point at which it is unacceptable for consumption.

The Food Standards Code of Food Standards Australia New Zealand, FSANZ, includes a standard that prescribes a date marking system for packaged food intended for retail sale or catering purposes.

Retailers usually print a 'Use by' date on steaks, roasts and other packaged fresh meats. Under normal circumstances of hygienic handling and storage at 4°C or colder, spoilage bacteria rather than pathogens grow on uncooked meat and meat products and the meat will be cooked by the consumer before they are consumed. Packages of such products could therefore bear a 'Best before' date rather than a 'Use by' one.

Processors must date-mark any pre-labelled packages of fresh meat. In addition, meat processors are being asked by retailers to provide dates for larger packs of meat such as vacuum packs that will eventually be either sold intact or sliced and prepared as smaller retail packs. Here, shelf-life of the large pack should take into account that retailers will expect a display life of two, perhaps three days from the retail packs prepared from it.

Increasingly, meat processors are being asked to show that their claimed shelf-lives for products have been validated. This Update discusses how the validation might be demonstrated.

## 2.1 General approaches to shelf-life estimation

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The term 'Shelf-life' is variously used for the:

- Point of retail display at which consumers decline to purchase; or
- Time to when the product no longer has an acceptable eating experience for the consumer; or
- Time to when consumption is no longer safe.

A shelf-life determination involves an experimental study of the deterioration of the food, culminating in identification of the point that marks the end of its shelf-life. It is important that you are clear about the shelf life that you wish to specify.

There are several established approaches for the gathering of shelf-life data on food products:

- Estimating shelf-life based on published data;
- Utilising known distribution times for similar products on the market;
- Using consumer complaints as the basis for determining whether a problem is occurring;
- Accelerated shelf-life testing; or
- Assessing changes that occur in trial packs under simulated commercial storage.

Relatively little information on shelf-lives of specific products is published. Many shelf-life data are proprietary and therefore not available. Estimates from the published literature, some of which are summarised in Meat Update information sheet 'Storage life of meat', September 2002, are rather old and may not relate closely enough to current processing and packaging systems or to current retailer or consumer expectations. The exception to this generalisation is that the food safety literature can often be used in circumstances where shelf-life is determined by an unacceptable safety risk.

Neither the utilisation of known distribution times nor the consumer complaint approach can be validated satisfactorily and accelerated testing has little application to meat products.

The most direct and common way to determine shelf-life is to carry out storage trials under controlled conditions that reflect those that the meat normally encounters during the usual course of distribution, retail display, and storage by the consumer. Selection of an appropriate, reliable approach to simulating quality loss that will occur during commercial distribution and storage is an important first step when using this approach.

Select conditions that you anticipate will cover most situations but not necessarily conditions of significant abuse. As an example, if the package carries the statement 'Keep refrigerated', it is unrealistic and inappropriate to undertake trials at 0°C; 4°C would be more realistic if a period of storage in the home is likely. Take into consideration the fact that both chilled and frozen meats will be subjected to temperature fluctuations, particularly during summer months. It is often advisable to determine the shelf-life at two temperatures – the recommended storage temperature and the maximum temperature expected under normal transport and storage conditions.

Of the categories of food spoilage that can occur – physical, chemical, and microbiological - the two principal spoilage mechanisms that affect shelf-life of meat are microbial growth and oxidation of myoglobin (browning) or lipids (rancidity).

## 2.2 Estimating shelf-life

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Before shelf-life testing can be carried out, it is important to establish which quality characteristics are important to the purchaser or consumer for the product under assessment. This may vary between products. Establishing the criteria of importance and defining the acceptable standards are policy matters for manufacturers and retailers to resolve. As stated earlier, variable quality characteristics to consider include:

Safety	Odour
Meat colour	Flavour
Overall appearance	Texture

Food safety shelf-life is limited by the presence of unacceptable numbers of pathogens on a meat or meat product and is a function of the initial level of contamination by the pathogens in question, along with time and temperature. It is common, however, to regard food safety as being compromised if the food has been subjected to conditions that permit growth of pathogens if the pathogens happened to be present.

Note that it is important not to rely on shelf-life evaluation to establish the microbiological safety of the product. In uncooked meats, it will not be the presence of pathogens that dictate shelf-life.

## 2.3 Measures of shelf-life

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In fresh meats that are stored in air, pseudomonads will dominate the total population of bacteria so a standard plate count is a good guide to the onset of spoilage.

For vacuum-packed meat however, total count is not a good index. As vacuum-packed meat is stored in the absence of oxygen, growth of pseudomonads, as strict aerobes, is restricted. Instead, after storage the bacterial population will consist mainly of lactic acid bacteria.

Consumer acceptability of meat and meat products, particularly frozen ones, can be affected by factors that are not microbiological (*Table 1*). They include:

- Meat colour and appearance;
- Rancidity caused by chemical oxidation of fats at low temperature;
- Changes in texture caused by extended enzymic activity or product drying during storage, eg freezer burn;
- Texture, flavour and odour changes caused by other chemical reactions occurring in the product during storage eg toughening from protein denaturation or colour and flavour changes from non-enzymic browning reactions.

Browning of meat is due to oxidation of the meat pigment myoglobin. Low pH meat - 5.5 and lower – seems to be more susceptible to colour deterioration. Development of browning can be followed instrumentally using a colour meter. If previous experience has told you what the causal products of odour and flavour spoilage are, they can be tested for using appropriate chemical analyses – gas chromatography combined with mass spectrophotometry for example.

Instrumental techniques are only useful if there is a good knowledge of the relationship between the levels of specific chemicals and consumer perceptions of spoilage of your product. If that knowledge is not available, information on the deterioration of quality has to be obtained by the use of taste panels using either trained technicians or untrained consumers.

## **2.4 Some specific examples**

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### **Raw meats - fresh**

Pathogen growth is most conveniently estimated in raw meats by predictive microbiology using a model such as that developed in Australia by the University of Tasmania and Meat & Livestock Australia. The criteria specified in the Export Control (Meat and Meat Products) Orders 2005 are appropriate for determining what would be deemed unacceptable temperature abuse that would compromise shelf-life.

In fresh meats that are stored in air e.g. in over-wrapped trays, as the numbers of pseudomonad bacteria reach around 100 million per cm<sup>2</sup> they produce a putrid odour and slime forms on the meat surface. The pseudomonads will dominate the total population of bacteria so a total count is a good guide to the onset of spoilage.

High microbial populations may not necessarily impair sensory characteristics but a pre-determined level of micro-organisms, together with factors such as sensory attributes is often used to indicate that the end of life has been reached. Total counts in excess of 1 million per cm<sup>2</sup> of product surface or per gram of mince or other comminuted product is often taken to indicate that spoilage is imminent and are often regarded as the end of acceptable shelf life.

**Table 1:** Suggested attributes to assess when estimating shelf-life of range of products

<b>Retail Meat package</b>	<b>Quality attribute</b>	<b>Nature of spoilage</b>	<b>End of shelf-life</b>	<b>Approach to estimating shelf-life</b>
Fresh meat on over-wrapped tray	Good pink-red 'bloom' Odour of fresh meat	Off-odours, off-flavours, stickiness, slime from bacteria. Discolouration	Loss of bloom, brown discolouration. Microbiological specification exceeded	Colour meter Colour panel Counts of total bacteria
Fresh/MAP – high oxygen	Good pink-red bloom Odour of fresh meat	Off-odours, off-flavours, slime from bacteria. Discolouration	Loss of bloom, brown discolouration. Microbiological specification exceeded	Colour meter, colour panel Counts of total bacteria Counts of specific bacteria
Vacuum pack	Purple meat colour, tight pack. Normal confinement odour	Sour, dairy off-odour, off-flavour, greening from microbial activity. Browning	Unacceptable, persistent confinement odour. Meat discoloured (brown, grey, and green) in intact pack.	Colour meter, colour panel Odour/taste panel Counts of specific bacteria
VP/ over-wrapped	Good pink-red bloom Odour of fresh meat Minimal drip	Off-odour, off-flavour (incl. sour, dairy odour) Browning	Loss of bloom, brown Sour odour, flavour Microbiological specification exceeded	Colour meter, colour panel Odour/taste panel Counts of total bacteria Counts of specific bacteria
VP/ MAP – high O <sub>2</sub>	Good pink-red bloom Odour of fresh meat Minimal drip	Off-odour, off-flavour (incl. sour, dairy odour) Browning	Loss of bloom, brown Sour odour, flavour	Colour meter, panel Odour/taste panel Counts of total bacteria Counts of specific bacteria
Frozen ground beef	Pink-red	Rancidity, Freezer burn	Rancid odour, flavour when cooked Surface desiccation, sponginess	Taste panel
Frozen lamb chops	Pink-red	Rancidity, Freezer burn	Rancid odour, flavour when cooked	Taste panel



## Raw meats in vacuum packs

Lactic acid bacteria grow slowly on vacuum-packed meat at chill temperatures to 10-100 million per gram after about 6 weeks storage. They will stay around this level for the rest of the life of the product. Signs of spoilage will not be evident until several weeks after the maximum population of bacteria is reached. When spoilage eventually becomes evident it will be due to cheesy or sour milk odours and flavours rather than the putrid odours caused by pseudomonads in air.

For vacuum-packaged fresh meat of normal pH, a total bacterial count is NOT a useful indication of the microbiological quality of the product. If the total count is made up of mostly lactic acid bacteria, counts of more than 10 million per g do not indicate incipient spoilage or any processing or storage problem. Only total counts in excess of 100 million per cm<sup>2</sup> would indicate the end of the product's shelf life.

If meat in vacuum packs has a pH greater than 5.9, off odours may be detected when the bacterial count is just over one million per cm<sup>2</sup> if:

- the storage temperature is 5-10°C; or
- there are traces of oxygen in the pack due to using a packaging film with a high oxygen transmission rate.

In such vacuum-packed meat there may be an increased growth of spoilage bacteria such as *Brochothrix thermosphacta*, *Shewanella putrefaciens*, and psychrotrophic enterobacteria. These bacteria will cause souring and off-odours. Selective counts of these organisms can be useful in identifying the limitations to storage life of such product.

## 2.5 Panel assessments

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Sensory techniques supported by statistical methods are frequently used to determine the time at which a product achieves the limit of acceptability. The determination of consumer acceptability is most reliably done by means of panels of 100 or more untrained tasters, an exercise that is usually cost-prohibitive for establishing shelf-life. To minimise the cost and time involved other approaches are:

1. An experienced sensory scientist determines the limit for acceptability of a given attribute and then uses a trained panel to measure the intensity of this attribute during storage
2. The acceptability assessed by a trained panel is correlated to that of untrained consumers.
3. An increasing number of untrained consumers are used to assess the deterioration during storage, concentrating the testing more heavily on samples that are close to the end of their shelf-life.

The first is the easiest to perform but does not give any information on consumer perceptions.

Techniques used for panelling include:

- Difference tests – paired comparisons and triangle tests are useful to compare stored product with fresh product. Errors can however occur because new fresh samples are used at each testing during the storage. This technique also has the drawback in that it says nothing about acceptability - just whether it differs from the fresh control. It can be used to compare a revised process or new packaging film with an existing one.
- Hedonic scoring – Consumers are asked to rate the acceptability of the product on some predetermined scale. Common scales include terms like: like very much, like a little, neither like or dislike, dislike a little and dislike very much. The limitation to this technique is that the acceptability can go up or down due to changes within the storage and panellists respond differently to these changes, eg rancidity, moisture loss.
- Quantitative descriptive analysis (QDA) is based on the ability of panellists to describe reliably their perceptions of a product's attributes. This requires screening of panellists and the development of a suitable sensory language. Sensory attributes are scored and give good information on which attributes change during storage. However results still have to be related to consumer acceptability.

Sensory assessments by panels should normally be designed and interpreted by a specialist. General procedures for shelf-life testing include:

1. Develop a testing protocol consisting of the specific objective, detailed test design which covers product, packaging and storage specifications and panelling procedures and includes the number of samples required.
2. Identify the key quality indicator/s from any previous studies or published literature. Any information on known distribution time or turnover time of the product would be useful here.
3. Establish the sampling frequency and duration of the testing based on experience from previous studies or published data. If the interval of sampling is too long, the risk of under or over estimating shelf-life increases. Determination of the end of the experiment must be based on some preset criterion such as minimum required commercial shelf-life or some specific organoleptic criterion of unacceptability.
4. All testing should be based on one common sample, if possible, to ensure consistency between panellists. There are a number of publications covering detailed procedures for effective sampling, taste panelling and analysis of data. It is important that specialist knowledge be obtained to ensure that the sampling and panelling will give meaningful results.
5. Reporting of the outcomes and recommendations along with the details of design and application of the experiment. This report is the validation of the chosen shelf life of the product and is an important document to support your HACCP based meat safety plan.

### 3 The process of setting guidelines

There is no single 'right' way to set guidelines for shelf-life. The process that we set out here requires you to think about the reason why you want to set some guideline, encourages you to think about the issues and presents two scientific approaches. One scientific approach is the use of predictive microbiology, which is suitable if you know a lot about the supply chain and have a lot of data. An alternative approach is to use existing benchmarks and general knowledge of the supply chain. In either case, the result is likely to be microbiological specifications and a program of testing.

We are using the word 'guidelines' here because we are providing general advice about shelf-life and the microbiological criteria relevant to shelf-life. If these criteria become part of a contractual arrangement between a supplier and a customer then they may be called a 'specification'. A microbiological criterion defines the acceptability of product in terms of the presence or absence or number of micro organisms in a lot of product (Codex Alimentarius Commission, 1997). The criterion consists of a number of elements that will be explained later in this document.

When considering microbiological guidelines for raw meat the following elements need to be considered:

#### 3.1 Decide a rationale for setting guidelines

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A retailer might set guidelines for one or more of the following reasons:

- Regulatory requirements
- Process validation and verification
- Reducing shrinkage
- Achieve shelf-life at time of consumption
- Provide confidence / due diligence

#### 3.2 Thinking behind setting a guideline

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Once deciding why we want guidelines for product entering the retailer's control what is the thinking we might use?

- *Do we currently have a problem* e.g. we shrink an unacceptable volume of product or there are customer complaints about product being "off" when they came to prepare the meal? If this is the case we need to investigate whether the problem lies with specific suppliers, with specific stores or with specific products. If the findings of this investigation indicate an across-the-board problem it will prompt us to examine microbiological levels in products from our suppliers. The probable outcome is that we will set guidelines which prompt some suppliers to improve their process.
- *We do not appear to have a problem with shrinkage or complaints.* Our thinking in this case would be to set a guideline which would almost never be failed by our suppliers.
- *Allow for random and occasional failure.* In every red meat abattoir/boning operation there are occasional high counts (TVCs and E. coli) for which there is no apparent reason.

- *All products will be consumed cooked.* Because red meat raw is almost never eaten raw the occasional presence of pathogenic organisms for which animals are carriers, such as *Salmonella*, is unavoidable, despite the best efforts of processors to prevent contamination.
- *Require information relevant to shelf-life and pathogens.* Two counts – total bacteria and an indicator of faecal contamination will suffice. See section 2.4 on testing for pathogens.

### **3.3 Gather data on microbiology of raw meat**

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In order to set realistic guidelines the current status of industry performance must be assessed. Relevant data include:

- Baseline studies undertaken on behalf of the red meat industry (MLA, 2005)
- Industry data gathered as part of the *E. coli Salmonella* Monitoring (ESAM) program undertaken by establishments registered with the Australian Quarantine and Inspection Service (AQIS).
- In-house data generated by suppliers (processors)

These data can be used to assess both average performance and outlier performance. As a basic principle, guidelines should accommodate the occasional outlier providing that there is no associated public health risk.

### **3.4 Gather data on handling by the retailer**

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After product leaves the processor, large retail operations have sophisticated transport, warehouse and distribution systems to stores. In-store, there may be further processing, together with refrigerated and shelf-storage. The retailer is responsible for maintaining the quality of the product for the majority of its shelf-life. For this reason, it is important that the retailer has good information on temperatures of product throughout each stage of storage. It is important to consider the length of time that product may spend at each stage in the distribution/retail chain. Data loggers inserted in product and air temperatures of transport vehicles and cool rooms provide a time course of temperature. As for microbiological quality of raw meat, it is important to consider the average times and temperatures, as well as the likely ranges.

### **3.5 Use existing benchmarks and expert opinion to estimate shelf-life**

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In setting actual numbers of organisms in the specification it is useful to compare the proposed numbers with those of other guidelines. If these guidelines are sufficient to ensure product shelf-life in other circumstances, then they could also be used in a specific supply chain. Also, these guidelines indicate the microbiological quality that's achievable by industry; there's little point requiring product to have unachievable quality. There are several sets of microbiological guidelines e.g. ESAM specifications, the European Union specifications and the microbiological guidelines which accompany the Australian meat standard (AS4696-2002).

### **3.6 Use predictive microbiology to estimate shelf-life**

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Time: temperature data can be used to predict growth of spoilage organisms (*Pseudomonas* Predictor) and *E. coli* (RI Calculator), the latter as an indicator of potential growth of faecal pathogens. The *Pseudomonas* Predictor has been used extensively to estimate potential growth (and shelf-life) of pork exported to Singapore. Under the Export Control (Meat and Meat Products) Orders, the RI calculator is used by each meat processor to validate chilling and holding procedures.

When temperature: time data through the supply chain are linked with baseline data gathered at the meat processor it becomes possible to predict the number of days for which the product will be microbiologically sound. The retailer can then assess the microbiological 'comfort factor' or margin which becomes important if a consumer inadvertently subjects product to temperature abuse, for example, by leaving meat products in the car on a hot day.

### **3.7 Assessing compliance with the guidelines**

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However the specifications are derived, a retailer will decide on the format in which data should be presented so that auditors or company representatives can assess performance. A time course graph or a summary of data in broad categories of TVC and *E. coli* count will be easy to interpret.

## 4 Data on raw meat and on supply chains

### 4.1 Characteristics of meat that influence spoilage

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During slaughter and dressing, spoilage and pathogenic micro organisms are transferred to the freshly-exposed carcass mainly from the hides despite the best efforts of processors to prevent this from occurring (Grau 1979). The rate and type of spoilage will depend on meat pH, temperature of storage and transport and packaging format.

The pH of meat depends on its glycogen stores at slaughter (Gill 1982). Usually in beef the pH reaches 5.5 but, when animals are stressed, glycogen is used up and the pH remains above 6. Called dark cutting or dry, firm, dark (DFD), high pH meat spoils more rapidly than normal meat because of its low glucose content. When glucose is low, bacteria switch to proteins as an energy source and their breakdown products, amines, make the meat smell putrid.

Normally, pork has a pH of 5.5-5.7 but when pigs are stressed before slaughter e.g. during transport, pale, soft exudative (PSE) meat occurs, the pH of which is similar to normal pork at 5.4.

Because high pH meat is vulnerable to spoilage it is not used for vacuum packing.

### 4.2 Organisms which spoil chilled meat

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Red meats and pork are spoiled by pseudomonads when packed aerobically, and by lactobacilli when packed anaerobically. Here are the main features of these spoilers.

#### ***Pseudomonas* spp. and other aerobic Gram negative organisms:**

- These are the most common spoilers of aerobically packaged red meats and pork and spoilage levels are reached within days.
- Spoilage occurs firstly, when proteins are broken down to amines (at around 10,000,000/g or /cm<sup>2</sup>) and secondly, when bacteria become so dense that slime is formed (at around 100,000,000/g or /cm<sup>2</sup>).
- In vacuum-packed meats, the oxygen level is very low and an atmosphere of CO<sub>2</sub> also builds up which inhibits the group.
- *Shewanella putrefaciens* is the major spoiler of high pH meat, even when it is vacuum packed, which it spoils at low levels (around 1,000,000/g or /cm<sup>2</sup>). (Cox 2001)

#### **Lactic acid bacteria (LAB):**

- This group is the major spoiler of meat in low oxygen environments such as vacuum and MAP products.
- Lactobacilli are tolerant of CO<sub>2</sub>.

- Spoilage is characterised by sour, “dairy” odours at around 100,000,000/g or /cm<sup>2</sup>.
- Because of their growth rate lactobacilli require around 100 days at 0°C to reach spoilage levels on vacuum-packed meats.

*Brochothrix thermosphacta* has similar growth characteristics to lactobacilli and is an occasional spoiler of vacuum-packed high pH meat

### 4.3 Microbiological Data which can help with guideline setting

We have available to us data from the recent baseline survey (MLA, 2005) and from the ESAM program (E. coli and Salmonella monitoring program run by AQIS in export abattoirs). These surveys give us a measure of overall industry performance at the abattoir and boning room levels and are a good starting point for setting actual numbers.

**Table 2:** Total Viable Count (TVC) on carcasses and boneless meat

		Log TVC/cm <sup>2</sup> (antilog)		
		Mean	95 <sup>th</sup> percentile	Maximum
<b>Carcasses</b>				
ESAM 2004	Steers/heifers	1.05 (11)	2.58 (380)	6.61 (3 981 000)
ESAM 2004	Cows/bulls	1.13 (14)	2.47 (295)	4.47 (29 510)
Baseline 3	Beef	1.33 (20)	2.81 (645)	5.84 (691 830)
ESAM 2004	Lambs	1.48 (30)	2.90 (795)	6.17 (1 479 000)
ESAM 2004	Sheep	1.59 (39)	2.55 (355)	5.19 (147 900)
Baseline 3	Sheep/lambs	2.28 (190)	3.67 (4680)	5.40 (251 200)
ESAM 2004	Pigs	1.81	2.91	
<b>Boneless</b>				
Baseline 3	Beef	1.19 (15)	2.70 (500)	5.49 (309 030)
Baseline 3	Sheep meat	1.81 (65)	3.18 (1510)	4.99 (97 700)

**Table 3:** Prevalence & concentration (cfu/cm<sup>2</sup>) of *E. coli* on carcasses & boneless meat

		Prevalence (%)	Log TVC/cm <sup>2</sup> (antilog)	
			95 <sup>th</sup> percentile	Maximum
<b>Carcasses</b>				
ESAM 2004	Steers/heifers	2.9	nd	1.51 (33)
ESAM 2004	Cows/bulls	6.7	-1.0 (0.08)	1.48 (308)
Baseline 3	Beef	4.9	0.89 (0.8)	1.72 (52)
ESAM 2004	Lambs	13.2	-0.18 (0.66)	6.0 (1 000 000)
ESAM 2004	Sheep	26.5	0.4 (2.7)	4.0 (10 000)
Baseline 3	Sheep/lambs	32.9	1.73 (53)	3.04 (1100)
ESAM 2004	Pigs	7.2		
<b>Boneless</b>				
Baseline 3	Beef	1.1	2.79 (616)	2.79 (616)
Baseline 3	Sheep meat	4.3	3.62 (4170)	4.48 (30 200)

nd = not detected

#### **4.4 Supply Chain information**

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The critical supply chain data required for shelf-life estimation and the development of microbiological criteria are the times and temperatures in the supply chain.

The time that product spends at each stage of the supply chain is needed. For example transport, storage, retail display times. It is also necessary to know the temperature at each step of the supply chain. Knowledge of the time: temperature history of the product will allow the growth of spoilage micro organisms to be estimated.

It is important, not only to know the most optimistic or the most likely times and temperatures. It is also necessary to know the longest times and the highest temperatures likely to be encountered. Data loggers should be used repeatedly at all stages of the supply chain to gain statistically satisfactory estimates of times and temperatures.

When considering setting specifications or microbiological criteria, it is also important to examine the status quo. Data on customer complaints, product returns etc. are a significant indicator of whether a change is required. If the product is meeting the expectations of the retailer and consumer, then there is no reason to set microbiological criteria tighter than the supplier is currently achieving. If there is room for improvement, then careful consideration needs to be given to the entire supply chain to determine whether a tighter criterion for the supplier is the best way of achieving an improvement.



## 5 Principles for setting microbiological criteria for meat safety & shelf-life

In general, the amount of sampling is related to the risk posed to the consumer. Risks are often defined in terms of risk to the consumer's health, but sampling may also be conducted to ensure that product will be acceptable to the consumer and not spoil. Public health risk increases in products which will be consumed:

- By the vulnerable (old, very young, pregnant, immuno-compromised)
- Without further treatment to reduce or eliminate the microbial hazard

Products to be consumed by vulnerable populations are sampled more stringently by increasing the number of samples and the size of the sample. Products which will be cooked before consumption are tested less stringently. The main point is that sampling must be related to risk of consumer illness or consumer rejection.

Testing product against microbiological criteria needs a sampling plan. A sample is taken to reflect the status of the entire batch or lot – the term “representative sample” is often used. In reality, there are always differences between the sample and the whole lot – that is, there is a possibility that the sample will present an outcome which is not representative of the lot. To reduce this risk, more than one sample unit is drawn from a lot, but this adds to the cost of sampling and testing.

The following terms are commonly employed for describing sampling plans:

- “n” is the number of sample units drawn
- “m” is the microbiological count which separates acceptable from marginally acceptable quality
- “M” is the microbiological count which separates marginally acceptable from defective quality
- “c” is maximum number of results allowed between “m” and “M”

### 5.1 Reasons for sampling

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Microbiological sampling may be done to satisfy one or more of the following reasons:

#### **Regulatory requirements**

When a controlling authority sets a sampling plan it takes into consideration risk to consumers - the term “risk-based approach” is often used. For example, AQIS set sampling plans for *Salmonella* on pig carcasses at:

$$n=55, c=6, m=\text{not detected in } 300\text{cm}^2$$

The plan involves sampling one carcass for every 5,000 head slaughtered. In a “window” of 55 tests, up to six may be positive for *Salmonella*. If more than six tests are positive within a window, more stringent sampling must be undertaken. This kind of sampling plan is suitable for monitoring of the hygienic standards of meat processing and is a good example of how sampling plans can be applied for regulatory purposes in the meat industry. It is not suitable for determining the suitability of a lot of product.

### Customer specifications

Customers can set their own specifications. For example, large hamburger chains require that *E. coli* O157:H7 is not detected in a lot of production. To satisfy this requirement, exporters test 25g samples of beef trim from each lot of production according to:

$$n=1, c=0, m=\text{not detected in 25g}$$

The essence of sampling to meet customer specifications is to that your own sampling should be at least as rigorous as that which the customer uses to test your product.

### Process validation and verification

When a process is developed a HACCP-based food safety plan should also be developed. The plan may be validated by a one-off, comprehensive sampling regime. For example, if the HACCP plan says that *Salmonella* will not be detected in final product, a large sampling survey is needed to give confidence that the process is valid. In such a survey the sampling for presence of *Salmonella* in product might be:

$$n=50, c=0, m=\text{not detected in 25g}$$

That is, fifty samples are tested over a period of time, of which none may be positive for *Salmonella*.

Once the process has been validated, it must be verified on a regular basis. For example, to verify a lot of production the following plan may be used:

$$n=1, c=0, m=\text{not detected in 25g}$$

That is, one sample is taken from each lot (which may be a shift or a period between work-breaks) and must be negative for *Salmonella*.

### Provide confidence

The phrases “*so I can sleep at night*” and “*so I won’t kill anyone*” are often used by managers to describe the sampling plan they think they need. In fact, such plans would send the company broke because, to have a high confidence level, a huge number of samples are required. In 1999, the American Meat Science Association (AMSA) gathered together 35 eminent meat microbiologists and posed key questions on meat sampling for them. One was: what sort of sampling is needed to have a high probability that the pathogen will be detected. The answers set out in *Table 4* for detecting *Salmonella* in minced meat when the prevalence is 0.1% (1 in 1,000 samples) reveal the huge number of samples required.

**Table 4:** Number of samples required to detect *Salmonella* in minced beef

Probability of detection (%)	Number of samples required
90	2303
95	2996
99	4605

The sample numbers (above) give a high level of confidence, but there wouldn’t be much product left for sale from the lot.

## 5.2 Types of sampling plan

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There are two kinds of attribute sampling plans (that is, sampling plans that classify product according to its attributes: acceptable or not acceptable), 2-class and 3-class. Two-class plans divide product into two classes and are essentially Pass/Fail. They are often used for dangerous micro organisms (pathogens). Three-class plans allow for a 'marginal' class, where product may not be considered to be optimal, but still acceptable occasionally. They are often used for spoilage organisms and micro organisms that indicate the standard of hygienic processing.

A 2-class plan for *Salmonella* in minced meat in the EU (European Commission, 2005) is:

$$n=5, c=0, m=\text{not detected in 25g}$$

The plan involves testing 5 samples of 25g for *Salmonella*. If all five samples are negative, the batch meets the standard. (But see the note about pathogen testing in section 5.4)

Three-class sampling plans include a "grey area" where some samples may exceed the acceptable limit (m) up to a marginally acceptable limit (M) which may be occasionally accepted, the number of occasions being defined by "c". No sample may exceed "M"; >M is a "no-go" area. Clearly "M" can only be allowed where the risk of illness is still remote.

A 3-class plan is for standard plate count (total viable count) in minced meat in the EU (European Commission, 2005) is:

$$n=5, c=2 m=10^5/\text{g}, M=10^6/\text{g}$$

Five samples are taken of which two may be between  $10^5$ - $10^6$ /g but none must be  $>10^6$ /g.

## 5.3 Shelf-life endpoints

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Odour formation in packages of meat is the primary means by which microbial activity brings shelf-life to an end. Not all bacteria have the same biochemical activity so that the Total Viable Count (TVC) is not always a good predictor of shelf-life. For example, Gram-positive bacteria, such as *Lactobacillus*, grow very well in refrigerated, vacuum-packed meats and counts around  $10^7$ /g (10 million/g) do not indicate spoilage. This is because the main waste product of *Lactobacillus* growth is lactic acid. Spoilage occurs when the counts reach  $10^8$ /g (100 million/g).

By contrast, Gram-negative bacteria such as *Pseudomonas*, *Alteromonas* and *Shewanella* are biochemically active against proteins and excrete waste products called amines which are associated with the odour of rotting flesh. When these organisms reach  $10^6$ /g (1 million/g) the product is close to spoilage level.

Setting a sampling plan for shelf-life therefore depends on the pH and on the likely microflora. If meats have pH>6, pseudomonads have an opportunity to become the dominant spoilage microflora.

Gram-negatives are inhibited by carbon dioxide and if this gas either accumulates or is flushed into the headspace pseudomonads will not become the dominant microflora and shelf-life will be extended.

## **5.4 Sampling for pathogens**

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In 1999 the American Meat Science Association report on microbiological testing stated that:

- The main purpose of sampling and microbiological testing should be to validate and verify process control with a HACCP system
- Pathogen testing (e.g. *Salmonella*) cannot assure food safety
- Pathogens will never be detected consistently when they are not randomly distributed throughout the lot or when they occur at a low incidence
- Testing of non-pathogens will allow validation and verification of processes
- Microbiological testing of foods in production is important but may be negated by handling and further processing later in the marketing chain

For these reasons it is recommended that specifications for pathogens not be used.

## 6 Setting guidelines for red meat products at entry to the retail system

In this section we take two different approaches to setting guidelines. They illustrate different thought processes using the available data and make different assumptions. The two approaches broadly reach the same conclusions for the two products examined.

In this section we take two examples:

- Gas flushed lamb packed for stir fry
- Lamb patties manufactured from vacuum-packed trim

The first example is chosen to represent a product with a potentially long retail life (7 days) under conditions which will allow growth of *Pseudomonas* (packing in aerobic film). The second example has conditions which inhibit *Pseudomonas* by using antimicrobials and by packing under vacuum.

### Gas flushed lamb

Lamb trim is packed under carbon dioxide for storage in the distribution chain and then packed either under modified atmosphere or in an over-wrapped tray prior to retail display. For much of its time in the marketing chain product is bulk-packed under CO<sub>2</sub>, a medium which prevents growth of Gram-negative spoilers such as pseudomonads. However, once meat is packed for retail, the packaging format defines shelf-life. If packed under modified atmosphere, the growth of pseudomonads will be suppressed. However, on product packed with aerobic film (over-wrap) pseudomonads will multiply.

### Lamb patties

Lamb trim is vacuum packed and stored at the patty making premises for up to 14 days when it is ground, antimicrobials (food acids and metabisulphite) are added, the patty is formed and packed under modified atmosphere rich in carbon dioxide.

An atmosphere of carbon dioxide is maintained for the entire life of the product post boning (vacuum then MA pack) preventing growth of *Pseudomonas*. Growth will be predominantly that of lactic acid bacteria.

The supply chains for the two products are shown in the *Tables 5 and 6*.

**Table 5:** Supply chain for gas flushed lamb trim

Location	Process	Storage time (day)	
		Most likely	Maximum
Abattoir chiller	Active chilling	1 <sup>(1)</sup>	3 <sup>(2)</sup>
Abattoir boning room	Boned, CO <sub>2</sub> flush	2	4
Distribution centre	Stored up to 15 days	10	19
Store	Held up to 6 days	13	25
Store	Open carton, repack as over-wrap	13	25
Store	Shelves for up to 7 days	16	32

**Table 6:** Supply chain for vacuum packed lamb trim used for patties

Location	Process	Storage time (day)	
		Most likely	Maximum
Abattoir chiller	Active chilling	1 <sup>(1)</sup>	3 <sup>(2)</sup>
Abattoir boning room	Boned, vacuum packed	2	4
Patty maker	Stored up to 14 days	9	18
Patty maker	Grind, add antimicrobials, form, pack under CO <sub>2</sub>	9	18
Distribution Centre	Up to 11 days	14	29
Store	Shelves for 1 day	15	30

(1)Overnight chill

(2)Weekend chill

## 6.1 Setting guidelines based on current abattoir performance

Taking the thinking expressed in section 4 (above) we are probably looking at:

- Total Viable Count (TVC) as an estimate of overall contamination level
- E. coli as an indicator of faecal contamination
- A 3-class approach

### 6.1.1 Approaches when setting guidelines

In developing our approach to setting a criterion we may know that we currently don't have much problem with shrinkage or returns/complaints because product is considered "off" when customers open the pack. This suggests that the microbiological quality of product and control through the chain is satisfactory to achieve the labelled shelf-life. So we may wish to set a guideline which will never or rarely affect most of our suppliers while, at the same time, prompting our "worst" suppliers to improve (it's worth noting though that even the worst are pretty good).

If we continue this approach we'll focus on the worst 5% of counts – that is, the counts which are between the 95<sup>th</sup> percentile and the maximum, which we can use as the basis for "m" and "M". This approach is similar to the approach taken for the development of performance criteria with the ESAM guidelines.

The microbiological guidelines which accompany the Australian meat standard (AS4696-2002) use broad bands with descriptors such as "Excellent", "Good", "Acceptable", "Marginal" and "Action Required" for the TVC and *E. coli* counts. From the viewpoint of a retailer wishing to gauge performance of a particular supplier it is logical to expect the vast bulk of test results at the abattoir to be in the "Excellent" or "Good" categories of the microbiological guidelines accompanying the Australian meat standard.

### 6.1.2 Setting a guideline for TVC

Tables 2 and 3 give us some prompts on numbers we can use for "m" and "M" and, in Table 7, are suggested values for "m" and "M" for TVC at the stage when product leaves the meat processor.

**Table 7:** Guideline for TVC on beef and sheep carcasses and boneless meat

	Log TVC/cm <sup>2</sup> or/g (antilog)	
	(m)	(M)
Beef carcasses/boneless meat	3 (1 000)	4 (10 000)
Sheep carcasses/boneless meat	3 (1 000)	4 (10 000)

It is suggested that suitable criteria for beef, pig and sheep carcasses and boneless meat (trim) be m=3, M=4. Such a guideline provides meat of high hygienic quality into the retailing system (“m” equates with the Excellent category and “M” with the Good category of the Guidelines which accompany the Australian Standard – see also Section 5.3) and should be achievable almost all the time.

Having set microbiological levels we need to specify the number of samples to be taken (n) plus the number of results (c) allowed between “m” and “M”.

In setting “n” (number of samples) n=15 is already in operation through the ESAM program. In setting “c”, based on the national baseline study, n=15, c=5 is a realistic criterion.

The complete guideline for TVC is: n=15, c=5, m=3 (1,000), M=4 (10,000)

### 6.1.3 Setting a guideline for *E. coli*

Microbiological criteria for *E. coli* relate to the hygienic processing of animals and the likelihood of contamination with pathogenic organisms such as *Salmonella*. Export abattoirs already have a guideline for *E. coli* based on a moving window of 15 samples, as part of the ESAM program (*Table 8*).

**Table 8:** ESAM guideline for *E. coli* prevalence & concentration for beef, sheep & pig carcasses

	n	c	m	M
Beef carcasses	15	3	0	20
Sheep carcasses	15	7	5	100
Pig carcasses (skin-on)	15	5	1	100

This guideline is aimed at identifying abattoirs with the worst 5% of sample windows (Vanderlinde *et al.* 2006). To set the guideline, data for beef, sheep and pig carcasses contained on the ESAM database were used.

### 6.1.4 Application of these guidelines in the supply chain

#### Gas flushed lamb

We’ve used microbial counts for ‘typical’ product processed in Australia and then calculated the increases in microbial count that may occur through the supply chain. We have taken into account times collected through the supply chain and made estimates of increases (*Table 9*).

If packed under modified atmosphere, spoilage over the regime set out in *Table 9* will not occur due to suppression of pseudomonads. However, on product packed with permeable film (over-wrap) pseudomonads will multiply approximately every 6.5

hours at 3°C. A critical factor is the proportion of pseudomonads within the total bacterial microflora to begin with. Grau (2001) estimates the proportion of psychrotrophic spoilers on carcass meat at 1% or less, a level used in Table 7.

It is estimated that the most likely residence time on retail shelves is 3 days which, in theory, will allow 11 divisions of *Pseudomonas* (11 x 6.5 hours = 3 days). For 7-day retail storage 25 divisions are predicted (25 x 6.5 hours = 7 days).

No lag phase is allowed for pseudomonads when the gaseous atmosphere in the pack changes from vacuum-pack to aerobic film pack. However, growth of pseudomonads in overwrap may be retarded by two factors:

- Residual CO<sub>2</sub> absorbed by the meat will take some time to dissipate
- The microflora, dominated by Gram-positives, will have some competitive effect on pseudomonads

For meat with a microbial loading around the mean established in the national baseline study (Phillips et al. 2006) and sold within 3 days pseudomonads will not reach a critical level.

However, for meat with a loading near the maximum established by Phillips et al. (1996), pseudomonads are predicted to reach a level associated with odour formation (10<sup>6</sup>/g) between 4 and 5 days.

It is suggested that for products which have a long (up to 7 days) retail shelf-life, modified atmosphere packaging be used.

### **Lamb patties**

As seen in Table 8, the most likely case is that the product will be retailed around 15 days after the carcass is chilled. An atmosphere of carbon dioxide is maintained for the entire life of the product post boning (vacuum then MA pack) preventing growth of *Pseudomonas*.

Growth will be predominantly that of lactic acid bacteria, which is estimated by Mano et al. (1995, 2000) at 2-3 log over 13 days on pork under 20% carbon dioxide at 7°C. In Table 8 the assumption is made of 1 log at 3°C over 13 days.

From Table 8 it can be judged that there is considerable shelf-life available to the customer because of the use of anti-microbial ingredients and MA packing.

### **6.1.5 Suitability of these guidelines in the supply chain**

From the examples worked in Tables 9 and 10 it can be seen that the microbiological criteria selected earlier in this section for TVC: n=15, c=5, m=3 (1,000), M=4 (10,000) for product as it leaves the meat processor and enters the retail chain generally cater for distribution and retailing requirements. The exception is when long (7 day) phases under aerobic conditions are part of the distribution/retailing regime.



**Table 9:** Gas flushed lamb trim used for stir fry lamb

Location	Process	Storage time (day)		log <sub>10</sub> TVC/cm <sup>2</sup> or /g		Pseudomonas/cm <sup>2</sup> or /g	
		Most likely	Maximum	Mean <sup>(3)</sup>	Maximum <sup>(4)</sup>	Mean <sup>(3)</sup>	Maximum <sup>(4)</sup>
Abattoir chiller	Active chilling	1 <sup>(1)</sup>	3 <sup>(2)</sup>	2.27 (186)	3.5 (3162)	2 <sup>(5)</sup>	32 <sup>(5)</sup>
Abattoir boning room	Boned, CO <sub>2</sub> flush	2	4	1.84 (70)	3.5 (3162)	0.7	32
Distribution centre	Stored up to 15 days	10	19	2.0 <sup>(6)</sup>	3.7 <sup>(6)</sup>	0.7	32
Store	Held up to 6 days	13	25	2.0 <sup>(6)</sup>	4.7 <sup>(7)</sup>	0.7	32
Store	Open carton, repack as over-wrap	13	25	2.5 <sup>(8)</sup>	5.2 <sup>(8)</sup>	0.7	32
Store	Shelves for up to 7 days	16	32	3.0 <sup>(9)</sup>	6.7 <sup>(10)</sup>	2000	1,000,000 <sup>(11)</sup>

(1) Overnight chill

(2) Weekend chill

(3) Mean count from Baseline 3 for chilled carcasses and for frozen lamb trim

(4) 90<sup>th</sup> percentile count from Baseline 3 for chilled carcasses and for frozen lamb trim

(5) Pseudomonads estimated at 1% of the total microflora (Grau, 2001)

(6) Shelf-life studies indicate no growth over 21 days because of the presence of CO<sub>2</sub>

(7) Shelf life studies indicate 1 log increase for vacuum-packed trim over 28 days

(8) Meat repacked for retail (assumed over-wrap). Half log scale allowed for knife work and repacking

(9) Most likely time of sale estimated at 3 days and a 0.5 log increase is allowed. Pseudomonads are not inhibited by over-wrap

(10) Maximum storage time 7 days - allow 1.5 log increase in TVC

(11) Odour formation reached after 4-5 day

**Table 10:** Vacuum packed lamb trim used for patties

Location	Process	Storage time (day)		log <sub>10</sub> TVC/cm <sup>2</sup> or /g		<i>Pseudomonas</i> /cm <sup>2</sup> or /g	
		Most likely	Maximum	Mean <sup>(3)</sup>	Maximum <sup>(4)</sup>	Mean <sup>(3)</sup>	Maximum <sup>(4)</sup>
Abattoir chiller	Active chilling	1 <sup>(1)</sup>	3 <sup>(2)</sup>	2.27 (186)	3.5 (3162)	2 <sup>(5)</sup>	32 <sup>(5)</sup>
Abattoir boning room	Boned, vacuum packed	2	4	1.84 (70)	3.5 (3162)	0.7	32
Patty maker	Stored up to 14 days	9	18	2.5 <sup>(6)</sup>	4.5 <sup>(6)</sup>	0.7	32
Patty maker	Grind, add antimicrobials, form, pack under CO <sub>2</sub>	9	18	3.0 <sup>(7)</sup>	5.0 <sup>(7)</sup>	0.7	32
DC	Up to 11 days	14	29	3.5 <sup>(8)</sup>	6.0 <sup>(9)</sup>	0.7	32
Store	Shelves for 1 day	15	30	4.0 <sup>(10)</sup>	6.5 <sup>(10)</sup>	0.7	32

- 1 Overnight chill
- 2 Weekend chill
- 3 Mean count from Baseline 3 for chilled carcasses and for frozen lamb trim
- 4 90<sup>th</sup> percentile count from Baseline 3 for chilled carcasses and for frozen lamb trim
- 5 Pseudomonads estimated at 1% of the total microflora (Grau, 2001)
- 6 Shelf-life studies indicate 0.5 log over 7 days of lactobacilli under 20% CO<sub>2</sub> and 1 log over 13 days (Mano et al. 1995, 2000)
- 7 Estimate 0.5 log increase for grinding and patty formation
- 8 Allow 0.5 log increase over 5 days (influence of anti-microbials and CO<sub>2</sub>)
- 9 Allow 1 log increase over 11 days (influence of anti-microbials and CO<sub>2</sub>)
- 10 Allow 0.5 log increase for retail display and customer handling

## 6.2 Setting guidelines based on variable initial contamination

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There is considerable variation between the times taken in the “best case” or usual chain and the maximum acceptable times. In order to provide useful data, these conditions were modelled using the Food Spoilage Predictor (University of Tasmania predictive model (Neumeyer, et al. 1997). This model is suitable for modelling growth of spoilage *Pseudomonas* spp. In situations where product is displayed under aerobic conditions after a period in MAP or vacuum packaged products, *Pseudomonas* growth will occur prior to modified atmosphere or vacuum packing and resume when removed from the packing for cutting and retail display.

This model does not account for the pH of the meat or meat products: however both Neumeyer *et al* (1997) and Widders *et al* (1994) have shown that differences in pH did not affect the growth or spoilage rate in a study where pork was inoculated with spoilage *Pseudomonas* spp.

It is important to consider the other assumptions made when using these models. These include:

- The temperature has been modelled as 2°C. There may be significant variation from this in a real supply chain.
- The TVC reported for the product in the chiller is predominantly made up of *Pseudomonas* spp.
- That there is negligible growth of *Pseudomonas* spp. in vacuum packaged or CO<sub>2</sub> flushed product and that, levels remain stationary under these atmospheric conditions rather than reduce.

The minimum requirements of the customer are 1-2 days storage in the home refrigerator. The “Customer” column is the available shelf life calculated by the model after prescribed display in retail at 2°C. Lag phase was calculated using information regarding generation times from Grau (1981) and relating this to lag phase according to the method outlined by Widders et al. (1994). Thus, for *Pseudomonas* the lag phase at 2°C is approximately 50 hours, while lag phase at 4°C is approximately 40 hours.

The major spoilage organism growing while the product is gas flushed or vacuum packed will be lactic acid bacteria. The typical growth of lactic acid bacteria on products packaged under gas or vacuum is described in section 6.1

The results of use the predictive models are shown in Table 11. These results suggest that, with the highest initial TVC (10<sup>3</sup>), less than two days of shelf life is available to the consumer, and there is only a consumer shelf-life if the product follows the more rapid path through the cool chain. The available shelf life improves in cases where the *Pseudomonas* spp. makes up less of the TVC, and in all cases the shelf life of the product in the hands of the consumer is improved if the path through the supply chain is shorter. Slow chilling does not make a large difference in the levels. Therefore, if a criterion were applying at the end of chilling it might be:

$$n=5, c=2, m=100 M=500$$

These criteria would be achieved by 95% of product included in Australian abattoir surveys.

The models are presented here in the knowledge that they are limited

- the actual temperatures at each phase of the chain are not known;
- the contribution that pseudomonads make to the TVC is unknown; and
- the effect (if any) of inhibitory substances produced by lactic acid organisms during MAP on the subsequent growth of aerobic spoilers (Nissen et al., 1996) is unclear

However, these models have been shown to be applicable in practice where product is stored under aerobic conditions. Their application is limited for products that are delivered to the customer under MAP. Collecting information on product shelf life over time and using this data to predict shelf life of product when shelf life trials are undertaken allows the models to be validated within a particular processor or supply chain.

**Table 11: Gas flushed Lamb Trim for stir fry**

Product is stored at 2°C throughout shelf-life. The Pseudomonas counts are estimated through the supply chain after various times (best case, worst case) at each stage of the supply chain. For each of four initial contamination levels, a best and worst case shelf-life is given after the product is purchased by the consumer.

<i>Pseudomonas</i> levels on product (log <sub>10</sub> cfu/g or /cm <sup>2</sup> )	Abattoir chiller (2°)		boning room (10°C transiently, then return to chiller under CO <sub>2</sub> )		Storage in DC under CO <sub>2</sub> (2°C)		Retail Outlet – presented under standard overwrap (2°C)		Customer (4°C)	
	Best case (24h)	Worse case (72h)	Best case (24h)	Worse case (24h)	Best case (192h)	Worse case (360h)	Best case (72 hrs)	Worse case (168 hrs)	Best case	Worse case
3.0	3.0	3.6	3.0	4.3	----	----	5.2	Spoiled at 3 days	1 d 14 h	-----
2.0	2.0	2.6	2.0	3.3	----	----	4.2	Spoiled at 5 days	2 d 12 h	-----
1.0	1.0	1.6	1.0	2.3	----	----	3.2	7.0	3 d 10 h	No S/L
-1.0	-1.0	-0.5	-1.0	0.25			1.2	5.5	5 d 6 hrs	1 d, 10 hrs

### 6.3 Relationship to other guidelines

There are other microbiological guidelines and criteria for red meat with which we can compare the suggested guidelines (above). As mentioned previously (Section 6.1.2) there are criteria in the microbiological guidelines which have been developed by Meat Standards Committee for establishments operating according to the Australian Standard (AS 4696:2002) for meat (*Table 12*).

**Table 12:** Microbiological guideline criteria for TVC and *E. coli* in red meats covered by the Australian Standard (AS 4696:2002)

Category	TVC/cm <sup>2</sup> or /g	<i>E. coli</i> /cm <sup>2</sup> or /g
Excellent	<1,000	Not detected
Good	1,000-10,000	1-10
Acceptable	10,000-100,000	10-100
Marginal (Action required)	100,000-1,000,000	100-1,000

The guidelines have four broad bands for TVC and *E. coli*. The Good band for TVC is analogous to the m-M zone of the suggested retail guidelines, above which the product is considered still acceptable but may not be adequate for products with long phases in aerobic packaging. For *E. coli* the Acceptable band is again similar to the m-M zone of the suggested retail guideline, with >100 cfu/cm<sup>2</sup> or /g falling into the 'Action Required' category. Thus it could be said that the levels of the microbiological guidelines are very similar to those suggested for retail red meat products. However, the guidelines are just that – guidelines – and have no sample number (n) or “c” to stipulate proportion of samples which can be in the Good zone.

The European Union (EU) has set microbiological criteria for foodstuffs including meats (European Commission, 2005). In *Table 13* are presented TVCs and Enterobacteriaceae criteria for cattle, sheep and pigs. All the samples collected on a day are averaged, and the action taken if M is exceeded is improvement in slaughter hygiene and review of process controls.

**Table 13:** EU criteria for TVC and *Enterobacteriaceae* for beef, sheep and pig carcasses (European Commission, 2005)

	N	c*	m**	M**
<b>TVC</b>				
Beef carcasses	5		3.5	5
Sheep carcasses	5		3.5	5
Pig carcasses	5		4	5
<b>Enterobacteriaceae</b>				
Beef carcasses	5		1.5	2.5
Sheep carcasses	5		1.5	2.5
Pig carcasses (skin-on)	5		2.0	3.0

\* no values for c are given

\*\* the mean of the log values of the counts on excised samples from warm carcasses

From *Table 13* it can be adduced that the EU believes pig carcasses are more heavily contaminated than beef and sheep, by allowing a higher “m”. Other factors of note are that testing must be done on tissue samples, rather than by sponging, and pre-

chill carcasses are sampled, compared with chilled carcasses for the Australian Standard and ESAM testing. As well, the EU stipulates testing for Enterobacteriaceae rather than for *E. coli* – a European tradition for which there is some merit. Together, these factors offset the fact that the EU criteria are rather less stringent than those suggested for retailers.

#### **6.4 Managing compliance with microbiological criteria**

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Once microbiological criteria are set, there is a need to ensure that they are complied with. The use of lot acceptance testing, as implied by the use of a sampling plan with defined values for  $n$ ,  $c$ ,  $m$  and  $M$  is not entirely suitable for meat processing, and would involve a huge increase in microbiological testing. The system of testing periodically is appropriate. Some systems (e.g. USA) require testing every so many carcasses, whereas other systems (e.g. EU) require more intensive testing every couple of weeks (rotating through different days to pick up differences that may occur). The aim is to gain confidence in the control of hygiene by the processor.

The choice of system for sampling and the frequency of testing is largely a matter of individual preference.

Once the system of sampling is established it's important that processors look at the numbers and take action if there is a trend towards the limit ( $M$ ) imposed by the criteria. This is most easily achieved by graphing the results as they are obtained and monitoring the trends.

## 7 Shelf-life Testing

Shelf-life testing is required by retailers in a number of situations:

- When new products are developed
- After changes in product formulation or processing e.g. preservatives used for patty manufactures
- When packaging is changed e.g. changing from vacuum to gas flushed packaging

Because a range of organisms need to be tested on several occasions during the shelf-life, microbiological testing can be very expensive. For this reason, it is important to plan the shelf life testing carefully. Things to consider include:

- Sampling intervals: It's usual to test on day zero (day of manufacture, about half-way through, and then on several days either side of the expected shelf-life.
- Temperature of testing: The temperature at which samples are stored during the shelf-life study should mirror those at which the product will be stored in the real situation. Including a data logger with the storage packs will give an accurate check of temperature and time.
- Microorganisms:
  - TVC provides a picture of the overall hygiene of the product.
  - Lactic acid bacteria are the predominant spoilers of vacuum packaged or MAP product.
  - *Pseudomonas spp* are spoilers of aerobically stored meat.
  - *Brochothrix* can be a problem in spoilage of high pH red meats, particularly where the product is vacuum packaged.
- Organoleptic parameters: Spoilage is more than just the microbiological aspects and product that is being shelf-life tested should be assessed for odour and colour changes and for slime production.

**Analysis of results:** The results of the shelf life studies provide valuable data for the processor or retailer, and these data can be used in predictive models to assess the impacts of temperature abuse or minor changes in formulation.



## 8 Appendix 1: Shelf-life testing: the claimable life of meat products

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Generally a food is considered to be past its shelf-life when it is no longer acceptable to the consumer. It can be that the colour, flavour, texture, aroma or nutrient content have deteriorated to the point that it is no longer acceptable. It can also be when it becomes a food safety issue, where the food product may make consumers ill.

Whilst shelf-life is usually equated with spoilage, for fresh meat particularly, the end of shelf-life might be reached before spoilage, as such, is evident. For example, the loss of bloom of mince or steaks or reaching a microbial count specified as an acceptable maximum by a retailer may be the determinant of retail shelf-life whereas spoilage as defined by off-odour and slime would be the point at which it is unacceptable for consumption.

The Food Standards Code of Food Standards Australia New Zealand, FSANZ, includes a standard that prescribes a date marking system for packaged food intended for retail sale or catering purposes.

Retailers usually print a 'Use by' date on steaks, roasts and other packaged fresh meats. Under normal circumstances of hygienic handling and storage at 4°C or colder, spoilage bacteria rather than pathogens grow on uncooked meat and meat products and the meat will be cooked by the consumer before they are consumed. Packages of such products could therefore bear a 'Best before' date rather than a 'Use by' one. On the other hand, for ready-to-eat (RTE) meat products, the shelf-life may be influenced by the growth of pathogens (e.g. *Listeria*), even at the recommended storage temperature and the date must be a 'Use by' one.

The reason for spoilage may be different for uncooked products compared with RTE ones; this needs to be taken into account when deciding how to determine and validate a claimed shelf-life. Determining the shelf-life of an RTE meat product may well involve microbiological assessment including, probably, testing for *Listeria monocytogenes*; determining the shelf-life of T-bone steaks will probably be based on assessment of colour stability and maybe odour during retail and home storage perhaps accompanied by some microbiological testing against specifications set by retailers.

Processors must date-mark any pre-labelled packages of fresh or processed meat. In addition, meat processors are being asked by retailers to provide dates for larger packs of meat such as vacuum packs that will eventually be either sold intact or sliced and prepared as smaller retail packs. Here, shelf-life of the large pack should take into account that retailers will expect a display life of two, perhaps three days from the retail packs prepared from it.

Increasingly, meat processors are being asked to show that their claimed shelf-lives for products have been validated. This Update discusses how the validation might be demonstrated.

## 8.1 General approaches to shelf-life estimation

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The term 'Shelf-life' is variously used for the:

- Point of retail display at which consumers decline to purchase; or
- Time to when the product no longer has an acceptable eating experience for the consumer; or
- Time to when consumption is no longer safe.

A shelf-life determination involves an experimental study of the deterioration of the food, culminating in identification of the point that marks the end of its shelf-life. It is important that you are clear about the shelf life that you wish to specify.

There are several established approaches for the gathering of shelf-life data on food products:

- Estimating shelf-life based on published data;
- Utilising known distribution times for similar products on the market;
- Using consumer complaints as the basis for determining whether a problem is occurring;
- Accelerated shelf-life testing; or
- Assessing changes that occur in trial packs under simulated commercial storage.

Relatively little information on shelf-lives of specific products is published. Many shelf-life data are proprietary and therefore not available. Estimates from the published literature, some of which are summarised in Meat Update information sheet 'Storage life of meat', September 2002, are rather old and may not relate closely enough to current processing and packaging systems or to current retailer or consumer expectations. The exception to this generalisation is that the food safety literature can often be used in circumstances where shelf-life is determined by an unacceptable safety risk.

Neither the utilisation of known distribution times nor the consumer complaint approach can be validated satisfactorily and accelerated testing has little application to meat products – probably being limited to long-life products such as beef jerky.

The most direct and common way to determine shelf-life is to carry out storage trials under controlled conditions that reflect those that the meat normally encounters during the usual course of distribution, retail display, and storage by the consumer. Selection of an appropriate, reliable approach to simulating quality loss that will occur during commercial distribution and storage is an important first step when using this approach.

Select conditions that you anticipate will cover most situations but not necessarily conditions of significant abuse. As an example, if the package carries the statement 'Keep refrigerated', it is unrealistic and inappropriate to undertake trials at 0°C; 4°C would be more realistic if a period of storage in the home is likely. Take into consideration the fact that both chilled and frozen meats will be subjected to temperature fluctuations, particularly during summer months. It is often advisable to determine the shelf-life at two temperatures – the recommended storage temperature and the maximum temperature expected under normal transport and storage conditions.

Of the categories of food spoilage that can occur – physical, chemical, and microbiological - the two principal spoilage mechanisms that affect shelf-life of meat are microbial growth and oxidation of myoglobin (browning) or lipids (rancidity).

## **8.2 Estimating shelf-life**

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Before shelf-life testing can be carried out, it is important to establish which quality characteristics are important to the purchaser or consumer for the product under assessment. This may vary between products. Establishing the criteria of importance and defining the acceptable standards are policy matters for manufacturers and retailers to resolve. As stated earlier, variable quality characteristics to consider include:

- Safety
- Meat colour
- Overall appearance
- Odour
- Flavour
- Texture

Food safety shelf-life is limited by the presence of unacceptable numbers of pathogens on a meat or meat product and is a function of the initial level of contamination by the pathogens in question, along with time and temperature. It is common, however, to regard food safety as being compromised if the food has been subjected to conditions that permit growth of pathogens if the pathogens happened to be present.

Note that it is important not to rely on shelf-life evaluation to establish the microbiological safety of the product. In this respect, the question that needs to be addressed is: “Will the product formulation and storage conditions control growth of pathogens during the designated shelf-life if they were present?” In this circumstance a HACCP analysis is necessary to identify which, if any, pathogens are relevant, and challenge testing may be required, particularly in the case of RTE meats. Such testing involves deliberate inoculation of the product with the pathogens that have been identified in HACCP or with indicator bacteria that are known behave similarly in the product to the pathogens.

In uncooked meats, and mostly with RTE meats, it will not be the presence of pathogens that dictate shelf-life.

## **8.3 Measures of shelf-life**

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In fresh meats that are stored in air, pseudomonads will dominate the total population of bacteria so a standard plate count is a good guide to the onset of spoilage.

For vacuum-packed meat however, total count is not a good index. As vacuum-packed meat is stored in the absence of oxygen, growth of pseudomonads, as strict aerobes, is restricted. Instead, after storage the bacterial population will consist mainly of lactic acid bacteria.

Consumer acceptability of meat and meat products, particularly frozen ones, can be affected by factors that are not microbiological (see Table 1). They include:

- Meat colour and appearance;
- Rancidity caused by chemical oxidation of fats at low temperature;
- Changes in texture caused by extended enzymic activity or product drying during storage, eg freezer burn;
- Texture, flavour and odour changes caused by other chemical reactions occurring in the product during storage eg toughening from protein denaturation or colour and flavour changes from non-enzymic browning reactions.

Browning of meat is due to oxidation of the meat pigment myoglobin. Low pH meat - 5.5 and lower – seems to be more susceptible to colour deterioration. Development of browning can be followed instrumentally using a colour meter. If previous experience has told you what the causal products of odour and flavour spoilage are, they can be tested for using appropriate chemical analyses – gas chromatography combined with mass spectrophotometry for example.

Instrumental techniques are only useful if there is a good knowledge of the relationship between the levels of specific chemicals and consumer perceptions of spoilage of your product. If that knowledge is not available, information on the deterioration of quality has to be obtained by the use of taste panels using either trained technicians or untrained consumers.

#### **8.4 Some specific examples**

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##### **Raw meats - fresh**

Pathogen growth is most conveniently estimated in raw meats by predictive microbiology using a model such as that developed in Australia by the University of Tasmania and Meat & Livestock Australia. The criteria specified in the Export Control (Meat and meat Products) Orders 2005 are appropriate for determining what would be deemed unacceptable temperature abuse that would compromise shelf-life.

In fresh meats that are stored in air e.g. in over-wrapped trays, as the numbers of pseudomonad bacteria reach around 100 million per cm<sup>2</sup> they produce a putrid odour and slime forms on the meat surface. The pseudomonads will dominate the total population of bacteria so a total count is a good guide to the onset of spoilage.

High microbial populations may not necessarily impair sensory characteristics but a pre-determined level of micro-organisms, together with factors such as sensory attributes is often used to indicate that the end of life has been reached. Total counts in excess of 1 million per cm<sup>2</sup> of product surface or per gram of mince or other comminuted product is often taken to indicate that spoilage is imminent and are often regarded as the end of acceptable shelf life.

Table 1: Suggested attributes to assess when estimating shelf-life of range of products

Retail meat package	Quality attribute	Nature of spoilage	End of shelf-life	Approach to estimating shelf-life
Fresh meat on over-wrapped tray	Good pink-red 'bloom' Odour of fresh meat	Off-odours, off-flavours, stickiness, slime from bacteria. Discolouration	Loss of bloom, brown discolouration. Microbiological specification exceeded	Colour meter Colour panel Counts of total bacteria
Fresh/MAP – high oxygen	Good pink-red bloom Odour of fresh meat	Off-odours, off-flavours, slime from bacteria. Discolouration	Loss of bloom, brown discolouration. Microbiological specification exceeded	Colour meter, colour panel Counts of total bacteria Counts of specific bacteria
Vacuum pack	Purple meat colour, tight pack. Normal confinement odour	Sour, dairy off-odour, off-flavour, greening from microbial activity. Browning	Unacceptable, persistent confinement odour. Meat discoloured (brown, grey, green) in intact pack.	Colour meter, colour panel Odour/taste panel Counts of specific bacteria
VP/ over-wrapped	Good pink-red bloom Odour of fresh meat Minimal drip	Off-odour, off-flavour (incl. sour, dairy odour) Browning	Loss of bloom, brown Sour odour, flavour Microbiological specification exceeded	Colour meter, colour panel Odour/taste panel Counts of total bacteria Counts of specific bacteria
VP/ MAP – high O <sub>2</sub>	Good pink-red bloom Odour of fresh meat Minimal drip	Off-odour, off-flavour (incl. sour, dairy odour) Browning	Loss of bloom, brown Sour odour, flavour	Colour meter, panel Odour/taste panel Counts of total bacteria Counts of specific bacteria
Sliced corned beef, cooked – vacuum pack	Pink Odour of corned beef	Souring. Slime, off-odour after pack opened Pathogen growth (e.g. <i>Listeria</i> )	Loss of pink colour. Souring. Microbiological specification exceeded	Colour meter, panel Taste panel Counts of total bacteria Counts of specific bacteria Challenge test – specific pathogen(s)
Frozen ground beef	Pink-red	Rancidity, Freezer burn	Rancid odour, flavour when cooked Surface desiccation, sponginess	Taste panel
Frozen lamb chops	Pink-red	Rancidity, Freezer burn	Rancid odour, flavour when cooked	Taste panel

## Raw meats in vacuum packs

Lactic acid bacteria grow slowly on vacuum-packed meat at chill temperatures to 10-100 million per gram after about 6 weeks storage. They will stay around this level for the rest of the life of the product. Signs of spoilage will not be evident until several weeks after the maximum population of bacteria is reached. When spoilage eventually becomes evident it will be due to cheesy or sour milk odours and flavours rather than the putrid odours caused by pseudomonads in air.

For vacuum-packaged fresh meat of normal pH, a total bacterial count is NOT a useful indication of the microbiological quality of the product. If the total count is made up of mostly lactic acid bacteria, counts of more than 10 million per g do not indicate incipient spoilage or any processing or storage problem. Only total counts in excess of 100 million per cm<sup>2</sup> would indicate the end of the product's shelf life.

If meat in vacuum packs has a pH greater than 5.9, off odours may be detected when the bacterial count is just over one million per cm<sup>2</sup> if:

- the storage temperature is 5-10°C; or
- there are traces of oxygen in the pack due to using a packaging film with a high oxygen transmission rate.

In such vacuum-packed meat there may be an increased growth of spoilage bacteria such as *Brochothrix thermosphacta*, *Shewanella putrefaciens*, and psychrotrophic enterobacteria. These bacteria will cause souring and off-odours. Selective counts of these organisms can be useful in identifying the limitations to storage life of such product.

## Cooked perishable meats

Cooking will normally destroy vegetative micro-organisms with only spores surviving. Post-processing contamination, however, will eventually lead to spoilage at the contaminated surfaces. Most commonly, spoilage of cured meats is caused by growth of lactic acid bacteria and normally becomes evident some time after the lactic bacteria reach their peak numbers. Green surface discolouration is caused by peroxide oxidation that is attributable to certain strains of these bacteria.

As stated earlier, determining the shelf-life of an RTE meat product may also involve challenge testing for *Listeria monocytogenes*.

## 8.5 Panel assessments

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Sensory techniques supported by statistical methods are frequently used to determine the time at which a product achieves the limit of acceptability. The determination of consumer acceptability is most reliably done by means of panels of 100 or more untrained tasters, an exercise that is usually cost-prohibitive for establishing shelf-life. To minimise the cost and time involved other approaches are:

1. An experienced sensory scientist determines the limit for acceptability of a given attribute and then uses a trained panel to measure the intensity of this attribute during storage

2. The acceptability assessed by a trained panel is correlated to that of untrained consumers.
3. An increasing number of untrained consumers are used to assess the deterioration during storage, concentrating the testing more heavily on samples that are close to the end of their shelf-life.

The first is the easiest to perform but does not give any information on consumer perceptions.

Techniques used for panelling include:

- Difference tests – paired comparisons and triangle tests are useful to compare stored product with fresh product. Errors can however occur because new fresh samples are used at each testing during the storage. This technique also has the drawback in that it says nothing about acceptability - just whether it differs from the fresh control. It can be used to compare a revised process or new packaging film with an existing one.
- Hedonic scoring – Consumers are asked to rate the acceptability of the product on some predetermined scale. Common scales include terms like: like very much, like a little, neither like or dislike, dislike a little and dislike very much. The limitation to this technique is that the acceptability can go up or down due to changes within the storage and panellists respond differently to these changes, eg rancidity, moisture loss.
- Quantitative descriptive analysis (QDA) – QDA is based on the ability of panellists to describe reliably their perceptions of a product's attributes. This requires screening of panellists and the development of a suitable sensory language. Sensory attributes are scored and give good information on which attributes change during storage. However results still have to be related to consumer acceptability.

Sensory assessments by panels should normally be designed and interpreted by a specialist. General procedures for shelf-life testing include:

1. Develop a testing protocol consisting of the specific objective, detailed test design which covers product, packaging and storage specifications and panelling procedures and includes the number of samples required.
2. Identify the key quality indicator/s from any previous studies or published literature. Any information on known distribution time or turnover time of the product would be useful here.
3. Establish the sampling frequency and duration of the testing based on experience from previous studies or published data. If the interval of sampling is too long, the risk of under or over estimating shelf-life increases. Determination of the end of the experiment must be based on some preset criterion such as minimum required commercial shelf-life or some specific organoleptic criterion of unacceptability.
4. All testing should be based on one common sample, if possible, to ensure consistency between panellists. There are a number of publications covering detailed procedures for effective sampling, taste panelling and analysis of data. It is important that specialist knowledge be obtained to ensure that the sampling and panelling will give meaningful results.

5. Reporting of the outcomes and recommendations along with the details of design and application of the experiment. This report is the validation of the chosen shelf life of the product and is an important document to support your HACCP based meat safety plan.

**Further reading**

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