Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods
HPA Draft Guidelines: Consultation from 01/12/08 to 20/02/09

Authorship
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Abbreviations

ACC  Aerobic colony count
ATP  Adenosine triphosphate
BRC  British Retail Consortium
CFA  Chilled Food Association
cfu  Colony forming units
EC  European Commission
EN  European Norm
EU  European Union
FBO  Food business operator
FSA  Food Standards Agency
GHP  Good hygiene practice
GID  Gastrointestinal disease
HACCP  Hazard analysis and critical control points
HPA  Health Protection Agency
HUS  Haemolytic Uraemic Syndrome
IBS  Irritable bowel syndrome
LACORS  Local Authorities Co-ordinators of Regulatory Services
PIF  Powdered infant formula
TTP  Thrombotic Thrombocytopaenic Purpura
UTI  Urinary tract infection
VT  Verocytotoxin
VTEC  Verocytotoxin-producing *Escherichia coli*
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Section 1 Introduction

1.0 Background

In pursuit of the Health Protection Agency’s (HPA) goal of preventing and reducing the incidence and consequences of infection, the HPA examines foods for public health purposes to help safeguard consumer health. These include samples submitted for surveillance and monitoring, as part of outbreak investigations or as Official Controls. The HPA has accumulated a wealth of expertise and data on the microbiological results thus generated and, crucially, on the interpretation of these results. This information was captured and promulgated in three previous sets of guidelines for practical use by Food Examiners and local authority enforcement officers. These revised guidelines have a different emphasis and are risk based focusing on public health and consumer protection.

Foodborne diseases of microbiological origin (most often infectious intestinal disease) can be caused by a variety of agents, which gain entry by the gastrointestinal tract. Table 1.0 lists examples of foodborne pathogenic agents. Symptoms of foodborne disease, which are not necessarily confined to diarrhoea and vomiting, are caused by viable organisms and/or by the toxins that they produce. The risk of disease from these agents varies depending on the pathogen, the dose, the host and the properties of the food matrix. The risk of disease can be influenced by age, immune status, underlying debilitating disease or stress factors, and the physiological state of the stomach and upper small intestine at the time of exposure to the agent. For these reasons a minimum infectious dose cannot be defined, although the risk of disease at low exposure for some agents is small.

The presence of foodborne pathogenic agents in ready-to-eat foods is significant and their absence is of paramount importance. With the exception of the aerobic and anaerobic bacterial spores, detection of foodborne pathogenic agents at any level is of concern and should be investigated with an urgency of response proportional to the level of contamination and risk to consumers. Although low numbers of pathogens, such as coagulase-positive staphylococci, Clostridium perfringens, Bacillus cereus, and Listeria monocytogenes, in ready-to-eat products probably represent a very low risk to immunocompetent people, their presence can suggest faults in the production or
subsequent handling of food which, if not controlled, could lead to an unacceptable increase in risk. There may also be a need for action when detecting low numbers of these organisms in ready-to-eat foods because there is variation in host susceptibility and interstrain differences in the pathogenicity of these bacteria.

**Table 1.0 Examples of foodborne pathogenic agents**

<table>
<thead>
<tr>
<th>Pathogenic agent</th>
<th>Found in foods or food components</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infections</strong></td>
<td></td>
</tr>
<tr>
<td>Bacterial, gastrointestinal</td>
<td><em>Bacillus cereus</em>, <em>Campylobacter</em> spp., <em>Clostridium perfringens</em>, verocytotoxin-producing <em>Escherichia coli</em> (VTEC), <em>Salmonella</em> spp., <em>Shigella</em> spp., <em>Yersinia enterocolitica</em>, <em>Vibrio cholerae</em>, <em>Vibrio parahaemolyticus</em></td>
</tr>
<tr>
<td>Bacterial, extra-gastrointestinal</td>
<td><em>Brucella</em> spp., <em>Cronobacter</em> spp., <em>Listeria monocytogenes</em>, <em>Mycobacterium bovis</em>, <em>Salmonella</em> Typhi and Paratyphi (enteric fever)</td>
</tr>
<tr>
<td>Viral, gastrointestinal</td>
<td>Norovirus, rotavirus</td>
</tr>
<tr>
<td>Viral, extra-gastrointestinal</td>
<td>Hepatitis A and E</td>
</tr>
<tr>
<td><strong>Intoxications (Microbiological toxins)</strong></td>
<td></td>
</tr>
<tr>
<td>Toxins produced by bacteria</td>
<td>Emetic toxin of <em>Bacillus cereus</em></td>
</tr>
<tr>
<td>Neurotoxins of <em>Clostridium botulinum</em></td>
<td></td>
</tr>
<tr>
<td>Enterotoxins of <em>Staphylococcus aureus</em></td>
<td></td>
</tr>
<tr>
<td>Histamine (scombrotokxin)</td>
<td></td>
</tr>
<tr>
<td>Toxins produced by algae</td>
<td>Paralytic shellfish poisoning, diarrhoeic shellfish poisoning toxins</td>
</tr>
<tr>
<td><em>Ciguatera</em> toxin</td>
<td></td>
</tr>
<tr>
<td>Toxins produced by fungi</td>
<td>Mycotoxins</td>
</tr>
<tr>
<td>Patulin</td>
<td></td>
</tr>
<tr>
<td><em>Ochratoxin</em></td>
<td></td>
</tr>
</tbody>
</table>

*a* For some agents listed routine food testing is not available. Not all the agents listed occur in the UK.

These revised guidelines are designed to help determine the microbiological safety of ready-to-eat foods and to indicate levels of bacterial contamination considered to be a public health risk. The interpretation of laboratory results in food microbiology is often the most difficult and complex aspect of the examination process. The purpose of the
original microbiological guidelines for ready-to-eat foods sampled at the point of sale\textsuperscript{2} was to standardise the interpretation of the results obtained from the microbiological (bacteriological) examination of foods by providing peer reviewed guidelines. Users of the guidelines should however be aware that the precision and reproducibility of many microbiological tests depends on many factors, some of which are outside the control of the laboratory. The act of sampling itself is the greatest contributory factor to the variability of a result for a particular sample as microorganisms are not usually homogenously distributed in a contaminated foodstuff. As a consequence it is unlikely that two successive samples taken from the same foodstuff will yield identical quantitative microbiological counts. The sample matrix itself, the packaging process, and the culturability of injured organisms will also contribute further to the lack of reproducibility between microbiological results. Results should therefore be interpreted in context taking such factors into account and in discussion with a Food Examiner. Criteria for other agents including viruses and enteric parasites are currently excluded; however as European Standard methods (EN) become available these may be included in the future.

1.1 Application of the guidelines

The guidelines are not intended to be prescriptive and have no legal standing in their own right. Food within the scope of the revised guidelines includes ready-to-eat food sampled at any point in the retail chain, i.e. retail, wholesale and food service sectors. This includes food components, such as herbs and spices, where they are added to foods without further cooking or processing.

As defined in Regulation (EC) No. 2073/2005 (as amended)\textsuperscript{5,6} “ready-to-eat food means food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level micro-organisms of concern”\textsuperscript{5}.

A definition of ‘retail’ is provided in Regulation (EC) 178/2002\textsuperscript{7} and means “the handling and/or processing of food and its storage at the point of sale or delivery to the final consumer, and includes distribution terminals, catering operations, factory canteens, institutional catering, restaurants and other similar food service operations, shops, supermarket distribution centres and wholesale outlets”. 

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Local Authorities and Port Health Authorities are responsible for food safety checks on imported foods at points of entry (e.g. Border Inspection Posts). The revised guidelines also apply to ready-to-eat imported food (as defined above), including both those from within the European Union (EU) as well as from countries outside of the EU.

1.2 Purpose and intended use of the guidelines
The purpose of establishing microbiological criteria, such as these guidelines, is to contribute to the provision of safe food products. These guidelines are for use by Food Examiners, food microbiologists and enforcement officers in identifying situations requiring investigation for public health or food safety reasons. The mere existence of criteria however cannot protect consumer health *per se*; of greater importance is the use of Good Hygienic Practice (GHP) and Hazard Analysis and Critical Control Point (HACCP) systems incorporating risk assessment by the food industry to ensure that microorganisms are eliminated or minimized to an extent that they cannot cause harm to human health\(^5\), and official controls to audit compliance by food business operators (FBOs)\(^9\). These guidelines do not take precedence over microbiological criteria within European or national legislation (see following section) but serve to complement legally enforceable standards and provide an indication of the microbiological safety for foods where standards currently do not exist.

1.3 Commission Regulation on microbiological criteria for foodstuffs
European or national regulations are a legal requirement and compliance is mandatory. A standard is a microbiological criterion contained in law, such as a regulation. As well as being an offence, products that do not comply with the standards are rejected as unfit for intended use. These guidelines have no legal basis but provide public health microbiological criteria based on evidence and expert opinion. Investigative action is required to identify and rectify the cause for those foodstuffs not compliant with public health or food safety microbiological criteria.

Microbiological criteria in the European Union has been harmonised in Community legislation by the European Commission Regulation on microbiological criteria for foodstuffs ([EC] No. 2073/2005 [as amended]) which came into force in January 2006\(^5,6\). It relates to the Regulation on the Hygiene of Foodstuffs ([EC] No. 852/2004) that also applies from January 2006\(^8\), and to the General Food Law Regulation ([EC] No.
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178/2002) that came into force in February 2002, although certain key provisions applied only from January 2005\(^7\). In addition, the Regulation laying down specific rules for food of animal origin ([EC] No. 853/2004\(^10\)) contains criteria for marine biotoxins, for live bivalve molluscs, and raw milk. Interpretative documents relating to the Regulation on microbiological criteria for foodstuffs have been produced by the FSA\(^11\) and the Chilled Foods Association (CFA) / British Retail Consortium (BRC)\(^12\). A definition of standard terms has also been published by the CFA\(^13\). These Regulations apply to all FBOs involved in the production and handling of food.

Two types of microbiological criteria are set out in Regulation (EC) No. 2073/2005 (as amended):

- Food safety criteria defining the acceptability of a product or a batch. They are applicable to foodstuffs placed on the market and throughout the shelf-life of the food.
- Process hygiene criteria defining the acceptability of the process. These include criteria for pathogens and indicator organisms, and apply only during the manufacturing process for the following foods: meat and meat products; milk and dairy products; egg and egg products; fish, shellfish and fishery products; vegetables and fruits and their products.

These guidelines should be used in conjunction with Regulations (EC) No. 2073/2005 (as amended; microbiological criteria for foodstuffs) and (EC) No. 178/2002 (General Food Law).

1.4 Risk based approach for use of microbiological criteria

Microbiological criteria should be targeted towards consumer health protection and help to quantify risks by determining hazards, critical limits and target levels for the assessment of the HACCP process. The use of microbiological criteria as risk management tools should only be applied where they can be shown to be effective\(^14\).

When assessing microbiological hazards associated with a specific food, only relevant agents (foodborne pathogens and toxins) should be targeted. The criteria for pathogenic micro-organisms and toxins included in these revised guidelines are therefore relevant only to ready-to-eat food (as defined above in section 1.1) samples at retail, wholesale,
in the food service sector, and as imported foods. Guideline microbiological criteria are also included here for bacteria that indicate possible poor hygiene and/or substandard practices.

Additionally, when using the microbiological criteria within these guidelines, the food type concerned (including intrinsic properties such as pH and water activity, and extrinsic properties such as temperature, packaging, and gas composition), the key processing factors, storage temperature, and shelf-life, should all be considered as well as the sampling framework and selection of microbiological tests.

1.5 Revision of the guidelines
These guidelines have been revised based upon the experience gained from the previous guidelines\textsuperscript{2-4}, microbiological and epidemiological evidence, analysis of data from the national LACORS/HPA microbiological food studies (including the UK contribution to the European Community Coordinated Food Control Programme) and other published surveys on the microbiological safety of foods, both in the UK and elsewhere.

1.6 Microbiological methodology and the practical use of these guidelines
Laboratory methods that allow rapid and accurate detection, identification and quantification of microbiological hazards enhance the ability to efficiently monitor and investigate contamination throughout the food chain. Methods are defined for Official Control sampling\textsuperscript{5,6,8}. However for public health investigations, for reasons of increased speed or sensitivity, different methods (as well as sample sizes) may be utilized.

Because of the change in emphasis in these guidelines (as compared to their earlier versions) additional essential information on the pathogenic agents, together with interpretation, public health action and evidenced based comment is now included.

1.7 Environmental samples
These guidelines do not include microbiological criteria for and interpretation of microbiological results from environmental samples. These important issues will be the subject of additional HPA guidance. However, taking appropriate and targeted environmental samples is recommended in these guidelines as part of the action
required for follow-up of microbiological results that are either unsatisfactory or potentially injurious to health.

As a guide environmental sampling is useful in the following situations:

- In an outbreak/incident investigation, environmental samples should be taken as soon as possible as part of the primary sampling exercise. Detection of pathogens in environmental samples is important because it may provide the only evidence to link a particular premise to an outbreak of infection;

- Environmental samples taken for hygiene indicators may be valuable during an investigation into poor microbiological results or during an inspection of a premises especially where there are concerns about the potential for cross contamination;

- Environmental sampling is also valuable as part of the follow-up to assess the effectiveness of deep cleaning of premises which have been shown to be contaminated with pathogens.
Section 2 Pathogens

2.0 Scope and application

As covered in section 1.1 these revised guidelines deal with ready-to-eat foods sampled from, for example retail, wholesale and food service sectors (i.e. ‘retail’ samples as defined by Regulation (EC) No. 178/2002). For some ready-to-eat foods that are taken as Official Control samples (sampled from production and/or on the market), please refer to the appropriate food safety or process hygiene criteria in Regulation (EC) No. 2073/2005 (as amended) for microbiological criteria and sampling plans.

There is evidence that investigation for the presence of pathogens in ready-to-eat food products contributes to food safety, however, the pathogens listed in the Tables 2.0 and 2.1 and Appendix are not equally applicable to all food groups. Interpretation of results should also be based on knowledge of the food product and the production process. The significance of the pathogenic microorganisms in ready-to-eat foods is discussed in the following sections and tables.

The Appendix provides further details on some of these pathogens including the most common foods associated with disease and the settings or locations most frequently associated with outbreaks of disease. The Appendix also identifies the most common routes of transmission, known risk host factors for more severe infection, the symptoms and possible consequences of infection, and their frequency as a cause of human illness in the UK.

2.1 Detection of pathogenic micro-organisms in ready-to-eat food

Detection of foodborne pathogenic bacteria in ready-to-eat food represents an unacceptable risk to health regardless of the number of bacteria present.

The pathogens identified in Table 2.0 should not be found in ready-to-eat food that has been adequately cooked and hygienically prepared. Table 2.0 details the likely cause of contamination along with the recommended actions if the pathogen is detected in ready-to-eat food.
2.2 Enumeration of pathogenic micro-organisms in ready-to-eat food

With the exception of the aerobic and anaerobic bacterial spores, small numbers of which are common in many foods, the pathogenic bacteria listed in Table 2.1 should not be present in ready-to-eat foods. Detection at any level is of concern and should always be investigated with an urgency of response being proportional to the level of contamination as shown in Table 2.1.

Although low numbers of the pathogens listed in Table 2.1 probably represent a low risk, their presence can suggest fault in the production and/or subsequent handling which, if not controlled, could lead to an unacceptable increase in risk. In addition pathogenic bacteria are often unevenly distributed in foods and the levels of contamination found, and therefore the subsequent interpretation placed on them, may vary between subsamples. Action may be justified when detecting low numbers of these organisms in ready-to-eat foods associated with food poisoning outbreaks or consumed by more vulnerable groups since these people may be both more susceptible and at greater risk of developing more serious disease.

2.3 Specialist and reference tests

Specialist and reference tests are available for many of these pathogens, the results of which will provide considerable added value to those from initial tests such as the potential of an isolate to cause disease and its likely severity. Specialist or reference tests are performed for:

- Verification of the results from the primary laboratory;
- Detection of the ability to produce a toxin or presence of a toxin gene;
- Comparative (fingerprinting or typing) analyses for strain characterisation;
- Typing of isolates to establish likely relationships between cultures from samples collected at different times or from different places, as well as for the identification of rare variants.

Specialist tests are also available where there is limited demand for primary testing such as those for uncommon pathogens, for example *Clostridium botulinum* neurotoxin, staphylococcal enterotoxin, and histamine, are not performed as part of the routine service from front-line laboratories, but usually as national or international specialties.
### Table 2.0 Guidance on the interpretation of results for detection of pathogens (the hazard) in ready-to-eat foods

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Result/25g</th>
<th>Risk Category</th>
<th>Interpretation</th>
<th>Cause</th>
<th>Action</th>
<th>Laboratory specialist and reference tests*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter spp. (thermotolerant)</td>
<td>Detected</td>
<td>High</td>
<td>UNSATISFACTORY and Potentially injurious to health and/ or unfit for human consumption*</td>
<td>Inadequate processing</td>
<td>Immediate investigation of: the food origin, production process and environment; re-sample food and environmental monitoring. Consider product recall.</td>
<td>Confirmation of identity, molecular typing.</td>
</tr>
<tr>
<td></td>
<td>Not detected</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cronobacter spp. (Enterobacter sakasakii)</td>
<td>Detected</td>
<td>High</td>
<td>UNSATISFACTORY and Potentially injurious to health and/ or unfit for human consumption*</td>
<td>Inadequate processing</td>
<td>Immediate investigation of: the food origin, production process and environment; re-sample food and environmental monitoring. Consider product recall.</td>
<td>Confirmation of identity, molecular typing.</td>
</tr>
<tr>
<td></td>
<td>Not detected</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli O157 (and other (verocytotoxin-producing E. coli (VTEC))</td>
<td>Detected</td>
<td>High</td>
<td>UNSATISFACTORY and Potentially injurious to health and/ or unfit for human consumption*</td>
<td>Inadequate processing</td>
<td>Immediate investigation of: the food origin, production process and environment; re-sample food and environmental monitoring. Consider product recall.</td>
<td>Confirmation of identity serotyping, phage typing verocytotoxin typing, molecular typing.</td>
</tr>
<tr>
<td></td>
<td>Not detected</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>Detected</td>
<td>High</td>
<td>UNSATISFACTORY and Potentially injurious to health and/ or unfit for human consumption*</td>
<td>Inadequate processing</td>
<td>Immediate investigation of: the food origin, production process and environment; re-sample food and environmental monitoring. Consider product recall.</td>
<td>Confirmation of identity serotyping, phage typing, anti-microbial resistance patterns, molecular typing.</td>
</tr>
<tr>
<td></td>
<td>Not detected</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>Detected</td>
<td>High</td>
<td>UNSATISFACTORY and Potentially injurious to health and/ or unfit for human consumption*</td>
<td>Cross contamination by food handler or faecal contamination</td>
<td>Immediate investigation of hygiene, cleaning and food handlers in outbreaks. Consider product recall.</td>
<td>Confirmation of identity, serotyping, molecular typing.</td>
</tr>
<tr>
<td></td>
<td>Not detected</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>Detected</td>
<td>High</td>
<td>UNSATISFACTORY and Potentially injurious to health and/ or unfit for human consumption*</td>
<td>Cross contamination by food handler Contaminated irrigation water</td>
<td>Immediate investigation of: the food origin, production process and environment; re-sample food and environmental monitoring. Consider product recall.</td>
<td>Confirmation of identity, serotyping (O1, O139) molecular typing.</td>
</tr>
<tr>
<td></td>
<td>Not detected</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a. It is common practice for 25g of food to be tested with the assumption that absence in 25g is SATISFACTORY. Testing of more or less food may however be indicated during outbreak investigations or when sampling based on Regulation (EC) No. 2073/2005 (as amended). Some ready-to-eat foods are taken as Official Control samples, please refer to the food safety or process hygiene criteria in Regulation (EC) No. 2073/2005 (as amended) for microbiological criteria and sampling plans.


c. All isolates should be sent to the reference laboratory for confirmation except for Campylobacter spp. where only those associated with outbreak investigations should be referred
Table 2.1  Guidance on the interpretation of results for enumeration of pathogens (the hazard) in ready-to-eat foods

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Result (cfu/g)</th>
<th>Risk category</th>
<th>Interpretation</th>
<th>Cause</th>
<th>Action</th>
<th>Laboratory specialist and reference tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacillus cereus</strong></td>
<td>&gt;10⁷</td>
<td>High</td>
<td>UNSATISFACTORY: Potentially injurious to health and/or unfit for human consumption*</td>
<td>Strong evidence for poor processing, poor quality raw materials, or poor temperature control</td>
<td>Immediately review temperature and time controls particularly for the storage of cooked foods. Re-sample food, raw food components and the food preparation environment. Consider product withdrawal and recall.</td>
<td>Confirmation of identity, typing</td>
</tr>
<tr>
<td></td>
<td>10³-10⁷</td>
<td>Moderate</td>
<td>UNSATISFACTORY</td>
<td>Likely evidence for poor processing, poor quality raw materials, or poor temperature control</td>
<td>Risk will increase proportional to the levels detected. Food may not become hazardous provided appropriate levels of control are applied. Review temperature and time controls particularly of cooking foods. Consider re-sampling food, raw food components and the food preparation environment.</td>
<td>Reported as presumptive B. cereus unless associated with an outbreak investigation</td>
</tr>
<tr>
<td></td>
<td>&lt;10³</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td>N/A</td>
<td>Reported, if present, as presumptive B. cereus</td>
</tr>
<tr>
<td><strong>Bacillus spp.</strong></td>
<td>&gt;10⁷</td>
<td>High</td>
<td>UNSATISFACTORY: Potentially injurious to health and/or unfit for human consumption*</td>
<td>Evidence for poor processing, poor quality raw materials, or poor temperature control</td>
<td>Immediately review temperature and time controls particularly for the storage of cooked foods. Re-sample food, raw food components and the food preparation environment. Consider product withdrawal and recall.</td>
<td>Confirmation of identity, typing</td>
</tr>
<tr>
<td>(other pathogenic Bacillus)</td>
<td>10³-10⁷</td>
<td>Moderate</td>
<td>UNSATISFACTORY</td>
<td>Likely evidence for poor processing, poor quality raw materials, or poor temperature control</td>
<td>Risk will increase proportional to the levels detected. Food may not become hazardous provided appropriate levels of control are applied. Review temperature and time controls particularly of cooking foods. Consider re-sampling food, raw food components and the food preparation environment.</td>
<td>Reported as presumptive Bacillus spp. unless associated with an outbreak investigation</td>
</tr>
<tr>
<td></td>
<td>&lt;10³</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td>N/A</td>
<td>Reported, if present, as presumptive Bacillus spp.</td>
</tr>
<tr>
<td><strong>Clostridium perfringens</strong></td>
<td>&gt;10⁷</td>
<td>High</td>
<td>UNSATISFACTORY: Potentially injurious to health and/or unfit for human consumption*</td>
<td>Strong evidence for poor processing, particularly during cooling period after cooking</td>
<td>Immediately review temperature and time controls. Re-sample food and the food preparation environment. Consider product withdrawal and recall.</td>
<td>Confirmation of identity, typing, pathogenicity (toxin gene detection)</td>
</tr>
<tr>
<td></td>
<td>10⁵-10⁷</td>
<td>Moderate</td>
<td>UNSATISFACTORY</td>
<td>Likely evidence for poor processing particularly cooling</td>
<td>Risk will increase proportional to the levels detected and the likelihood of subsequent growth in the absence of appropriate levels of control. Review temperature and time controls particularly cooling and storage practices in place to prevent growth. Consider re-sampling food and the food preparation environment.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;10⁵</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td>N/A</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Level</th>
<th>Category</th>
<th>Risk</th>
<th>Immediate Action</th>
<th>Long-term Action</th>
<th>Confirmation of Identity, Typing, Pathogenicity, Enterotoxin Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Listeria monocytogenes</strong></td>
<td>$&gt;10^3$</td>
<td>High</td>
<td>UNSATISFACTORY: Potentially injurious to health and/or unfit for human consumption&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Strong evidence for poor processing, temperature control, or over extension of shelf-life</td>
<td>Immediate investigation of: the food origin, production process and environment; re-sample food and environmental monitoring. Consider product withdrawal and recall.</td>
<td>Confirmation of identity, serotyping, molecular typing. For foods in high risk category, all isolates should be sent for reference testing.</td>
</tr>
<tr>
<td></td>
<td>$10^1 - 10^3$</td>
<td>Moderate</td>
<td>UNSATISFACTORY</td>
<td>Likely evidence for poor processing and/or poor quality raw materials</td>
<td>Risk will increase proportional to the levels detected and the likelihood of subsequent growth under normal storage conditions. Review quality of raw materials, food preparation environment (including cleaning), cooking, temperature and shelf life controls. Consider re-sampling food and environmental monitoring.</td>
<td>Where moderate risk is identified, consideration should be given for referral of isolates, particularly where associated with persistent problems or as part of outbreak investigations.</td>
</tr>
<tr>
<td></td>
<td>$&lt;10^3$</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Staphylococcus aureus and other coagulase-positive staphylococci</strong></td>
<td>$&gt;10^4$</td>
<td>High</td>
<td>UNSATISFACTORY: Potentially injurious to health and/or unfit for human consumption&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Strong evidence for poor handling and temperature control.</td>
<td>Immediately review food handling as well as temperature and time controls. Re-sample food, food preparation environment and food handlers. Consider product withdrawal and recall.</td>
<td>Confirmation of identity, typing, pathogenicity (toxin gene detection), enterotoxin detection in food and food remnants from cases of suspected food poisoning.</td>
</tr>
<tr>
<td></td>
<td>$10^1 - 10^4$</td>
<td>Moderate</td>
<td>UNSATISFACTORY</td>
<td>Likely evidence for poor handling, process and temperature control.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$&lt;10^4$</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vibrio parahaemolyticus</strong></td>
<td>$&gt;10^3$</td>
<td>High</td>
<td>UNSATISFACTORY: Potentially injurious to health and/or unfit for human consumption&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Strong evidence for poor processing.</td>
<td>Immediate investigation of the food origin, review cooking and subsequent temperature and time controls. Re-sample processed (cooked) food, raw food components (particularly marine products) and the food preparation environment. Consider product withdrawal and recall.</td>
<td>Confirmation of identity, typing.</td>
</tr>
<tr>
<td></td>
<td>$10^1 - 10^3$</td>
<td>Moderate</td>
<td>UNSATISFACTORY</td>
<td>Likely evidence for poor processing or cross-contamination.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$&lt;10^3$</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Confirmation of identity, typing, pathogenicity (toxin gene detection), enterotoxin detection in food and food remnants from cases of suspected food poisoning.
a. Some ready-to-eat foods are taken as Official Control samples, please refer to the food safety or process hygiene criteria in Regulation (EC) No. 2073/2005 (as amended) for microbiological criteria and sampling plans.
c. Detected in 25g by enrichment for long shelf life foods capable of supporting the growth of the bacterium such as soft ripened cheese, sliced meats, smoked fish and pate.
d. Not detected in 25g by enrichment for long shelf life foods capable of supporting the growth of the bacterium.
2.4 Additional guidance on use of microbiological criteria for detection of pathogenic micro-organisms

The additional guidance below should be read in conjunction with Table 2.0 and Appendix I.

2.4.1 *Campylobacter* spp. (thermotolerant)
Disease is caused by the ingestion of viable thermotolerant *Campylobacter* spp. The most common species of *Campylobacter* isolated from cases of foodborne disease are *C. jejuni* and *C. coli* but illness has also been associated with other thermotolerant species. *Campylobacter* spp. are unable to grow outside the mammalian gut (including in food). Adequate cooking of food will kill the bacterium and a reduction in numbers may occur following freezing.

This is the most common cause of bacterial gastrointestinal disease (GID) in the UK. Most cases of infection are sporadic and the outbreaks of foodborne infection that do occur are difficult to identify. The food vehicle for the majority of infections remains unidentified.

2.4.2 *Cronobacter* (*Enterobacter sakazakii*)
Illness is caused by the ingestion of viable *Cronobacter sakazakii* which, together with other *Cronobacter* species, has recently been re-classified from *Enterobacter sakazakii*\(^{15}\). Investigations of several outbreaks of *C. [E.] sakazakii* infection occurring in neonatal intensive care units worldwide have shown outbreaks to be associated with milk based powdered infant formulas (PIF)\(^{16}\). PIF is not a sterile product, *Cronobacter* spp. may be found in the process environment and may be present in PIF as a result of post process contamination. Poor practices in the preparation and storage of prepared formula feeds have been implicated as a cause of infection, however making up formula using water that is at least 70°C will destroy the bacterium if present.

2.4.3 *Escherichia coli* O157 and other verocytotoxin-producing *E. coli* (VTEC)
The most important *Escherichia coli* from a food safety perspective are verocytotoxin-producing *E. coli* (VTEC) and infection is caused by ingestion of live bacteria. This group of bacteria produce powerful toxins (verocytotoxins 1 (VT1) and 2 (VT2)). Despite the relatively low number of cases of VTEC infection compared with that of *Salmonella*
and Campylobacter, the potentially fatal consequence of this disease particularly in the young and old give it high public health significance. It is estimated that VTEC O157 infection is the cause of approximately 70% of the cases of renal failure in young children. The consumption of very low numbers of VTEC O157 (as few as 10) in food is sufficient to cause infection, however adequate cooking will kill the bacterium.

Most infections in the UK are due to a single serotype of VTEC, i.e. O157, but other serotypes have been associated with sporadic cases of illness or outbreaks of foodborne disease. In continental Europe and Australia a broader range of VTEC serotypes are reported. Other serotypes of VTEC that have been associated with disease in humans include O26, O103, O111 and O145.

2.4.4 Salmonella spp.
Salmonella infection is caused by ingestion of live bacteria. Infection occurs in all age groups, however host factors may increase the susceptibility to infection, for example those using antacid treatment to reduce the acidity of the stomach are more vulnerable to infection. In the UK, an estimated 35 foodborne outbreaks of infection occur per year. Adequate cooking will kill the bacterium, however low numbers of Salmonella in foods are known to have caused infection.

Around 2500 serotypes of Salmonella have been described. These serotypes can be further characterised using specialist methods to identify strains. Typing Salmonella species in this way is fundamental for national and international surveillance and outbreak detection.

2.4.5 Shigella spp.
Shigellosis is caused by ingestion of live bacteria and most cases in the UK are due to Shigella sonnei, however cases due to Sh. flexneri, Sh. boydei and Sh. dysenteriae also occur. In contrast to the most common foodborne pathogenic agents, shigellosis is exclusively a human disease. Illness can result following the ingestion of very low numbers of bacteria (as low as 10 cells depending on host susceptibility) and it is therefore easily spread from person to person particularly amongst young children. Infection can occur in all ages and there is an association between infection and travel to areas where hygiene is poor.
The majority of cases in the UK are acquired as a result of person to person spread and occasionally by eating food contaminated by infected food handlers, or through the consumption of vegetable or fruit crops irrigated with untreated water or contaminated by infected crop workers. Adequate cooking will kill the bacterium.

### 2.4.6 *Vibrio cholerae*

Cholera is caused by the ingestion of live *Vibrio cholerae*. In the UK all cases are associated with foreign travel particularly to the Indian sub-continent. Two serogroups of *V. cholerae*, O1 and O139, have been identified as a cause of outbreaks of infection. Cholera is an extremely virulent disease that affects both children and adults. It is associated with a rapid onset of severe diarrhoea. Case fatality rates of 1-10% have been reported and this is dependent largely on access to healthcare and treatment by proper rehydration.

Foodborne transmission occurs through consumption of crops cultivated in untreated water, through washing or handling foods which receive no further processing or by the consumption of raw/undercooked seafood. Food commercially imported from countries where *V. cholerae* is endemic are rarely implicated in outbreaks of cholera in importing countries. Food produced under good manufacturing practices pose only a negligible risk for cholera transmission and the bacterium is killed by adequate cooking.

### 2.5 Additional guidance on use of microbiological criteria for enumeration of pathogenic micro-organisms

The additional guidance below should be read in conjunction with Table 2.1 and Appendix I.

#### 2.5.1 *Bacillus cereus*

Large numbers of *B. cereus* are needed to cause illness either by releasing toxin into the food prior to consumption (emetic syndrome) or by producing a different toxin in the gut after eating the food (diarrhoeal syndrome). The emetic syndrome is particularly associated with farinaceous products such as rice and pasta dishes. A wider range of foods have been implicated with the diarrhoeal syndrome including meat products, soups, vegetables, puddings and sauces.
B. cereus is a diverse group of bacteria which are widespread in the environment, therefore all foods and food ingredients are likely to be contaminated by the spores of this bacterium. The spores may survive the cooking process, hence people are frequently exposed to low numbers of B. cereus through food without becoming ill. Minimum growth temperatures for B. cereus vary between 4°C and 12°C with an upper limit of around 50°C although some psychrotrophic strains occur. Not all strains produce toxins that cause either the emetic or diarrhoeal disease. The emetic and diarrhoeal toxins are distinct; the emetic toxin that is pre-formed in food is both acid and heat stable. Hence foods can be toxic in the absence of viable B. cereus.

2.5.2 Bacillus spp. (other pathogenic Bacillus)
Illness is caused by the B. subtilis group (including B. subtilis, B. licheniformis, B. pumilis and B. amyloliquifaciens) and occurs less frequently than B. cereus gastroenteritis. Symptoms are similar to those from B. cereus and include acute-onset vomiting often followed by diarrhoea, as well as diarrhoea accompanied infrequently by vomiting. Illness is strain and possibly species dependent. Illness follows the consumption of a wide variety of poorly stored cooked foods containing large numbers of Bacillus cells (10⁵ to 10⁹ cfu/g or more) and includes food prepared from poultry, meat, vegetables, and farinaceous products such as rice and bread. The temperature range for growth is similar to B. cereus (see above). The exact mechanisms and toxins produced by this group are less well understood than for B. cereus but some may be associated with preformed toxin, and some with viable organisms. Not all of the B. subtilis group have the potential to cause disease, indeed some natural fermentations rely on this group of bacteria resulting in safe products with very high levels of these bacteria.

Spices such as pepper often carry a significantly high load of Bacillus species, usually in the spore form. Although these are not normally regarded as ready-to-eat foods they may be added to a ready-to-eat food as a garnish or seasoning, albeit as a very small proportion of the finished product. However, depending on the nature of the food to which they are added, outgrowth is possible and may then pose a health risk. Levels in spices exceeding 10⁶ per gram are therefore regarded as unsatisfactory. If high levels of Bacillus spp. are found in ready-to-eat foods such as ready meals it is worth investigating whether any spices such as pepper have been added since the main cooking process, for example to the mashed potato topping.
HPA Draft Guidelines: Consultation from 01/12/08 to 20/02/09

2.5.3 *Clostridium perfringens*
Illness is caused by the ingestion of large numbers of vegetative bacteria, the bacterium sporulates in the lower small intestine, produces enterotoxin which causes diarrhoea. This enterotoxin is not produced in foods. Spores are common in the environment and may survive the cooking process such that low level contamination of the final product may occasionally occur. Control is achieved by preventing spore germination and growth in food and rapid cooling, adequate cold storage and adequate reheating of food are of paramount importance. *C. perfringens* will grow between 15°C and 52°C with virtually no growth below 12°C. Not all *C. perfringens* produce enterotoxin and these non-toxigenic isolates (irrespective of the numbers of bacteria present) will not produce disease. However the presence of high numbers of non-toxigenic *C. perfringens* in a ready-to-food is unsatisfactory and indicates poor processing, particularly during cooling.

2.5.4 *Listeria monocytogenes*
Illness is caused by the ingestion of live bacteria. *Listeria monocytogenes* is relatively common in the food environment and in raw foods. Growth of this bacterium following both post process contamination of cooked or processed foods or in raw foods probably represent the greatest risk for disease transmission. *L. monocytogenes* can grow between <0°C to 45°C, albeit slowly at refrigeration temperatures. The bacterium is killed by adequate cooking. Unrefrigerated foods and those chilled for extended periods are at increased risk of allowing significant growth, particularly if the chiller temperature is suboptimal. Vulnerable groups (pregnant women, the immunosuppressed, and the elderly) are at particular risk of infection, hence consumption of lower levels may be of greater risk when eaten by these groups. All *L. monocytogenes* should be considered as potentially pathogenic.

In refrigerated long shelf life foods such as soft ripened cheese, pâté, smoked fish, and cooked sliced meat, the presence of *L. monocytogenes* at any level may be of significance, due to the potential for growth during storage. For these products it is therefore recommended that an enrichment method be used, in addition to enumeration, to ensure that there is an absence of the bacterium in 25g portions of these foods.
2.5.5  *Staphylococcus aureus* and other coagulase-positive staphylococci

Illness due to *Staphylococcus aureus* is caused by enterotoxins which are preformed in food. Not all *S. aureus* contain enterotoxin genes and therefore have the potential to cause food poisoning. Although most cases of infection are due to *S. aureus*, other coagulase- positive *Staphylococcus* species (e.g. *S. intermedius*) can also produce enterotoxins and cause disease.

Adequate cooking will kill the bacteria, however, some protection is afforded in dry, high-fat and high-salt foods. The toxins are heat stable and can survive some normal cooking processes including boiling, hence toxin can be present in the absence of viable organisms. Most coagulase-positive staphylococci grow between 7°C and 48°C with no growth at refrigeration temperatures. Many people carry *S. aureus* and contamination of foods after processing by food handlers can occur. Toxin production starts at 10°C and storage of foods below this should prevent its development.

In foods such as ripened cheeses, *Staphylococcus aureus* levels are highest 2–3 days after initial production and may reduce significantly during storage. It is therefore important that the producer arranges for products to be periodically sampled and enumerated for this group of bacteria during this period. If levels exceed $10^5$ cfu/g at any point there is a significant risk that *S. aureus* may produce enterotoxins that will remain in the cheese regardless of the remaining recoverable level of this organism. Cheese products sampled at retail with coagulase-positive staphylococci levels in excess of $10^3$ cfu/g should be regarded with suspicion and further investigation is warranted.

2.5.6  *Vibrio parahaemolyticus*

This organism is a marine bacterium found in coastal and estuarine waters. It is a rare cause of illness in the UK and is most frequently associated with the ingestion of live *V. parahaemolyticus* in uncooked imported seafoods or ingestion of foods cross-contaminated with seafood. Growth has been reported between 14°C and 40°C and therefore does not occur in seafoods stored at proper refrigeration temperatures, however, freezing does not destroy the organisms. Many isolates appear unable to produce the toxin responsible for causing the disease. *V. parahaemolyticus* is killed by most heat treatments.
Section 3   Hygiene Indicator Organisms

3.0   Scope and application
As covered in section 1.1 these revised guidelines deal with ready-to-eat foods sampled from retail, wholesale and food service sectors (i.e. placed on the market). For some ready-to-eat foods that are taken as Official Control samples (sampled from production and/or on the market), please refer to the appropriate food safety or process hygiene criteria in Regulation (EC) No. 2073/2005 (as amended) for microbiological criteria and sampling plans\(^5,6\).

Indicator organisms can be used to help assess the microbial safety of a food product. In general terms, the presence of organisms, such as those in Table 3.0, indicates the failure in process control(s) and/or the potential for the presence of pathogens. The rationale for using an indicator organism for determining food product safety is that an indicator organism tends to be present in higher numbers and is quick and easy to identify.
### Table 3.0 Guidance on the interpretation of results for hygiene indicator organisms in ready-to-eat foods

<table>
<thead>
<tr>
<th>Hygiene Indicator</th>
<th>Results (cfu/g)</th>
<th>Interpretation</th>
<th>Comment</th>
<th>Cause</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enterobacteriaceae</strong></td>
<td>&gt;10⁴</td>
<td>UNSATISFACTORY</td>
<td>Members of this group occur in the environment as well as the gut of man and animals. Their presence at these levels suggests an overall poor general hygiene status of a food product. These bacteria are not reliable indicators of contamination by faecal pathogens in a food.</td>
<td>Poor hygiene due to undercooking, or cross contamination from raw meat, staff or food contact surfaces as well as poor temperature and time control.</td>
<td>Review cooking and all hygiene procedures including cleaning. Re-sample food and undertake environmental monitoring of food preparation environment.</td>
</tr>
<tr>
<td></td>
<td>10² - ≤10⁴</td>
<td>BORDERLINE</td>
<td>Interpret in conjunction with test results from other microbiological parameters but detection in several foods or other areas of the food production environment raises concern.</td>
<td>Possible evidence of poor hygiene due to undercooking, or cross contamination from raw meat, staff or food contact surfaces as well as poor temperature and time control.</td>
<td>Review cooking and all hygiene procedures including cleaning. Consider re-sampling food and the food preparation environment. Action should be proportional to the levels detected.</td>
</tr>
<tr>
<td></td>
<td>&lt;10²</td>
<td>SATISFACTORY</td>
<td></td>
<td>N/A</td>
<td>None</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>&gt;10⁴</td>
<td>UNSATISFACTORY</td>
<td>Originates from the intestinal tract of man and animals indicating contamination and growth (depending on the level detected) at some stage of the process. The detection of <em>E. coli</em> is not a reliable indicator that faecal pathogens are present in the food and results should be interpreted in conjunction with test results from other microbiological parameters. Repeated or widespread detection in several foods or environmental sites highlights an increased food safety risk.</td>
<td>Poor hygiene due to undercooking, or cross contamination from raw food especially meat, staff or food contact surfaces as well as poor temperature and time control.</td>
<td>Review cooking and all hygiene procedures including cleaning. Re-sample food and undertake environmental monitoring of the food preparation environment.</td>
</tr>
<tr>
<td></td>
<td>10² - ≤10⁴</td>
<td>BORDERLINE</td>
<td>Although <em>E. coli</em> should not be detected in ready-to-eat foods, low levels may occasionally be found. Repeated or widespread detection in several foods or areas of the food production environment suggests an increased food safety risk.</td>
<td>Possible evidence of poor hygiene due to undercooking, or cross contamination from raw food especially meat, staff or food contact surfaces, as well as poor temperature and time control.</td>
<td>Review cooking and all hygiene procedures including cleaning. Consider re-sampling food and the food preparation environment. Action should be proportional to levels detected.</td>
</tr>
<tr>
<td></td>
<td>≤20</td>
<td>SATISFACTORY</td>
<td></td>
<td>N/A</td>
<td>None</td>
</tr>
</tbody>
</table>
| Listeria spp. (not L. monocytogenes) | >10^5 | UNSATISFACTORY | With very rare exceptions, L. monocytogenes is the only pathogen for humans. Detection of other Listeria species at this level signifies a risk that L. monocytogenes could multiply in the food. Listeria can grow, albeit slowly, at refrigeration temperatures, and its presence in foods with a long shelf life at this level suggest action is needed. Caution should be taken for foods likely to be consumed by vulnerable groups in whom the risk of Listeria is increased, e.g. foods served in hospitals.

10 - ≤10^3 | BORDERLINE | May become a problem especially in foods capable of supporting growth of Listeria (see above). Caution should be taken for foods intended to be fed to vulnerable groups in whom the risk of listeria is increased, e.g. foods served in hospitals.

<10^2 | SATISFACTORY | Strong evidence for poor processing, or poor temperature control including suboptimal operation of refrigerators, or over extension of shelf life.

<10 | N/A | None |

**Listeria spp. (not L. monocytogenes)**

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10^5</td>
<td>Review factory hygiene (including cleaning) together with temperature and shelf life controls. Re-sample food and the food preparation environment, particularly plant and machinery.</td>
</tr>
<tr>
<td>10 - ≤10^3</td>
<td>Review quality of raw materials, factory hygiene (including cleaning), temperature and shelf life controls. Consider re-sampling food and the food preparation environment, particularly plant and machinery. Consider sending isolates for reference tests. Action should be proportional to levels detected.</td>
</tr>
<tr>
<td>&lt;10^2</td>
<td>None</td>
</tr>
</tbody>
</table>

a. The criterion listed for Enterobacteriaceae does not apply to fresh fruit and vegetables or food that contains fresh fruit and vegetables as ingredients i.e. sandwiches, because these food types can contain high levels of Enterobacteriaceae as part of their normal micro-flora.

b. Some ready-to-eat foods are taken as Official Control samples, please refer to the food safety or process hygiene criteria in Regulation (EC) No. 2073/2005 (as amended) for microbiological criteria and sampling plans.

c. According to Regulation (EC) No. 2073/2005 (as amended) the limit for E. coli in live bivalve molluscs and live echinoderms, tunicates and gastropods placed on the market during their shelf-life (e.g. raw oysters intended to be eaten raw) is 230 MPN/100g flesh and intra-valvular liquid (food safety criterion) using ISO TS 16649-3.

d. Detected in 25g by enrichment for long shelf life foods capable of supporting the growth of the bacterium such as soft ripened cheese, sliced meats, smoked fish and pâté.

e. Not detected in 25g by enrichment for long shelf life foods capable of supporting the growth of the bacterium.
3.1 Additional guidance on use of microbiological criteria for hygiene indicator organisms

The additional guidance below should be read in conjunction with Table 3.0.

3.1.1 Enterobacteriaceae

The Enterobacteriaceae family is a large group of bacteria with similar properties that is used to assess the general hygiene status of a food product. This group includes species that originate from the intestinal tract of animals and humans as well as plants and the environment. All Enterobacteriaceae are killed by the heat processes used in food production and should be readily removed from the factory, equipment and surfaces by appropriate cleaning procedures. Their presence therefore signifies inadequate cleaning or post-processing contamination.

Some fish (particularly scombroid fish, e.g. mackerel and tuna) may contain histamine (scombrotoxin) formed by bacterial growth and spoilage (including by some Enterobacteriaceae) if they are not processed properly or stored at an adequate refrigeration temperature. Ingestion of fish with high histamine levels is toxic, and maximum permissible levels of <200 or <400 mg/kg of histamine are set by EU legislation.

3.1.2 Escherichia coli

Escherichia coli belongs to the Enterobacteriaceae family and is used as a faecal indicator to assess the hygiene status of a food product. Escherichia coli are killed by the heat processes used in food production and should be readily removed from the factory, equipment and surfaces by appropriate cleaning procedures. Although some strains are pathogenic and not all of them are detected by currently available laboratory methods, these are generally believed to be very uncommon in ready-to-eat foods. Specific tests for the detection of pathogenic strains are required when illness is suspected.

_E. coli_ is often found in cheese made from raw milk. Regulation (EC) No. 2073/2005 (as amended) has no criteria for _E. coli_ in cheese made from raw milk. A routine test for VTEC O157 is therefore recommended (please refer to Table 2.0 and section 2.4.3 for information on VTEC O157).
3.1.3 *Listeria* species

*Listeria* spp. are able to grow at normal refrigeration temperatures but are killed by temperature regimes such as 74°C for two minutes. This organism shows a greater resistance to heat than the Enterobacteriaceae. In foods that have undergone such a heat treatment the presence of *Listeria* spp. indicates undercooking or post-process contamination, hence their presence can be used as an indicator to assess the hygienic status. *Listeria* spp. are also environmental contaminants that can survive in both food processing premises and on equipment if inappropriate hygiene measures are used; these organisms are less sensitive to the cleaning procedures used in food processing environments than Gram-negative bacteria.

In certain refrigerated long shelf life foods such as soft ripened cheese, pâté, smoked fish, and cooked sliced meat, the presence of *Listeria* spp. at any level may be of significance, due to the potential for growth during storage. For these products it is therefore recommended that an enrichment method be used, in addition to enumeration, to ensure that there is an absence of *Listeria* spp. in 25g of food.
Section 4 Aerobic Colony Counts

4.0 Scope and application

The Aerobic Colony Count (ACC), also known as the Total Viable Count or Standard Plate Count, is an indicator of quality not safety. Immediate action in response to high ACCs is not usually warranted except for shelf-stable canned or bottled food products immediately after opening (Group 1, Table 4.0). The level will depend initially on the type and duration of processing that the food has received during production (see Table 4.0). Thereafter the level will depend on the way it is handled and stored. For example, immediately after a pasteurisation heat process, products will normally have an ACC of below $10^6$ cfu/g, whilst a more rigorous heat process such as grilling, roasting or baking will result in counts below $10^3$ cfu/g. For canned products that are microbiologically stable at ambient temperature, viable micro-organisms are usually absent but occasional bacterial spores may survive, depending on the severity of the heat process. Products that have received a desiccating process will be stable whilst remaining dry, but may contain relatively high numbers of bacteria that can multiply following rehydration.

Microbes are inevitably introduced during slicing, packaging, portioning and other manipulations but this can be minimised by good hygiene, both of personnel and of equipment. The type of packaging may then influence the rate of microbial growth, for example vacuum packaging will prevent the growth of obligate aerobic organisms due to the exclusion of oxygen. The temperature of refrigeration for non-ambient stable products also influences the microbial growth rate; storage below 8°C will prevent growth of most food-borne pathogens but not of spoilage organisms such as psychrotrophic pseudomonads; a lower refrigeration temperature will reduce the rate of growth further and help to extend shelf life. As the duration of storage increases the aerobic colony count also increases; this will also occur if refrigeration temperatures are poorly controlled or if the food is frequently taken in and out of refrigeration.

An ACC of less than $10^6$ cfu/g is usually associated with a mixed flora. Above this level there is usually a predominant organism, and the acceptability and organoleptic quality of the food will depend on which type of organism predominates. In meat products for example the flora frequently consists almost entirely of lactic acid bacteria (mainly lactobacilli and streptococci), a group of organisms that will grow well at refrigeration
temperatures. Spoilage will eventually occur at a level of around $10^9$ cfu/g due to the production of lactic acid. If the predominant organism or group of organisms consists of Gram-negative bacilli, spoilage is likely to be noticeable at $10^7$ – $10^8$ cfu/g; pseudomonads tend to produce taste taints, discoloration, and slime whilst other Gram-negative bacilli frequently produce slime. Yeasts may cause spoilage at slightly lower levels ($10^6$ – $10^7$ cfu/g) due to acid and gas production. If high levels of *Bacillus* spp. are found this may be due to the addition of pepper or other spices after any heat treatment; investigations of the full preparation process is needed. If ACCs are high it is therefore important to identify the predominant organism type in order to fully interpret the significance of the level. Tests by the laboratory for catalase and oxidase production and a Gram stain are usually sufficient to achieve the differentiation needed to interpret results.

For raw, ready-to-eat food commodities such as salad vegetables, ACCs are likely to be much higher, between $10^6$ and $10^8$ cfu/g. This will tend to limit their shelf life as spoilage will usually be visible. This also applies to products such as rice or pasta salads containing raw vegetables. If these products are dried (e.g. herbs), the ACC per gram appears to increase due to the weight of water being removed. Raw meat and fish, eaten untreated or cold smoked, will also have ACCs of around $10^6$ – $10^7$ cfu/g, whereas marinated products are likely to have lower counts due to the acidity of the marinade. Some food commodities such as ripened cheeses and fermented meats, fish and vegetables are produced by adding starter cultures of bacteria; the predominant organisms are therefore the starter bacteria and other bacteria are usually present only in low numbers due to the acidity produced during the fermentation.

This diversity of food products and the production methods used means that a good understanding of the product type is needed in order to fully interpret the ACC. Guidance is given in Table 4.0 but careful consideration should be given to the type of food being tested and whether it is truly ready-to-eat or an ingredient that requires a further heating process before consumption. The stage of shelf life should also be considered; if sampled at the point of production ACC are likely to categorise foods as “satisfactory”, whereas if sampled at the end of shelf life an ACC can normally be expected to approach the upper “borderline” limit. If used correctly ACCs can provide useful information about the general quality and remaining shelf life of the food in question, and
thus highlight potential problems of storage and handling since production; however they are not deemed a priority in a risk based analysis.
Table 4.0. Guidance on the interpretation of results for aerobic colony count levels in various ready-to-eat foods and components

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Examples</th>
<th>Satisfactory</th>
<th>Borderline</th>
<th>Unsatisfactory</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Ambient stable canned and bottled foods immediately after removal from container</td>
<td>Canned tuna, canned salmon, corned beef, soups, stews, desserts, fruit</td>
<td>&lt;10</td>
<td>N/A</td>
<td>See note d</td>
</tr>
<tr>
<td>2 Foods cooked immediately prior to sale or consumption</td>
<td>Takeaway food, burgers, kebabs, sausages, pizza, ready meals (cook/chill &amp; cook/freeze)</td>
<td>&lt;10³</td>
<td>10³ - &lt;10⁵</td>
<td>≥10⁵</td>
</tr>
<tr>
<td>3 Cooked foods chilled but with minimum handling prior to sale or consumption; canned pasteurised foods requiring refrigeration</td>
<td>Whole pies, sausage rolls, samosas, flans, quiches, chicken portions; canned ham, desserts, cakes with dairy cream</td>
<td>&lt;10⁴</td>
<td>10⁴ - 10⁷</td>
<td>≥10⁷</td>
</tr>
<tr>
<td>4 Powdered foods, bakery and confectionery products without dairy cream</td>
<td>Soup powders, milk powder, powdered dairy products, other foods ready to eat after reconstitution or warming, cakes without cream</td>
<td>&lt;10⁴</td>
<td>10⁴ - 10⁶</td>
<td>≥10⁶</td>
</tr>
<tr>
<td>5 Cooked foods chilled but with some handling prior to sale or consumption</td>
<td>Sliced meats, cut pies, pate, sandwiches without salad, hot smoked fish (mackerel, etc.)</td>
<td>&lt;10³</td>
<td>10³ - &lt;10⁷</td>
<td>≥10⁷ See note e</td>
</tr>
<tr>
<td>6 Long shelf life food products requiring refrigeration</td>
<td>MAP or vacuum packed products; meat, fish, fruit and vegetables</td>
<td>&lt;10⁵</td>
<td>10⁵ - 10⁸</td>
<td>≥10⁸</td>
</tr>
<tr>
<td>7 Non-fermented dairy products and dairy desserts</td>
<td>Most milk and butter, cream, ice cream, fresh cheese (cottage/cream cheese), trifle with dairy cream</td>
<td>&lt;10³</td>
<td>10³ - 10⁷</td>
<td>≥10⁷</td>
</tr>
<tr>
<td>8 Raw RTE meat and fish, cold smoked fish</td>
<td>Sushi, smoked salmon, gravlax</td>
<td>&lt;10⁸</td>
<td>10⁸ - 10⁹</td>
<td>See note f</td>
</tr>
<tr>
<td>9 Preserved food products – pickled, marinated or salted</td>
<td>Pickled or salted fish, cooked shellfish in vinegar, vegetables in vinegar or oil, herbs, spices</td>
<td>N/A</td>
<td>N/A</td>
<td>See note f</td>
</tr>
<tr>
<td>10 Dried foods</td>
<td>Fruits, berries, vine fruits, nuts, sunflower seeds, herbs, spices, dried fish</td>
<td>N/A</td>
<td>N/A</td>
<td>See note f</td>
</tr>
<tr>
<td>11 Fresh fruit and vegetables, products containing raw vegetables.</td>
<td>Whole fruit, pre-prepared fruit salads, vegetable crudités, salads, sandwiches with salad, mixed commodity salads containing raw vegetables</td>
<td>N/A</td>
<td>N/A</td>
<td>See note f</td>
</tr>
<tr>
<td>12 Fermented, cured and dried meats, fermented vegetables, ripened cheeses</td>
<td>Continental sausages/salamis, jerky, sauerkraut, olives, bean curd, cheddar, stilton, brie, fermented milk drinks and butter, yoghurt, etc.</td>
<td>N/A</td>
<td>N/A</td>
<td>See note f</td>
</tr>
</tbody>
</table>

a. Satisfactory: No action required
b. Borderline: Consider the source of the food (producer/retailer etc.) and the stage of shelf life before determining action. If other samples from the same source are also of borderline quality further investigation may be appropriate.
c. Unsatisfactory: Consider investigating reasons for high count
d. Food Group 1
   • Most products are normally sterile when sampled from the container but if they are consumed after subsequent further preparation then assess them as Group 5.
   • These products are "Unsatisfactory" if spore forming anaerobes are present but these require special tests for detection and enumeration. Spore forming aerobes are also usually absent in foods that have been cooked in their container but low levels may occur in canned fish products.
e. Food Group 5
   • Determine the predominant micro-organism. "Unsatisfactory" if the predominant organism is > $10^6$ yeasts, >$10^7$ Gram negative bacillus or Bacillus spp., or >$10^8$ lactic acid bacteria.
f. Food Groups 8-12
   • ACCs not routinely performed. For spoilage investigation, "Unsatisfactory" if the predominant organism is > $10^6$ yeasts, >$10^7$ Gram negative bacillus or Bacillus spp., or >$10^8$ lactic acid bacteria.
Section 5  How to use the Guidelines

5.0  Introduction

The primary purpose of these guidelines is to assess the microbiological safety of ready-to-eat food at any point in the retail chain, i.e. retail, wholesale, catering, and port of entry. Guidelines for pathogens also apply to food poisoning investigations in all other settings including the domestic environments.

These guidelines are for use by Food Examiners, food microbiologists and enforcement officers to assess the microbiological safety and hygiene quality of food samples collected as follows:

- During predefined sampling programmes such as the LACORS/HPA studies;
- Samples taken at or during food inspections;
- Samples taken to confirm previous adverse findings in order to determine the scale of microbiological contamination;
- Samples collected during investigations of outbreaks of disease;
- Complaints relating to microbiological spoilage.

All of the above types of samples are usually single samples and are not associated with any formal sampling plan. Any follow up studies which require testing under the regulations should be done in accordance with the requirements of the regulations. Follow up testing is best done in conjunction with advice from a Food Examiner or other appropriately qualified food microbiologist to ensure that the most appropriate testing, which may include environmental sampling, is performed.

These guidelines should not be used to interpret microbiological parameters which are part of statutory regulations. However, for public health purposes additional or supplementary test parameters not covered by the regulations may be performed on these samples. In this case these guidelines can be used to assess these additional parameters on ready-to-eat foods not covered by the regulations. Food samples taken at the producer premises as part of an inspection would normally be expected to give satisfactory results for all parameters investigated and any deviation should be investigated.
In the absence of statutory criteria or industry standards then these guidelines may be helpful to food companies producing ready-to-eat foods. In these situations it is the responsibility of the company microbiologist or equivalent to ensure that the guidelines are fit for purpose for their products.

5.1 Pathogens
Detection of pathogens in ready-to-eat foods is very uncommon. It is therefore essential that appropriate action is taken in a timely way.

- Action must always be taken when pathogens are detected in any ready-to-eat food; the action taken must be proportional to the risk to health;
- Since the presence of pathogens indicates a potential problem with the production process, follow-up of other foods collected from the same or related environments should include investigation for other pathogens that may also survive poor processing;
- Some pathogens represent a greater risk at lower levels of contamination (e.g. E. coli O157, Salmonella and Campylobacter) and must be absent from ready-to-eat foods; detection requires immediate action. The level of contamination for some other pathogens (e.g. Staphylococcus aureus, Bacillus cereus and Listeria monocytogenes), and the context of the sample origin, may influence the level of response;
- Detection of pathogens in ready-to-eat food that have not been collected as part of an outbreak investigation should lead to a review of community acquired cases to identify if an increase has occurred.

5.2 Hygiene Indicator Organisms
The presence of indicator bacteria in ready-to-eat food, although not inherently a hazard in itself, is indicative due to undercooking, cross-contamination, poor cleaning and/or temperature and time control. Indicator bacteria may also be associated with an increased likelihood of the presence of pathogens.

- There are a number of recommended actions listed in Table 3.0 that could be taken in response to an unsatisfactory result;
HPA Draft Guidelines: Consultation from 01/12/08 to 20/02/09

- Indicator bacteria will be recovered more frequently than pathogens and therefore any action taken in relation to these organisms should be proportionate;
- Several foods from the same premises with borderline levels of indicators should prompt further investigation;
- It is recommended that any proposed actions should be discussed with a Food Examiner.

5.3 Aerobic Colony Counts
Aerobic colony counts (ACCs) do not directly contribute towards a safety assessment of ready-to-eat food. However, they can be used as part of a general quality assessment including that of long shelf life foods, and as part of a programme of shelf-life testing carried out by the food producer.

- If an ACC is above the expected level a determination of the constituent organisms and their level is needed before any follow-up investigation is instigated;
- High counts may suggest quality issues and possible poor temperature control and these should be investigated.

5.4 Notification of pathogens in food samples
These guidelines recommend that detection of pathogens in ready to eat food samples, at levels considered to be potentially injurious to health, should be notified immediately to the competent authorities so that public health action can be implemented. Such information would be used for the purpose of preventing, protecting against, controlling or providing a public health response to the incidence or spread of infection or contamination. Although this is not currently mandatory this is likely to change in 2009.

The Health Protection (Notification) [and (Miscellaneous Provisions)] and Public Health (Infectious Diseases) (Amendment) (England) Regulations 2009 are currently in draft and available for consultation. These will come into force on [1st October] 2009. These Regulations will contain standing national requirements for laboratories that test food samples (whether in the public or independent sector) to notify detection of pathogens to the competent authority.
The HPA guidelines for assessing the microbiological safety of ready-to-eat food will assist laboratories in this notification requirement of pathogens recovered from food.
HPA Draft Guidelines: Consultation from 01/12/08 to 20/02/09

References


<table>
<thead>
<tr>
<th>Hazard</th>
<th>Food types most often associated with human infections</th>
<th>Common settings / locations linked with outbreaks</th>
<th>Major routes of transmission</th>
<th>Known host risk factors for severe infection</th>
<th>Symptoms, severity and sequelae</th>
<th>No. of reported human cases in UK in 2007*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em> spp. (thermotolerant)</td>
<td>Poultry, red meat, milk and dairy products made with un-pasteurised milk or post-pasteurisation contaminated milk, drinking water</td>
<td>Consumption of food prepared outside the home (e.g., Barbecues) Consumption of untreated water/milk on holiday (e.g., farm, cottage, caravan site)</td>
<td>Foodborne Cross contamination Zoonotic Waterborne</td>
<td>Age (&lt;5 yrs or &gt;60 yrs) Immune status Proton pump inhibitors</td>
<td>Diarrhoea, headache, abdominal pain; usually lasts 2-7 days Irritable bowel syndrome (IBS) most common sequelae, reactive arthritis, Guillain-Barré syndrome</td>
<td>57,815</td>
</tr>
<tr>
<td><em>Cronobacter</em> (Enterobacter sakasakii)</td>
<td>Powdered infant formula; made up / stored formula</td>
<td>Neonatal special care / Intensive Care Units</td>
<td>Foodborne Cross contamination</td>
<td>Pre-term delivery or immuno-compromised Neonates</td>
<td>CNS infections, Meningitis, necrotising enterocolitis bacteraemia, urinary tract infection (UTI), wound infections.</td>
<td>0</td>
</tr>
<tr>
<td><em>Escherichii coli</em> O157 (and other verocytotoxin-producing E. coli (VTEC))</td>
<td>Under cooked beef, milk and dairy products, salad vegetables, drinking water</td>
<td>Pre-school / nurseries Zoonotic (gettin farms) Domestic home Institutional settings Consumption of untreated water/milk on holiday (e.g., farm, cottage, caravan site)</td>
<td>Foodborne Cross contamination Environmental exposure Person-Person Zoonotic Waterborne</td>
<td>Age (&lt;5 yrs (HUS) or &gt;60 yrs (TTP))</td>
<td>Diarrhoea, vomiting, abdominal pain, haemorrhagic colitis, lasts 2 weeks in uncomplicated cases, can be fatal; Haemolytic Uraemic Syndrome (HUS), Thrombotic Thrombocytopaenic Purpura (TTP)</td>
<td>1,149</td>
</tr>
<tr>
<td><em>Salmonella</em> spp. (non Typhi/Paratyphi)*</td>
<td>Eggs, poultry, pork, beef, dairy products, seeds, herbs, salad vegetables chocolate</td>
<td>Consumption of food prepared outside the home Foreign travel</td>
<td>Foodborne Cross contamination Person-Person Zoonotic</td>
<td>Reduced immune status</td>
<td>Diarrhoea, vomiting, abdominal pain, fever; lasts several days to 3 weeks, and in severe cases death Septicaemia and inflammation of the abdominal wall, reactive arthritis</td>
<td>13,802</td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>Salad vegetables</td>
<td>Pre-schools / nurseries Institutional settings Foreign travel</td>
<td>Person-Person Foodborne (intrinsic)</td>
<td>Age (&lt;5 yrs)</td>
<td>Diarrhoea, vomiting, bacillary dysentery; last average of 4 to 7 days; HUS, Toxic Megacolon.</td>
<td>1,638</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>Seafood, drinking water</td>
<td>Foreign travel particularly to the Indian subcontinent</td>
<td>Waterborne Foodborne</td>
<td></td>
<td>Diarrhoea, vomiting, severe dehydration, leg cramps</td>
<td>47</td>
</tr>
</tbody>
</table>

*a, Data provided by Health Protection Agency, Health Protection Scotland, Communicable Disease Surveillance Centre Northern Ireland
b, S. Typhi and S. Paratyphi cause enteric fever (typhoid fever and paratyphoid) which is almost exclusively acquired abroad through the ingestion of heavily contaminated food and water
c, Cases being infants of less than one year
d, Most cases identified are person to person
e, All foreign travel associated
f, The Table is not inclusive and features, other than that described, may occur but are generally considered uncommon.
<table>
<thead>
<tr>
<th>Hazard</th>
<th>Food types most often associated with human infections</th>
<th>Common settings / locations linked with outbreaks</th>
<th>Major routes of transmission</th>
<th>Known risk host factors for severe infection/intoxication</th>
<th>Symptoms, severity and sequelae&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of reported human cases in UK in 2007&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacillus cereus</strong></td>
<td>Cooked rice (emetic syndrome) Cooked meats, poultry and vegetables, soups, spices (diarrhoeal syndrome)</td>
<td>Commercial catering</td>
<td>Foodborne</td>
<td>Unknown</td>
<td>Vomiting (emetic syndrome) Diarrhoea (diarrhoeal syndrome) Usually mild and short-lived, lasts ~ 1 day</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Bacillus spp. (other pathogenic Bacillus)</strong></td>
<td>Cooked meats, poultry and vegetables</td>
<td>Commercial catering</td>
<td>Foodborne</td>
<td>Unknown</td>
<td>Vomiting and diarrhoea Usually mild and short-lived, lasts ~ 1 day</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Clostridium perfringens</strong></td>
<td>Cooked meat, gravy and stock</td>
<td>Commercial and institutional catering</td>
<td>Foodborne, Non-foodborne infection (i.e. person-to-person and antibiotic-associated infections occur in elderly)</td>
<td>Most people are probably susceptible</td>
<td>Diarrhoea, abdominal pain; Usually mild and short-lived, lasts ~ 1 day, but diarrhoea longer and more severe in elderly Dehydration in severe cases</td>
<td>73&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Listeria monocytogenes</strong></td>
<td>Commercially prepared sliced meats, pâté, soft cheese, sandwiches, smoked fish (with extended and refrigerated shelf lives)</td>
<td>Hospitals</td>
<td>Foodborne, Cross contamination (zoonotic, person to person, environmental)</td>
<td>Age (&gt;60 yrs), Pregnancy Newborn infants Immunosuppression Antacid treatment</td>
<td>Non-invasive: diarrhoea, fever, headache, muscle pain Invasive: Fever and severe systemic infections possible (septicaemia and meningitis, miscarriage, and abortion). High case fatality rate.</td>
<td>261&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus and other coagulase-positive staphylococci</strong></td>
<td>Processed meats, poultry, fish, shellfish, and dairy products.</td>
<td>Commercial and institutional catering</td>
<td>Foodborne, Food handlers Cross contamination</td>
<td>Most people are susceptible</td>
<td>Nausea and vomiting, last 1 – 2 days, may be very acute Abdominal cramps and diarrhoea Collapse in very severe cases</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Vibrio parahaemolyticus</strong></td>
<td>Fish and shellfish</td>
<td>Various</td>
<td>Foodborne</td>
<td>Most people are probably susceptible</td>
<td>Diarrhoea</td>
<td>41&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data provided by Health Protection Agency, Health Protection Scotland, Communicable Disease Surveillance Centre Northern Ireland

<sup>b</sup> Many foodborne infections are not reported

<sup>c</sup> The Table is not inclusive and features, other than that described, may occur but are generally considered uncommon.