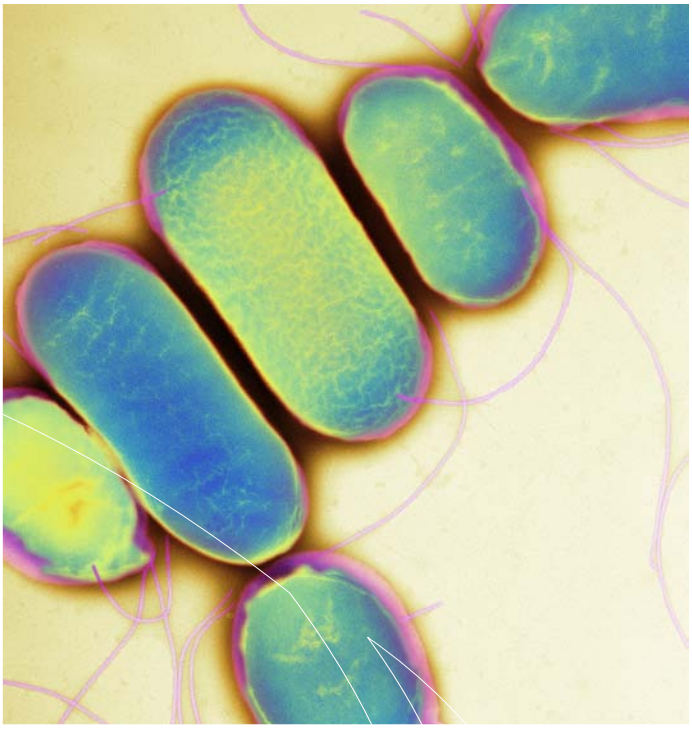
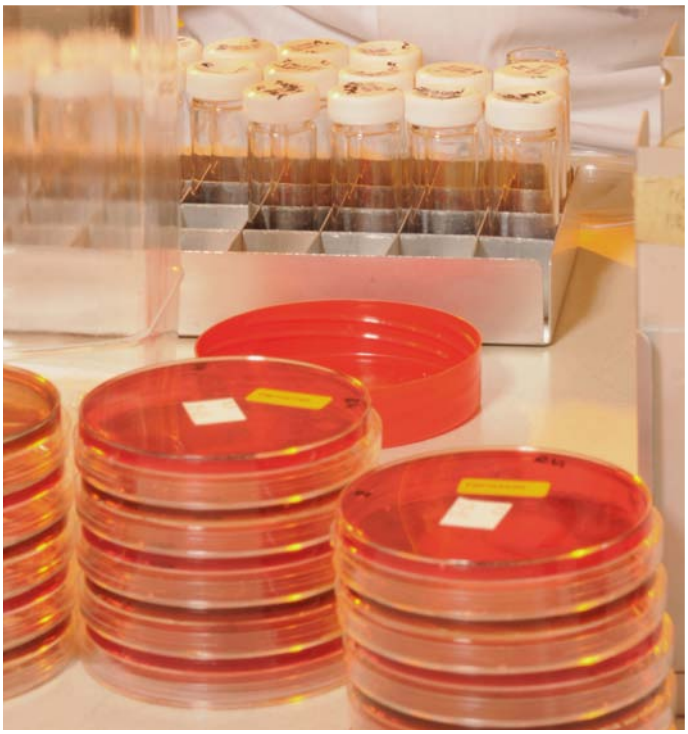
Health Protection Agency

Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market





Authorship

These guidelines for assessing the microbiological safety of ready-to-eat foods have been revised and written by a Health Protection Agency Working Group and chaired by Professor Eric Bolton.

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Citation

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Abbreviations

ACC	Aerobic colony count
BRC	British Retail Consortium
CFA	Chilled Food Association
cfu/g	Colony forming units per gram
EC	European Commission
EN	European Norm
EU	European Union
FBO	Food business operator
FSA	Food Standards Agency
g	Gram
GHP	Good hygiene practice
HACCP	Hazard analysis and critical control point
HPA	Health Protection Agency
HUS	Haemolytic uraemic syndrome
IBS	Irritable bowel syndrome
ISO	International Organization for Standardization
Kg	Kilogram
LACORS	Local Authorities Co-ordinators of Regulatory Services
MAP	Modified atmosphere packaging
Mg	Milligram
MPN	Most probable number
NPHS	National Public Health Service for Wales
TTP	Thrombotic thrombocytopaenic purpura
UHT	Ultra high temperature
UK	United Kingdom
VT	Verocytotoxin
VTEC	Verocytotoxin-producing *Escherichia coli*
Yrs	Years

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SECTION 1

Introduction

1.1 Purpose of the guidelines

In pursuit of the Health Protection Agency's (HPA) goal of preventing and reducing the incidence and consequences of infection[1], the HPA examines foods from Local and Port Health Authorities to help safeguard consumer health. These foods include samples submitted for surveillance and monitoring, for official control purposes, and those tested as part of outbreak investigations. From this work the HPA has accumulated a wealth of data both on the microbiological results and, crucially, on their interpretation. This information was captured and promulgated in three previous sets of these guidelines[2-4] for practical use by Food Examiners and Local Authority Enforcement Officers. These revised guidelines supersede those previously issued and have a different emphasis focusing on public health and consumer protection. Additional information on the bacteria that cause foodborne disease and those that act as hygiene indicators, on interpretation of test results, comments on poor practices that are likely to have contributed to adverse results and suggested appropriate public health actions, are now included.

The use of microbiological criteria as risk management tools should only be applied when they can be shown to be effective and can contribute to the provision of safe products[5-7]. Microbiological testing alone cannot guarantee the safety of food and microbiological criteria should be used to support Good Hygienic Practice (GHP) and Hazard Analysis and Critical Control Point (HACCP) systems. The food industry has a duty to ensure that micro-organisms are eliminated or minimized to the extent that they cannot cause harm to human health[8], and official controls are in place to audit compliance by food business operators (FBOs)[9].

1.2 Scope of the guidelines

Food within the scope of the revised guidelines includes ready-to-eat food sampled within the retail chain, e.g. retail, wholesale, distribution and food service sectors (as defined by Regulation (EC) No. 178/2002[10]). This includes food components, such as herbs and spices, where they are added to foods without further cooking or processing. The guidelines for pathogens also apply to food poisoning investigations in all settings including domestic environments. Criteria are also applied for bacteria that indicate possible poor hygiene and/or substandard practices. In some circumstances these guidelines may also be used to assess more fully the safety and quality of food taken from the producer's premises. Although potable water is now defined as food this matrix is not addressed in these guidelines as there is relevant legislation and guidance that covers this commodity[11-14]. For some ready-to-eat foods (sampled from production and/or on the market) statutory criteria exist and these food safety or process hygiene criteria are laid down in Regulation (EC) No. 2073/2005 (as amended) including sampling plans, analytical methods, and corrective actions[15,16].

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

These guidelines do not take precedence over microbiological criteria within European or national legislation (see section 1.4) but serve to complement legally enforceable standards and provide an indication of the microbiological safety for foods where standards currently do not exist. Investigative action is required to identify and rectify the cause for those foodstuffs not compliant with microbiological food safety criteria and/or where there is a perceived risk to public health. These guidelines should therefore not be used to interpret the results of microbiological parameters which are part of statutory regulations. To safeguard public health, however, additional tests on ready-to-eat foods not covered by the regulations may be considered appropriate. Food samples taken at producer premises as part of inspections by local enforcement officers would be expected to give satisfactory results for all parameters and any deviation should be investigated.

1.3 Intended use of the guidelines

These guidelines are for use by Food Examiners and enforcement officers in identifying situations requiring investigation for public health or food safety reasons, and are applicable to the following types of samples:

- Samples collected during predefined sampling programmes such as the Local Authorities Co-ordinators of Regulatory Services (LACORS)/HPA national microbiological food studies[17];
- 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 suspected outbreaks of disease;
- Samples submitted after complaints.

All of the types of samples listed above 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[18,19] or other appropriately qualified food microbiologist to ensure that the most appropriate testing, which may include environmental sampling, is performed.

When using the microbiological criteria within these guidelines, the food type concerned (including its 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.

1.4 Commission Regulation on microbiological criteria for foodstuffs

European or national regulations are a legal requirement and compliance is mandatory. Microbiological criteria in the EU have been harmonised in Community legislation by the European Commission (EC) Regulation on microbiological criteria for foodstuffs ([EC] No. 2073/2005 [as amended]) which came into force in January 2006[15,16]. This supports the Regulation on the Hygiene of Foodstuffs ([EC] No. 852/2004) that also applies from January 2006[8], and the General Food Law Regulation ([EC] No. 178/2002) that came into force in February 2002, although certain key provisions applied only from January 2005[10].
In addition, the Regulation laying down specific rules for food of animal origin ([EC] No. 853/2004[20]) 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 Food Standards Agency (FSA)[21] and the Chilled Food Association (CFA) / British Retail Consortium (BRC)[22]. A definition of standard terms has also been published by the CFA[23]. 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) and include criteria for both pathogens and indicator organisms:

- 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 apply only during the manufacturing process.

Failure to meet the criteria alone is not an offence but the specific corrective action must be carried out to comply fully with the Regulation. Microbiological criteria are intended to assist with validating and verifying HACCP-based food safety management systems.

SECTION 2

Pathogens

2.1 Introduction

Examination for the presence of pathogens in ready-to-eat food products contributes to food safety[24-30]. However, the pathogens listed in Tables 1 to 3 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 and care must be taken when interpreting results obtained in the absence of this information. The significance of the pathogenic micro-organisms in ready-to-eat foods is discussed in the following sections and tables.

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

Detection of the foodborne pathogenic bacteria shown in Table 1 in ready-to-eat food represents an unacceptable risk to health regardless of the number of bacteria present. The pathogens listed in Table 1 should not be found in ready-to-eat food that has been adequately prepared. Table 1 details the likely cause of contamination along with the suggested actions if the pathogen is detected in ready-to-eat food.

2.2.1 *Campylobacter* species (thermotolerant)

This is the most common cause of bacterial gastrointestinal infections in the UK. Most cases of disease are sporadic and the outbreaks of foodborne infection that do occur are difficult to identify. The route of transmission for the majority of infections remains unidentified. The consumption of low numbers of *Campylobacter* in food is sufficient to cause infection[31,32].

Disease is caused by the ingestion of viable thermotolerant *Campylobacter* species. 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* species are unable to grow in food, they are killed by heat and a reduction in numbers has also been observed following freezing of contaminated foodstuffs. Cross contamination of ready-to-eat foods in the food preparation environment is an important route of transmission[33,34].

2.2.2 *Escherichia coli* O157 and other verocytotoxin-producing *E. coli* (VTEC)

The most important *Escherichia coli* from a food safety perspective are the verocytotoxin-producing *E. coli* (VTEC). 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 the elderly give it a high public health significance. It is estimated that VTEC infection is the cause of approximately 70% of the cases of renal failure in children[35]. The consumption of very low numbers of viable VTEC in food is sufficient to cause infection[36].

Not all cases of VTEC are foodborne (Table 3) and different transmission routes can occur within the same outbreak. Most infections in the United Kingdom (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, infections from a broader range of VTEC serotypes are reported. The other serotypes of VTEC that have been associated most frequently with disease in humans include O26, O103, O111 and O145[37,38].

2.2.3 *Salmonella* species

Salmonella infection is caused by ingestion of viable bacteria. Infection occurs in all age groups, however host factors may increase the susceptibility to infection, for example treatment to reduce the acidity of the stomach, are more vulnerable to infection. The infectious dose for *Salmonella* species is usually quite large[32]. However, data from outbreaks has shown that consumption of low numbers of *Salmonella* in food together with the mode of delivery of the bacterium to the gastrointestinal tract can cause infection and this is particularly evident with high fat / low water activity foods, such as chocolate, fermented meats, cheese and snacks, in which the organism can survive for long periods of time[39-51]. Application of good hygiene and temperature and time control during food preparation is also important to prevent cross-contamination and multiplication in foods or ingredients that are able to support its growth[24,52-54].

Around 2,500 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 essential for national and international surveillance and identification of outbreaks[24,44-49,55,56]. The emergence of new *Salmonella* strains or strains that have anti-microbial drug resistance contributes to the concern regarding imported food. Anti-microbial drug resistant *Salmonella* infections are associated with an increased hospitalization rate, morbidity, and mortality[57].

2.2.4 *Shigella* species

Shigellosis is caused by ingestion of viable bacteria and most cases in the UK are due to *Shigella sonnei*. Infection due to other *Shigella* species including *Sh. flexneri, Sh.*

boydii and *Sh. dysenteriae* also occur. In contrast to the most common foodborne pathogenic agents, shigellosis is exclusively a human disease. 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. Illness can result following the ingestion of very low numbers of viable bacteria (as low as 10 cells depending on host susceptibility) and it is therefore easily spread from person to person particularly amongst young children[32]. Infection can occur in all ages and there is an association between infection and travel to areas where hygiene is poor.

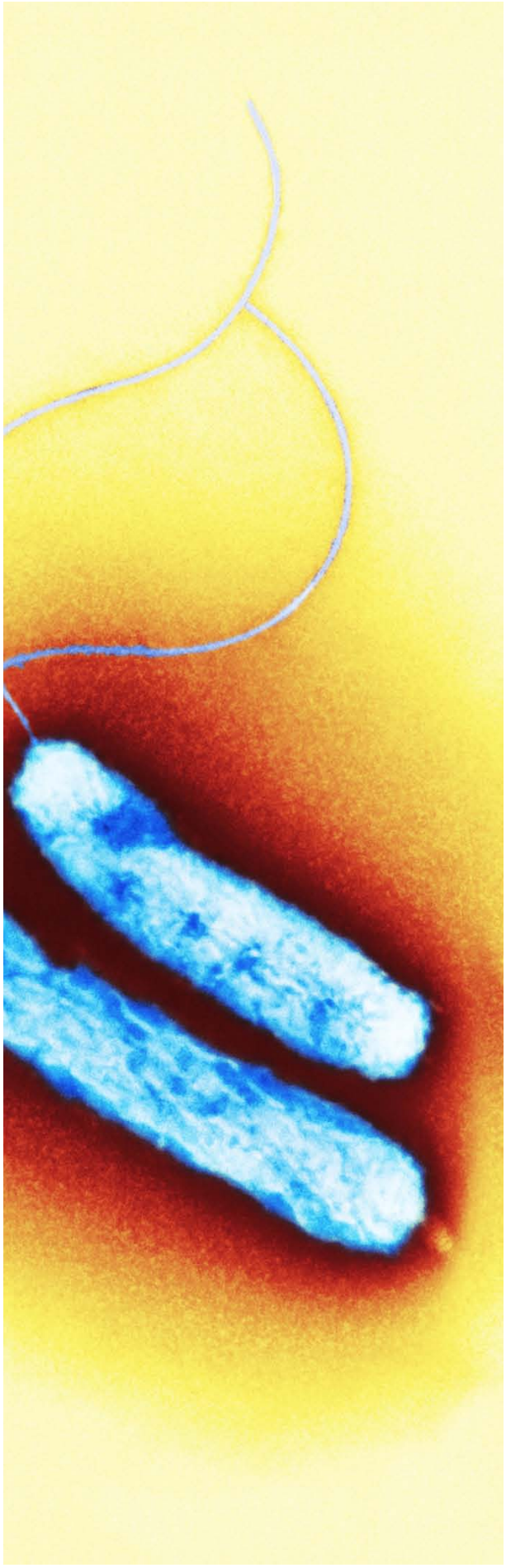
2.2.5 *Vibrio cholerae*

Cholera is caused by the ingestion of viable *Vibrio cholerae*. Two serogroups of *V. cholerae*, O1 and O139 have been identified as causing outbreaks. 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, or irrigated by, untreated water, through washing or handling foods which receive no further processing or by the consumption of raw or undercooked seafood. Foods that are commercially imported from countries where *V. cholerae* is endemic have been, albeit rarely, implicated in outbreaks of cholera and the potential for foodborne transmission from imported food remains. In the UK, all cases are associated with foreign travel particularly to the Indian sub-continent. Food produced under good manufacturing practices pose only a negligible risk for cholera transmission and the bacterium is killed by adequate cooking.

2.3 Enumeration of pathogenic micro-organisms in ready-to-eat food

Although low numbers of the pathogens listed in Table 2 probably represent a low risk, their presence can suggest fault(s) in the production and/or subsequent handling which, if not controlled, could lead to an unacceptable increase in risk. Contamination by the pathogens listed in Table 2 should always be investigated with an urgency of response which is proportional to the level of contamination and type of food as shown in Table 2. 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 sub-samples. The detection of low numbers of these organisms in ready-to-eat foods



which are thought to be associated with food poisoning outbreaks or which are consumed by more vulnerable groups warrants further investigation by the enforcement authority in consultation with the Food Examiner. Vulnerable people are generally more susceptible to these infections and are at greater risk of developing more serious disease.

2.3.1 *Bacillus cereus*

Large numbers of *Bacillus cereus* are needed to cause illness either by releasing toxin into the food prior to consumption (emetic syndrome) or by producing a different toxin or toxins 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.

Bacillus 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 is pre-formed in food and is both acid and heat stable. Hence foods can be toxic in the absence of viable *B. cereus*.

2.3.2 *Bacillus* species (other pathogenic *Bacillus*)

Illness is caused by the *Bacillus 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* (10^5 to 10^9 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 section 2.3.1). 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 which rely on production of very high levels of these bacteria result in safe products.

Spices and spice products such as pepper and curry paste 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 10^6 cfu/g are therefore regarded as unsatisfactory. If high levels of *Bacillus* spp. are found in ready-to-eat foods, the possibility that spices such as pepper have been added after the main cooking process, for example to the egg mayonnaise or mashed potato topping, should be investigated.

2.3.3 *Clostridium perfringens*

Clostridium perfringens is found in the gut and thus indicates faecal contamination although spores commonly occur in the environment. It is uncommon to detect this organism in properly handled ready-to-eat foods. Illness is caused by the ingestion of large numbers of viable vegetative bacteria, which sporulate in the lower small intestine and 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 foodborne 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.

2.3.4 *Listeria monocytogenes*

Illness is caused by the ingestion of live bacteria. *Listeria monocytogenes* occurs commonly in the environment and in raw foods, and consequently will occur in some food production environments. Growth of this bacterium following both post-process contamination of cooked or processed foods or in raw foods probably represents 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 chilled temperatures are suboptimal. Vulnerable groups (pregnant women, the immunosuppressed, the elderly, and many patients in

hospitals) are at particular risk of infection, hence consumption of low levels of *L. monocytogenes* may be of greater risk when eaten by these groups.

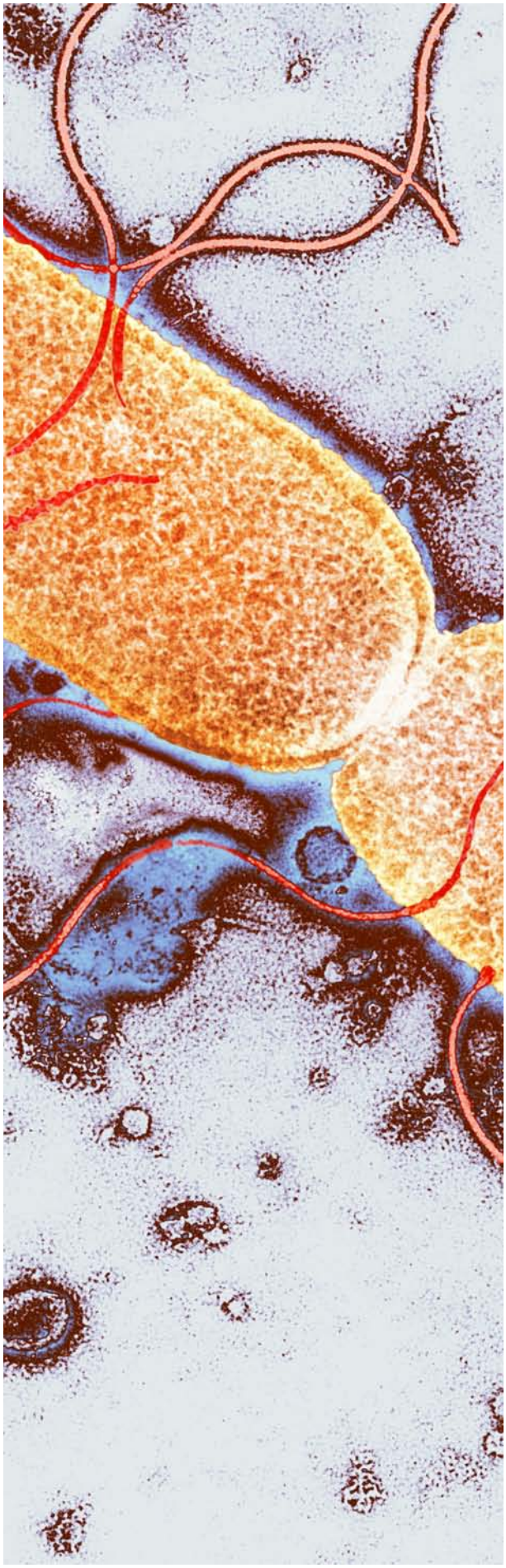
In refrigerated high risk foods such as soft ripened cheese, pâté, smoked fish, and cooked sliced meat, where there is a potential for growth during storage, and in foods likely to be served to vulnerable groups (such as those served in hospital), the presence of *L. monocytogenes* at any level may be of public health significance and should be investigated. For these high risk food products and to assess the public health significance 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 (Table 2).

Food safety criteria for *L. monocytogenes* in Regulation (EC) No. 2073/2005 (as amended)[16] are applicable to three categories of ready-to-eat foods: absence of *L. monocytogenes* in 25g is required in some foods, e.g. ready-to-eat foods intended for infants and those for special medical purposes; while for other ready-to-eat foods (including those able to support growth or not of *L. monocytogenes) L. monocytogenes* should not exceed 10^2 cfu/g within the shelf-life. However investigation on public health grounds may be justified in products with levels of less than 10^2 cfu/g, especially in high risk foods, those likely to be consumed by vulnerable groups and those which are defined in Regulation (EC) No. 2073/2005 that do not support the growth (based on a shelf life of less than 5 days) but where growth may occur under poor temperature and time control.

2.3.5 *Staphylococcus aureus* and other coagulase-positive staphylococci

Illness due to *Staphylococcus aureus* is caused by enterotoxins which are preformed in food. Only some *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 foodborne disease.

Adequate cooking will kill the bacterium, however some protection is afforded in dry, high-fat and high-salt foods. Staphylococcal enterotoxins are heat-stable and can survive some normal cooking processes including boiling, hence active 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 and fermented meat products, *S. aureus* levels are highest 2–3 days after initial production and may reduce significantly during storage. If levels exceed 10^5 cfu/g at any time during the life of a food, there is a risk of sufficient enterotoxin to cause illness that will remain in the food product regardless of subsequent recoverable levels of this organism. However 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, for example by arranging for checks of the producer's test records. If levels exceed 10^4 cfu/g, isolates should be sent to the Reference Laboratory for enterotoxin gene testing. If levels exceed 10^5 cfu/g in any product or if the food is associated with possible staphylococcal food poisoning, the food (if available) should be tested for enterotoxin and the strain for enterotoxin gene detection.

The only food safety criterion for staphylococci in Regulation (EC) No. 2073/2005 (as amended) is for an absence of staphylococcal enterotoxins in cheese, milk powder and whey powder in product placed on the market during their shelf life[16]. This Regulation has process hygiene criteria with limits of between 10 and 10^5 coagulase positive staphylococci/g in cheese, milk and whey powder during manufacture, and if values of $>10^5$ cfu/g are detected, the batch should be tested for staphylococcal enterotoxins. However, since assays for enterotoxin detection are not rapid, can be insensitive for some food matrices and do not detect all types of staphylococcal enterotoxins, public health actions should not be delayed pending results.

2.3.6 *Vibrio parahaemolyticus*

Vibrio parahaemolyticus 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 *Vibrio parahaemolyticus* in uncooked imported seafood or ingestion of foods cross-contaminated with seafood. Growth has been reported between 14°C and 40°C and therefore does not occur in seafood stored at proper refrigeration temperatures; however freezing does not destroy the organism but it is killed by most heat treatments. Many isolates appear unable to produce the toxin responsible for causing the disease.

2.4 Foodborne pathogens and risk of disease

Further details on some of these pathogens including the most common foods associated with them and the settings or locations most frequently associated with outbreaks of disease are provided in Table 3. This table also identifies the most common routes of transmission, known host risk factors for more severe infection, the symptoms and possible consequences of infection, and their frequency as a cause of human illness in the UK.

Foodborne diseases of microbiological origin can be caused by a variety of agents, which gain entry by the gastrointestinal tract. 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. Host risk factors include 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 agents that may cause illness in ready-to-eat foods is a significant risk to consumer health 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 proportionate to the level of contamination and risk to consumers. Although low numbers of pathogens, such as coagulase-positive staphylococci, *C. perfringens, B. cereus,* and *L. monocytogenes,* in ready-to-eat products probably represent a very low risk to immunocompetent people, they are more significant for the immunocompromised and vulnerable groups. Low levels may be due to natural contamination of raw materials used in those foods, but usually their presence suggests faults in the production or subsequent handling of food which 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.

2.5 Specialist and reference tests

Specialist and reference tests are available for many foodborne pathogens and their toxins, the results of which will provide considerable added value to those from initial tests and to epidemiological investigations. Specialist or reference tests are performed for:

- Verification of the microbiological results from the primary laboratory;
- Identification of rare or unusual pathogens;
- Comparative (fingerprinting or typing) analyses for strain characterisation to establish likely relationships between cultures from samples collected during outbreaks and at different times or from different places in the food chain;

- Detection of toxins, and/or the potential to produce a toxin;
- Distinction, where possible, between non-pathogenic and pathogenic variants of the same species;
- Assessment of the likely disease severity;
- Detection of additional bacterial pathogens as well as viruses (e.g. norovirus) and parasites.

Tests for *Cronobacter (Enterobacter) sakazakii, Clostridium botulinum* neurotoxin, staphylococcal enterotoxins, *Bacillus* toxins, histamine and shellfish toxins, norovirus and parasites are usually only available at national or international reference laboratories. Given the specialist and complex nature of some of these tests, results may not be available as quickly as primary tests. Hence public health actions and interventions should not be delayed pending the results of specialist and reference tests.

SECTION 3

Hygiene Indicator Organisms

3.1 Introduction

The presence of indicator bacteria in ready-to-eat food, although not inherently a hazard, can be indicative of poor practice that may be one or more of the following:

- poor quality of raw materials or food components;
- undercooking;
- cross-contamination;
- poor cleaning;
- poor temperature and time control.

Indicator bacteria may be associated with an increased likelihood of the presence of pathogens[58]. Indicator organisms are useful in the assessment of food product safety because they tend to be present in higher numbers than most pathogens and are relatively quick and easy to identify.

There are a number of recommended actions listed in Table 4 that could be taken in response to an unsatisfactory result for indicator bacteria. 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.

3.2 Enterobacteriaceae

The Enterobacteriaceae family is a group of bacteria 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 in heat treated foods therefore signifies inadequate cooking or post-processing contamination. High levels of these bacteria are expected in some food commodities such as salad vegetables. The use of sanitising rinses may reduce but not entirely remove these organisms.

Some Enterobacteriaceae can contribute to the formation of histamine (scombrotoxin) in foods such as scombroid fish (e.g. mackerel and tuna) and occasionally some cheeses if these are not processed properly and/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 (depending on the type of product) are set by Regulation (EC) No. 2073/2005 (as amended)[16].

3.3 *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. Occasional strains are pathogenic; these are rarely found in ready-to-eat foods although not all can be recovered by currently available detection laboratory methods. Specialist methods to detect pathogenic strains are required when illness is suspected.

Escherichia coli may sometimes be found in soft, mould-ripened or washed-rind cheese made from raw milk. Although Regulation (EC) No. 2073/2005 (as amended) has no criteria for *E. coli* in cheese made from raw milk[16] it is recommended that these cheese types be routinely tested for *E. coli* and investigation undertaken if a change in trend is detected. It is also recommended that a risk assessment is performed to assess the need for periodic monitoring for VTEC O157. Tests should be urgently applied where there is epidemiological evidence linking VTEC infection with specific foods (please refer to Table 1 and section 2.2.2 for information on VTEC O157).

3.4 *Listeria* species

Listeria spp. are able to grow at normal refrigeration temperatures but are killed by temperature regimes such as 70°C for two minutes. These organisms show 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. Their presence can be used as an indicator to assess the hygienic status of a food product. *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 many other bacteria.

The term Listeria spp. is fully inclusive of all *Listeria* spp., including *L. monocytogenes*. The occurrence of these bacteria at any level may therefore be of significance in certain refrigerated high risk foods (e.g. soft ripened cheese, pâté, smoked fish, cooked sliced meats) due to the potential for growth during storage in some of these products. For these products it is therefore recommended that an enrichment method be used, in addition to enumeration, to check that there is an absence of *Listeria* spp. in 25g of food (Table 4).

SECTION 4

Aerobic Colony Counts

4.1 Introduction

The Aerobic Colony Count (ACC), also known as the Total Viable Count or Standard Plate Count, is an indicator of quality, not safety, and cannot directly contribute towards a safety assessment of ready-to-eat food.

4.2 ACC levels in various ready-to-eat foods

ACCs can be used as part of a general quality assessment including that of extended shelf-life foods; as such it can be used 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.

Immediate action in response to high ACCs is not usually warranted except for shelf-stable canned or bottled food products immediately after opening (Category 1, Table 5). The level will depend initially on the type and duration of processing that the food has received during production (see Table 5). 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 10^4 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 occasionally thermotolerant spores may survive, depending on the severity of the heat process. Products that have received a drying 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 should 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 retard 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 foodborne pathogens (with the notable exceptions of *Listeria monocytogenes* and *Yersinia enterocolitica*) 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), which can 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 bacteria, spoilage is likely to be noticeable at 10^7 – 10^8 cfu/g; pseudomonads tend to produce taints, discolouration, and slime whilst other Gram-negative bacteria 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 may occur relatively rapidly and will usually be visible. This also applies to products such as rice or pasta salads containing raw vegetables. If products are dried (e.g. herbs), the ACC per gram appears to increase due to the volume 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 unless they have been spoiled. Some food commodities such as fermented meats, fish, vegetables and most types of cheese 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 5 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 ACCs 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.

SECTION 5

Supplementary Advice on Use of the Guidelines

5.1 Microbiological methodology

Laboratory methods that allow rapid and accurate detection, identification and quantification of microbiological hazards enhance the ability to monitor and investigate contamination throughout the food chain. Methods are defined for Official Control sampling[15,16,20,59]. However for public health investigations and for reasons of increased speed or sensitivity, different methods (as well as sample sizes) may be utilised as long as they have been validated according to internationally acceptable protocols and their use authorised by the Competent Authority.

The interpretation of laboratory results in food microbiology is often the most difficult and complex aspect of the examination process. Users of these guidelines should 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. Sampling itself is the greatest contributory factor to the variability of a result for a particular sample as micro-organisms are not usually homogenously distributed in a contaminated foodstuff. The sample matrix itself, the type of packaging, and the culturability of injured organisms will also contribute further to the variation of reproducibility between microbiological results. Results should therefore be interpreted in context taking such factors into consideration. 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.

5.2 Environmental samples

These guidelines do not include microbiological criteria for and interpretation of microbiological results from environmental samples. Sampling the food environment will be the subject of additional HPA guidance. However, taking appropriate and targeted environmental samples is recommended in these guidelines for unsatisfactory results on ready-to-eat foods and should also be considered for borderline values. Testing the food environment makes a positive and additional contribution to food safety.

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;
- For hygiene indicators 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;
- 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 6

Tables

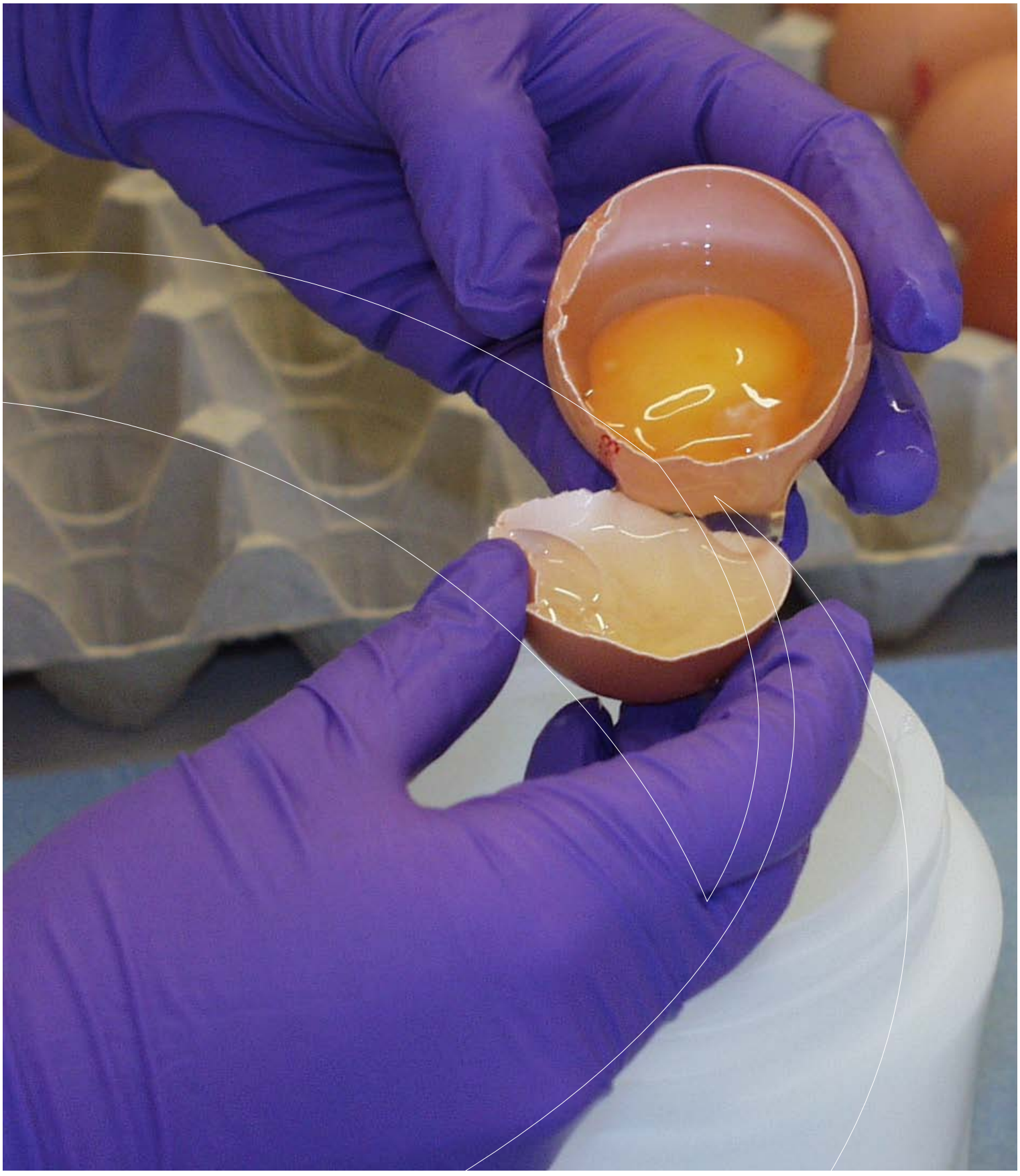


Table 1. Guidance on the interpretation of results for detection of bacterial pathogens (the hazard) in ready-to-eat foods placed on the market.

Hazard	Result/25g[a]	Microbiological Risk Category	Interpretation	Likely Cause	Suggested Actions (Not exclusive) NB: Perform risk assessment before any further action	Additional information including laboratory specialist and reference tests[c] Actions should not be delayed pending results of specialist tests.
Campylobacter spp. (thermotolerant)	Detected	High	**UNSATISFACTORY: Potentially injurious to health and/ or unfit for human consumption[b]**	Inadequate processing Cross contamination	Immediate investigation of: the food origin, production process and environment; take investigative food samples and consider environmental monitoring.	Confirmation of identity, molecular typing.
	Not detected	Low	SATISFACTORY		N/A	
Escherichia coli O157 (and other verocytotoxin-producing *E. coli* (VTEC))	Detected	High	**UNSATISFACTORY: Potentially injurious to health and/ or unfit for human consumption[b]**	Inadequate processing Cross contamination	Immediate investigation of: the food origin, production process and environment; take investigative food samples and consider environmental monitoring.	Confirmation of identity serotyping, phage typing verocytotoxin typing, molecular typing.
	Not detected	Low	SATISFACTORY		N/A	
Salmonella spp.	Detected	High	**UNSATISFACTORY: Potentially injurious to health and/ or unfit for human consumption[b]**	Inadequate processing Cross contamination	Immediate investigation of: the food origin, production process and environment; take investigative food samples and consider environmental monitoring.	Confirmation of identity, serotyping, phage typing, anti-microbial resistance patterns, molecular typing. Regulation (EC) No. 2073/2005 (as amended) contains microbiological criteria for some specific food / *Salmonella* combinations and the requirements to be complied with by FBOs.
	Not detected	Low	SATISFACTORY		N/A	
Shigella spp.	Detected	High	**UNSATISFACTORY: Potentially injurious to health and/ or unfit for human consumption[b]**	Cross contamination by food handler or faecal contamination of raw product	Immediate investigation of hygiene, cleaning, and food handlers in outbreaks.	Confirmation of identity, serotyping, molecular typing.
	Not detected	Low	SATISFACTORY		N/A	
Vibrio cholerae (O1 and O139)	Detected	High	**UNSATISFACTORY: Potentially injurious to health and/ or unfit for human consumption[b]**	Inadequate processing Cross contamination by food handler Contaminated irrigation water	Immediate investigation of: the food origin, production process and environment; take investigative food samples and consider environmental monitoring.	Confirmation of identity, serotyping, molecular typing.
	Not detected	Low	SATISFACTORY		N/A	

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 is 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.
b, Regulation (EC) No. 178/2002, Article 14 Food safety requirements.
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. Guidance on the interpretation of results for enumeration of bacterial pathogens (the hazard) in ready-to-eat foods placed on the market

Hazard	Result (cfu/g)[a]	Microbiological Risk Category	Interpretation	Likely Cause	Suggested Actions (Not exclusive) NB: Perform risk assessment before any further action	Additional information including laboratory specialist and reference tests[c] Actions should not be delayed pending results of specialist tests.
Bacillus cereus	$>10^5$	High	**UNSATISFACTORY: Potentially injurious to health and/ or unfit for human consumption[b]**	Strong evidence for poor processing, poor quality raw materials, or poor temperature control	Immediately review temperature and time controls particularly for the storage of cooked foods. Take investigative samples of food, raw food components and the food preparation environment.	Not all strains produce toxins and are able to produce gastrointestinal disease, Confirmation of identity, molecular typing
	10^3 - $\leq 10^5$	Moderate	BORDERLINE	Likely evidence for poor processing, poor quality raw materials, or poor temperature control	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 for cooked foods. Consider taking investigative samples of food, raw food components and the food preparation environment.	Reported as presumptive *B. cereus* unless associated with an outbreak investigation when confirmation and typing are performed
	$<10^3$	Low	SATISFACTORY		N/A	Reported, if present, as presumptive *B. cereus*
Other pathogenic *Bacillus* spp. *(B.subtilis* group*)*	$>10^5$	High	**UNSATISFACTORY: Potentially injurious to health and/ or unfit for human consumption[b]**	Strong evidence for poor processing, poor quality raw materials, or poor temperature control.	Immediately review temperature and time controls particularly for the storage of cooked foods. Take investigative samples of food, raw food components and the food preparation environment.	Not all strains produce gastrointestinal disease. Confirmation of identity, molecular typing High levels occur in specific fermented products and do not represent a public health risk
	10^3 - $\leq 10^5$	Moderate	BORDERLINE	Likely evidence for poor processing, poor quality raw materials, or poor temperature control	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 taking investigative samples of food, raw food components and the food preparation environment.	Reported as presumptive *Bacillus* spp. unless associated with an outbreak investigation when confirmation and typing are performed
	$<10^3$	Low	SATISFACTORY		N/A	Reported, if present, as presumptive *Bacillus* spp.

Clostridium *perfringens*	>10^4	High	**UNSATISFACTORY: Potentially injurious to health and/ or unfit for human consumption**[b]	Strong evidence for poor processing, particularly during cooling period after cooking, the use of left over food, or from stocks and gravies	Immediately review temperature and time controls. Take investigative samples of food and the food preparation environment.	Confirmation of identity, typing, pathogenicity (toxin gene detection)
	10 - ≤10^4	Moderate	BORDERLINE	Likely evidence for poor processing particularly cooling	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 taking investigative samples of food and the food preparation environment.	Refer isolates associated with an outbreak investigation
	<10	Low	SATISFACTORY		N/A	
Listeria monocytogenes	>10^2	High	**UNSATISFACTORY: Potentially injurious to health and/ or unfit for human consumption**[b]	Strong evidence for poor processing, environmental or cross-contamination during production or at point of sale, poor temperature control or inappropriate length of shelf-life	Immediate investigation of: the food origin, production process and environment. Take investigative samples of food and environmental monitoring.	Refer isolates for confirmation of identity, serotyping, molecular typing.
	10 - ≤10^2 [c]	Moderate	BORDERLINE	Likely evidence for poor processing and/or poor quality raw materials	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 taking investigative samples of food and environmental monitoring. In refrigerated high risk foods where there is a potential for growth during storage, and in foods likely to be served to vulnerable groups (such as that served in hospital) the presence of *L. monocytogenes* at any level may be of significance and should be investigated.	Consider referral of isolates, particularly where associated with persistent contamination or as part of outbreak investigations. For foods in high risk categories[c], refer isolates for reference testing. Regulation (EC) No. 2073/2005 (as amended) contains microbiological criteria for some specific food / *L. monocytogenes* combinations and the requirements to be complied with by FBOs.
	<10^d	Low	SATISFACTORY		N/A	

***Staphylococcus aureus* and other coagulase-positive staphylococci**	>10^4	High	**UNSATISFACTORY: Potentially injurious to health and/ or unfit for human consumption[b]**	Strong evidence for poor handling and temperature control.	Immediately review food handling as well as temperature and time controls. Take investigative samples of food, food preparation environment and food handlers.	Not all strains are capable of producing toxin and causing disease. Confirmation of identity, typing, pathogenicity (toxin gene detection) of isolates, Consider enterotoxin detection in food and food remnants from cases of suspected food poisoning, or where high levels (>10^5 cfu/g) may have occurred at any stage in the food chain.
	20 - ≤ 10^4	Moderate	BORDERLINE	Likely evidence for poor handling, process and temperature control.	Risk will increase proportional to the levels detected and the likelihood of subsequent growth in the absence of appropriate levels of control. Review handling as well as processing controls, especially if there opportunities for growth of staphylococci during processing or maturation of the product. Consider taking investigative samples of food, food preparation environment and food handlers.	Consider referral of isolates, particularly where associated with outbreak investigations or where there is die off of the bacterium during storage. Regulation (EC) No. 2073/2005 (as amended) contains microbiological criteria for some specific food / coagulase-positive staphylococci combinations and the requirements to be complied with by FBOs
	<20	Low	SATISFACTORY		N/A	
Vibrio parahaemolyticus	>10^3	High	**UNSATISFACTORY: Potentially injurious to health and/ or unfit for human consumption[b]**	Strong evidence for poor processing.	Immediate investigation of the food origin, review cooking and subsequent temperature and time controls. Take investigative samples of processed (cooked) food, raw food components (particularly marine products) and the food preparation environment.	Confirmation of identity, typing
	20 - ≤ 10^3	Moderate	BORDERLINE	Likely evidence for poor processing or cross-contamination.	Risk will increase proportional to levels detected. Food may not become hazardous provided appropriate levels of control are applied. Consider taking investigative samples of processed (cooked) foods, raw food components (particularly marine products) and the food preparation environment.	Consider referral of isolates, particularly where associated with outbreak investigations
	<20	Low	SATISFACTORY		N/A	

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.
b, Regulation (EC) No. 178/2002, Article 14 Food safety requirements. Not applicable to foods fermented with *Bacillus* spp.
c, Detected in 25g by enrichment for high risk foods capable of supporting the growth of *L. monocytogenes* such as some soft ripened cheeses, sliced meats, smoked fish and pâtés.
d, Not detected in 25g by enrichment for high risk foods capable of supporting the growth of *L. monocytogenes*.

Table 3. Major features of foodborne diseases due to selected pathogens

Hazard	Food types most often associated with human infections	Microbiological Risk Category	Major routes of transmission	Known host risk factors for severe infection	Symptoms, severity and sequelae[a]	No. of reported human cases in UK in 2007[b]
Bacillus cereus	Cooked rice (emetic syndrome) Cooked meats, poultry and vegetables, soups, spices (diarrhoeal syndrome)	Commercial catering	Foodborne	Unknown	Vomiting (emetic syndrome) Diarrhoea (diarrhoeal syndrome) Usually mild and short-lived, lasts ~ 1 day	Many foodborne infections are not reported
Bacillus spp. (other pathogenic *Bacillus*)	Cooked meats, poultry and vegetables	Commercial catering	Foodborne	Unknown	Vomiting and diarrhoea Usually mild and short-lived, lasts ~ 1 day	Many foodborne infections are not reported
Campylobacter spp. (thermotolerant)	Poultry, red meat, milk and dairy products made with unpasteurised milk or post-pasteurisation contaminated milk, untreated drinking water	Consumption of food prepared outside the home Barbecues Consumption of untreated water/milk on holiday (e.g. farm, cottage, caravan site)	Foodborne Cross-contamination Zoonotic Waterborne	Age (<5 yrs or >60 yrs) Reduced immune status Antacid treatment	Diarrhoea, headache, abdominal pain; usually lasts 2-7 days Irritable bowel syndrome (IBS) most common sequelae, reactive arthritis, Guillain-Barré syndrome	57,815
Clostridium perfringens	Cooked meat, gravy and stock	Commercial and institutional catering	Foodborne, Non-foodborne infection (i.e. person-to-person and antibiotic- associated infections occur in elderly)	Most people are probably susceptible	Diarrhoea, abdominal pain; Usually mild and short-lived, lasts ~ 1 day, but diarrhoea longer and more severe in elderly Dehydration in severe cases	73
Escherichia coli O157 (and other verocytotoxin-producing *E. coli* (VTEC))	Under cooked beef, milk and dairy products made with unpasteurised milk or post-pasteurisation contaminated milk, salad vegetables, untreated drinking water	Pre-school / nurseries Zoonotic (petting farms) Domestic home Institutional settings Consumption of untreated water/milk on holiday (e.g. farm, cottage, caravan site)	Foodborne Cross-contamination Environmental exposure Person-to-person Zoonotic Waterborne	Age (<5 yrs (HUS) or >60 yrs (TTP))	Diarrhoea, vomiting, abdominal pain, haemorrhagic colitis, lasts 2 weeks in uncomplicated cases, can be fatal; Haemolytic Uraemic Syndrome (HUS), Thrombotic Thrombocytopaenic Purpura (TTP)	1,149

Listeria monocytogenes	High risk foods, such as sliced meats, pâté, soft cheese, sandwiches, smoked fish	Hospitals and the community	Foodborne Cross-contamination	Age (>60 yrs), Pregnancy Newborn infants Immunosuppression Antacid treatment	Non-invasive: diarrhoea, fever, headache, muscle pain Invasive: fever and severe systemic infections possible (septicaemia and meningitis, and miscarriage). High case fatality rate.	261
Salmonella spp. (non Typhi/Paratyphi)	Eggs, poultry, pork, beef, dairy products, seeds, herbs, salad vegetables, chocolate	Consumption of food prepared outside the home Foreign travel	Foodborne Cross-contamination Person-to-person Zoonotic	Reduced immune status	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	13,802
Shigella spp.	Salad vegetables	Pre-schools / nurseries Institutional settings Foreign travel	Person-to-person Foodborne	Age (<5 yrs)	Diarrhoea, vomiting, bacillary dysentery; last average of 4 to 7 days; HUS, Toxic megacolon.	1,638[c]
Staphylococcus aureus and other coagulase- positive staphylococci	Processed meats, poultry, fish, shellfish, and dairy products.	Commercial and institutional catering	Foodborne Food handlers Cross-contamination	Most people are susceptible	Nausea and vomiting, last 1 – 2 days, may be very acute Abdominal cramps and diarrhoea Collapse in very severe cases	Many foodborne intoxications are not reported
Vibrio cholerae	Imported seafood, untreated drinking water	Foreign travel particularly to the Indian subcontinent	Waterborne Foodborne	Most people are probably susceptible	Diarrhoea, vomiting, severe de-hydration, leg cramps	47[d]
Vibrio parahaemolyticus	Imported seafood	Various	Foodborne	Most people are probably susceptible	Diarrhoea	41

a, The Table is not inclusive and features, other than that described may occur but are generally considered uncommon.
b, Data provided by Health Protection Agency, Health Protection Scotland, Communicable Disease Surveillance Centre Northern Ireland
c, Most cases identified are person-to-person
d, All foreign travel associated

Table 4. Guidance on the interpretation of results for hygiene indicator organisms in ready-to-eat foods placed on the market

Hygiene Indicator	Result (cfu/g)	Interpretation	Comment	Likely Cause	Suggested Actions (Not exclusive)
Enterobacteriaceae[a]	$>10^4$	UNSATISFACTORY	Members of this group occur in the environment as well as the gut of humans 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.	Poor hygiene due to undercooking, or cross contamination from raw meat, food handlers or food contact surfaces as well as poor temperature and time control.	Review cooking and all hygiene procedures including cleaning. Take investigative samples of food and undertake environmental monitoring of food preparation environment.
	10^2 - $\leq 10^4$	BORDERLINE	Interpret in conjunction with test results from other microbiological parameters but detection in several foods or other areas of the food production environment should be investigated.	Possible evidence of poor hygiene due to undercooking, or cross contamination from raw meat, food handlers or food contact surfaces as well as poor temperature and time control.	Review cooking and all hygiene procedures including cleaning. Consider taking investigative samples of food and the food preparation environment. Action should be proportional to the levels detected.
	$<10^2$	SATISFACTORY		N/A	Regulation (EC) No. 2073/2005 (as amended) contains microbiological criteria for some specific food / Enterobacteriaceae combinations and the requirements to be complied with by FBOs
Escherichia coli[b,c,d]	$>10^2$	UNSATISFACTORY	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 *E. coli* can signify a risk that faecal pathogens are present. 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.	Poor hygiene due to undercooking, or cross contamination from raw food especially meat, food handlers or food contact surfaces as well as poor temperature and time control.	Review cooking and all hygiene procedures including cleaning. Take investigative samples of food and undertake environmental monitoring of the food preparation environment.
	20 - $\leq 10^2$	BORDERLINE	Although *E. coli* 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.	Possible evidence of poor hygiene due to undercooking, or cross contamination from raw food especially meat, food handlers or food contact surfaces, as well as poor temperature and time control.	Review cooking and all hygiene procedures including cleaning. Consider taking investigative samples of food and the food preparation environment. Action should be proportional to levels detected.
	<20	SATISFACTORY		N/A	Regulation (EC) No. 2073/2005 (as amended) contains microbiological criteria for some specific food / *E. coli* combinations and the requirements to be complied with by FBOs

***Listeria* spp. (not *L.monocytogenes*)**	**$>10^2$**	UNSATISFACTORY	With very rare exceptions, species other than *L. monocytogenes* are not pathogenic to 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 an extended shelf life at this level suggests follow-up action. Remedial action should be taken for foods likely to be consumed by vulnerable groups in whom the risk of listeriosis is increased, e.g. foods served in hospitals	Strong evidence for poor processing, or poor temperature control including suboptimal operation of refrigerators, or over extension of shelf life.	Review factory hygiene (including cleaning) together with temperature and shelf life controls. Take investigative samples of food and the food preparation environment, particularly plant and machinery. Consider sending isolates for reference tests
	10 - $\leq 10^2$ [e]	BORDERLINE	May become a problem especially in foods capable of supporting growth of *Listeria* (see above). Remedial action should be taken for foods intended to be fed to vulnerable groups in whom the risk of listeriosis is increased, e.g. foods served in hospitals.	Possible evidence for poor processing or poor quality raw materials. Indicate process has the potential to allow contamination by *L. monocytogenes*.	Review quality of raw materials, factory hygiene (including cleaning), temperature and shelf life controls. Consider taking investigative samples of food and the food preparation environment, particularly plant and machinery. Consider sending isolates for reference tests. Action should be proportional to levels detected
	<10[f]	SATISFACTORY		N/A	

a, The criterion listed for Enterobacteriaceae does not apply to fresh fruit and salad vegetables or food that contains fresh fruit and vegetables as ingredients e.g. sandwiches, as these food types can contain high levels of Enterobacteriaceae as part of their normal micro-flora. The criterion does not apply to cheeses ripened using a culture of *Hafnia alvei* or *Proteus vulgaris*.

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/100 g flesh and intra-valvular liquid (food safety criterion) using ISO TS 16649-3.

d, Criterion does not apply to cheeses made from raw milk

e, Detected in 25g by enrichment for high risk foods capable of supporting the growth of *Listeria spp.* such as some soft ripened cheese, sliced meats, smoked fish and pâté

f, Not detected in 25g by enrichment for high risk foods capable of supporting the growth of *Listeria* spp.

Table 5. Guidance on the interpretation of results for aerobic colony count levels in various ready-to-eat foods and components placed on the

	Food Category	Examples	Result (cfu/g)		
			Satisfactory[a]	Borderline[b]	Unsatisfactory[c]
1	Ambient stable canned, bottled, cartoned and pouched foods immediately after removal from container	Canned products such as tuna, salmon, corned beef, soups, stews, desserts, fruit; UHT products	<10	Not Applicable	See note d
2	Foods cooked immediately prior to sale or consumption	Takeaway food, burgers, kebabs, sausages, pizza , ready meals (cook/chill & cook/freeze) after regeneration	$<10^3$	10^3 - $<10^5$	$\geq 10^5$
3	Cooked foods chilled but with minimum handling prior to sale or consumption; canned pasteurised foods requiring refrigeration	Whole pies, sausage rolls, samosas, flans, quiches, chicken portions; canned ham, pasteurised foods including fruit juice and soups; desserts	$<10^4$	10^4- $<10^7$	$\geq 10^7$
4	Bakery and confectionery products without dairy cream, powdered foods	Cakes without dairy cream, soup powders, milk powder, powdered dairy products, other reconstituted powdered foods ready to eat after reconstitution or warming	$<10^4$	10^4 - $<10^6$	$\geq 10^6$
5	Cooked foods chilled but with some handling prior to sale or consumption	Sliced meats, cut pies, pâté, sandwiches without salad, hot smoked fish (mackerel, etc.), molluscs, crustaceans and other shellfish out of shell	$<10^5$	10^5 - $<10^7$	$\geq 10^7$ See note e
6	Non-fermented dairy products and dairy desserts, mayonnaise and mayonnaise based dressings, cooked sauces	Most milk and butter, cream, ice cream, fresh cheese (mascarpone, paneer), trifle with dairy cream, satay, cakes with dairy cream	$<10^5$	10^5 - $<10^7$	$\geq 10^7$
7	Food mixed with dressings, dips, pastes	Coleslaw, dips, taramasalata, houmous	$<10^6$	10^6 - $<10^7$	$\geq 10^7$
8	Extended shelf life food products requiring refrigeration	MAP or vacuum packed products, e.g. meat, fish, fruit and vegetables	$<10^6$	10^6 - $<10^8$	$\geq 010^8$ See note e
9	Raw ready-to-eat meat and fish, cold smoked fish	Sushi, smoked salmon, gravalax	$<10^6$	10^6 - $<10^7$	See note f
10	Preserved food products –pickled, marinated or salted	Pickled or salted fish, cooked shellfish in vinegar, vegetables in vinegar or oil, herbs, spices	Not Applicable	Not Applicable	See note f
11	Dried foods	Fruits, berries, vine fruits, nuts, sunflower seeds, herbs, spices, dried fish	Not Applicable	Not Applicable	See note f
12	Fresh fruit and vegetables, products containg raw vegetables	Whole fruit, pre-prepared fruit salads, vegetable crudités, salads, sandwiches with salad, mixed commodity salads containing raw vegetables	Not Applicable	Not Applicable	See note f
13	Fermented, cured and dried meats, fermented vegetables, ripened cheeses	Continental sausages/salamis, jerky, sauerkraut, olives, bean curd, cheddar, stilton, brie, fermented milk drinks and butter, yoghurt, etc	Not Applicable	Not Applicable	See note f

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 Category1

- Most products are normally sterile when sampled from the container but if they are consumed after subsequent further preparation then assess them as Category 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 Category 8

- 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 Categories 9 -13

- ACCs not routinely performed. For spoilage investigation, **"Unsatisfactory"** if the predominant organism is > 10^6 yeasts, >10^7 Gram negative bacilli or *Bacillus* spp., or >10^8 lactic acid bacteria unless added as a processing aid.

Glossary

Aerobic – conditions in which oxygen is present

Anaerobic – conditions in which oxygen is absent.

Antimicrobial resistance - the ability of micro-organisms of certain species to survive or even to grow in the presence of a given concentration of an antimicrobial agent, that is usually sufficient to inhibit or kill micro-organisms of the same species (Directive 2003/99/EC[60]).

a_w – the water activity (a_w) of a food is a measure of availability of water for the metabolic activity and growth of micro-organisms.

Batch - a group or set of identifiable products obtained from a given process under practically identical circumstances and produced in a given place within one defined production period (Regulation (EC) No. 2073/2005).

Borderline – test results that are not unsatisfactory but are also not satisfactory, are on the upper limit of acceptability and which indicate the potential for development of public health problems and of unacceptable risk.

Bacterial spores (endospores) - exist in a free state and are a tough, dormant form that are very resistant to desiccation, heat, and a variety of chemical and radiation treatments that are otherwise lethal to vegetative bacteria. The genera of Gram-positive bacteria, *Bacillus* and *Clostridium*, produce endospores which are released from a bacterial cell.

Competent authority - the central authority of a Member State competent for the organisation of official controls or any other authority to which that competence has been conferred (Regulation (EC) No. 882/2004).

Contamination – the presence or introduction of a hazard (Regulation (EC) No. 852/2004).

Disease – any change from a normal physiological state or function.

Emetic – causes vomiting.

Fermentation – conversion of a carbohydrate, such as sugar, by micro-organisms into an acid or an alcohol.

Flora - the bacteria and other micro-organisms that normally are found in a food.

Food business operator - the natural or legal persons responsible for ensuring that the requirements of food law are met within the food business under their control (Regulation (EC) No. 178/2002).

Food examiner - a person who possesses the requisite qualifications and experience to carry out examinations for the purposes of the Food Safety Act[18].

Food safety criterion – criterion defining the acceptability of a product or a batch of foodstuff applicable to products placed on the market (Regulation (EC) No. 2073/2005).

Foodborne outbreak - an incidence, observed under given circumstances, of two or more human cases of the same disease and/or infection, or a situation in which the observed number of cases exceeds the expected number and where the cases are linked, or are probably linked, to the same food source (Directive 2003/99/EC[60]).

Imported food – non-UK produced foods which are imported from other countries within or outside the European Union. Import means the release for free circulation of food or the intention to release food for free circulation (Regulation (EC) No. 882/2004).

Microbiological criterion – criterion defining the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of micro-organisms, and / or on the quantity of their toxins / metabolites, per unit(s) of mass, volume, area or batch (Regulation (EC) No. 2073/2005).

Modified atmosphere packaging (MAP) – removal of air from a food package and replacement with a strictly controlled gaseous mixture of carbon dioxide, oxygen, and/or nitrogen, and then hermetically sealed.

Morbidity – effect of disease.

Mortality – death as a result of disease.

Official control - any form of control that the competent authority or the Community performs for the verification of compliance with feed and food law, animal health and animal welfare rules (Regulation (EC) No. 882/2004).

Pasteurisation – a form of heat treatment that kills vegetative pathogens and spoilage microorganisms in milk and other foods e.g. for milk a common pasteurisation process is 71.7°C for 15 seconds.

Pathogen – a micro-organism that has the capacity to cause disease, i.e. has the property of pathogenicity.

pH – the relative acidity or alkalinity of a food.

Potable water - water intended for drinking or use in food preparation.

Process hygiene criterion – criterion indicating the acceptable functioning of the production process. Such a criterion is not applicable to products placed on the market. It sets an indicative contamination value above which corrective actions are required in order to maintain the hygiene of the process in compliance with food law (Regulation (EC) No. 2073/2005).

Psychrotroph - a micro-organism that can grow at temperatures between -1°C and 5°C and have an optimum growth temperature in the mesophilic range (20-30°C)

Ready-to-eat food – 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 (Regulation (EC) No. 2073/2005).

Retail – 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 (Regulation (EC) No. 178/2002).

Risk - a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard (Regulation (EC) No. 178/2002).

Risk assessment - a scientifically based process consisting of four steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation (Regulation (EC) No. 178/2002).

Sample - a set composed of one or several units or a portion of matter selected by different means in a population or in an important quantity of matter, which is intended to provide information on a given characteristic of the studied population or matter and to provide a basis for a decision concerning the population or matter in question or concerning the process which has produced it (Regulation (EC) No. 2073/2005).

Satisfactory – test results indicating good microbiological quality

Shelf-life – the period preceding the "Use by" or the minimum durability date (Directive 2000/13/EC[61]).

Sporulation – the process by which some bacteria are able to produce endospores to enhance their survival under adverse conditions (see bacterial spores).

Symptoms – manifestation or evidence of disease.

Thermotolerant – able to survive high temperatures.

Toxin – a poisonous substance with the capacity to cause disease.

Unsatisfactory – for pathogens, test results at levels which indicate a product that is potentially injurious to health and/or unfit for human consumption and require immediate remedial action. For hygiene indicators, test results that require remedial action.

Vegetative bacteria – a bacterial cell which is capable of actively growing; multiplication occurs by division of the cell into two.

Viable - capable of living, developing, or germinating in favourable environmental conditions.

Vulnerable groups – population of persons more susceptible or more likely to develop foodborne disease, sometimes of greater severity; these groups include pregnant women, the elderly, young babies, children and people with weakened immune systems.

Zoonosis - any disease and/or infection which is naturally transmissible directly or indirectly between animals and humans (Directive 2003/99/EC[60]).

Zoonotic agent – any virus, bacterium, fungus, parasite or other biological entity which is likely to cause a zoonosis (Directive 2003/99/EC[60]).

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