UK Health Security Agency

Guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market

Interpretation of test results generated by UKHSA Food Water and Environmental Microbiology Services Laboratories

2024

Contents

Greater than, less than symbols

Throughout this document the following symbols are used:

< less than
≤ less than or equal to
> greater than
≥ greater than or equal to

1. Introduction

These guidelines were completed in 2024. Since the previous edition in 2009 (1), the UK has left the EU and this has resulted in transfer of legal responsibility from EU to UK legislation through Statutory instruments, particularly The Food and Feed Hygiene and Safety (Miscellaneous Amendments) (England) Regulations 2020: UK Statutory Instrument 2020 Number 1410 (2). The statutory instruments refer to EU legislation, and much remains the same or very similar such as legal obligations for food safety of food business operators and microbiological criteria. EU references have been updated to reflect the law in force, in all new or amended guidance published since the transition.

1.1 Purpose of the guidelines

In food legislation, food business operators (FBOs) have obligations to produce and serve safe food and ensure that microorganisms are eliminated or minimised to an acceptable level to the extent that they cannot cause harm to human health and that food is fit for human consumption (3). Food safety management systems are essential to produce safe food, including the application of good hygienic practice (GHP) and hazard analysis and critical control point (HACCP) systems (4, 5, 6), and to ensure that official controls are in place to audit legislative compliance by FBOs (7). Microbiological testing provides important information for verification of food safety management systems, although testing alone does not guarantee the safety of food.

To interpret the results of microbiological testing, criteria are used to define the acceptability of a product, a batch of foodstuffs or a process. This is based on the absence, presence or number of microorganisms, and/or on the quantity of their toxins or metabolites, per units of mass, volume, area or batch when tested by a specified or equivalent method (6). These are known as microbiological criteria. 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 (4, 5, 8, 9).

Within the UK Health Security Agency (UKHSA), 3 laboratories deliver the Food, Water and Environmental Microbiology Services (FWEMS). These laboratories examine food samples that are collected by local authorities or port health authorities under standardised conditions (6, 10). Food samples are submitted for public health investigations including as part of outbreak investigations, for Official Control (7) purposes or for surveillance and monitoring. The FWEMS laboratories therefore contribute towards the response to foodborne threats to health and work to provide authoritative and practical expert advice to a range of stakeholders (government, local government, the NHS and the public) such as that described here for ready-to-eat (RTE) foods placed on the market.

Since the publication of the last edition of these guidelines, there has been an accumulation of much additional data on the microbiological testing of foods from the FWEMS (11 to 37).

Crucially, staff in FWEMS have further experience of interpreting the results following microbiological examination of food. The guidelines are for practical use within UKHSA as well as by local authority and port health authority enforcement officers. These guidelines provide a framework for standardisation of both interpretation of laboratory results as well as advice on what remedial actions can be recommended by staff (including Food Examiners) in the UKHSA FWEMS laboratories, with a focus on public health and consumer protection.

These updated guidelines have been reviewed, revised, and supersede those previously issued. They include 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. These guidelines have been expanded to include information on statutory requirements such as for Shiga toxin-producing *Escherichia coli* (STEC) testing (38) as well as advice on sampling, transport and test selection.

Within the requirements of the Food Safety Act 1990 (39), a Food Examiner is required “to carry out examinations for the purposes of this Act”. The qualifications for Food Examiners are defined in the Food Safety (Sampling and Qualifications) (England) Regulations 2013 (41). UKHSA employs experienced microbiologists with the necessary qualifications to act as Food Examiners who assist authorised officers from local authorities and port health authorities in the enforcement of the Food Safety Act (6, 10, 39). This assistance is likely to involve the provision of advice, receiving and testing of samples, issuing of reports and certificates of examination and by providing witness statements and testimony to assist prosecutions.

Others (including Food Examiners outside UKHSA) may find the approaches, interpretation and advice published here helpful for their activities. However, there are differences in legislation and practices between the devolved administrations of the UK, for example, EU food law is entirely applicable in Northern Ireland. Consequently, local use, experience and interpretation may result in differing opinions and interpretation which are outside the scope and opinions outlined here. Great care is needed when using the interpretations outlined here for results generated by other laboratories outside of UKHSA where methodology may be different to those utilised within UKHSA (for example, nature of the enrichment broth or isolation agar, incubation time or temperature) and therefore may produce different results, particularly in terms of test sensitivity.

1.2 Scope of the guidelines

Food within the scope of these revised guidelines includes RTE food sampled within the retail chain, for example, the retail, wholesale, distribution and food service sectors. RTE food is defined in Regulation (EC) 2073/2005 (40) as “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, microorganisms of concern”. This, on occasion, may present difficulties when, for example, a food not generally consumed without

cooking is consumed raw. However, this definition does include food components, such as herbs and spices, where they are added to foods without further cooking or processing. These guidelines for pathogens also apply to foodborne disease investigations in all settings, including where food is collected from domestic environments.

Criteria are applied to RTE foods at the point of sale and relate to results for the detection of bacteria or bacterial groups that indicate the presence of pathogens, possible poor hygiene and/or substandard practices. In some circumstances, these guidelines may also be used to assess the safety and quality of food taken from the producer's premises. For some RTE foods (sampled from production and/or on the market), statutory microbiological criteria exist. These food safety or process hygiene criteria (including sampling plans, analytical methods, and corrective actions) are laid down in EU Regulation (EC) 2073/2005 and subsequent amendment regulations (40), as well as those specified elsewhere in legislation (7, 47). Although potable and bottled water are defined as food (including when used as a food ingredient), these matrices are not addressed in these guidelines as there is relevant legislation and guidance already available including The Water Supply Regulations, 2016 (42) and The Natural Mineral Water, Spring Water and Bottled Drinking Water Regulations 2007 (43) and its amendment from 2009 (44).

Port health authorities are responsible for physical checks on imported foods at designated points of entry (for example, border control posts) which may involve sampling and submission for microbiological testing. Requirements for sampling and testing of imported foods of non-animal origin are set out in Regulation (EC) 2017/625 (7, 59).These revised guidelines apply to RTE imported food from countries outside Great Britain including those from the European Union (EU) and from Northern Ireland where EU food law still applies (2).

These guidelines do not take precedence over microbiological criteria within UK legislation (see section 1.7) but serve to be consistent with and complement legally enforceable standards, as well as providing an indication of the microbiological safety of foods where other standards currently do not exist or are not explicit. Investigative and corrective actions are likely to be 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. To safeguard public health, additional tests on RTE foods not covered by the regulations may be considered appropriate. Food samples prepared in a premises that are taken as part of inspections by enforcement officers would be expected to give satisfactory results for all parameters and any deviation from satisfactory results should be investigated.

Criteria for other agents including viruses and enteric parasites are currently excluded, however as European Standards (EN) and other internationally recognised methods are available, some of these may be included in future revisions of these guidelines.

1.3 Commission regulation on microbiological criteria for foodstuffs

Compliance with legal regulation is a mandatory requirement. Microbiological criteria in the EU have been harmonised in Community legislation by the European Commission Regulation on Microbiological Criteria for Foodstuffs, Regulation (EC) 2073/2005 as amended (40). This supports the Regulation on the Hygiene of Foodstuffs, Regulation (EC) 852/2004 as amended (3), and the General Food Law Regulation (EC) 178/2002 as amended (45). In addition, the regulation laying down specific rules for food of animal origin Regulation (EC) 853/2004 as amended (46) contains microbiological criteria for live bivalve molluscs, and raw milk is regulated by the Food Safety and Hygiene (England) Regulations 2013 (47).

These regulations apply to all FBOs involved in the production and handling of food. Interpretative documents relating to the regulation on microbiological criteria for foodstuffs have been produced by the EU as well as the Food Standards Agency (FSA) (48) and the Chilled Food Association (CFA) / British Retail Consortium (BRC).

Microbiological criteria are intended to assist with validating and verifying HACCP-based food safety management systems. Corrective action must be carried out when results do not fully comply with the regulation. Two types of microbiological criteria are set out in Regulation (EC) 2073/2005 (40) including criteria for food safety and process hygiene:

1.3.1 Food safety criteria

Food safety criteria define the acceptability of a product or a batch and these UKHSA RTE guidelines are designed to be consistent with legislative criteria. Food safety criteria are applicable to foodstuffs placed on the market and are applied throughout the shelf-life of a product. If tests indicate that the criteria are not met, the food business operator will not be able to place the food on the market and in some cases, a product recall or withdrawal may be required. When food safety criteria are not met, the food safety management procedures should be reviewed to ensure that products comply in the future. Food safety criteria are defined by a 2-class plan, that is, results are either satisfactory or unsatisfactory. Food business operators have a legal requirement to inform the competent authority (usually the local authority) of unsatisfactory results in food. Similarly, the official laboratory also has a legal obligation under the official control regulations (7) to inform the competent authorities responsible for designating the testing, where the results of a test indicate a risk to human health (usually the local authority).

1.3.2 Process hygiene criteria

Process hygiene criteria define the acceptability of the process (40). These apply at specific stages of processing, manufacturing, and handling until the food is placed on the market, and are not applicable to products placed on the market. Process hygiene criteria set an indicative

acceptability value for both pathogens and indicator organisms above which corrective actions are required in order to ensure that the hygiene of the process is compliant with food law. These criteria are outside the scope of the UKHSA guidelines because of the stage of application within the food chain. However, process hygiene results can help understand the level of control of food safety management systems of a food business operator, during outbreak or incident investigations. Process hygiene criteria use a 3-class plan (unsatisfactory, acceptable/borderline or satisfactory) as well as 2-class plans.

A value of ‘m’ is defined as a threshold below which samples are considered as satisfactory and ‘M’ as a value above which samples are unsatisfactory and are present in both food safety criteria and process hygiene criteria. Since multiple samples are usually required, an unsatisfactory result can also be defined when a specified number of samples have results falling between m and M: results are interpreted as borderline when m is exceeded but not sufficiently to be classified as unsatisfactory. For example, the process hygiene criterion 2.1.8 for *E. coli* in meat preparations at the end of the manufacturing process requires 5 samples (n) to be tested with unsatisfactory results if the *E. coli* level exceeds 5,000 cfu/g (M) in any sample or if more than 2 (c) of the 5 samples have levels between 500 cfu/g (m) and 5,000 cfu/g (M) (40). If a process hygiene criterion is exceeded, this should prompt the FBO to review the current procedures to improve production hygiene and may also prompt further testing. For example, under criterion 2.2.3, when detecting more than 10^5 coagulase-positive staphylococci /g in cheese made from raw milk, the cheese batch must be tested for staphylococcal enterotoxins under food safety criterion 1.21. FBOs are encouraged to trend process hygiene results, and an upwards trend can suggest a potential loss of effectiveness of their food safety management systems.

Where samples generate unsatisfactory or borderline results for indicator organisms according to the interpretation in the UKHSA RTE guidelines, a review of all hygiene procedures is recommended, and this review includes results and trends from process hygiene testing. Consideration should also be given to the likely changes in results of microbiological testing (for levels of *E. coli* and Enterobacteriaceae) for food samples collected during manufacture as compared to those placed on the market. Examples of these final considerations are given in tables 2a and 2b.

1.4 Intended use of the guidelines

These guidelines are intended for use by Food Examiners within UKHSA as well as enforcement officers in local authorities and port health authorities in identifying situations requiring investigation for public health or food safety reasons. Samples can be collected under the following circumstances:

- during investigations of suspected outbreaks of disease
- following complaints
- during food hygiene inspections

- to confirm previous adverse findings and to determine the scale of microbiological contamination
- during predefined sampling programmes such as part of UKHSA co-ordinated microbiological food studies
- routine or ad hoc checks

Single samples are often collected and are not associated with any formal sampling plan. However, follow-up actions that require testing to demonstrate legislative compliance should be done in accordance with the requirements of the appropriate regulations (that is, including multiple samples from a batch where specified in the legislation). Follow-up testing plans are best designed in conjunction with advice from a Food Examiner (6, 10, 41) or other appropriately qualified food microbiologist to ensure that the most appropriate food and environmental sampling is performed.

Test result interpretations derived from these guidelines will support risk assessment specific for the food type under examination. Such risk assessments should also consider the intrinsic properties of the food such as pH, salt content and water activity, as well as the extrinsic properties such as food packaging, including gas composition of modified atmosphere packaging, key processing factors such as storage temperature and shelf-life. All these factors should be considered as well as the sampling framework, the selection of microbiological tests and, in some instances, the intended final consumer. Predictive modelling (using computer models such as Combase) may be helpful in determining whether intrinsic factors such as pH, salt content and water activity are likely to limit the growth of pathogenic bacteria during the shelf-life of the food product.

1.5 Sampling and transport of samples

In order to carry out the appropriate microbiological examination of food and provide a meaningful interpretation of test results, it is essential that samples are collected in a suitable manner using the correct equipment. Sampling itself is probably the greatest contributory factor to the variability of a result as microorganisms are not usually homogeneously distributed in a contaminated foodstuff. Although a detailed consideration of sampling is beyond the scope of this document, some general considerations are given below.

The sampling procedure may vary depending on the type of food, and the reason for sampling. If food-handling practices within a catering premises are being investigated, it may be appropriate to sample the food using the utensils that would normally be used for handling or serving the food. However, if a sub-sample of food is to be examined as supplied by the producer, the sample should be collected using sterile utensils.

When sampling food it is recommend that:

1. At least 100 grams of food is required, unless an alternative quantity has previously been agreed with the laboratory. Larger samples may be necessary in specific instances where a more sensitive detection limit is required for a pathogen of concern.
2. Where intact foods are to be examined, the whole sample in its original packaging is placed inside a food-grade plastic bag.
3. For aseptic sampling of open packs, take a portion of the food using appropriate sterile utensils. This will normally be a representative portion of all components but may be a specific portion such as a core sample, surface sample or filling. Place the food sample into a sterile food-grade plastic bag or sterile jar, taking care not to allow the sample to touch the outside or top edge of the container.
4. Label the container with the location, sample details, sender's reference, sampling officer and date and time of sampling. Record the sender's reference and any other relevant information, such as the reason for and place of sampling, temperature of storage, type of packaging and type of sample, country of origin and durability dates on the laboratory submittal form. When a secure chain of evidence is required, place the container in an evidence bag or into another food-grade bag sealed with a tamper evident tag.

If it is not possible to immediately commence testing, samples can be stored in a tamper-evident cool-box overnight, provided that it is properly packed with an adequate number of frozen ice packs (at least 10% of the total cool box volume) or transferred to a secure fridge or cold-room, and submitted to the laboratory as early as possible on the following day.

A calibrated temperature logging device should be placed between food samples. Food Examiners and other staff within each UKHSA FWEMS laboratory will be able to advise on the correct packing of insulated boxes for refrigerated sample transport. The following should also be considered:

- store fresh or refrigerated samples in a cool box between 2°C and 8°C taking care to keep raw foods in a separate box from RTE foods
- hot products should not be included in the same transport container as ambient, chilled or frozen products
- frozen products should be either transported at or below -15°C or transported in a refrigerated cool box (0°C to 8°C) as they will be defrosted on arrival for testing at the laboratory
- some ambient-stable products do not require refrigerated transport (for example, powdered products and cans), and it is acceptable to transport these at ambient temperatures. It is good practice to use tamper evident containers and ensure that they are not exposed to temperatures above 40°C as this may affect their microbiological content

The Food Law Practice Guidance (England) (10) section 4.6.12 requires that for "food samples, the temperature of transport must be monitored, and recorded on receipt at the laboratory" and that "the temperature of storage must be such as to minimise microbial change, and be monitored using a calibrated thermometer or other similar device" so that "samples for examination reach the laboratory in a condition microbiologically unchanged from that existing

when the sample was taken" (section 4.6.15). Furthermore, ISO/TS 17728:2015 on sampling techniques for microbiological analysis of food and feed samples (50) states that "Transport time to the laboratory should be as short as possible and should be no more than 24 hours and in controlled temperature conditions to ensure maintenance of sample integrity. All necessary steps shall be taken to avoid changes to the intrinsic microbiota and these should be documented".

Testing of RTE food samples requiring refrigeration will usually commence within 24 hours of receipt; however, a longer time period may be acceptable provided the food sample is tested within its shelf life and within 36 hours of collection. Such samples should be held at 1°C to 5°C in the laboratory until work commences. Frozen samples are thawed in a refrigerator at 1°C to 5°C prior to testing. If testing cannot begin within an appropriate time on the day of receipt, it may be appropriate to keep the sample frozen at less than -18°C until such time as the sample can be processed, but this will depend on the target organisms being investigated and will need to be agreed with a Food Examiner. Where shelf life studies are required, ambient stable or refrigerated foods should be maintained at appropriate storage temperatures in a controlled environment until testing commences.

In certain circumstances, such as where food fails to comply with food safety requirements of the Food Safety Act 1990 (39), or during the investigation of a food poisoning outbreak, Authorised Officers may submit food samples with the intention that formal enforcement action and/or legal proceedings will ensue if an adverse microbiological result is obtained. These foods are termed formal samples and are handled and examined in such a way as to comply with the procedures laid down in the Food Safety (Sampling and Qualifications) Regulation 2013 (41), and the Food Standards Agency Food Law Practice Guidance 2021 (10). When it is anticipated that a formal sample is to be taken, the Authorised Officer should notify the relevant Food Examiner in advance to get advice on appropriate samples, sampling technique, tests to be carried out and to ensure prompt receipt and handling on arrival at the laboratory. Upon notification that formal samples are likely to be submitted to the laboratory, the Food Examiner is also responsible for ensuring that appropriate advice and guidance on all aspects of transport is provided to ensure that the authorised officer carrying out sampling follows all necessary procedures.

1.6 Microbiological methodology

Laboratory methods used in FWEMS laboratories are generally designed either for detection (that is, to determine whether the specified microbiological target is present in a specified weight or volume of the sample) or for enumeration, where the number of bacterial cells (or colony forming units) of the specified microbiological target is determined per g or mL of food. The final reported result relies on the detection of a particular target in pure culture which is often the subject of further tests for confirmation of identity and characterisation. The use of one or both approaches (detection or enumeration) enhances the ability to monitor and investigate contamination throughout the food chain. Test methods are specified for Official Control sampling (3, 6, 38, 46) and are defined in ISO standards. Within some of the ISO standard

methods, alternative methods are available when it can be demonstrated that equivalent results are achieved. In order to achieve a greater level of standardisation within the UKHSA, FWEMS laboratories use national standard methods that comply with ISO standards and are fit for purpose for Official Control work. However, for public health investigations, and for reasons of increased speed or sensitivity, different methods or sample sizes may be utilised providing they have been validated or verified appropriately (that is, establishment of the performance characteristics of a method and provision of objective evidence that the performance requirements for a specified intended use are fulfilled). In practice, additional methods used in FWEMS are usually based on the ISO methods specified for Official Control, with clear justifications for any deviations which are usually minor technical changes in the method that are not expected to affect the result (for example, additional confirmatory tests, change in kit manufacturer, constricted temperature ranges). It is common practice for 25g of food to be tested with the assumption that absence of a specific target organism in 25g is satisfactory. Testing of more (or less) food may however be done during outbreak investigations or when sampling is based on Regulation (EC) 2073/2005 as amended (40). Some RTE foods are taken as Official Control samples, and note should be taken of the testing requirements for the food safety or process hygiene microbiological criteria in Regulation (EC) 2073/2005 as amended (40). Formal samples will be processed in the same way as all other samples, however additional assurance will be provided for evidential purposes and to enable compliance with legislative requirements.

To ensure that the results obtained following testing of a food can be properly interpreted, it is essential that the testing methodology and the test selection is appropriate. Test selection can be relatively straightforward when investigating a food poisoning incident where the hazard is known or suspected, and the recovery of the specific pathogen is one of the major reasons for testing. In contrast, considering appropriate microbiological testing of a food for the purpose of assessing safety and quality during routine monitoring can be challenging due to the diversity of the food types encountered. Guidance is given in Appendix 1 and Appendix 2 for test selection in a range of food types in the absence of further information. This guidance is based on the physico-chemical nature of the food matrix, the amount of processing involved in production and information on the distribution (both prevalence and levels) of specific groups of bacteria. A Food Examiner will be able to give further advice on the use of these and suitable deviations for specific situations.

Accreditation is a formal recognition of a laboratory's competence to conduct testing. For Official Controls, the competent authorities designate laboratories that operate, and are assessed and accredited, in accordance with the relevant European Standards (7), which for the UKHSA FWEMS laboratories is EN ISO/IEC 17025 'General requirements for the competence of testing and calibration laboratories' (51). Compliance with this standard is assessed by the United Kingdom Accreditation Service (UKAS) and accreditation relates to individual tests or groups of tests. The schedule of accredited tests of any accredited laboratory can be obtained from the UKAS website.

1.7 Interpretation of results

The interpretation of laboratory results in food microbiology is often the most difficult and complex aspect of the examination process. All laboratories estimate the uncertainties associated with testing (also known as measurement uncertainty), however users of the guidelines should be aware that the precision and reproducibility of microbiological tests also depend on other factors, some of which are outside the control of the laboratory. Microbiological testing will also have limits of sensitivity, hence the use of the wording ‘not detected’ (or ‘less than 20’) as opposed to ‘not present’. As previously discussed in section 1.4, sampling itself is likely to be the greatest contributory factor to the variability of a result for a particular sample as microorganisms are not usually homogeneously distributed in a contaminated foodstuff. The sample matrix, the type of packaging, and the ability to culture injured microorganisms will also contribute further to the reproducibility between microbiological results. Results should therefore be interpreted in context, taking such factors into consideration.

1.7.1 Polymerase chain reaction (PCR)

The use of PCR as part of microbiological testing of food samples has been introduced by UKHSA FWEMS laboratories for different target pathogens. There are 3 approaches for the use of PCR: the first is to act as a negative screen for pathogens in enrichment broths to allow a focused testing of presumptive positive samples and the second is as a more rapid confirmation tool for presumptive positive isolates. The third approach, used for STEC, is where there is no alternative culture method and the PCR method will provide information on the presence of genetic markers which encode virulence factors associated with human infection. The use of PCR to detect STEC (together with genes associated with a higher likelihood of causing disease) is a requirement in the amended microbiological criteria for sprouted seeds in Regulation (EC) 209/2013 (38). It is anticipated that PCR targeted against a greater range of microbiological hazards will be required by legislation for Official Control testing in the future. There may be technical reasons for an inability to confirm the presence of a specific pathogen by culture, for example, there may be inhibition by the background microbiota (for example, STEC is more difficult to isolate in the presence of high levels of generic *E. coli*) or PCR techniques may be more sensitive than conventional culture (25). Furthermore, organisms containing target genes may be non-viable, toxin genes may occur in other organisms which do not cause disease or even in phages (viruses) which have been induced as a result of the food matrix or growth in culture media. Therefore, while PCR is a powerful tool that improves the laboratory’s ability to detect pathogens, interpretation of results for the detection of a specific pathogen which has not been confirmed by conventional culture-based microbiological testing is problematic and will be reported as ‘presumptive’ detection. Food Examiners will assist in the interpretation of results obtained using PCR technologies.

1.7.2 Pathogens

The presence of any potentially pathogenic bacteria in RTE food represents a risk to health. Pathogens are not generally found in RTE food that has been adequately prepared, particularly

those that have been properly cooked and have not been subject to cross-contamination. Investigation should always be considered where contamination with pathogens has been demonstrated. These investigations should be carried out with an urgency of response proportional to the level of contamination, the scale of distribution and the population at risk. For each target described here, the individual parts of the Table 1 series give details of the possible causes of contamination and suggest the appropriate action to take when a pathogen is detected in a RTE food. The detection of low numbers of some groups of pathogens is interpreted as of low risk to consumers, although their presence may indicate faults in the production process and/or subsequent handling of a food product which, if not controlled, could lead to an unacceptable increase in risk. Pathogenic bacteria are often unevenly distributed in foods, therefore the levels of contamination found, and the subsequent interpretation of the significance of this, may vary between sub-samples. The detection of low levels of pathogens in RTE foods warrants consideration, and a risk assessment of the intended use of the specific food will be necessary. Low levels of pathogens can be crucial in understanding contamination routes within a food chain implicated in an outbreak. In addition, more stringent criteria may be justified for foods served to vulnerable people since they are likely to be both more susceptible and at greater risk of developing more serious disease.

1.7.3 Hygiene indicator organisms

The significance of hygiene indicator bacteria in RTE food is included in the scope of these Guidelines. Although these bacteria are not generally an inherent hazard, their presence, particularly above specified levels, is generally agreed to indicate one or more of the following:

- faecal contamination
- poor hygiene, inadequate food handling or cross-contamination
- substandard conditions or inadequate practices for processing (particularly temperature and time control) which may result in undercooking
- use of low quality raw materials or food components
- unsatisfactory cleaning practices

Tests for hygiene indicator organisms in RTE foods are often a more rapid and effective way of assessing hygiene than the detection of pathogens, since detection of the pathogens can be more time consuming and complex than that for the indicators. Although the detection of pathogens is often performed in parallel with indicator organisms, pathogens in food are often heterogeneously distributed and present in low numbers making detection difficult. Furthermore, the enumeration of indicator organisms is routinely used to verify effective implementation of food safety management systems such as good agricultural practices (GAP), good manufacturing practices (GMP), good hygiene practices (GHP) and hazard analysis critical control point systems (HACCP). However, it should be emphasised that testing should never be relied upon as a food safety management strategy, but rather should complement and verify existing management systems.

Indicator organisms for food microbiology can include coliform bacteria, Enterobacteriaceae, *Escherichia coli* or species of *Listeria* other than *L. monocytogenes*. The presence of indicator organisms does not necessarily indicate pathogenic organisms are present in the same food sample; however this is usually indicative of loss of control of the food safety management system, and increased risk of pathogen presence. Therefore, the detection of an indicator organism in a food raises concerns that a pathogen may also be present, if not in the tested sample, then in previous or subsequent batches of this food. Below are some examples of associations between indicator organisms, pathogens and public health metrics that have been recently reported:

- there was a significant association between increasing levels of indicator *E. coli* and elevated levels of coagulase positive staphylococci, detection of STEC genes (*stx*) by PCR but decreased probability of isolation of STEC in cheese made from unpasteurised milk (25)
- a significant association between the presence of *Salmonella* species and increasing levels of *E. coli* in imported edible leaves (24)

There are a number of recommended actions listed in the Table 2 series that could be considered in response to an unsatisfactory result for the presence of indicator bacteria. Several foods from the same premises with borderline levels of hygiene indicators should prompt further investigation. Food Examiners will be able to discuss likely reasons for these results which may help inform corrective actions and provide supporting evidence which may be suitable to form part of formal enforcement actions. It is advised, however, that prosecutions based only on results of indicator organisms are less likely to be successful.

1.7.4 Aerobic colony counts

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 usually directly contribute towards a safety assessment of RTE food. ACCs have been used in previous editions of these Guidelines and are widely used elsewhere as part of a general microbiological quality assessment and will also allow an informed judgement about the adequacy of heat treatment, processing failures, storage conditions or the level of post-process contamination (55, 56). Since ACC testing in UKHSA laboratories is usually performed together with detection of pathogens and indicator organisms, consistent interpretation of ACC results has proved a useful addition to the scope of these guidelines. ACC results that fail to comply with the guideline levels stated here cannot be used alone to support a product recall or to take legal action against a business but can be used to highlight possible deficiencies in processing or food preparation practices, storage conditions or shelf-life of the food being produced.

1.8 Reporting of results

Test reports are issued by UKHSA FWEMS for all samples submitted for testing and are the only authoritative reports of the test results. Test reports will also state the accreditation status

of the tests performed, and if results are reported for non-accredited tests, this is clearly stated on the report. Results from other laboratories or sources are outside the control of UKHSA and should be used (and interpreted) with an awareness of any differences in the laboratory methods and procedures that were applied.

1.8.1 Formal samples

These may be taken either at the time of a routine inspection of a food premises or on subsequent visits if officers feel that there is a need to verify the quality and safety of food sold from that premises, and with a view to pursuing legal action if the results show an offence has been committed. Formal samples are taken in accordance with The Food Safety (Sampling and Qualifications) Regulations 2013 (41) and tested in an Official Laboratory (7). Food Examiners will ensure that formal samples are stored, examined, results verified, a report and certificate of examination issued, and the sample eventually discarded according to laboratory procedures. Reports of testing formal samples will be generated with reference to appropriate legislation or guidance and signed by an authorised signatory, usually a Food Examiner. Certificates of examination can also be produced by the Food Examiner in accordance with the requirements of the Food Safety (Sampling and Qualifications) Regulations 2013 (41). On request, the Food Examiner will prepare a witness statement in relation to the samples submitted for examination which can be used as part of a prosecution, and they can also advise on a suitable person to provide an expert witness statement.

1.9 Secondary specialist and reference tests

Specialist and reference tests are available for foodborne pathogens and their toxins, the results of which will provide considerable added value to those from initial testing during epidemiological investigations. Specialist and reference tests are usually only available at national or international reference laboratories and because of the specialist and complex nature of these tests, results may not be available as quickly as results of primary tests. Public health actions and interventions should be taken based on the primary results and must not be delayed pending the results of specialist and reference tests. Public health responses may change following the results of secondary tests such as the detection of a specific toxin, a variant of a pathogen more likely to cause serious disease or a previously unrecognised association with an outbreak. Food Examiners will be able to advise on the availability of these specialist tests, how to access them and what additional actions to take. They can also advise on the suitability of food samples for specialist testing as well as transportation.

Specialist or reference tests may be performed for:

- comparative analyses for strain characterisation (typing) to establish likely relationships between cultures from patients and from samples collected during outbreaks and at different times or from different places in the food chain – this is now often achieved by whole genome sequencing (WGS)

- detection of toxins, and/or the ability or potential to produce toxin (presence of toxin genes) likely to influence disease or disease severity
- distinction, where possible, between non-pathogenic and pathogenic variants of the same species
- confirmation of the microbiological results from the primary laboratory
- identification of viruses and parasites or unusual pathogens

Some specialist tests are specified in legislation such as detection of:

- *Cronobacter* species, Regulation (EC) 2073/2005 as amended (40)
- staphylococcal enterotoxins, Regulation (EC) 2073/2005 as amended (40)
- histamine (scombrotoxin), Regulation (EC) 2073/2005 as amended (40)
- marine biotoxins in live bivalve molluscs: Regulation (EC) 853/2004 as amended (46)
- *Trichinella* species in meat: Regulation (EC) 2015/1375 (57)

Other tests may be required on an ad hoc basis such as detection of *Clostridium botulinum* neurotoxin, *Bacillus* species toxins, norovirus and parasites. There may be limited or no availability in the UK for some tests, particularly for diseases rarely occurring in the UK.

1.10 Environmental samples

Environmental samples are outside the scope of these guidelines and microbiological criteria for interpretation of microbiological results from environmental samples are covered by separate UKHSA guidance (49). Taking appropriate and targeted environmental samples is recommended when unsatisfactory results from RTE foods are found and should also be considered for borderline values. Results from testing of the food processing environment can make a substantial contribution to food safety in the following situations:

- during an outbreak or 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 microbiological evidence to link a particular site to an outbreak of foodborne disease
- during an investigation when poor microbiological results have been found or during an inspection of premises, to help identify a source of contamination, or where there are concerns about the potential for cross contamination
- as part of a follow-up investigation to assess the effectiveness of deep cleaning of premises that have been shown to be contaminated with pathogens

2. Detection of pathogens

2.1 Introduction

Examination for the presence of pathogens in RTE food products contributes to the management of food safety (21, 28, 30, 23, 33, 34). Symptoms of foodborne disease are not only confined to gastroenteric symptoms (for example, diarrhoea and vomiting) and may include systemic infection (for example, septicaemia, meningitis, jaundice). The collection of information on symptoms during public health investigations can be important to determine which tests are applied to foods. In addition, because certain pathogens are disproportionately associated with specific food groups, these are reflected in the suggested sample testing algorithms (Appendix 1 and Appendix 2). 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 microorganisms in RTE foods is discussed in the following sections and the Table 1 series. Details on some of these pathogens are provided in this section including the most common foods associated with them and the settings or locations more frequently associated with outbreaks of disease. The most common routes of transmission, the known host risk factors for more severe infection, the symptoms and possible consequences of infection, and their frequency as a cause of human illness, are also detailed.

Large studies of infectious intestinal disease have been used to estimate the reporting rates in the community for each case reported to national surveillance. So, for example, for every case of human *Campylobacter*, norovirus, *Salmonella*, STEC and *Clostridium perfringens* reported to national surveillance, it was estimated that there were 9, 288, 5, 7 and 2,519 cases of these infections respectively occurring in the community (53). Foodborne diseases of microbiological origin can be caused by a variety of microbiological agents (viable organisms and/or toxins produced by microorganisms) which gain entry to the rest of the body via the gastrointestinal tract following consumption. The dose response (the likelihood of disease at different levels of exposure) is often poorly understood for bacterial pathogens. However, the likelihood of disease will be influenced by 3 main factors, each with major uncertainties:

1. Interactions between the food matrix and the pathogen: The food matrix may interact with the pathogen, in some instances altering the organism's physiological state and its ability to cause disease. Some food matrices, including those high in fat (for example, chocolate) may increase survival of some pathogens through the stomach and increase susceptibility of the host to diseases.
2. The pathogen (virulence): Within an individual species of bacteria, there may be variation in the ability to cause disease. For some species this is poorly understood. However, for the purposes of almost all legislation and guidance (including these guidelines), species or groups of pathogens are considered equally: for example, all

Salmonella species as well as all *Listeria monocytogenes* should be regarded as capable of causing disease.

3. The host: Host risk factors can also affect the susceptibility to foodborne disease. These include age, immune status, underlying disease, pregnancy, stress factors, and the physiological state of the stomach and intestines at the time of exposure to the agent: for example, the use of antacids by patients will increase susceptibility to infection by reducing stomach acidity and therefore its protective effect.

It is generally agreed that there is a dose response, in that there is a direct relationship between the level of exposure from food and the outcome, that is, the greater the numbers of organisms ingested in food, the more likely that disease will develop. At low exposures, there is still a risk of developing disease, albeit this risk for some agents is small. Because of the range of very different food types and matrices associated with food-borne disease, as well as the complexity and variation in the disease process and variation in susceptibility within the human population, there is unlikely to be a single (or indeed simple) infective dose for an individual pathogen. Consequently, a minimum infectious dose cannot be defined, although general conclusions on the likelihood of disease occurring after a specific exposure can be inferred from prior knowledge of the individual agent.

The presence of foodborne agents that cause illness in RTE foods is a significant risk to consumers' health and ensuring that they are absent is of paramount importance. Detection of foodborne pathogenic agents at any level is of concern and should be investigated with an urgency of response proportionate to the agent, level of contamination and risk to consumers. Any level of *Campylobacter, Salmonella* and STEC are considered as unacceptable in a RTE food. Low numbers of certain pathogens such as coagulase-positive staphylococci, *C. perfringens, B. cereus*, and *L. monocytogenes* in RTE products can represent a risk, even to immunocompetent people, but the risk is generally low: higher levels of these organisms are much more likely to present a significant risk of illness. However, these risks are of much greater concern for the immuno-compromised and vulnerable groups. Low levels may be due to natural contamination of raw materials used in foods, but usually their presence suggests faults in the production or subsequent handling of food which, at a later stage of the life of the food, could lead to an unacceptable increase in risk. There may also be a need for action when detecting low numbers of these organisms in RTE foods because there is variation in host susceptibility and inter-strain differences in the pathogenicity of these bacterial species.

2.2 Other pathogens and microbiological toxins

A range of other bacteria, viruses and parasites, as well as toxic metabolic by-products of microbiological origin, can also cause foodborne disease, but are not covered in detail in these guidelines. Examples of these include:

- viruses: norovirus, rotavirus, hepatitis A virus, hepatitis E virus

- bacteria causing disease less commonly in the UK: *Brucella* species, *Coxiella burnetii* (causative agent of Q fever), *Cronobacter* species, other pathogenic *E. coli* (enterotoxigenic, enteroinvasive, enteropathogenic and enteroaggregative), *Mycobacterium bovis*, *Shigella* species (including *S.sonnei, S. flexneri, S. boydii* and *S. dysenteriae*)
- parasites: *Anisakis* species, *Fasciola hepatica*, *Cyclospora cayetanensis*, *Toxoplasma gondii*, *Cryptosporidium* species, *Giardia* species, *Taenia solium* and *T. saginata*, *Trichinella spiralis*
- toxic metabolites: aflatoxins, botulinum toxin, marine biotoxins (DSP, PSP, ciguatera, ASP), scombrotoxin, staphylococcal enterotoxins

The diseases caused by most of these agents are rare in the UK and consequently there is a low demand for detection of these agents in food. UKHSA Food Examiners will be able to advise on the availability of tests and what samples are applicable to be tested.

Statutory microbiological criteria for some of these (for example, *Cronobacter* species, scombrotoxin, marine toxins, staphylococcal enterotoxins and *Trichinella* species) are briefly mentioned in section 1.9.

3. Hygiene indicator organisms

3.1 Enterobacteriaceae

Enterobacteriaceae are a family of bacteria that are used to assess the general hygiene status of a food product. This family includes species that originate from the intestinal tract of animals and humans, as well as from plants and the environment. Therefore, these bacteria are not reliable indicators of contamination by faecal pathogens in a food. All Enterobacteriaceae are killed by the usual processes used in food production (including cooking and pasteurisation) and should be readily removed from equipment, surfaces and food production environments by appropriate cleaning procedures. Their presence in heat-treated foods signifies inadequate cooking or post-processing contamination. High levels of these bacteria can occur in some uncooked or unprocessed food commodities such as salad vegetables, due to their natural microbiota as well as cross-contamination from the environment (for example, via water run-off from agricultural land and untreated irrigation water) during primary production, including at harvest. The use of sanitising rinses may reduce but not entirely remove these organisms, which may contribute to spoilage of such food products if allowed to multiply. The presence of Enterobacteriaceae should be interpreted in conjunction with test results from other microbiological parameters but detection in several foods from the same premises or in other areas of the food production environment should be investigated. When Enterobacteriaceae are present at high levels (>10^4 cfu/g) in cooked or processed foods, it suggests an overall poor general hygiene status of a food product. These criteria do not apply to certain cheeses ripened using starter cultures containing *Hafnia alvei* or *Proteus vulgaris.* Further information can be found in Table 2a.

Coliform bacteria (coliforms) are a sub-group within the Enterobacteriaceae family and are used in some guidance and statutory criteria as a hygiene indicator. However, Enterobacteriaceae are increasingly used in preference to coliforms as they represent a broader group of organisms that also includes pathogenic species such as Salmonella. There is an exception with raw drinking milk as specified in the Food Safety and Hygiene (England) Regulations 2013 (47) which requires that it must meet the standard of less than 100 cfu/ml for coliforms (as well as a plate count of less than or equal to (≤) 20,000 cfu/ml).

Some Enterobacteriaceae can contribute to the formation of histamine (scombrotoxin) in foods such as scombroid fish (for example, mackerel and tuna) and occasionally fermented foods such as cheeses and salami if these are not processed properly and/or stored at the correct temperature. Ingestion of fish with high histamine levels is harmful, and maximum permissible levels of less than (<) 200 or <400 mg/kg of histamine (depending on the type of product) are set by Regulation (EC) 2073/2005 as amended (40). Histamine testing in food is not performed by UKHSA FWEMS laboratories but testing for this toxin may be required during investigations of illness and is provided by public analysts. Illness is typically characterised by flushing of the

face and upper body, severe headache, palpitations, abdominal cramps and diarrhoea, usually within 10 to 60 minutes of consumption of associated food products.

Regulation (EC) 2073/2005 as amended (40) includes process hygiene criteria for Enterobacteriaceae with limits (M) of:

- 10 cfu/ml for pasteurised milk and other pasteurised dairy products at the end of the manufacturing process (n=5, c=0, m=M)
- 10 cfu/g for milk powder and whey powder at the end of the manufacturing process (n=5, c=0, m=M)
- 100 cfu/g for ice cream and frozen dairy products at the end of the manufacturing process (n=5, c=2, m=10 cfu/g)
- absence in 10g of dried infant formulae and dried dietary foods for special medical purposes intended for infants below 6 months of age (n=10, c=0, m=M) and dried follow-on formulae at the end of the manufacturing process (n=5, c=0, m=M)
- 100 cfu/g in egg products at the end of the manufacturing process (n=5, c=2, m=10 cfu/g)

Where samples generate unsatisfactory or borderline results for indicator organisms according to the interpretation in these guidelines, a review of all hygiene procedures is recommended, and this review includes results and trends from process hygiene testing during manufacture. Consideration should also be given to the likely changes in Enterobacteriaceae levels for food samples collected during manufacture compared to those placed on the market.

3.2 *Escherichia coli*

Escherichia coli belongs to the coliform bacteria group and is therefore classified within the Enterobacteriaceae family. *E. coli* is widely used for assessing the hygiene status of food products and is an indicator of either human or animal faecal contamination. The organism is killed by heat processes used in food production (including pasteurisation) and should be readily removed from the factory, equipment and surfaces, by appropriate cleaning procedures. The presence of *E. coli* in a final product therefore indicates contamination of starting materials or ingredients, cross-contamination during manufacture or of the final product, with high levels suggesting that growth has occurred. Further information can be found in Table 2b.

E. coli may sometimes be found in soft, mould-ripened or washed-rind cheese made from raw milk. Although Regulation (EC) 2073/2005 as amended (40) does not include criteria for *E. coli* in cheese made from raw milk, it is recommended that these cheese types be routinely tested for *E. coli* in order to detect adverse trends or unusually elevated levels. Guidance for raw cheese enforcement from the Scottish Food Enforcement Group (54) recommended that a target level of <100 cfu/g is achievable for some cheese types, and where this is exceeded, further evidence should be provided to verify food safety.

The *E. coli* criteria in these UKHSA RTE guidelines are not applicable to live bivalve molluscs and live echinoderms, tunicates and marine gastropods placed on the market during their shelf-life for which the food safety criteria in Regulation (EC) 2073 as amended (40) must be applied.

Regulation (EC) 2073/2005 as amended (40) includes process hygiene criteria for *E. coli* with limits (M) of:

- 1,000 cfu/g for cheese made from milk or whey that has undergone heat treatment, sampled at the time during the manufacturing process when the *E. coli* count is expected to be the highest (n=5, c=2, m=100 cfu/g)
- 100 cfu/g for butter and cream made from raw milk or milk that has undergone a lower heat treatment than pasteurisation at the end of the manufacturing process (n=5, c=2, m=10 cfu/g)
- 10 MPN/g for shelled and shucked products of cooked crustaceans and molluscan shellfish at the end of the manufacturing process (n=5, c=2, m=1 MPN/g)
- 1,000 cfu/g for pre-cut fruit and vegetables and unpasteurised fruit and vegetable juices at the end of the manufacturing process (n=5, c=2, m=100 cfu/g)

These criteria are applicable during or at the end of the manufacturing process, and *E. coli* levels in these foods should not increase between manufacturing and being placed on the market.

The standard tests used to enumerate indicator *E. coli* in food and environmental samples involve the detection of β-glucuronidase enzyme activity. Whilst the majority of *E. coli* produce this enzyme, this cannot be used for the detection of pathogenic strains such as *E. coli* O157 (STEC O157) and some other STEC. Different methods are required for the detection of the pathogenic *E. coli* and these have greater sensitivity than those used to enumerate generic *E. coli*. Methods to detect STEC in foods are used during outbreak investigations or where there is a specific concern (please refer to Table 1e for information on STEC).

3.3 *Listeria* species

Listeria species are able to grow at temperatures below 0°C and above 40°C but are killed by temperatures such as those used for pasteurisation, although they show a greater resistance to heat than the Enterobacteriaceae. The presence of *Listeria* species in foods that have undergone heat treatment indicates undercooking or post-process contamination. *Listeria* species are also environmental contaminants that can survive in both food processing premises and on equipment if adequate hygiene measures are not used. These organisms readily form biofilms and are less susceptible to the cleaning procedures used in food processing environments than many other bacteria and may become established in the food processing environment, particularly in moist areas such as drains and wet floors. They may also be found around the feet or base of equipment which is not easily moved, where thorough cleaning underneath is difficult.

The term '*Listeria* species' is fully inclusive of all species including *L. monocytogenes*, and failures in processes that allow the presence and growth of other *Listeria* species will be equally permissive to the presence and growth of *L. monocytogenes*. Therefore, the detection of these organisms should be seen as an indication of an increased risk of *L. monocytogenes* and, in some cases, growth of other *Listeria* species may actually mask the presence of *L. monocytogenes* in a sample. For high-risk food products that will allow the growth of *Listeria* species during their shelf-life, it is recommended that an enrichment (detection) method be used in addition to enumeration as this is more sensitive and can ensure that there is an absence of *Listeria* species in 25g of food (Table 2c).

4. Aerobic colony counts

If used correctly, ACCs can provide useful information about the general quality and shelf-life of the food being tested, and thus highlight potential problems of storage and post production handling. However, they are not deemed a priority in a risk-based analysis. Elevated ACCs should indicate the following interpretations and follow-up actions:

- if an ACC is above the expected level, a determination of the predominant constituent organisms and their respective levels is needed before any follow-up investigation is instigated
- high counts may suggest quality or hygiene issues and possible poor temperature control and these should be investigated

In an effort to simplify the interpretation of these levels, the interpretation of ACCs used here (Table 3) is based on the categorisation of foods into broad groups based on the level of handling, intrinsic and extrinsic properties, and effects of processing and storage on the microorganisms present, and the stage of production.

It must be noted that, due to the diversity of food preparation practices, some of the types of foods submitted may be applicable to more than one food category. For example, rice could be categorised as category 2 if hot when collected, category 3 if collected from a chilled batch that has not been extensively handled, category 5 if it has been portioned and other cooked foods added or category 12 if RTE raw vegetables have been added to create a rice salad product. Furthermore, knowledge of a specific production process may result in selection of a different category to that suggested in Table 3. An example of this would be that homemade coleslaw produced on a very small scale with a short shelf-life may be more appropriately placed in category 12 while coleslaw produced commercially on a large scale with a long shelf-life would be placed in category 7. Examples of foods belonging to each category are provided in the tables however, a good understanding of the product type, preparation process and the stage of production at the time of sampling is needed in order to fully interpret the ACC.

An ACC result of greater than (>) 10^6 cfu/g is usually associated with a predominant organism, and the acceptability and organoleptic quality of the food will depend on what type of organism predominates. In meat products, for example, the microbiota frequently consists almost entirely of lactic acid bacteria (mainly lactobacilli and streptococci), which 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 to 10^8 cfu/g. Pseudomonads tend to produce taints, discolouration and slime, whilst other Gram-negative bacteria frequently produce slime only. Yeasts may cause spoilage at slightly lower levels (10^6 to 10^7 cfu/g) due to acid and gas production. *Bacillus* species can cause spoilage problems such as ‘ropiness’ in bread and ‘bittiness’ in milk. However, if high levels of *Bacillus* species are found in other food products, this may be due to the addition of pepper or other spices after heat treatment, as dried spices

have been frequently shown to be contaminated with relatively high numbers of *Bacillus* spores. If elevated *Bacillus* levels are found, investigation of the full preparation process is recommended. If ACCs are high, it is therefore important to identify the predominant organism type to allow interpretation of the significance of the ACC result that is observed. Tests by the laboratory for catalase and oxidase production and a Gram-stain are usually sufficient to achieve the differentiation needed to interpret results.

Advice is available from a Food Examiner and further explanation on the different categories of foods is given below. Examples of foods belonging to each category is provided in Table 3.

Category 1

Category 1 foods include canned, bottled or poached products that are microbiologically stable at ambient temperatures. These foods will typically not contain viable microorganisms. Occasionally, thermotolerant spores may survive the production process. Samples found to have an elevated level of thermotolerant spores are not normally considered to be a cause for concern provided that the pH and/or water activity (a_w) is sufficiently low to prevent outgrowth of the spores. Further testing to determine the pH/a_w of category 1 food would be recommended to enable interpretation of the result when counts are higher than expected. Immediate action in response to high ACCs is not usually warranted except for shelf-stable canned or bottled food products immediately after opening.

Category 2

Category 2 foods include those that have been subjected to a rigorous heating process such as grilling, roasting or baking. These foods, if processed effectively, will have an ACC of $<10^3$ cfu/g. This category is applicable only if the sample is collected immediately after processing, that is, collected whilst hot.

Category 3

Category 3 foods include cooked food that has been chilled but has not been extensively handled and where no procedure to disrupt the structural integrity of the food has been applied following the cooking process (including products collected immediately after a pasteurisation heat process).

Category 4

Bakery products, confectionery and powdered foods following reconstitution.

Category 5

Category 5 foods include cooked food that is chilled followed by handling that disrupts the structural integrity of the food (for example, slicing, shucking or portioning). These types of products usually have higher ACC counts than food that is subjected to minimum handling. The

introduction of bacteria to these products can, however, be minimised by good hygiene, both of personnel and of equipment.

Category 6

Non-fermented dairy products (including pasteurised milk), butter, cream, dairy desserts and ice-cream. These food types will have higher ACC counts as they are not highly processed and are often subject to handling as part of the preparation process. (Note that there is a specific standard for raw milk in the Food Safety and Hygiene (England) (Amendment) Regulation 2016, which requires that it must meet the standard of ≤20,000 cfu/ml (as well as a coliform count of <100 cfu/ml)).

Category 7

Food mixed with dressings, dips or pastes and those that have been produced with ingredients that have been lightly processed and/or subjected to handling. Consequently, they are associated with a high ACC count.

Category 8

Products with an extended shelf-life. The type of packaging used for these foods (for example, modified atmosphere packaged and vacuum packaged food) can influence the rate of microbial growth and this packaging is often used to extend product shelf-life. Vacuum packaging will retard the growth of obligate aerobic organisms due to the exclusion of oxygen. The temperature of refrigeration for perishable products also influences the microbial growth rate; storage below 8°C will prevent growth of most foodborne pathogens (with the notable exceptions of *L. 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 the product shelf-life. As the duration of storage increases, the ACC also increases, and this will occur more rapidly if refrigeration temperatures are poorly controlled or if the food is frequently taken in and out of refrigeration.

Category 9

Raw meat and fish, eaten uncooked or cold-smoked. These products naturally have ACCs of around 10^6 to 10^7 cfu/g. Where counts are $>10^7$ cfu/g then an assessment of the predominant microbiota is required to interpret the result.

Categories 10 to 13

Foods which are not routinely examined to determine the ACC. These food categories include preserved products, dried foods, RTE fresh fruit and vegetables and fermented products that are known to have naturally high ACCs. The intrinsic properties of preserved and dried foods, including low pH and low water activity (a_w), control bacterial outgrowth and the ACC would only be examined as part of an investigation of spoilage of these foods. RTE food commodities such as salad vegetables have naturally high ACCs due to the microbiota acquired from the

environment during growth and harvest. This will limit their shelf-life as spoilage may occur relatively rapidly. This also applies to any product containing RTE uncooked fruit or vegetables as an ingredient. Fermented foods are those produced by adding starter cultures of bacteria or those where the natural microbiota in the ingredients are encouraged to grow in order to achieve a natural fermentation. The predominant organisms are therefore the starter bacteria, and other bacteria are usually present only in low numbers due to the acidity produced during food fermentation. The ACC of these products is naturally high and only relevant in the context of food spoilage.

The stage of shelf-life must also be considered when interpreting the ACC of a food. If sampled at the point of production, ACC should be well within the ‘satisfactory’ levels described in Table 3 whereas if food is sampled at the end of shelf-life an ACC can normally be expected to approach the upper ‘borderline’ levels.

5. Tables

This section contains the tables relating to pathogens, hygiene indicators and Aerobic Colony Counts as describe above.

The pathogen tables (Table 1 series) give details of aetiology, incidence of infection, symptoms, host risk factors, exposures, the typical incubation period, transmission and recommended control measures. A table to facilitate interpretation of results for each pathogen is also included suggesting likely causes and suggested action to take. Where pathogens are detected at injurious levels in a RTE food, immediate action is recommended to review the process and take further investigative samples of food, raw food components and the food preparation environment.

The hygiene indicator tables (Table 2 series) give details of likely causes and suggested actions. Where samples generate unsatisfactory or borderline results for indicator organisms according to the interpretation in the UKHSA RTE guidelines, a review of all hygiene procedures is recommended, and this review may include results and trends from process hygiene testing during manufacture. Consideration should also be given to the likely changes in the levels of hygiene indicators for food samples collected during manufacture compared to those placed on the market.

The Aerobic Colony Count table (Table 3) below include details of foods belonging to each category. The list of foods is not exhaustive and other food types can be included depending on the intrinsic and extrinsic characteristics of a food.

Table 1a.i. *Bacillus cereus* in RTE foods placed on the market

Aetiological agent and incidence of infection	*Bacillus cereus* group comprises at least 8 species of spore forming bacteria most of which are found widely within the environment. Disease is caused by toxin produced in food prior to ingestion as well as consumption of viable organisms. Food poisoning is typically caused by *B. cereus* and possibly by *B. thuringiensis which is also a member of this group*. Incidence is unknown: cases are likely to occur but be undiagnosed due to rapid onset, short duration and mild symptoms.
Symptoms and host risk factors [note 1]	Symptoms usually mild and short-lived lasting about 12 to 24 hours. Host risk factors for severe infection are unknown. Emetic syndrome (preformed toxin): vomiting, nausea with some diarrhoea. Diarrhoeal syndrome (viable organisms): diarrhoea and abdominal pain.
Exposure and incubation period	Emetic: disease after exposures to >10^5 cfu/g followed by 0.5 to 5 hours incubation. Diarrhoeal: disease after exposures to >10^5 cfu/g, followed by 8 to 16 hours incubation.
Transmission	All cases are foodborne, often from commercial catering environments. Spores likely to survive food processing (including cooking); temperature and time abuse of food can lead to germination and sufficient growth of the organism to cause disease. For the emetic syndrome, disease results from production of emetic toxin in the food prior to consumption and is often associated with farinaceous products such as rice. Diarrhoeal syndrome results from ingestion of viable organisms which produce diarrhoeal toxin(s) in the gut and is associated with a wider range of products including meat products, soups, vegetables, puddings and sauces. Not all strains produce toxins. The emetic toxin is pre-formed in food and is both acid and heat stable so foods may be toxic in the absence of viable *B. cereus*.
Control measures	The most important control measure is temperature control followed by the need to ensure cross-contamination does not occur. Rapid chilling of food to below 4 to 5°C after cooking or holding above 60°C is recommended. Reheating food to a temperature of above 75°C. *B. cereus* grows between 10 and 50°C, although some will grow at 4°C.
Additional information	Due to the widespread nature of these bacteria in the environment, all foods and food ingredients have the potential to be contaminated by spores. As spores may survive the cooking process, people are frequently exposed to low numbers of *B. cereus* through food without becoming ill. Currently laboratory tests cannot reliably distinguish pathogenic and non-pathogenic strains. *B. thuringiensis* is used as an insecticide, and high levels can therefore occur in some food products of non-animal origin. Microbiological criteria exist for *B. cereus* in dried infant formulae and dried dietary foods for special medical purposes intended for infants below 6 months of age (40). *B. cereus* are reported by UKHSA laboratories as 'presumptive *B. cereus'* unless associated with an outbreak when confirmation and typing is performed. Counts >10^5 cfu/g may be sent for confirmation of identity and typing if linked to cases of illness. Actions should not be delayed pending results of specialist tests.

[Note 1] Features other than those described may occur but are generally considered less common than those listed.

Table 1a.ii. Interpretation of results for enumeration of *Bacillus cereus* from RTE foods placed on the market [note 1]

Result (cfu/g)	Interpretation	Likely cause	Suggested actions (not exclusive)
$>10^5$	Unsatisfactory: Potentially injurious to health and/or unfit for human consumption	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.
10^3 to $\leq 10^5$	Borderline	Likely evidence for poor processing, poor quality raw materials, or poor temperature control	Risk will increase proportionally 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.
$<10^3$	Satisfactory	n/a	n/a

[Note 1] See additional information in Table 1bi on bacillus in spices. This may be equally applicable to *B. cereus*.

Table 1b.i. *Bacillus* species (including *B. subtilis* group) in RTE foods placed on the market

Aetiological agent and incidence of infection	A group of spore forming bacteria most of which are found widely within the environment. Food poisoning reported from *B. subtilis* group *(B. subtilis, B. licheniformis, B. pumilis,* and *B. amyloliquifaciens*) which occur less frequently than *B. cereus* food poisoning. Incidence unknown: cases likely to occur and not be diagnosed due to mildness and short duration of symptoms.
Symptoms and host risk factors [note 1]	Similar symptoms to *B. cereus*. Acute onset vomiting often followed by diarrhoea but can also be diarrhoea accompanied infrequently by vomiting. Usually mild and short lived, lasting 24 to 36 hours. Illness is strain and possibly species dependant. Host risk factors for severe infection are unknown.
Exposure and incubation period	Disease after exposures to over 10^5 cfu/g followed by 2 to 15 hours incubation.
Transmission	All cases are foodborne. Foods prepared from cooked meat, poultry and vegetables and farinaceous products such as rice and bread are most commonly associated with disease in commercial catering. Spores likely to survive food processing (including cooking) and temperature and time abuse of food can lead to germination and sufficient growth of the organism to cause disease. The exact mechanisms and toxins produced by this group are less well understood than for *B. cereus* but some may be associated with pre-formed toxin and some with viable organisms.
Control measures	As with *B. cereus* the most important control measure is temperature control followed by the need to ensure cross contamination does not occur. *B. subtilis* group grow at 10 to 50°C although some grow at 5 to 9°C.
Additional information	Not all of the *B. subtilis* group have the potential to cause disease; some natural fermentations which rely on production of very high levels of these bacteria result in safe products. This is specifically seen in soya products where *B. licheniformis* and *B. subtilis* occur, leading to difficulty in interpreting results (particularly with certain imported foods) unless it is known that these organisms are part of the production procedure. Spices and spice products (for example, pepper and curry paste) often carry a high load of *Bacillus* spores. Although not normally regarded as RTE foods, these products may be added to a RTE 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 these bacteria may then pose a health risk. Levels in spices exceeding 10^6 cfu/g are therefore regarded as unsatisfactory. If high levels of *Bacillus* species are found in RTE foods, the possibility that spices such as pepper have been added after the main cooking process should be investigated. There are no statutory microbiological criteria for *Bacillus* species. Where *Bacillus* species counts are equal to or less than 10^5 cfu/g, these are reported by UKHSA FWEMS laboratories as presumptive *Bacillus* species unless associated with an outbreak when confirmation and typing are performed. Counts of more than 10^5 cfu/g may be sent for confirmation of identity and typing. Actions should not be delayed pending results of specialist tests.

[Note 1] Features other than those described may occur but are generally considered less common than those listed.

Table 1b.ii. Interpretation of results for enumeration of *Bacillus* species (including *B. subtilis* group) from RTE foods placed on the market [note 1]

Result (cfu/g)	Interpretation	Likely cause	Suggested actions (not exclusive)
$>10^5$	Unsatisfactory: potentially injurious to health and/ or unfit for human consumption	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.
10^3 to $\leq 10^5$	Borderline	Likely evidence for poor processing, poor quality raw materials, or poor temperature control	Risk will increase proportionally to the levels detected. Food may not become hazardous provided appropriate levels of control are applied. Review temperature and time controls particularly for cooking foods. Consider taking investigative samples of food, raw food components and the food preparation environment.
$<10^3$	Satisfactory	n/a	n/a

[Note 1] Levels of 10^6 cfu/g in spices and spice products should be regarded as unsatisfactory (see notes in additional information Table 1bi).

Table 1c.i. *Campylobacter* species in RTE foods placed on the market

Aetiological agent and incidence of infection	A group of bacteria common in the intestinal tracts of birds and other animals. In humans, 90% of infections are caused by *Campylobacter jejuni* and most of the remaining by *C. coli*. Most common cause of bacterial gastrointestinal infection in the UK. Approximately 60,000 cases reported annually with approximately 9-fold under-ascertainment in the UK (53). Most cases of disease are recorded as sporadic, but outbreaks do occur.
Symptoms and host risk factors [note 1]	Diarrhoea (sometimes with blood), headache, abdominal pain; usually lasts 2 to 7 days. Sequelae include irritable bowel syndrome (most common), reactive arthritis and Guillain-Barré syndrome. Risk of infection increased for the very young and elderly and individuals with reduced immunity.
Exposure and incubation period	Disease likely at low exposures followed by 1 to 3 days incubation but up to 10 days can occur.
Transmission	Acquired through ingestion of viable organisms. Mostly foodborne and linked to undercooking of raw poultry meat/offal and cross-contamination from raw poultry to RTE foods. Less frequently acquired via water or by direct contact with animals. Person-to-person spread is rare. Foods most often associated with infection include chicken, chicken and duck liver pâté and parfait, unpasteurised milk and dairy products, untreated drinking water. Transmission is linked to consumption of food prepared outside the home, barbecues, inadequately treated drinking water supplies, contact with untreated waters, for example, during outdoor leisure activity and holiday on caravan/farm sites.
Control measures	The organism is unable to grow in food and is killed by heat treatment equivalent to pasteurisation. While freezing can reduce their numbers, viable campylobacters may still be present if the initial contamination level was high. Adequate cooking and application of good hygiene control during food preparation (for example, raw poultry) is important to prevent cross-contamination and ensure effective heat-treatment.
Additional information	Isolates from outbreaks should be submitted to the Reference Laboratory for confirmation and typing. Microbiological criteria exist for Campylobacter spp. in carcases after chilling (40) as process hygiene criteria with limits of 1,000 cfu/g in a proportion of carcases.

[Note 1] Features other than those described may occur but are generally considered less common than those listed

Table 1c.ii. Interpretation of results for detection of *Campylobacter* species from RTE foods placed on the market

Result in 25g [note 1]	Interpretation	Likely cause	Suggested actions (not exclusive)
Detected	Unsatisfactory: potentially injurious to health and/ or unfit for human consumption	Inadequate processing and/or cross contamination	Immediate investigation of the food origin, production process and environment. Take investigative food samples and consider environmental monitoring.
Not detected	Satisfactory	n/a	n/a

[Note 1] Testing of more or less food may be indicated during outbreak investigations.

Table 1d.i. *Clostridium perfringens* in RTE foods placed on the market

Aetiological agent and incidence of infection	Member of genus of anaerobic spore forming bacteria. *C. perfringens* is widespread in the environment and in the faeces of animals. Likely to be underdiagnosed due to the short duration of symptoms. Estimated to be approx. 90,000 cases in the community per year.
Symptoms and host risk factors [note 1]	Diarrhoea and abdominal pain usually lasting no more than 24 hours. Vomiting is rare. Host risk factors for disease not known but can be more serious in the elderly.
Exposure and incubation period	High likelihood of illness when exposed to $>10^6$ vegetative cells followed by an incubation period of 8 to 22 hours (usually 12 to 18 hours) which allows sporulation of the bacterium in the lower intestine and the production of enterotoxin which causes diarrhoea.
Transmission	Ingestion of contaminated food which has been subjected to inadequate temperature control after cooking allowing the germination of spores and multiplication of vegetative cells. *C. perfringens* diarrhoea is also transmitted by non-foodborne routes including person to person and antibiotic associated disease, especially in the elderly. Outbreaks are often associated with institutions which cater for large numbers of people such as hospitals, schools and hotels, where storage and temperature control of pre-prepared food has been inadequate. Most commonly associated foods include cooked meat and poultry dishes, leftover food, stocks and gravies. Seasonally associated with times of increased institutional catering such as Christmas dinners.
Control measures	The growth range of *C. perfringens* is between 15 and 52°C with no growth below 12°C. Control is achieved by preventing both the germination of spores and the growth of vegetative cells by rapidly cooling cooked food, adequate cold storage of cooked food followed by adequate reheating.
Additional information	Spores of *C. perfringens* in food can survive cooking. Slow cooling and unrefrigerated storage allows germination to form vegetative cells. Not all strains of *C. perfringens* contain the enterotoxin gene which, when expressed, forms a protein that causes food poisoning. Isolates from outbreaks should be sent for confirmation, typing and determination of the presence of enterotoxin genes. The presence of high numbers of non-toxigenic strains, although not pathogenic, should still be considered unsatisfactory in RTE food as they are indicative of poor processing, particularly cooling. There are no statutory microbiological criteria for *C. perfringens.*

[Note 1] Features other than those described may occur but are generally considered less common than those listed.

Table 1d.ii. Interpretation of results for enumeration of *Clostridium perfringens* from RTE foods placed on the market

Result (cfu/g)	Interpretation	Likely cause	Suggested actions (not exclusive)
$>10^4$	Unsatisfactory: potentially injurious to health and/ or unfit for human consumption	Strong evidence for poor temperature and time control particularly during cooling after cooking. Use of leftover food, stocks or gravies	Immediately review temperature and time controls. Take investigative samples of food and the food preparation environment.
10 to $\leq 10^4$	Borderline	Likely evidence for poor processing particularly cooling	Risk will increase proportionally 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.
<10	Satisfactory	n/a	n/a

Table 1e.i. Shiga-like toxin-producing *Escherichia coli* (STEC) of O157 and other O-serotypes in RTE foods placed on the market

Aetiological agent and incidence of infection	Members of a group of bacteria which are widespread in the enteric tracts of animals and containing shiga-toxin (*stx*) genes. The majority of STEC infections diagnosed in the UK are caused by serotype O157 followed by O26. Detection methods are increasingly identifying more cases due to non-O157 STEC. All STEC should be considered as pathogenic for humans and capable of causing at least diarrhoea. Based on the analysis of the *stx* subtypes, certain STEC subtypes may be associated with severe illness, that is, haemolytic uraemic syndrome (HUS), bloody diarrhoea (BD) and/or hospitalisation. Although *stx2a* showed the highest rates of HUS, hospitalisation and BD, all other *stx* subtypes or combinations thereof, for which there was sufficient data, are also associated with at least one of these severe illness outcomes. There are around 800 cases diagnosed annually in England and Wales.
Symptoms and host risk factors [note 1]	Symptoms can range from asymptomatic to mild gastroenteritis through to severe BD. On rare occasions, STEC infections cause serious conditions: HUS; and thrombotic thrombocytopenic purpura (TTP). Cases of HUS and TTP usually require hospitalisation and can be fatal. Variable duration of illness. Gastrointestinal illness may last from a few days up to a week while more severe disease may last longer. Disease more likely to develop in children and the elderly. Asymptomatic carriage is rare.
Exposure and incubation period	1 to 6 day incubation period. Low dose exposures are likely to cause disease.
Transmission	Acquired through ingestion of viable organisms. Most cases are foodborne, transmission also by consumption of untreated water, direct contact with animals or natural environments and person-to person spread. Foods frequently associated with infection include undercooked red meats, for example, beefburgers; salads and other leafy greens; unpasteurised milk and dairy products; fermented meats. Linked to foods prepared outside the home, untreated water or milk (for example, associated with leisure activity, farm/caravan sites), children's nurseries and petting farms. Animal reservoirs include cattle, sheep, goats, pigs, horses, farmed deer, dogs, rabbits and geese.
Control measures	Killed by heat-treatment (equivalent to pasteurisation). May survive low pH (3.6 to 4.0), drying in food and fermentation processes, and for extended times in natural environments (for example, cow pats). Can grow in foods (7 to 46 ºC) and survives well at chill temperatures. Hygiene controls during food preparation (for example, raw beef) are important to prevent cross contamination.
Additional information	Also known as Vero-cytotoxin producing *E. coli* (VTEC). There is a requirement in European food law (Regulation (EC) 209/2013) for sprouted seeds to be tested for the presence of STEC O157, O26, O111, O103, O145 and O104.

[Note 1] Features other than those described may occur but are generally considered less common than those listed.

Table 1e.ii. Interpretation of results for detection of *Escherichia coli* O157 and other shigatoxin-producing *E. coli* (STEC) from RTE foods placed on the market

Result in 25g [note 1]	Interpretation	Likely cause	Suggested actions (not exclusive)
Detected [note 2]	Unsatisfactory: potentially injurious to health and/or unfit for human consumption	Inadequate processing and/or cross contamination Contamination of untreated raw products (for example, salads).	Immediate investigation of: the food origin, production process and environment; take investigative food samples and consider environmental monitoring.
Not detected	Satisfactory	n/a	n/a

[Note 1] Testing of more or less food may be indicated during outbreak investigations or when sampling is based on Regulation (EC) 2073/2005 as amended (40). There can be occasions of presumptive detection of STEC which are not confirmed by the isolation of the bacterium. It is not possible to interpret presumptive detection by PCR in the absence of isolation of an STEC in pure culture. Detection by PCR alone could indicate that background bacteria have caused interference with the isolation of STEC by culture. Alternatively, *stx* genes may potentially be present in species of bacteria other than *E. coli* ; or *stx* genes present in dead STEC organisms or free *stx*-containing phage may have been detected.

[Note 2] All STEC should be considered as pathogenic for humans and capable of causing at least diarrhoea and although stx2a showed the highest rates of HUS, hospitalisation and BD, all other stx subtypes or combinations thereof were also associated with at least one of these severe illness outcomes (58).

Table 1f.i. *Listeria monocytogenes* in RTE foods placed on the market

Aetiological agent and incidence of infection	Widespread in the environment and has transient residency in the intestinal tracts of animals. Almost all cases of listeriosis are due to *L. monocytogenes*. There are approximately 150 to 200 cases per year. Infection is potentially life threatening and listeriosis is the biggest single cause of death from a foodborne illness in Europe.
Symptoms and host risk factors [note 1]	Severe systemic infection including meningitis, septicaemia, pregnancy complications and still birth and other organs may become invaded. Healthy adults and pregnant women may only experience mild influenza-like symptoms or febrile gastroenteritis. The elderly, immunocompromised and the unborn infant are most at risk. The most common patient group are those over 60 years of age with underlying illness.
Exposure and incubation period	Disease likely at high exposures but will vary between different vulnerable groups, and may follow an incubation period of less than 24 hours to over 3 months.
Transmission	Acquired through ingestion of viable organisms in food. A wide range of RTE foods (often with extended refrigerated shelf lives) which allow the growth of this bacterium such as sandwiches (served in hospital), soft ripened cheese, pâté, smoked fish, butter and cooked sliced meat. Melon has been associated with cases in the USA. Foods which do not support the growth of the bacterium have also been associated with transmission and includes frozen sweetcorn and ice-cream. Various settings, particularly where vulnerable groups consume RTE foods capable of supporting the growth of the bacterium. The most common food vehicle linked with illness in England is pre-prepared sandwiches eaten in hospital.
Control measures	Killed by heat (including pasteurisation). Unrefrigerated foods and those stored chilled for extended periods are at increased risk of allowing growth, particularly if chilled temperatures are suboptimal. *L. monocytogenes* occurs commonly in the environment and in raw food: post-process contamination is a major risk from harbourage sites in food production environments. The organism can grow in food at temperatures between less than 0°C and 45°C

[Note 1] Features other than those described may occur but are generally considered less common than those listed

Table 1f.ii. Interpretation of results for detection and enumeration of *Listeria monocytogenes* from RTE foods placed on the market

Result (cfu/g)	Interpretation	Likely cause	Suggested actions (not exclusive)
>100 [note 1]	Unsatisfactory: potentially injurious to health and/or unfit for human consumption	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.
Detected [note 2] up to ≤100	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 storage conditions. When detected at ≤100 cfu/g it is also necessary to know whether the manufacturer has evidence to show that >100 cfu/g is not likely to be exceeded throughout the shelf-life of the product. [note 1] Review quality of raw materials, food preparation environment (including cleaning), cooking, temperature and shelf-life controls. Consider taking investigative samples of food and environmental swabs. 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.
Not detected <10/20	Satisfactory	n/a	n/a

[Note 1] Regulation (EC) 2073/2005 (as amended) (40) has a criterion for absence in 25g in RTE foods intended for infants and foods for special medical purposes placed on the market during their shelf life.
[Note 2] Includes where the organism has been detected by enrichment only in a 25g sample and at less than 10/20 cfu/g.

Table 1g.i. *Salmonella* species in RTE foods placed on the market

Aetiological agent and incidence of infection	*Salmonella enterica* are enteric bacteria which occur in a wide range of animals. All salmonellae can cause human disease but the serovars *Salmonella* Enteritidis and *S*. Typhimurium are the most common. *Salmonella* species are the second most common cause of bacterial gastrointestinal infection in the UK. Around 8,000 cases are diagnosed annually.
Symptoms and host risk factors [note 1]	Diarrhoea, vomiting, abdominal pain, fever; with illness lasting from several days to 3 weeks. Sequelae include septicaemia, inflammation of the abdominal wall and reactive arthritis. Infection occurs in all age groups; host factors may increase the susceptibility to infection, such as reduced immune status.
Exposure and incubation period	Exposure to relatively large numbers of bacteria likely to cause illness in healthy adults but exposure to lower levels important for the young and immune-suppressed. Incubation period is usually 12 to 48 hours.
Transmission	Salmonella infection is caused by ingestion of viable bacteria. Transmission is most often foodborne but direct transmission through contact with animals and person-to-person occurs. Foods commonly associated with infection include inadequately cooked eggs and poultry, or products containing these ingredients, such as egg mayonnaise. Many other foods have been linked to disease including pork, beef, dairy products, seeds, herbs, salad, vegetables, fruit, coconut, spices, nuts, fruit juice, chocolate and snack products. Consumption of food prepared outside the home and foreign travel are also risk factors. Some high fat or low water activity foods can protect Salmonella from processes such as heating as well as stomach acidity and increase chances of infection.
Control measures	Killed by heat-treatment (equivalent to pasteurisation); dried products may lead to greater heat tolerance. Organisms may survive low pH (3.6 to 4.0); drying and fermentation processes; extended times in natural environments and in frozen or dry foods (for example, chocolate, desiccated coconut). Salmonella may grow in foods over the temperature range 6 to 48°C and at pH 3.7 to 9.5 under otherwise ideal conditions. Application of good hygiene is important to prevent cross-contamination; temperature and time control are necessary during food preparation to prevent multiplication and ensure adequate heat-killing.
Additional information	Regulation (EC) 2073/2005 (as amended) (40) contains food safety and process hygiene criteria for some specific food and Salmonella combinations and the requirements to be complied with by FBOs.

[Note 1] Features other than those described may occur but are generally considered less common than those listed.

Table 1g.ii. Interpretation of results for detection of *Salmonella* species from RTE foods placed on the market

Result in 25g [note 1]	Interpretation	Likely cause	Suggested actions (not exclusive)
Detected	Unsatisfactory: potentially injurious to health and/or unfit for human consumption	Inadequate processing. Cross contamination. Contamination of raw foods and food ingredients.	Immediate investigation of: the food origin, production process and environment. Take investigative food samples and consider environmental monitoring.
Not detected	Satisfactory	n/a	n/a

[Note 1] Testing of more or less food may be indicated during outbreak investigations or when sampling is based on Regulation (EC) 2073/2005 (as amended) (40).

Table 1h.i. *Staphylococcus aureus* and other coagulase-positive staphylococci in RTE foods placed on the market

Aetiological agent and incidence of infection	Staphylococci are a group of bacteria common in the skin and mucous membranes of humans and animals. Most cases of staphylococcal food poisoning are due to enterotoxin produced by *S. aureus* from humans, although other animals are also carriers of this bacterium. Other coagulase-positive *Staphylococcus* species (for example, *S. intermedius*) can also produce enterotoxins and cause foodborne disease. Only some *S. aureus* contain enterotoxin genes and therefore have the potential to cause food poisoning. Incidence unknown but many cases likely to occur without being diagnosed due to short duration of symptoms which are usually mild.
Symptoms and host risk factors [note 1]	Acute, rapid onset nausea and vomiting, duration 1 to 2 days. Abdominal cramps and diarrhoea may occur. Most people susceptible. Symptoms due to ingestion of enterotoxin which is rapidly expelled, and long term effects are not reported.
Exposure and incubation period	Intoxication likely when $>10^5$ cfu/g has occurred at some time in the life of the food and >1 µg of enterotoxin is ingested. The incubation period is 2 to 6 hours.
Transmission	Foodborne, association with cross-contamination from food handlers or the environment. 30 to 50% of people carry *S. aureus* on their skin or mucous membranes which can also cause cutaneous infection. Contamination of foods after processing by food handlers is the most likely cause. Some domestic animals (sheep and goats) carry enterotoxigenic *S. aureus* which cause food poisoning, particularly in dairy products made from milk from these animals. The toxin is stable after some food processes including mild heat, reduction of pH and drying.
Control measures	Good hygiene of food handlers to prevent contamination. Adequate temperature control to prevent growth and cooking to kill viable cells. Good dairy hygiene during milking, particularly for unpasteurised milk. Commercially prepared processed and proteinaceous products most often implicated in food poisoning; for example, dairy and confectionery (milk, cream filled cakes, bakery products, cheese, ice cream), cooked meats and pasta. Optimal growth between 35 to 37°C (range 7 to 45°C) and will grow in high salt (> a_w 0.87). Bacterium killed by heat, but enterotoxins are heat-stable and can survive some normal cooking processes including limited boiling: biologically active toxin can therefore be present in cooked food in the absence of viable organisms.
Additional information	The only food safety criterion for staphylococci in Regulation (EC) 2073/2005 (as amended) (40) is for an absence of staphylococcal enterotoxins in cheese, milk powder and whey powder in product placed on the market during their shelf life. This regulation includes process hygiene criteria with limits of between 10 and 10^5 coagulase positive staphylococci (CPS)/g in cheese, milk and whey products 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 (and are not widely available in the UK), public health actions should be based on unsatisfactory CPS enumeration results and not be delayed pending staphylococcal enterotoxin results.

[Note 1] Features other than those described may occur but are generally considered less common than those listed.

Table 1h.ii. Interpretation of results for enumeration of *Staphylococcus aureus* and other coagulase-positive staphylococci from RTE foods placed on the market

Result (cfu/g)	Interpretation	Likely cause	Suggested actions (not exclusive)
$>10^4$	Unsatisfactory: potentially injurious to health and/ or unfit for human consumption	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.
20 to $\leq 10^4$	Borderline [note 1]	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 are opportunities for growth of staphylococci during processing or maturation of the product. Consider taking investigative samples of food, food preparation environment and food handlers.
<20	Satisfactory [note 1]		n/a

[Note 1] Since the organism may have grown and then died off, enterotoxin can be present even when there are satisfactory or borderline levels of *Staphylococcus aureus* and other coagulase-positive staphylococci in a RTE food placed on the market. Therefore, when associated with typical symptoms, toxin testing or direct microscopic examination of the food homogenate for Gram-positive cocci may be helpful.

Table 1i.i *Vibrio cholerae, V. parahaemolyticus* and *V. vulnificus* in RTE foods placed on the market

Aetiological agent and incidence of infection	*Vibrio* species are a diverse group of bacteria common in marine and estuarine environments. *V. cholerae*, *V*. *parahaemolyticus* and *V. vulnificus* infections are very rare in the UK. Almost all cases are travel-associated.
Symptoms and host risk factors [note 1]	*V. cholerae* (serogroups O1 and O139) causes cholera, which is characterised by profuse, watery diarrhoea. *V. parahaemolyticus* causes a milder gastroenteritis with symptoms including diarrhoea, cramps and nausea. *V. vulnificus* causes gastroenteritis but can also cause skin and soft tissue lesions with subsequent septic shock and bloodstream infections particularly in immunocompromised individuals. Foreign travel is a risk factor.
Exposure and incubation period	Infection generally after high exposures and onset usually within 24 hours (*V. parahaemolyticus*) or 2 to 3 days (*V. cholerae*) after consumption of contaminated food.
Transmission	For *V. cholerae*, consumption of untreated water and contaminated foods – usually imported (for example, shellfish). For all other species, consumption of raw or undercooked imported sea-foods particularly those produced in warmer areas of the world and imported to the UK (or foods cross-contaminated with seafood) and where the bacteria have been allowed to grow. Outbreaks are very rare in the UK. For *V. vulnificus*, exposure of damaged skin to contaminated seawater.
Control measures	The organisms are killed by adequate cooking. Food produced using good manufacturing practices pose only a negligible risk for transmission of *Vibrio* species. Other species such as *V. metschnikovii* and *V. alginolyticus* are occasionally isolated from seafood in the UK. Whilst these are not usually considered to be pathogenic, their presence may be seen as an indication of hygiene concerns such as cross-contamination and/or inadequate cooking.
Additional information	There are no statutory microbiological criteria for *Vibrio* species

[Note 1] Features other than those described may occur but are generally considered less common than those listed.

Table 1i.ii Interpretation of results for detection of *Vibrio cholerae* O1 and O139 from RTE foods placed on the market

Result in 25g [note 1] [note 2]	Interpretation	Likely cause	Suggested actions (not exclusive)
Detected	Unsatisfactory: potentially injurious to health and/or unfit for human consumption.	Inadequate processing. Cross contamination.	Immediate investigation of the food origin, production process and environment; take investigative food samples and consider environmental monitoring.
Not detected	Satisfactory		n/a

[Note 1] Testing of more or less food may be indicated during outbreak investigations.
[Note 2] Perform a risk assessment before any further action.

Table 1i.iii Interpretation of results for enumeration of *Vibrio parahaemolyticus* and *Vibrio vulnificus* from RTE foods placed on the market

Result (cfu/g) [note 1]	Interpretation	Likely cause	Suggested actions (not exclusive) [note 2]
$>10^3$	Unsatisfactory: potentially injurious to health and/or unfit for human consumption	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.
20 to $\leq 10^3$	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.
<20	Satisfactory		n/a

[Note 1] Testing of more or less food may be indicated during outbreak investigations.
[Note 2] Perform a risk assessment before any further action.

Table 1j.i *Yersinia enterocolitica* and *Y. pseudotuberculosis* in RTE foods placed on the market

Aetiological agent and incidence of infection	*Yersinia* species are a diverse group of bacteria belonging to the Yersiniaceae family of bacteria. Yersiniosis, which is caused by the enteric bacterial pathogens *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* is the third most commonly reported zoonotic infection in Europe (60). In the UK, diagnosis of yersiniosis is typically only attempted in patients with severe illness where yersiniosis is suspected or where immunological sequelae are evident following a gastrointestinal illness. In 2020 it was estimated that around 7,500 *Y. enterocolitica* infections go undiagnosed in England annually (60). The apparently low incidence of yersiniosis in England is probably due to limited laboratory testing.
Symptoms and host risk factors [note 1]	Gastrointestinal symptoms that can last for 1 to 3 weeks. Following infection some patients go on to develop secondary immunological complications, including erythema nodosum, arthritis, Reiter's disease and glomerulonephritis. *Y. enterocolitica* and *Y. pseudotuberculosis* have also been associated with causing a number of other primary acute infections, including mesenteric lymphadenitis, terminal ileitis and pseudo-appendicitis.
Exposure and incubation period	The infectious dose is thought to be high at 10^8 to 10^9 cells, the incubation time is about 3 to 7 days but can be between 1 and 11 days.
Transmission	Transmission from animals to humans can occur via direct contact with animals or with their environment. Pigs are the animal most associated with transmission of pathogenic *Y. enterocolitica* although most human gastrointestinal infections are foodborne. Outbreaks of yersiniosis associated with the consumption of contaminated meat, dairy products, and salad vegetables have been reported.
Control measures	This organism will grow at refrigeration temperatures. The organisms are killed by adequate cooking. Food produced under good manufacturing practices pose only a negligible risk for transmission of *Yersinia* species. The presence of *Yersinia* species in RTE food may be seen as an indication of hygiene concerns such as cross-contamination, inadequate cooking and/or poor storage.
Additional information	*Y. enterocolitica and Y. pseudotuberculosis* are conventionally identified using biochemical tests to genus and species level. *Y. pseudotuberculosis* is a homogeneous species, whereas *Y. enterocolitica* is heterogeneous, comprising 6 biotypes. Historically, biotype 1A was regarded as being non-pathogenic in humans and biotype 1B was regarded as highly pathogenic. Pathogenicity can also be linked to particular serotypes of *Y. enterocolitica*, with O:3, O:8, O:9 and O:5,27 being the serotypes most commonly associated with human illness. Virulent strains carry virulence genes ystA, invA and ail. Currently there are no statutory microbiological criteria for *Yersinia* species

[Note 1] Features other than those described may occur but are generally considered less common than those listed.

Table 1j.ii Interpretation of results for detection of *Yersinia enterocolitica* or *Yersinia pseudotuberculosis* from RTE foods placed on the market

Result in 25g [note 1] [note 2]	Interpretation	Likely cause	Suggested actions (not exclusive)
Detected [note 3]	Unsatisfactory: potentially injurious to health and/or unfit for human consumption	Inadequate processing. Cross contamination. Poor temperature control.	Immediate investigation of the food origin, production process and environment; Consider the Enterobacteriaceae count obtained from the sample and virulence characteristics; take investigative food samples and consider environmental monitoring.
Not detected	Satisfactory	n/a	n/a

[Note 1] Testing of more or less food may be indicated during outbreak investigations.

[Note 2] Perform a risk assessment before any further action.

[Note 3] Levels of Yersinia that are likely to cause harm are not fully understood, but investigation should be undertaken where it is detected in RTE food.

Table 2a. Interpretation of results for enumeration of Enterobacteriaceae from RTE foods placed on the market

Result (cfu/g)	Interpretation	Likely cause	Suggested actions (not exclusive)
$>10^4$	Unsatisfactory	Poor hygiene due to undercooking, or cross contamination from raw ingredients, staff 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 up to $\leq 10^4$	Borderline	Possible evidence of poor hygiene due to undercooking, or cross contamination from raw ingredients, staff 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	n/a

Table 2b. Interpretation of results for enumeration of *Escherichia coli* from RTE foods placed on the market

Result (cfu/g)	Interpretation	Likely cause	Suggested actions (not exclusive)
$>10^2$	Unsatisfactory	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.	Review cooking and all hygiene procedures including cleaning. Take investigative samples of food and undertake environmental monitoring of the food preparation environment.
20 up to $\leq 10^2$	Borderline	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.	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	n/a

Table 2c. Interpretation of results for detection and enumeration of *Listeria* species. (not *L. monocytogenes*) from RTE foods placed on the market

Result (cfu/g)	Interpretation	Likely cause	Suggested actions (not exclusive)
$>10^2$	Unsatisfactory	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.
Detected [note 1] up to $\leq 10^2$	Borderline	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.
Not detected <20	Satisfactory	n/a	

[Note 1] Includes where the organism has been detected by enrichment only in a 25g sample and at <20 cfu/g.

Table 3. Guidance on the interpretation of results for aerobic colony count levels in category 1 to 13 RTE foods placed on the market

Food category		Examples	Result (cfu/g)		
			Satisfactory [note 1]	Borderline [note 2]	Unsatisfactory [note 3]
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, canned fruit; UHT products, jams, chutneys, sauces such as pasta sauce.	<10	n/a	Check pH and a_w [note 4]
2	Foods cooked immediately prior to sale or consumption	Takeaway food without salad, burgers, kebabs, sausages, pizza, cook or chill and cook or freeze after regeneration.	$<10^3$	10^3 to $<10^5$	$\geq 10^5$
3	Cooked foods chilled but with minimum handling prior to sale or consumption	Whole pies, sausage rolls, samosas, flans, quiches, chicken portions, canned ham, pasteurised foods including fruit juice, soups, sauces, desserts, rice.	$<10^4$	10^4 to $<10^7$	$\geq 10^7$
4	Bakery and confectionery products without dairy cream; powdered foods	Cakes without dairy cream; soup powders, milk powder, powdered dairy products; other powdered foods that will be RTE after reconstitution or warming.	$<10^4$	10^4 to $<10^6$	$\geq 10^6$
5	Cooked foods chilled but with some handling prior to sale or consumption	Sliced meats, cut pies, pâté, sandwiches without salad, hot smoked fish (for example mackerel), molluscs, cooked crustaceans and other shellfish out of shell.	$<10^5$	10^5 to $<10^7$	$\geq 10^7$ determine predominant flora [note 5]
6	Non-fermented dairy products, butter, dairy desserts, cooked sauces [note 6]	Pasteurised milk, butter, cream, ice cream, fresh cheese (mascarpone, paneer), trifle with dairy cream, cakes with dairy cream, cooked chilled sauces and gravy	$<10^5$	10^5 to $<10^7$	$\geq 10^7$

Food category		Examples	Result (cfu/g)		
			Satisfactory [note 1]	Borderline [note 2]	Unsatisfactory [note 3]
7	Food mixed with dressings, dips, pastes	Coleslaw (mass produced only), dips, taramasalata, houmous, sandwich fillings containing mayonnaise.	$<10^6$	10^6 to $<10^7$	$\geq 10^7$
8	Extended shelf-life food products requiring refrigeration	MAP or vacuum packed products, for example, sliced meats, unsmoked fish.	$<10^6$	10^6 to $<10^8$	$\geq 10^8$ determine predominant flora [note 5]
9	Raw RTE meat and fish, cold smoked fish	Sushi containing raw RTE fish, smoked salmon, gravadlax.	$<10^6$	10^6 to $<10^7$	Indicative of spoilage if the predominant organism: [note 7]
10	Preserved food products: pickled, marinated or salted	Pickled or salted fish, cooked shellfish in vinegar, vegetables in vinegar or oil, honey, jam and chutney out of open jars.	n/a		ACCs not usually performed. For spoilage investigation, consider investigating the reasons for an elevated level if the predominant organism: [note 7]
11	Dried foods	Fruits, berries, vine fruits, nuts, sunflower seeds, herbs, spices, dried fish, biltong, jerky, insects.			
12	Fresh fruit and vegetables, products containing raw vegetables.	Whole fruit, pre-prepared fruit salads, vegetable crudités, salads, sandwiches with salad, mixed commodity salads containing raw vegetables.			
13	Fermented, cured and dried meats, fermented vegetables, ripened cheeses	Continental sausages or salamis, sauerkraut, olives, bean curd, cheddar, stilton, brie, fermented milk drinks and butter, yoghurt and products containing the above.			

Notes

[Note 1] Satisfactory: no action required.

[Note 2] Borderline: consider the source of the food (for example, producer or retailer) 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.

[Note 3] Unsatisfactory: consider investigating the reasons for this level.

[Note 4] Food category 1:

- most products are 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 and the pH (>4.4) or a_w (>.0.92) of the food is unable to control potential growth of these bacteria.

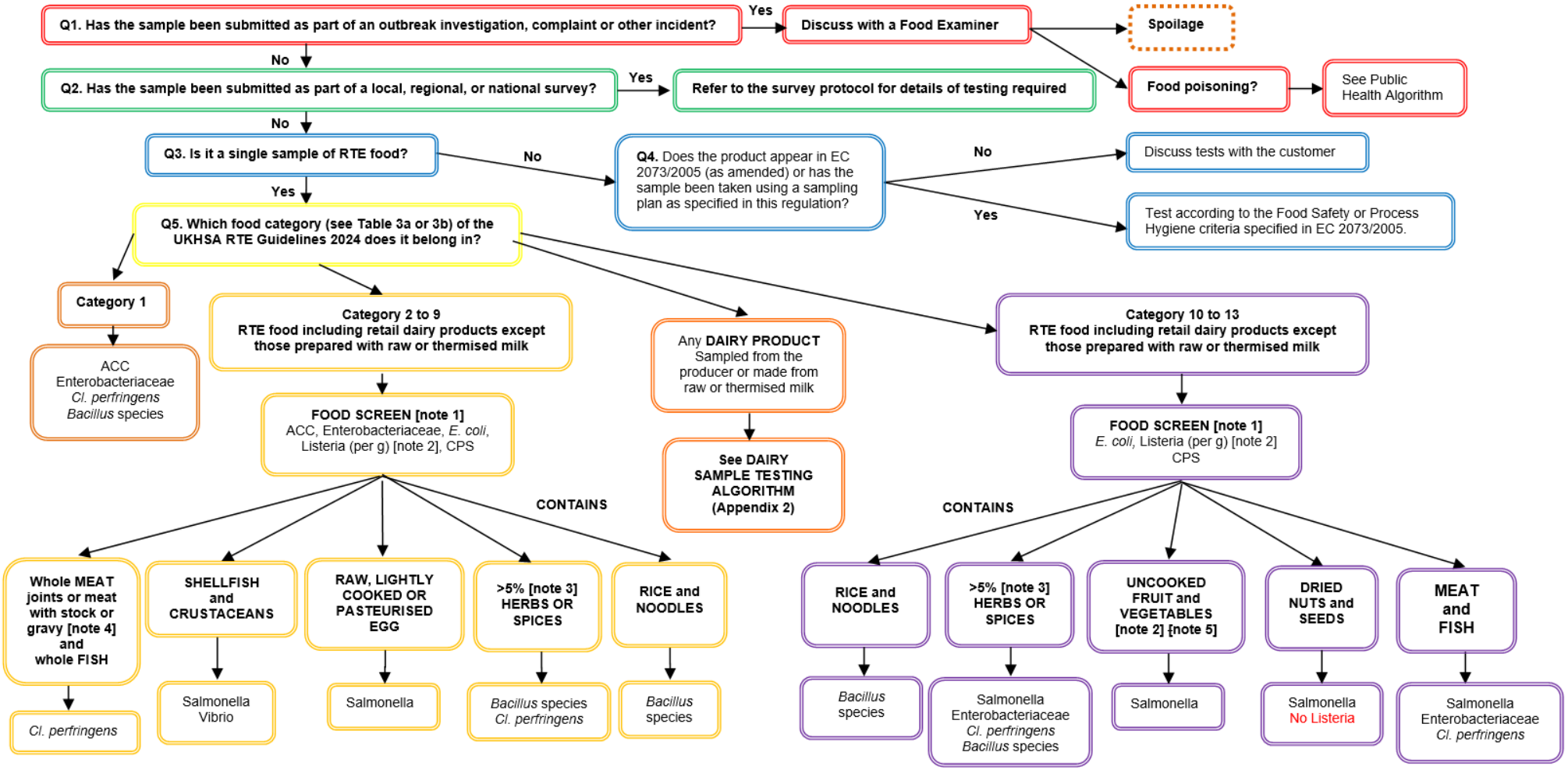
[Note 5] Food categories 5 and 8. Determine the predominant micro-organism. ‘Unsatisfactory’ if the predominant organism is $>10^6$ yeasts, $>10^7$ Gram negative bacillus or *Bacillus* species, or $>10^8$ lactic acid bacteria.

[Note 6] Food category 6. Separate standards exist for raw milk in the Food Safety and Hygiene (England) Regulations 2013 (47), requires that it must meet the standard of ≤20,000 cfu per ml.

[Note 7] is $>10^6$ yeasts, $>10^7$ Gram negative bacilli or *Bacillus* species, or $>10^8$ lactic acid bacteria unless added as a processing aid.

Appendices

Appendix 1. UKHSA FWEMS food sample testing algorithm



Notes

[Note 1] If the product is dried or frozen consider using the MPN method for *E. coli*.

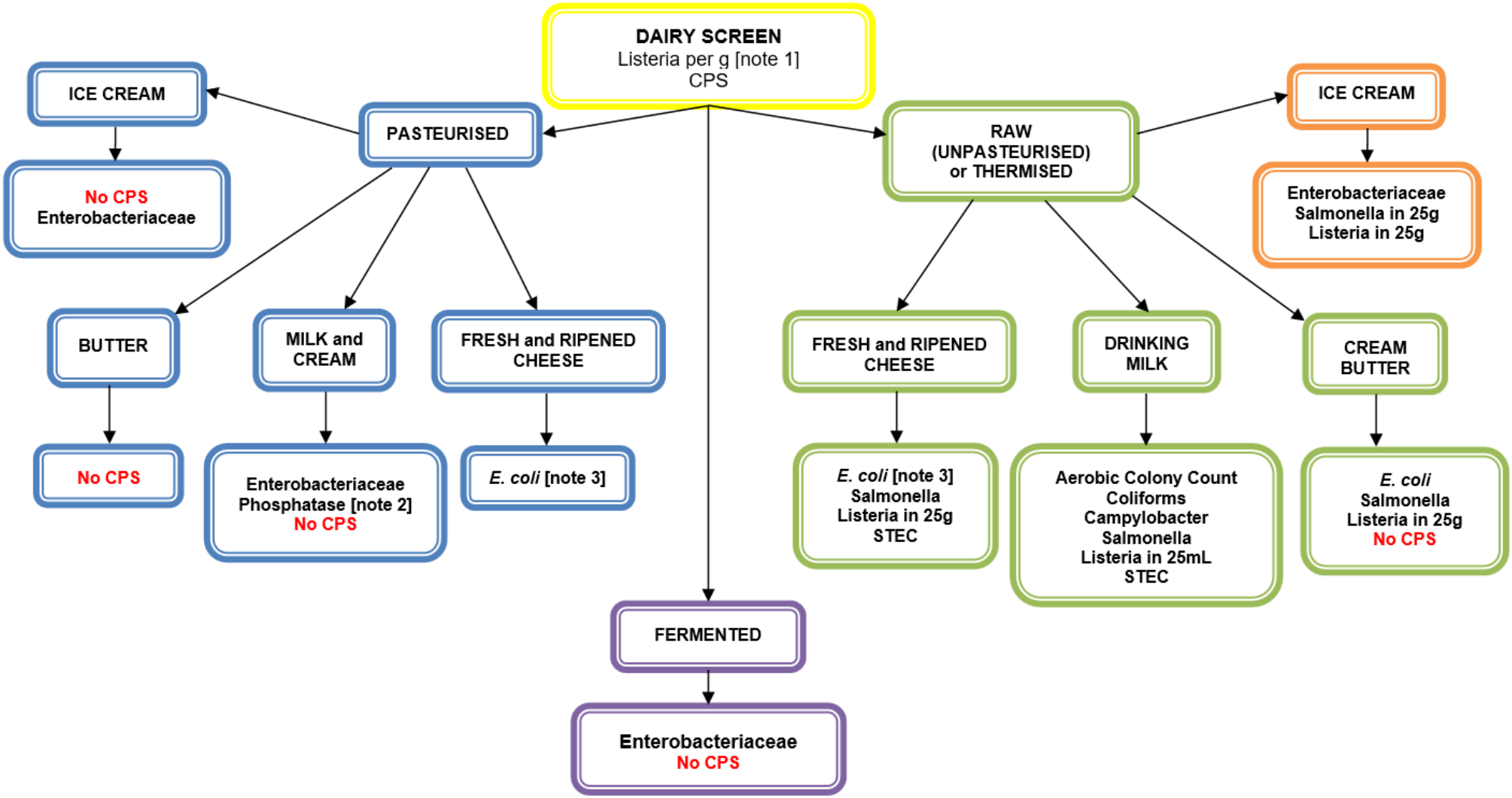
[Note 2] If the product has been sampled as part of an outbreak investigation, has been sampled from the producer, has a shelf-life of >4 days or is for consumption by high risk groups perform detection in 25g.

[Note 3] Test products with less than 5%, if herbs or spices are an unprocessed garnish or additive.

[Note 4] Test for Campylobacter if the sample is a poultry pâté or parfait.

[Note 5] RTE sprouted seeds should not be tested for *E. coli* or Coagulase positive staphylococci (CPS) but should otherwise be tested in accordance with the algorithm with inclusion of Shiga toxin producing *E. coli* (STEC) and Listeria in 25g.

Appendix 2. UKHSA FWEMS dairy sample testing algorithm



Notes

[Note 1] Test 25g or 25ml if sample has a shelf-life of more than 4 days or if it is deemed able to support the growth of Listeria (pH >than 4.4, a_w >0.92 or >pH5.0 / a_w 0.94 in combination).

[Note 2] Phosphatase testing should not be carried out on whipped or clotted cream. There are no statutory limits for alkaline phosphatase in cream.

[Note 3] If the product is ripened consider using the MPN method for *E. coli*.

Appendix 1a. Text version of the FWEMS food sample testing algorithm

Question 1
Has the sample been submitted as part of an outbreak investigation, complaint or other incident?

- If yes, the testing requirement will be discussed with a Food Examiner and a decision made as to what testing is performed this may include consideration of spoilage organisms or the likely organisms that cause food poisoning. The Food Examiner may look at other sources of information, for example, a public health algorithm.

- If no, go to question 2.

Question 2
Has the sample been submitted as part of a local, regional or national survey?

- If yes, refer to the survey protocol for details of testing required.

- If no, go to question 3.

Question 3
Is it a single sample of RTE food?

- If no, go to question 4.

- If yes, go to question 5.

Question 4
Does the product appear in EC 2073/2005 (as amended) or has the sample been taken using a sampling plan as specified in this regulation?

- If no, discuss tests with the customer.

- If yes, test according to the food safety or process hygiene criteria specified in EC 2073/2005

Question 5
Which food category (see Table 3) of the UKHSA RTE guidelines 2024 does the food belong in?

- If category 1, test for ACC, Enterobacteriaceae, *Cl. perfringens* and *Bacillus* species.

- If categories 2 to 9 (RTE food including retail dairy products except those prepared with raw or thermised milk) perform a food screen [including ACC, Enterobacteriaceae, *E. coli*, Listeria (per g) and coagulase positive staphylococci (CPS). If the product is dried or frozen consider using the MPN method for *E. coli*. If the product has been sampled as part of an outbreak investigation, has been sampled from the producer, has a shelf-life of more than 4 days or is for consumption by high risk groups, perform Listeria detection in 25g.

- If the product contains whole meat joints or meat with stock or gravy and whole fish also test for *Cl. perfringens*, if the sample is a poultry pâté or parfait also test for Campylobacter.

- If the product contains shellfish and crustaceans also test for Salmonella and Vibrio.

- If the product contains raw, lightly cooked or pasteurised egg, also test for Salmonella.

- If the product contains more than 5% herbs or spices or less than 5% herbs or spices as an unprocessed garnish or additive, also test for *Bacillus* species and *Cl. perfringens*.

- If the product contains rice and noodles also test for *Bacillus* species.

Any dairy product from any food category that has been sampled from the producer or made from raw or thermised milk will be tested using the dairy sample testing algorithm in Appendix 2. Go to Appendix 2.

- If categories 10 to 13 RTE food including retail dairy products except those prepared with raw or thermised milk, perform a food screen including *E. coli,* Listeria (per g) and CPS. If the product is dried or frozen consider using the MPN method for *E. coli*. If the product has been sampled as part of an outbreak investigation, has been sampled from the producer, has a shelf-life of more than 4 days or is for consumption by high risk groups perform Listeria detection in 25g.

- If the product contains rice and noodles also test for *Bacillus* species

- If the product contains more than 5% herbs or spices or less than 5%, herbs or spices as an unprocessed garnish or additive also test for Salmonella, Enterobacteriaceae, *Bacillus* species and *Cl. perfringens*.

- If the product contains uncooked fruit and vegetables also test for Salmonella. Foods containing raw fruit or vegetables are not tested for Enterobacteriaceae, RTE sprouted seeds should not be tested for *E. coli* or CPS but should otherwise be tested in accordance with the algorithm with inclusion of Shiga toxin producing *E. coli* (STEC) and Listeria in 25g.

- If the product is dried nuts and seeds, also test for Salmonella but do not include Listeria.

- If the product is meat and fish also test for Salmonella, Enterobacteriaceae and Cl perfringens.

Appendix 2a. Text version of the FWEMS dairy sample testing algorithm

Dairy products from any food category that have been sampled from retail except those made from raw or thermised milk will be tested using the food sample testing algorithm as described in Appendix 1.

Start by performing a dairy screen on all samples including *Listeria* per g and CPS. Test 25g or 25ml if the sample has a shelf-life of more than 4 days or if it is able to support the growth of Listeria (pH >4.4, aw >0.92 or >pH5.0 / a_w 0.94 in combination).

- If the dairy product is pasteurised adjust testing of the following sample types as follows:

- for ice cream do not test for CPS but add Enterobacteriaceae
- for butter do not test for CPS
- for milk and cream do not test for CPS but add Enterobacteriaceae and Phosphatase. Phosphatase testing should not be carried out on whipped or clotted cream samples. There are no statutory limits for alkaline phosphatase in cream.
- for fresh and ripened cheese add *E. coli.* If the product is ripened consider using the MPN method for *E. coli*.

- If the dairy product is fermented, do not test for CPS but add Enterobacteriaceae.

- If the dairy product is raw (unpasteurised) or thermised, adjust testing of the following sample types as follows:

- for ice cream also include Enterobacteriaceae, *Salmonella* and *Listeria* in 25g
- for cream and butter do not test for CPS but include *E. coli*, *Salmonella* and Listeria in 25g
- for drinking milk also include aerobic colony counts, coliforms, Listeria, Salmonella, Campylobacter and STEC in 25mL
- for fresh and ripened cheese add E. coli, Salmonella, Listeria and STEC in 25g. If the product is ripened consider using the MPN method for *E. coli*

Abbreviations

Abbreviation	Meaning
ACC	aerobic colony count
a_w	water activity
BD	bloody diarrhoea
BRC	British Retail Consortium
CFA	Chilled Food Association
cfu/g	colony forming units per gram
cfu/ml	colony forming units per millilitre
CPS	coagulase positive staphylococci
EC	European Commission
EN	European Standard
EU	European Union
FBO	food business operator
FSA	Food Standards Agency
FWEMS	Food Water and Environmental Microbiology Services
g	gram
GAP	good agricultural practices
GHP	good hygiene practice
GMP	good manufacturing practices
HACCP	hazard analysis and critical control point
HUS	haemolytic uraemic syndrome
ISO	International Organization for Standardization
MAP	modified atmosphere packaging
MPN	most probable number
pH	acidity or alkalinity
PHE	Public Health England
RTE	ready-to-eat
STEC	Shiga toxin-producing *Escherichia coli*
TTP	thrombotic thrombocytopenic purpura
UHT	ultra-high temperature
UKFSS	UK Food Surveillance System
VTEC	Verocytotoxin producing *Escherichia coli*

Glossary

Term	Meaning
A_w	The water activity (a_w) of a food is a measure of the availability of water for the metabolic activity and growth of microorganisms.
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 (40).
Borderline	One or more test results that are not unsatisfactory but are also not satisfactory and are on the upper limit of acceptability. Borderline results indicate the potential for development of public health problems and of unacceptable risk and should be investigated.
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 competent for the organisation of official controls or any other authority to which that competence has been conferred (7).
Contamination	The presence or introduction of a hazard (3).
Disease	Any change from a normal physiological state or function.
Emetic	Causes vomiting.
Fermentation	Conversion of a carbohydrate, such as sugar, by microorganisms into an acid or an alcohol.
Final consumer	The ultimate consumer of a foodstuff who will not use the food as part of any food business operation or activity (45).
Food	Any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans (45).
Food business operator	The natural or legal person or persons responsible for ensuring that the requirements of food law are met within the food business under their control (45).
Food Examiner	A person who possesses the requisite qualifications and experience to carry out microbiological examinations for the purposes of The Food Safety (Sampling and Qualifications) (England) Regulations 2013 (41).
Food safety criterion	Criterion defining the acceptability of a product or a batch of foodstuff applicable to products placed on the market (40).

Term	Meaning
Foodborne outbreak	An incidence, observed under given circumstances, of 2 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) (61).
Formal sample	Samples taken as part of a routine inspection of a food premises or at a later date if officers feel that there is a need to justify the quality and safety of food sold from that premises and with a view to pursuing legal action if the results show an offence has been committed. Formal samples are taken in accordance with The Food Safety (Sampling and Qualifications) Regulations 2013 (41) and tested in an Official Laboratory (7). Food Examiners will ensure that the formal samples are stored, examined, results verified, a report and certificate of examination are issued and the sample eventually discarded according to laboratory procedures.
Hazard	An incidence, observed under given circumstances, of 2 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) (61).
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) 2017/625) (7).
Measurement uncertainty	Parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand.
Microbiological criterion	Criterion defining the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of microorganisms, and/or on the quantity of their toxins or metabolites, per unit of mass, volume, area or batch (40).
Microbiota	The community of commensal, symbiotic and pathogenic microorganisms within a given environment: also known as biota or flora.
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.

Term	Meaning
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) 2017/625 (7).
Pasteurisation	A form of heat treatment that kills vegetative pathogens and spoilage microorganisms in milk and other foods, for example, for milk a common pasteurisation process is 71.7°C for 15 seconds.
Pathogen	A micro-organism that has the capacity to cause disease, that is, 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 and meeting the minimum requirements laid down in Council Directive (EU) 2020/2184 (52).
Placing on the market	The holding of food or feed for the purpose of sale, including offering for sale or any other form of transfer, whether free of charge or not, and the sale, distribution, and other forms of transfer themselves (45)
Process	Any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes (3).
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) 2073/2005) (40).
Psychrotroph	A microorganism that can grow at temperatures between -1°C and 5°C and have an optimum growth temperature in the mesophilic range (20°C to 30°C).
Ready-to-eat (RTE) food	Food intended by the producer or the manufacturer for direct human consumption without the need for further cooking or other processing effective to eliminate or reduce to an acceptable level microorganisms of concern (Regulation (EC) 2073/2005) (40).
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 (45).
Risk	A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard (45).

Term	Meaning
Risk assessment	A scientifically based process consisting of 4 steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation (45).
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 (40).
Satisfactory	All test results indicating good microbiological quality.
Shelf-life	The period preceding the 'Use by' or the minimum durability date (40).
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.
Thermised milk	A generic description of a range of sub-pasteurisation heat treatments (for example, 57°C to 68°C for 10 to 20 seconds) that markedly reduce the number of spoilage bacteria in milk.
Toxin	A poisonous substance with the capacity to cause disease.
Unsatisfactory	For pathogens, one or more test results at levels which indicate a product that is potentially injurious to health and/or unfit for human consumption and requires immediate remedial action. For hygiene indicators unsatisfactory results do not mean that the batch of food is unsafe, however pathogens may be present and remedial action is required.
Vegetative bacteria	A bacterial cell which is capable of actively growing. Multiplication occurs by division of the cell into 2.
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.
Zoonotic infection	Any disease and/or infection which is naturally transmissible directly or indirectly between animals and humans (Directive 2003/99/EC) (61).

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UKHSA is responsible for protecting every member of every community from the impact of infectious diseases, chemical, biological, radiological and nuclear incidents and other health threats. We provide intellectual, scientific and operational leadership at national and local level, as well as on the global stage, to make the nation health secure.

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