



Food Safety
AUTHORITY OF IRELAND

3

GUIDANCE NOTE

Guidelines for the Interpretation of Results of Microbiological Testing of Ready-to-Eat Foods Placed on the Market (Revision 2)

Guidance Note No. 3
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of Microbiological Testing of Ready-to-Eat Foods
Placed on the Market
(Revision 2)

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I. PURPOSE OF THIS GUIDANCE NOTE

This document provides guideline microbiological limits for ready-to-eat foods placed on the market. These guideline limits can be used if legal microbiological criteria (Appendix I) do not exist for a particular combination of food and microorganism.

This Guidance Note is intended to be used by:

- Food business operators
- Enforcement officers, and
- Laboratories performing microbiological testing on ready-to-eat foods

Food business operators may also use the limits presented in this Guidance Note when they are carrying out testing as a means to validate or verify that their procedures based on HACCP (Hazard Analysis Critical Control Point) and good hygiene practice (GHP) are working effectively.

2. ACKNOWLEDGEMENT

The microbiological limits presented in this revision of Guidance Note No. 3 were adapted from the UK's Health Protection Agency Guidelines (2009)¹, taking into account, legal microbiological criteria set in Commission Regulation (EC) No 2073/2005, as amended².

While the Food Safety Authority of Ireland's (FSAI's) Scientific Committee endorsed the use of the UK's 2009 guidelines, they also provided a Scientific Opinion (Appendix 2) on the appropriate microbiological guidelines to set for coagulase-positive staphylococci, *E. coli* and Enterobacteriaceae, for which there was a discrepancy between guideline limits set in the first edition of Guidance Note No.3 and in the UK document, and legal process hygiene criteria set in Commission Regulation (EC) No 2073/2005, as amended.

¹ Guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market (available at: www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1259151921557)

² Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs, as amended (available at www.fsai.ie)

3. HOW TO USE THIS GUIDANCE NOTE

Before using this Guidance Note, users should check if legal microbiological criteria (Appendix I) have been set for the food/microorganism combination of interest. Legal microbiological criteria take precedence over the guideline limits set in this Guidance Note.

If relevant legal microbiological criteria do not exist, the microbiological limits presented in this Guidance Note can be used to assess the acceptability of ready-to-eat food when placed on the market. The food may be sampled by a food business operator or by an enforcement officer as part of official control activities.

The Guidance Note provides microbiological limits (and states the appropriate action to take based on results) for:

- Pathogens and microbial toxins (Section 4)
- Hygiene indicators (Section 5), and
- Aerobic colony counts (Section 6)

Further information on each microorganism, toxin or test is provided in Section 7.

It is not necessary to test all ready-to-eat foods for all parameters listed in this Guidance Note. In order to obtain meaningful information, and to make best use of resources, only tests relevant to a particular food should be carried out. The decision on what analysis should be carried out on a particular ready-to-eat food should be based on the significance of each microorganism, microbial toxin or test to that food. On the other hand, it may be necessary to test for additional microorganisms or microbial toxins for which guideline limits are not set in this Guidance Note.

3.1 Ready-to-Eat Food

The limits presented in this Guidance Note only apply to ready-to-eat food when placed on the market. Commission Regulation EC No 2073/2005, as amended, defines ‘ready-to-eat food’ 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 micro-organisms of concern;’

3.1.1 Cooking before consumption

Food which the producer, manufacturer or packer intends to be thoroughly cooked before consumption, is not considered ready-to-eat. Thoroughly cooking food so that its core reaches a temperature of 75°C (or equivalent time-temperature combination), is considered effective to achieve a 6-D reduction³ in the number of *Listeria monocytogenes* cells. *L. monocytogenes* is regarded as the most heat resistant non-spore-forming foodborne pathogen. Therefore, cooking procedures that are effective to destroy *L. monocytogenes* will destroy other non-spore-forming pathogens should they be present in the food.

Alternative cooking time/temperature combinations may be used by the food business operator so long as they achieve the same lethal effect as 75°C instantaneously. Scientifically accepted alternative time/temperature combinations include: 70°C for 2 minutes, 67°C for 5 minutes and 64°C for 12 minutes and 37 seconds⁴.

3.1.2 Reheating before consumption

Food which the producer, manufacturer or packer intends to be thoroughly reheated before consumption is not considered ready-to-eat. Thoroughly reheating previously cooked food to achieve a core temperature of $\geq 70^{\circ}\text{C}$ ⁵ is also considered effective to eliminate microorganisms of concern or reduce them to an acceptable level. Foods which are intended to be reheated for palatability purposes but will not reach a core temperature of at least 70°C, are considered ready-to-eat.

³ A 6D process is a heat process which reduces a particular microorganism type by 6 Log₁₀ cycles

⁴ For other equivalent time/temperature combinations, see the FSAI’s Guidance Note No.20: Industrial Processing of Heat-Chilled Foods (Section 3.8) – available at www.fsai.ie

⁵ Reheating temperature cited in I.S. 340:2007 (Hygiene in the catering sector) and I.S. 341: 2007 (Hygiene in retailing and wholesaling) – available at www.nsai.ie

3.1.3 Washing before consumption

Most salad leaves and many herbs are consumed without cooking. Even if salad leaves and herbs are labelled with an instruction to wash before consumption, they are considered ready-to-eat. Although washing can reduce microbial contamination on the plant's surface, it is not effective to eliminate microorganisms of concern or reduce them to an acceptable level because some pathogens can become internalised within the plant's tissue.

3.2 Food Placed on the Market

The limits presented in this Guidance Note apply to ready-to-eat food when placed on the market. According to Regulation (EC) No 178/2002, 'placing on the market' means:

'...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;'

Therefore, the limits presented in this Guidance Note can apply to food sampled in:

- Supermarkets
- Convenience stores
- Food stalls
- Distributors
- Wholesalers
- Catering establishments
- Manufacturers/packers/primary producers (when product is ready for sale or distribution)
- Point of import

The guideline limits for pathogens may also be applied *with caution* to foods sampled from domestic premises as part of food poisoning investigations. Caution should always be taken when interpreting the results of microbiological examination of food samples taken from domestic premises as the food may not have been handled and stored appropriately. A single temperature measurement taken at the time of sampling does not verify adequate temperature control since purchase.

3.3 Single Samples

The limits in Guidance Note No.3 apply to single samples (n=1) which are not associated with any formal sampling plan. Taking single samples is considered a practical approach for official sampling because multiple samples from the same batch of food may not be available, particularly when food is sampled at retail level.

3.3.1 Sampling by food business operators

Although the limits in this Guidance Note apply to single samples, food business operators should be aware that they will have more confidence in their test results if they increase the stringency of their sampling plan, for example testing n=5 instead of n=1. This is because the chance of a contaminated batch of food being missed decreases as the number of samples tested increases.

The following two ICMSF (International Commission on Microbiological Specifications for Foods) publications⁶ provide advice on setting appropriate sampling plans:

1. **Microorganisms in Foods 7: Microbiological Testing in Food Safety Management** (ICMSF, 2002)
2. **Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance** (ICMSF, 2011)

3.4 Analytical Method

It is important that the analytical method used is able to detect the microorganism of concern in the food being tested. Where possible, laboratories should use methods which have been validated for the food commodity concerned by international organisations such as:

- ISO www.iso.org
- AFNOR www.afnor.org
- MICROVAL www.microval.org
- NORDVAL www.nmkl.org

Where this is not possible, laboratories should use methods for which the reliability has been statistically established in ring trials between several laboratories.

⁶ Available to purchase at: www.icmsf.org/publications/books.html

3.5 Laboratory Accreditation

Accreditation is the formal recognition of a laboratory's competence to conduct testing in compliance with the international standard ISO 17025. Compliance with this standard requires laboratories to demonstrate competence, impartiality and integrity.

The Irish National Accreditation Board (INAB) is the national body with responsibility for accreditation of laboratories in Ireland. Each accredited laboratory is issued with a certificate of accreditation that lists the analytical tests (including the basis of the method and relevant food matrix) for which the laboratory is accredited. A laboratory's accreditation status can be checked on INAB's website at: www.inab.ie

Laboratories operating outside of Ireland are not accredited by INAB, but by other accreditation bodies such as the United Kingdom Accreditation Service (UKAS). Accreditation provided by these accreditation bodies is equivalent if they are signatory to the European Co-operation for Accreditation (EA) multi-lateral agreement for testing.

3.5.1 Testing on behalf of food business operators

It is best practice that the laboratory analysing samples for food business operators is accredited to conduct the analytical method on the required food matrix, but this is not a legal requirement.

A laboratory may be accredited for some but not all of the analytical test methods it offers to its clients. If accreditation of the specific method and matrix is not available, it is best practice to choose a laboratory that is accredited to use the relevant method in other food matrices or a laboratory that has been accredited for a broad range of test methods.

3.5.2 Testing for official control purposes

Regulation (EC) No 882/2004 (Article 12) requires that laboratories which analyse food for official control purposes must be designated by the competent authority and operate and be assessed and accredited in accordance with the following European standards:

- a. EN ISO/IEC 17025 on general requirements for the competence of testing and calibration laboratories
- b. EN ISO/IEC 17011 on conformity assessment – general requirements for accreditation bodies accrediting conformity assessment bodies⁷

⁷ In 2008, EN/ISO IEC 17011 replaced EN 45002 on general criteria for the assessment of testing laboratories and EN 45003 on calibration and testing laboratory accreditation.

The European Commission has published 'Guidance Note on official controls, under Regulation (EC) No 882/2004, concerning microbiological sampling and testing of foodstuffs'⁸. The document provides guidance on:

- Official control sampling
- Requirements of official laboratories
- Analytical methods and accreditation requirements for testing official control samples, and
- Interpreting microbiological test results

3.6 Interpreting Results

Test results may be interpreted as satisfactory, borderline or unsatisfactory, depending on the detection/non-detection or number of microorganisms in a food sample, or the detection/non-detection of a microbial toxin.

- ✓ **Satisfactory** means the test results indicate the food sample meets with the guideline limit.
- ✓ **Borderline** means that the test results indicate the food sample meets the guideline limit, but that it may be approaching unsatisfactory.
- ✗ **Unsatisfactory** means that the test results indicate the sample of food exceeds the guideline limit. If the result of a pathogen or microbial toxin test is unsatisfactory, the ready-to-eat food is considered unsafe to eat.

For some test parameters, test results will either be 'satisfactory' or 'unsatisfactory', with no 'borderline' result. For example, *Salmonella* test results are either satisfactory (*Salmonella* not detected) or unsatisfactory (*Salmonella* detected). There is no 'borderline' result for a *Salmonella* test.

⁸ Available at www.fsai.ie

3.7 Action required if Test Results are Borderline or Unsatisfactory

No action is required if the test results are satisfactory. If test results are borderline, or unsatisfactory, the action required differs depending on whether the result is for a:

- Pathogen or toxin test (Section 4)
- Hygiene indicator test (Section 5), or
- Aerobic colony count test (Section 6)

3.8 Sample Designation

Samples are designated according to the complete set of microbiological test results associated with each sample for each parameter listed in Section 4 (Pathogens and Microbial Toxins), Section 5 (Hygiene Indicators) and Section 6 (Aerobic Colony Counts) of this Guidance Note as follows:

- ✓ If the results for all microbiological parameters tested for a sample are satisfactory, the overall designation for that sample is **Satisfactory**
- ✓ If the result for one or more microbiological parameters tested for a sample is borderline, the overall designation for that sample is **Borderline**
- ✗ If the result of one or more microbiological parameters tested for a sample is unsatisfactory, the overall designation for that sample is **Unsatisfactory**. If the result of a pathogen or microbial toxin test is unsatisfactory, the ready-to-eat food is considered unsafe to eat
- ✗ If the result of one or more microbiological parameters tested for a sample is borderline, and the result of one or more microbiological parameters tested for that same sample is unsatisfactory, the overall designation for that sample is **Unsatisfactory**. If the result of a pathogen or microbial toxin test is unsatisfactory, the ready-to-eat food is considered unsafe to eat

4. PATHOGENS AND MICROBIAL TOXINS

Table 1 lists guideline microbiological limits for pathogens and staphylococcal enterotoxin in ready-to-eat food when placed on the market. The absence of a pathogen or microbial toxin from Table 1 does not imply that it is insignificant to food safety. In addition, the pathogens and microbial toxins listed are not relevant to all ready-to-eat foods.

Table 2 lists the actions that should be taken if results are borderline and Table 3 lists the actions that should be taken if results are unsatisfactory.

Table 1. Guideline limits for certain pathogens and microbial toxins in ready-to-eat food placed on the market

Pathogen ^(a) /toxin ^(b)	Result Based on cfu/g or absence/presence in 25g ^(c)		
	Satisfactory No action required	Borderline See Table 2 for action required	Unsatisfactory See Table 3 for action required
<i>Bacillus cereus</i> and other pathogenic <i>Bacillus</i> spp. ^(d)	<10 ³	10 ³ - ≤10 ⁵	>10 ⁵
<i>Campylobacter</i> spp.	Not detected	Borderline result not applicable	Detected
<i>Clostridium perfringens</i>	<10	10 - ≤10 ⁴	>10 ⁴
Coagulase-positive staphylococci ^(e)	<20	20 - ≤10 ⁴	>10 ⁴ ^(f)
<i>Salmonella</i> spp. ^(g)	Not detected	Borderline result not applicable	Detected
<i>Shigella</i> spp.	Not detected	Borderline result not applicable	Detected
Staphylococcal enterotoxin ^(h)	Not detected	Borderline result not applicable	Detected
Verotoxigenic <i>E. coli</i> (VTEC) ⁽ⁱ⁾	Not detected	Borderline result not applicable	Detected
<i>Vibrio cholerae</i> (O1 and O139)	Not detected	Borderline result not applicable	Detected
<i>Vibrio parahaemolyticus</i>	<20	20 - ≤10 ³	>10 ³

^(a) Guideline limits for *L. monocytogenes* in ready-to-eat food are not set in Guidance Note No.3 because Commission Regulation (EC) No 2073/2005, as amended, sets legal microbiological criteria for *L. monocytogenes* in all ready-to-eat food

^(b) Commission Regulation (EC) No 2073/2005, as amended, sets legal microbiological criteria for histamine in fish and fishery products

^(c) Although laboratories commonly require 25g of food for each analytical test, a larger or smaller portion of food may be required for some tests or a smaller portion may be all that is available during food poisoning investigations. Check with the laboratory before sampling

^(d) Using the reference method ISO 7932, the result is reported as 'presumptive *B. cereus*'. The term 'presumptive' is used to acknowledge the fact that it is difficult to distinguish *B. cereus* from other closely related but less commonly encountered *Bacillus* species, such as *B. anthracis*, *B. thuringiensis*, *B. weihenstephanensis* and *B. mycoides*

^(e) Note: satisfactory/borderline levels of coagulase-positive staphylococci in food do not guarantee that toxin is not present. The organisms may have grown to sufficient levels to produce enterotoxins and then died off

^(f) For cheeses made from raw milk placed on the market, unsatisfactory is >10⁵ cfu/g. This reflects the ≤10⁵ limit set in the process hygiene criterion (applicable during the manufacturing process) for food category 2.2.3 in Commission Regulation (EC) No 2073/2005, as amended (see Appendix 2 for Opinion of the Scientific Committee)

^(g) Commission Regulation (EC) No 2073/2005, as amended sets legal criteria for *Salmonella* spp. in certain food categories. Refer to the Regulation for relevant food categories and associated sampling plans

^(h) Commission Regulation (EC) No 2073/2005, as amended sets a legal criterion requiring absence of staphylococcal enterotoxins in certain cheeses, milk powder and whey powder. Refer to food category 1.21 of the Regulation for details of the criterion and associated sampling plans

⁽ⁱ⁾ VTEC is also referred to as shiga-toxin producing *E. coli* (STEC). Commission Regulation (EC) No 2073/2005, as amended sets a legal criterion for STEC serogroups O157, O26, O111, O103, O145 and O104:H4 in sprouts. See food category 1.29 of the Regulation, for details of the criterion and associated sampling plans

4.1 Borderline Results

Borderline results are applicable to four pathogens listed in this Guidance Note:

1. *Bacillus cereus* and other pathogenic *Bacillus* species
2. *Clostridium perfringens*
3. Coagulase positive staphylococci, and
4. *Vibrio parahaemolyticus*

If the result of pathogen test is borderline, the batch of food is not considered unsafe under Article 14, Regulation 178/2002, but the food business operator should investigate the cause of the elevated levels and take measures as part of their HACCP-based procedures and GHP to prevent counts in this batch of food (or subsequent batches) reaching unsatisfactory levels. Table 2 outlines the action required when a borderline result is received for a pathogen.

4.2 Unsatisfactory Results

For most pathogens and microbial toxins, their presence in a ready-to-eat food is unsatisfactory and presents risk to health. The result of testing being categorised as:

1. Satisfactory (not detected) or
2. Unsatisfactory (detected)

For some pathogens, the classification depends on the number of microorganisms counted because, for these pathogens, numbers must exceed a certain threshold before the test result is considered unsatisfactory and the food presents a risk to health.

If an unsatisfactory result is received for a pathogen or microbial toxin test, the batch of food is usually⁹ considered unsafe (Article 14, Regulation 178/2002) and should be withdrawn/recalled from the market (Article 19, Regulation 178/2002). Table 3 outlines the action required when an unsatisfactory result is received for a pathogen or toxin test.

⁹ For coagulase positive results, food is unsafe if staphylococcal enterotoxin has been detected. For *Bacillus* spp. food is unsafe if the species identified is pathogenic

Table 2. Action required when results of pathogen or microbial toxin tests are borderline

Pathogen or toxin	Action required for borderline result
<i>Bacillus cereus</i> and other pathogenic <i>Bacillus</i> spp.	At the levels detected, these pathogens are not considered injurious to health. However, the risk will increase proportional to the levels detected and the likelihood of subsequent growth.
<i>Clostridium perfringens</i>	
Coagulase-positive staphylococci ^(a)	<p>While the batch of food is not considered unsafe (Article 14, Regulation 178/2002) the food business operator should investigate the cause of the elevated levels and take measures as part of their HACCP-based procedures and GHP to prevent counts in this batch of food (or subsequent batches) reaching unsatisfactory levels.</p> <p>The food business operator should:</p> <ol style="list-style-type: none"> 1. Investigate the cause of the borderline result 2. Carry out the necessary actions to prevent numbers reaching unsatisfactory levels, for example: <ol style="list-style-type: none"> a. Review cooking times/temperatures to ensure adequate heat treatment b. Review refrigeration times/temperatures to ensure adequate refrigeration c. Verify that they have set a safe shelf-life for the food d. Review selection and origin of raw materials e. Review cleaning and sanitising procedures to ensure they are effective f. Review staff hygiene practices to ensure they are adequate g. Review staff training to ensure that staff are appropriately trained h. Review and update their HACCP-based procedures and GHP i. Test further samples of food and samples from the food preparation environment <p>Competent authorities should:</p> <ol style="list-style-type: none"> 1. Ensure the food business operator immediately takes the necessary actions detailed above 2. Carry out additional sampling as necessary 3. Carry out a detailed inspection of the premises, food production and handling processes to determine if hygiene practices are inadequate, as necessary
<i>Vibrio parahaemolyticus</i>	
<i>Campylobacter</i> spp.	
<i>Salmonella</i> spp.	
Verocytogenic <i>E. coli</i> (VTEC)	
<i>Shigella</i> spp.	
Staphylococcal enterotoxin	
<i>Vibrio cholerae</i> (O1 and O139)	A borderline result is not applicable to these pathogens; test results are either satisfactory or unsatisfactory.

^(a) If food is suspected to have caused staphylococcal food poisoning, it should be tested for the presence of staphylococcal enterotoxin. If staphylococcal enterotoxin is detected, the food is considered unsafe (Article 14, Regulation 178/2002) and should be withdrawn/recalled from the market (Article 19, Regulation 178/2002), i.e. the actions described in Table 3 for food business operators and competent authorities, should be carried out.

Table 3. Action required when results of pathogen or microbial toxin tests are unsatisfactory

Pathogen or toxin	Action required for borderline result
<i>Campylobacter</i> spp.	Detection in a ready-to-eat food is potentially injurious to health. The batch of food is considered unsafe (Article 14, Regulation 178/2002) and should be withdrawn/recalled from the market (Article 19, Regulation 178/2002).
<i>Clostridium perfringens</i>	
<i>Salmonella</i> spp.	<p>The food business operator must:</p> <ol style="list-style-type: none"> 1. Notify the competent authorities according to the FSAI's: <ol style="list-style-type: none"> a. Code of Practice No.5: Food incidents and alerts, and b. Guidance Note No.10: Product Recall and Traceability 2. Withdraw/recall the food from the market (as appropriate) according to the protocol in Guidance Note No.10 3. Notify trade customers and/or consumers (as appropriate) according to the protocol in Guidance Note No.10 4. Investigate the cause of the unsatisfactory result and carry out the actions necessary to prevent the unsatisfactory result from reoccurring, for example: <ol style="list-style-type: none"> a. Review cooking times/temperatures to ensure adequate heat treatment b. Review refrigeration times/temperatures to ensure adequate refrigeration c. Verify that they have set a safe shelf-life for the food d. Review selection and origin of raw materials e. Review cleaning and sanitising procedures to ensure they are effective f. Review staff hygiene practices to ensure they are adequate g. Review staff training to ensure that staff are appropriately trained h. Review and update their HACCP-based procedures and GHP i. Test further samples of food and samples from the food preparation environment <p>Competent authorities should:</p> <ol style="list-style-type: none"> 1. Ensure the food business operator immediately takes the necessary actions detailed above 2. Carry out additional sampling as necessary 3. Carry out a detailed inspection of the premises, food production and handling processes to determine if hygiene practices are inadequate, as necessary
<i>Shigella</i> spp.	
Staphylococcal enterotoxin	
<i>Vibrio cholerae</i> (O1 and O139)	
<i>Vibrio parahaemolyticus</i>	
Verocytogenic <i>E. coli</i> (VTEC)	
<i>Bacillus cereus</i> and other pathogenic <i>Bacillus</i> spp.	As not all <i>Bacillus</i> spp. cause illness, the organism should be identified (the result is reported as 'presumptive <i>B. cereus</i> ' using the ISO 7932). If the species is pathogenic, the batch of food is considered unsafe (Article 14, Regulation 178/2002) and must be withdrawn/recalled from the market (Article 19, Regulation 178/2002). The actions described above for food business operators and competent authorities should be carried out.
Coagulase-positive staphylococci	Not all strains of coagulase positive staphylococci are capable of producing toxin and causing disease. The food should be tested for the presence of staphylococcal enterotoxin. If staphylococcal enterotoxin is detected, the food is considered unsafe (Article 14, Regulation 178/2002) and must be withdrawn/recalled from the market (Article 19, Regulation 178/2002). The actions described above for food business operators and competent authorities should be carried out.

4.3 Reference Laboratory Testing

Specialist or reference tests are available which help provide extra information on foodborne pathogens. These tests are usually performed in specialist or national reference laboratories in order to:

- Verify the results from a primary laboratory
- Detect more unusual pathogens, viruses and parasites
- Detect toxins, or the genes for toxin production
- Perform typing of isolates, e.g. serotyping, phage typing, antimicrobial resistance typing or genetic fingerprinting
- Assess the pathogenic potential of an isolate

Testing for norovirus, Hepatitis A, parasites and toxins, e.g. *Clostridium botulinum* neurotoxin, staphylococcal enterotoxins, *Bacillus* toxins, shellfish toxins and histamine, may only be available at national or international reference laboratories. These tests are complex to perform and so results are not quickly available. If food is suspected to contain these contaminants, public health actions shouldn't be delayed pending test results.

5. HYGIENE INDICATORS

Table 4 lists guideline limits for hygiene indicator microorganisms. Table 5 outlines suggested actions if results are borderline or unsatisfactory.

An unsatisfactory result for a hygiene indicator test does not mean that the batch of food is considered unsafe under Article 14, Regulation 178/2002. However, unsatisfactory results represent unsatisfactory levels of microbial contamination and indicate that pathogens may be present. The food business operator should investigate the cause of the elevated levels and take measures as part of their HACCP-based procedures and GHP to in order to ensure levels in subsequent batches of food are satisfactory. For borderline results, the action carried out should be proportional to the levels detected.

Table 4. Guideline limits for certain indicator microorganisms in ready-to-eat foods sampled when placed on the market

Hygiene Indicator		Result Based on cfu/g or presence in 25g		
		Satisfactory No action required	Borderline See Table 5 for action required	Unsatisfactory See Table 5 for action required
Enterobacteriaceae ^(a) ^(b) ^(c)		<10 ²	10 ² - ≤10 ⁴	>10 ⁴
<i>E. coli</i> ^(d)		<20	20 - ≤10 ²	>10 ²
<i>Listeria</i> spp. (other than <i>L. monocytogenes</i>)	Foods that can't support the growth of <i>Listeria</i> spp. ^(e)	<10	10 - ≤10 ²	>10 ²
	Foods that can support the growth of <i>Listeria</i> spp.	Not detected	Borderline result not applicable	Detected

^(a) These limits for Enterobacteriaceae do not apply to fresh fruit and salad vegetables – or food that contains raw fresh fruit and vegetables, e.g. sandwiches, salad bowls, coleslaw etc. – because these foods can naturally contain high levels of Enterobacteriaceae as part of their normal micro-flora

^(b) These limits for Enterobacteriaceae do not apply to cheeses ripened using a culture of *Hafnia alvei* or *Proteus vulgaris* as these bacteria are both members of the Enterobacteriaceae family

^(c) See Appendix 2 for Opinion of the Scientific Committee. Because Commission Regulation (EC) No 2073/2005 sets some process hygiene criteria for Enterobacteriaceae (which apply at the manufacturing stage) that are stricter than the limits presented in this table, the Enterobacteriaceae limits in this table do not apply to the following categories of food if sampled when placed on the market:

1. Pasteurised milk and other pasteurised liquid dairy products (food category: 2.2.1)
2. Milk powder and whey powder (food category 2.2.7)
3. Ice-cream (containing milk ingredients) and frozen dairy desserts (food category 2.2.8)
4. Dried infant formulae and dried dietary foods for special medical purposes intended for infants below six months of age (food category 2.2.9)
5. Dried follow-on formulae (food category 2.2.10), and
6. Egg products (food category 2.3.1)

^(d) See Appendix 2 for Opinion of the Scientific Committee. Because Commission Regulation (EC) sets some process hygiene criteria for *E. coli* that are more lenient than the levels presented in this table, the following *E. coli* limits should be applied to the three food categories listed below if they are sampled when placed on the market: **satisfactory** <100 cfu/g; **borderline** 10²-10³ and **unsatisfactory** >10³:

1. Cheeses made from milk or whey that has undergone heat treatment (food category 2.2.2)
2. Ready-to-eat pre-cut fruit and vegetables (food category 2.5.1)
3. Ready-to-eat unpasteurised fruit and vegetable juices (food category 2.5.2)

^(e) Types of ready-to-eat foods that are automatically considered unable to support the growth of *Listeria* spp. are those:

1. With a shelf-life less than five days
2. With a pH ≤4.4
3. With a water activity ≤0.92
4. With a combined pH ≤5.0 and water activity ≤0.94
5. With a growth potential is ≤0.5 log₁₀ cfu/g
6. Which are frozen (but only while the product remains frozen)

Table 5. Action required when results of hygiene indicator tests are unsatisfactory or borderline

Hygiene Indicator	Unsatisfactory Result	Borderline Result
<i>E. coli</i>	<p>Although the batch of food is not considered unsafe (Article 14, Regulation 178/2002) unsatisfactory results represent unsatisfactory levels of microbial contamination and indicate that pathogens may be present.</p> <p>Food business operators should:</p> <ol style="list-style-type: none"> 1. Investigate the cause of the unsatisfactory result 2. Carry out the necessary actions to ensure counts in subsequent batches of food are at satisfactory levels, for example: <ol style="list-style-type: none"> a. Review cooking times/temperatures to ensure adequate heat treatment b. Review refrigeration times/temperatures to ensure adequate refrigeration c. Review selection and origin of raw materials d. Review cleaning and sanitising procedures to ensure they are effective e. Review staff hygiene practices to ensure they are adequate f. Review staff training to ensure that staff are appropriately trained g. Review and update their HACCP-based procedures and GHP h. Test further samples of food and samples from the food preparation environment 	<p>The counts are approaching unsatisfactory levels. The action carried out should be proportional to the levels detected. If results are close to unsatisfactory, food business operators are advised to carry out the investigations and actions recommended for unsatisfactory results in order to ensure counts in subsequent batches of food are at satisfactory levels.</p>
Enterobacteriaceae		
<i>Listeria</i> spp.		

6. AEROBIC COLONY COUNTS

Table 6 lists aerobic colony count (ACC) limits for various categories of ready-to-eat food. The ACC test is a generic test that counts organisms which grow under aerobic conditions at mesophilic temperatures on a particular growth medium. The counts provide useful information to assess a food's quality or its remaining shelf-life.

6.1 Action required for Borderline or Unsatisfactory Results

The ACC test cannot be used to assess the safety of food. An unsatisfactory result for the ACC test does not mean that the batch of food is considered unsafe (Article 14, Regulation 178/2002). However, an unsatisfactory result represents unsatisfactory levels of microbial contamination. The food business operator should investigate the cause of the elevated levels and take measures as part of their procedures based on HACCP and GHP in order to return levels in subsequent batches of food to satisfactory levels, for example:

1. Review **cooking** times/temperatures to ensure adequate heat treatment
2. Review **refrigeration** times/temperatures to ensure adequate refrigeration
3. Review the **shelf-life** they set to check that it is appropriate
4. Review selection and origin of **raw materials**
5. Review **cleaning and sanitising** procedures to ensure they are effective
6. Review **staff hygiene** practices to ensure they are adequate
7. Review **staff training** to ensure that staff are appropriately trained
8. Review and update their **procedures based on HACCP and GHP**
9. **Test further samples** of food and samples from the food preparation environment

For borderline results, the action carried out should be proportional to the levels detected.

Table 6. Guidance on the interpretation of results for ACC in ready-to-eat foods when placed on the market

Food category		Examples	ACC result (cfu/g)		
			Satisfactory	Borderline	Unsatisfactory
1	Ambient stable canned, bottled, cartoned and pouched foods immediately after removal from container	Canned products such as tuna, salmon, corned beef, soups, stews, desserts, fruit; UHT products	<10	Not applicable	See footnote ^(a)
2	Foods cooked immediately prior to sale or consumption	Takeaway food, burgers, kebabs, sausages, pizza, ready-meals (cook/chill and cook/freeze) after regeneration	<10 ³	10 ³ - <10 ⁵	≥10 ⁵
3	Cooked foods chilled but with minimum handling prior to sale or consumption; canned pasteurised foods requiring refrigeration	Whole pies, sausage rolls, samosas, flans, quiches, chicken portions; canned ham, pasteurised foods including fruit juice and soups; desserts	<10 ⁴	10 ⁴ - <10 ⁷	≥10 ⁷
4	Bakery and confectionery products without dairy cream, powdered foods	Cakes without dairy cream, soup powders, milk powder, powdered dairy products, other reconstituted powdered foods ready-to-eat after reconstitution or warming	<10 ⁴	10 ⁴ - <10 ⁶	≥10 ⁶
5	Cooked foods chilled but with some handling prior to sale or consumption	Sliced meats, cut pies, pâté, sandwiches without salad, hot smoked fish (mackerel, etc.), molluscs, crustaceans and other shellfish out of shell	<10 ⁵	10 ⁵ - <10 ⁷	≥10 ⁷
6	Non-fermented dairy products and dairy desserts, mayonnaise and mayonnaise-based dressings, cooked sauces	Most milk and butter, cream, ice-cream, fresh cheese (cream cheese, mascarpone, paneer), trifle with dairy cream, cakes with dairy cream, satay	<10 ⁵	10 ⁵ - <10 ⁷	≥10 ⁷
7	Food mixed with dressings, dips, pastes	Coleslaw, dips, taramasalata, humous	<10 ⁶	10 ⁶ - <10 ⁷	≥10 ⁷

Food category		Examples	ACC result (cfu/g)		
			Satisfactory	Borderline	Unsatisfactory
8	Extended shelf-life food products requiring refrigeration	MAP or vacuum packed products, e.g. meat, fish, fruit and vegetables	<10 ⁶	10 ⁶ - <10 ⁸	≥10 ⁸ See footnote ^(b)
9	Raw ready-to-eat meat and fish, cold smoked fish	Sushi, smoked salmon, gravalax	<10 ⁶	10 ⁶ - <10 ⁷	See footnote ^(c)
10	Preserved food products-pickled, marinated or salted	Pickled or salted fish, cooked shellfish in vinegar, vegetables in vinegar or oil, herbs, spices	Not applicable	Not applicable	See footnote ^(c)
11	Dried foods	Fruits, berries, vine fruits, nuts, sunflower seeds, herbs, spices, dried fish	Not applicable	Not applicable	See footnote ^(c)
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	Not applicable	Not applicable	See footnote ^(c)
13	Fermented, cured and dried meats, fermented vegetables, ripened cheeses	Continental sausages/ salamis, jerky, sauerkraut, olives, bean curd, cheddar, stilton, brie, fermented milk drinks and butter, yoghurt, etc	Not applicable	Not applicable	See footnote ^(c)

^(a) Food category 1: These products are unsatisfactory if spore forming anaerobes are present but these require special tests for detection and enumeration. Spore forming aerobes are also usually absent in foods that have been cooked in their container but low levels may occur in canned fish products. Most Food Category 1 foods are sterile if sampled direct from the container, but if they undergo further preparation before consumption then they should be assessed as Food Category 5

^(b) Determine the predominant micro-organisms. "Unsatisfactory" if the predominant organisms is >10⁶ yeast, >10⁷ Gram negative bacilli or *Bacillus* spp., or >10⁸ lactic acid bacteria

^(c) ACCs not routinely performed. For spoilage investigation, "Unsatisfactory" if the predominant organisms is >10⁶ yeast, >10⁷ Gram negative bacilli or *Bacillus* spp., or >10⁸ lactic acid bacteria unless added as a processing aid

7. INFORMATION SPECIFIC TO EACH MICROORGANISM OR TOXIN

This section provides background information about each microorganism and microbial toxin for which a limit is specified in this Guidance Note (listed in alphabetical order).

7.1 Aerobic Colony Count

Aerobic colony count (ACC), also known as total viable count or standard plate count, is a generic test that counts the number of microorganisms that grow under aerobic conditions at mesophilic temperatures on a particular growth medium. The test can provide useful information to assess the quality of a food (hygiene, organoleptic) or its remaining shelf-life.

As the ACC test does not differentiate aerobic microorganisms or indicate the presence of pathogens, unsatisfactory results do not directly relate to the safety of a food. The expected ACC level varies depending on the type of food. A higher than expected level (unsatisfactory) should be investigated because this can indicate:

- Quality issues, and/or
- Poor temperature control

Thirteen categories of ready-to-eat food are listed in Table 6. Although a food may fit into more than one category, the ACC result should be assessed against the limits presented for the category into which the food best fits, based on the type of product, the processing it has received and the potential for microbiological growth during storage. For example, coleslaw best fits into Food Category 7: 'Food mixed with dressing, dips, pastes'.

For some food categories, testing for ACC is generally not applicable. However, in the case of spoilage investigation, guidelines on interpreting results are provided in Table 7. When the ACC level is $>10^6$ cfu/g, there is usually a predominant microorganism, while ACC levels $<10^6$ cfu/g are usually associated with a mixed flora¹⁰.

¹⁰ Health Protection Agency (2009) Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market. Available at: www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1259151921557

Table 7. Significance of predominant microorganism identified with high ACC counts in food spoilage investigations (Source: HPA, 2009)

Predominant Microorganism	Significance
Lactic acid bacteria (mainly lactobacilli and streptococci)	Spoilage occurs at 10^9 due to formation of lactic acid. Grow well at refrigeration temperatures. Frequently predominate in meat products.
Gram negative bacteria	Spoilage occurs at $10^7 - 10^8$. Frequently produce slime. Pseudomonads also produce taints and discolouration.
Yeasts	Spoilage occurs at $10^6 - 10^7$ due to acid and gas production.
<i>Bacillus</i> spp.	Levels $>10^5$ food safety issue if species is pathogenic. High levels may be due to addition of pepper or other spices after heat treatment.

7.2 *Bacillus cereus*

Bacillus species produce spores allowing them to survive harsh conditions such as cooking, drying and pasteurisation. As *Bacillus cereus* is widespread in the environment, low levels of *B. cereus* spores or cells are found on virtually all foods including: vegetables, cereals, spices, meat, meat products, pasteurised liquid egg, milk, dairy products and dried foods. Spices and spice products in particular can carry a high load of *Bacillus* spp. and may be a source of *B. cereus* spores.

Almost all types of food have been associated with *B. cereus* food poisoning. However, the majority of cases have been linked to heat treated foods which have been subjected to time temperature abuse during storage and handling, e.g. cooked rice left for extended periods at room temperature. As *B. cereus* spores survive normal cooking temperatures, the spores can germinate leading to growth of vegetative cells if the food is cooled slowly and left at room temperature. *B. cereus* causes illness by producing toxins in food or the small intestine.

B. cereus causes two types of foodborne disease:

1. Emetic (vomiting) food poisoning – following consumption of food containing pre-formed toxin. The toxin may be formed once *B. cereus* numbers in the food exceed 10^5 cfu/g
2. Diarrhoeal food poisoning – following consumption of food containing high numbers of *B. cereus* cells (usually $>10^6$), which then go on to produce diarrhoeal toxin in the small intestine, or sometimes in the food

The emetic syndrome is usually associated with starchy (farinaceous) foods such as rice and pasta dishes. As the emetic toxin is both acid- and heat-stable, foods can contain the toxin even if *B. cereus* cells are not detected. The emetic toxin's heat resistance is of concern when food is cooked in advance and left at room temperature for an extended period of time before reheating, because reheating will not inactivate this toxin.

Diarrhoeal syndrome is usually associated with a wider range of foods including: meat products, soups, vegetables, puddings and sauces.

To control the growth of *B. cereus*, cooked food (unless its pH and/or water activity prevents *B. cereus* growth) should be kept hot (63°C or above) or cooled rapidly and refrigerated (5°C or less) to prevent the growth of vegetative cells. See FSAI Guidance Note No.15¹¹ and Guidance Note No.20¹² for best practice regards chilling and storing cooked foods.

7.2.1 *Bacillus* species, other than *Bacillus cereus*

Not all *Bacillus* species are toxigenic – some fermented foods rely on the production of very high levels of *Bacillus* species but remain safe to eat. However, some species do produce toxins when numbers exceed 10⁵ cfu/g. In addition to *Bacillus cereus*, foodborne illness has been reported due to *B. subtilis*, *B. licheniformis*, *B. pumilis* and *B. amyloliquifaciens*.

7.3 *Campylobacter* spp.

Campylobacter spp. can be found in the intestinal tracts of many animals and can therefore contaminate raw meat and poultry, raw milk and water supplies. *Campylobacter jejuni* and *C. coli* are the most common species isolated from cases of human campylobacteriosis, but other *Campylobacter* species, such as *C. lari*, have caused illness.

Most cases of *Campylobacter* illness are sporadic with few outbreaks reported, making food vehicles more difficult to identify. Campylobacteriosis has been most often associated with undercooked poultry (and cross-contamination from raw poultry to ready-to-eat foods), raw milk, contaminated water supplies, fresh produce and bivalve molluscs.

Campylobacter spp. do not normally grow in food (they need special atmospheric conditions for growth) but low numbers are sufficient to cause disease. Adequate cooking and prevention of cross-contamination are important control measures for *Campylobacter*. *Campylobacter* are killed by normal cooking temperatures and freezing has been shown to reduce numbers.

¹¹ Guidance Note No.15: Cook-Chill Systems in the Food Service Sector. Available at: www.fsai.ie/resources_publications.html

¹² Guidance Note No.20: Industrial Processing of Heat-Chill Foods. Available at: www.fsai.ie/resources_publications.html

7.4 *Clostridium perfringens*

Clostridium perfringens forms spores which can persist in the environment and contaminate a wide range of raw foods. Most outbreaks of *C. perfringens* food poisoning are caused by meat dishes (including stews and gravies) that have been subjected to time/temperature abuse after cooking. The spores survive normal cooking temperatures but can germinate and rapidly multiply if the food is cooled slowly and left at room temperature for extended periods. Illness is caused when large numbers of *C. perfringens* are eaten in the food and then produce toxin in the small intestine. The illness is not caused by the production of toxin in food.

To control *C. perfringens* spore germination and subsequent growth, cooked food (unless its pH and/or water activity prevents *C. perfringens* growth) should be kept hot (63°C or above) or cooled rapidly and refrigerated (5°C or less) to prevent the growth of vegetative cells. See FSAI Guidance Note No.15¹³ and Guidance Note No.20¹⁴ for best practice regards chilling and storing cooked foods.

7.5 Coagulase-positive Staphylococci and Staphylococcal Enterotoxins

Commission Regulation (EC) No 2073/2005, as amended, sets legal microbiological criteria for staphylococcal enterotoxin in certain cheeses, milk powder and whey powder when placed on the market during their shelf-life (food category 1.21).

Staphylococcus aureus is the most common species of coagulase-positive staphylococci to cause illness, although illness has been caused by other species including *S. intermedius* and *S. hyicus*. Staphylococcal food poisoning is caused by the production of enterotoxins in food. Not all strains of *S. aureus* or species of coagulase-positive staphylococci can produce enterotoxins. The pathogenic potential of an isolate can be confirmed by testing for the presence of the genes for enterotoxins production.

Many healthy people carry *S. aureus* on their skin and in their nose and throat and the microorganisms can be isolated from boils, skin infections and wound infections. Therefore, foods commonly implicated in staphylococcal food poisoning are those that have been contaminated via physical handling and then subjected to time/temperature abuse, e.g. sandwiches, ham, potato salads, cream- or custard filled pastries, cold deserts, etc.

¹³ Guidance Note No.15: Cook-Chill Systems in the Food Service Sector. Available at: www.fsai.ie/resources_publications.html

¹⁴ Guidance Note No.20: Industrial Processing of Heat-Chill Foods. Available at: www.fsai.ie/resources_publications.html

There is a risk of sufficient enterotoxin to cause illness being produced when:

1. *S. aureus* counts in the food exceed 10^5 cfu/g and
2. The temperature of the food is between 10 and 45°C

S. aureus are also carried by animals and can contaminate raw meat and raw milk. Cheese made from raw milk is at risk of containing staphylococcal enterotoxins if *S. aureus* numbers are allowed to multiply to toxin-producing levels during production. During cheese maturation, *S. aureus* numbers may drop because of unfavourable conditions, but this does not guarantee that staphylococcal enterotoxins are not present in the cheese.

While *S. aureus* is heat sensitive and is killed by adequate cooking or pasteurisation, the enterotoxin is heat resistant. Reheating cooked food may not destroy toxin that has been formed in food because a temperature of 100°C for at least 30 minutes is necessary to inactivate the toxin. Therefore, the risk of staphylococcal food poisoning is reduced by minimising handling of foods, using good hand hygiene and maintaining good temperature control by keeping foods cold (5°C or less) and hot foods hot (63°C or above).

If coagulase positive staphylococci are detected in food at levels $>10^5$, or the food is suspected to have caused staphylococcal food poisoning, the food should be tested for the presence of enterotoxins. The presence of staphylococcal enterotoxins in food renders the food unsafe and it should be withdrawn or recalled. Tests for staphylococcal enterotoxins may only be available in reference laboratories. If the presence of staphylococcal enterotoxins is suspected, health actions and interventions should not be delayed pending the confirmed results.

7.6 Enterobacteriaceae

Enterobacteriaceae are a broad group of bacteria that includes species originating from plants, the environment and the intestinal tract of animals and humans. The Enterobacteriaceae group includes pathogenic species, e.g. *E. coli*, *Salmonella*, *Shigella* and *Yersinia*, and those responsible for food spoilage, e.g. *Citrobacter freundii*, *Klebsiella* spp, *Providencia rettgeri*, *Enterobacter agglomerans*. Other members include: *Edwardsiella*, *Erwinia*, *Escherichia*, *Hafnia*, *Proteus* and *Serratia*.

Enterobacteriaceae are useful indicators of hygiene, process failure and post-processing contamination of heat processed foods. The presence of Enterobacteriaceae in heat-processed foods indicates inadequate cooking or post-processing contamination. This is because all Enterobacteriaceae are killed by heat processing and should be readily removed from the factory, equipment and surfaces by appropriate cleaning procedures. Some Enterobacteriaceae can contribute to histamine formation in susceptible foods – such as mackerel, tuna and some cheeses – if they are not properly processed or stored.

Unsatisfactory results, although not inherently a hazard can indicate:

- Poor quality of raw materials or food components
- Inadequate heat processing
- Cross-contamination
- Inadequate cleaning and sanitisation
- Poor temperature and time control
- Increased likelihood of the presence of pathogens

As fresh fruit, vegetables and salad vegetables (or foods which contain these as ingredients, e.g. sandwiches) naturally contain high levels of Enterobacteriaceae, it is not useful to test these foods for Enterobacteriaceae.

7.7 *E. coli*

Commission Regulation (EC) No 2073/2005, as amended, sets legal microbiological criteria for *E. coli* in live bivalve molluscs, and live echinoderms, tunicates and gastropods when placed on the market during their shelf-life (food category 1.25). Potable water is legally required to be free from *E. coli* (see Appendix I of this Guidance Note for applicable legislation).

E. coli is part of the Enterobacteriaceae family (Section 7.6). The *E. coli* test is used to assess the hygiene status of a food product and can indicate that faecal pathogens could be present. *E. coli* are killed by adequate heat processing and should not be present in heat processed food. *E. coli* are also removed from the food processing environment by effective cleaning and sanitisation.

E. coli counts of <20 cfu/g food are considered satisfactory. Unsatisfactory results, although not inherently a hazard, can indicate:

- Faecal contamination of raw materials
- Inadequate heat processing
- Cross-contamination
- Inadequate cleaning and sanitisation
- Poor temperature and time control
- Increased likelihood of the presence of pathogens

See Section 7.11 for information on verotoxingenic *E. coli* (VTEC) which is pathogenic.

7.8 *Listeria monocytogenes*

This Guidance Note does not set guideline limits for *L. monocytogenes* in ready-to-eat food because Commission Regulation (EC) No 2073/2005, as amended, sets legal microbiological criteria for *L. monocytogenes* in all ready-to-eat food when placed on the market during their shelf life. If *L. monocytogenes* is detected or enumerated in ready-to-eat food, compliance of the batch of food must be assessed against the legal limits set in Commission Regulation (EC) No 2073/2005, as amended. The legal limit is either '100 cfu/g' or 'absence in 25g' depending on the category of ready-to-eat and/or stage of sampling. See food categories 1.1, 1.2 and 1.3 in the Regulation for details of the criteria and associated sampling plans.

L. monocytogenes has been found on a wide range of foods including unpasteurised milk, soft and semi-soft cheese, deli meats, vegetables, fish products and seafood. The organism can persist in food production facilities and contaminate food after processing. Because *L. monocytogenes* can grow at refrigeration temperatures, it is of concern in chilled, ready-to-eat foods – especially if those foods have a long shelf-life.

Consuming food containing *L. monocytogenes* at levels > 100 cfu/g is considered a food safety risk¹⁵. However, older people, adults with compromised immune systems and pregnant women may become infected after consuming lower numbers because their immune system is weakened.

As *L. monocytogenes* is widespread in the environment, it is likely to be present in many raw foods. This risk can be controlled through adequate cooking and refrigeration, setting a safe shelf-life for chilled, ready-to-eat food, avoiding cross-contamination and implementing a strict cleaning and sanitisation procedure in establishments producing ready-to-eat foods to avoid post-processing contamination. Individuals vulnerable to *L. monocytogenes* infection are advised against eating certain ready-to-eat foods unless they are thoroughly heated^{16,17}.

7.8.1 *Listeria* spp. (other than *L. monocytogenes*)

Of the *Listeria* spp., only *L. monocytogenes* is considered to be a significant human pathogen. *Listeria* spp. are environmental contaminants capable of persisting in food processing environments including food processing equipment if effective cleaning and sanitation measures are not in place. *Listeria* spp. show greater heat resistance than Enterobacteriaceae, but are killed by adequate cooking. Therefore, the detection of *Listeria* spp. in ready-to-eat food can indicate inadequate heat-treatment or post-processing contamination from the production environment.

¹⁵ Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health on *Listeria monocytogenes* (1999)
Available at: http://ec.europa.eu/food/fs/sc/scv/out25_en.pdf

¹⁶ Pregnancy and *Listeria*: available at: www.safefood.eu/Publications/Consumer-info/Pregnancy-and-Listeria.aspx

¹⁷ Reducing the Risk of Food Poisoning: Information for People who are Particularly Vulnerable.
Available at www.fsai.ie/resources_publications.html

Because the presence of *Listeria* spp. could indicate that *L. monocytogenes* is present, it is advisable to set stricter guideline criteria for foods that can support the growth of *Listeria* spp. than foods that can't support the growth of *Listeria* spp. (see Table 4). The detection test (which gives a detected/not detected result) is more sensitive than the enumeration test (which gives a count result) since the detection test includes an enrichment step that should increase the chance of detecting *Listeria* spp. if present.

7.9 *Salmonella* spp.

Commission Regulation (EC) No 2073/2005, as amended, sets legal microbiological criteria for *Salmonella* in a wide range of foods when placed on the market during their shelf-life, including: fresh poultry meat, minced meat, meat preparations and meat products, mechanically separated meat, gelatine and collagen, dairy products (including infant formula and follow-on formula), egg products, live bivalve molluscs and live echinoderms, tunicates and gastropods, sprouted seeds, ready-to-eat pre-cut fruit and vegetables and unpasteurised juices. Refer to the Regulation for relevant food categories and associated sampling plans.

A wide range of animals can carry *Salmonella* leading to the contamination of animal products such as meat, poultry, eggs or raw milk. *Salmonella* infection has also been linked to other fresh and processed foods, including: fruit juice, tomatoes, lettuce, herbs, melons, sprouted seeds, spices, nuts, peanut butter, chocolate and powdered infant formula. Food processing environments can become contaminated with *Salmonella* acting as a source of food contamination.

There are over 2,500 *Salmonella* serotypes reported, all of which are considered pathogenic. *S. Typhimurium* and *S. Enteritidis* cause most human cases of salmonellosis in Ireland.

The most common manifestation of *Salmonella* infection is gastroenteritis, caused by consuming live bacteria in food. Although the infectious dose is thought to be large, outbreaks have been caused after consuming low numbers of *Salmonella* in high-fat/low-water activity foods, e.g. chocolate, cheese, ice-cream, that appear to protect *Salmonella* from stomach acid. The infectious dose is also believed to be lower in those most susceptible to *Salmonella* infection, i.e. infants, the elderly and individuals who are immunocompromised.

Adequate cooking and temperature control, and prevention of cross-contamination are important control measures for *Salmonella*. For foods which will not be cooked before consumption, such as fresh fruit and vegetable, contamination at the growing and distribution stages should be prevented through good agricultural practice.

7.10 *Shigella* spp.

Shigellosis is exclusively a human disease with the majority of cases via person-to-person spread by the faecal-oral route. Foodborne shigellosis is caused by eating food contaminated by infected food handlers or irrigation water contaminated with human faeces. Only a small number of bacteria are required to cause infection. *Shigella sonnei*, *S. flexneri*, *S. boydii* and *S. dysenteriae* are the species most often involved in illness.

Shigella is easily destroyed by heat. Salads and fresh herbs are the foods most often involved in shigellosis outbreaks. Strict personal hygiene to prevent contamination and temperature control to prevent growth of *Shigella* on foods are important control steps for shigellosis.

7.11 Verotoxigenic *E. coli* (VTEC)¹⁸

Commission Regulation (EC) No 2073/2005, as amended, sets legal microbiological criteria for STEC (VTEC) serogroups O157, O26, O111, O103, O145 and O104:H4 in sprouts when placed on the market during their shelf-life (food category 1.29).

From a food safety perspective, VTEC are the most important pathogenic *E. coli*. VTEC can cause potentially life-threatening illness. It causes haemorrhagic colitis (severe illness involving bloody diarrhoea), haemolytic uraemic syndrome (HUS – a kidney disease that can lead to renal failure) and thrombotic thrombocytopenic purpura (TPP – an extension of HUS with additional fever and neurological symptoms). The infectious dose is thought to be as low as two cells. Therefore, ready-to-eat foods containing VTEC are a food safety risk.

More than 200 VTEC serotypes have been isolated from humans. While *E. coli* O157, O26, O103, O111 and O145 are the serotypes most frequently associated with human disease, many other serotypes are pathogenic. *E. coli* O104:H4 caused a large multinational outbreak in 2011 associated with the consumption of sprouts.

While cattle are thought to be a major reservoir of VTEC, a wide range of animals have been found to excrete VTEC in their faeces, including goats, horses, dogs, cats, deer, rats, seagulls and houseflies. Person-to-person spread, contaminated water supplies and animal contact are all important transmission routes. A wide range of foods have also been associated with VTEC infection including: beef; lamb; fermented sausages; raw milk and dairy products or re-contaminated pasteurised dairy products; salads; sprouts; fruit juices; and vegetables.

¹⁸ Also referred to as shiga-toxin producing *E. coli* (STEC)

Adequate cooking and prevention of cross-contamination are important control measures for VTEC. For foods which will not be cooked before consumption, such as fresh fruit and vegetable, contamination at the growing and distribution stages should be prevented through good agricultural practice.

7.12 *Vibrio cholerae* (O1 and O139)

Two serotypes of *Vibrio cholerae* – O1 and O139 – cause cholera which can lead to epidemics and pandemics. The illness causes profuse, watery diarrhoea ('rice water' stools) resulting in extreme water loss and salt imbalance which can lead to death. Cholera has a mortality rate of 50-70%.

Cholera is a cause of illness and mortality in areas with poor sanitation and hygiene and is endemic in the Indian subcontinent, Asia and Africa. It is usually transmitted person-to-person or via contaminated/untreated water supplies. Foodborne transmission occurs when fruit, vegetables and other foods become contaminated by irrigation water, the use of 'night soil' as fertiliser, infected food handlers, contaminated food containers, or flies.

V. cholerae is also controlled through good hygienic practice and adequate cooking.

7.13 *Vibrio parahaemolyticus*

V. parahaemolyticus causes a mild form of gastroenteritis (although it can begin with violent pains). The illness is usually self-limiting with death being rare.

V. parahaemolyticus requires a certain level of salt to grow (halophile), therefore it is almost exclusively associated with marine environments and fish, shellfish and other seafoods. *V. parahaemolyticus* is killed by heating, so these foods are of most risk when consumed raw or undercooked – or when they lead to cross-contamination of ready-to-eat foods. *V. parahaemolyticus* usually occurs in low numbers in these foods, but temperature abuse will allow rapid growth.

APPENDIX I. FOODS FOR WHICH MICROBIOLOGICAL CRITERIA HAVE BEEN SET IN LEGISLATION¹⁹

Microbiological criteria have been set in legislation for various foods, some of which are ready-to-eat foods

Type of Food	European Legislation	Irish Legislation
Ready-to-eat foods Carcases Fresh poultry meat Minced meat, meat preparations, meat products Mechanically separated meat Gelatine and collagen Dairy products Egg products Live bivalve molluscs Fishery products Cooked crustaceans and molluscan shellfish Pre-cut fruit and vegetables (ready-to-eat) Unpasteurised fruit and vegetable juices (ready-to-eat) Sprouts and sprouted seeds	Commission Regulation (EC) No 2073/2005, as amended	S.I. No. 474 of 2012, as amended, for food businesses supervised by the Health Service Executive S.I. No. 432 of 2009, as amended, for food businesses supervised by the Department of Agriculture, Food and Marine, the Sea-Fisheries Protection Authority, local authorities or for food businesses that are supervised by the Health Service Executive and approved under Regulation (EC) No 853/2004
Raw milk	Regulation (EC) No 853/2004, as amended	S.I. No. 432 of 2009, as amended
Drinking water Spring water 'Other' water	Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption	S.I. No. 225 of 2007, as amended S.I. No. 122 of 2014
Natural mineral water	Directive 2009/54/EC of the European parliament and of the Council of 18 June 2009 on the exploitation and marketing of natural mineral waters	S.I. No. 225 of 2007 as amended

In addition, Commission Regulation (EC) No 669/2009, as amended, requires increased level of official controls (including laboratory analysis) on imports of certain feed and food of non-animal origin. Regulation (EC) No 854/2004, as amended, lays down microbiological criteria which competent authorities use to classify live bivalve mollusc production and relaying areas.

¹⁹ List does not take into account, amendments made or new legislation introduced after 9 May 2014. Legislation is available to download at: www.fsai.ie/legislation/food_legislation.html

**APPENDIX 2.
OPINION OF SCIENTIFIC
COMMITTEE ON THE REVISION
OF GUIDANCE NOTE No. 3**

Background

The Food Safety Authority of Ireland (FSAI) is currently revising Guidance Note 3²⁰ which sets national guideline criteria for the microbiological analysis for ready-to-eat foods sampled at the point-of-sale. The guideline criteria are being revised to take account of:

- Commission Regulation (EC) No 2073/2005²¹, which lays down legal microbiological criteria for foodstuffs, and
- Revised UK guidelines²², issued by the Health Protection Agency (HPA), for assessing the microbiological safety of ready-to-eat foods placed on the market

There are discrepancies between guideline limits (applicable at point-of-sale) and legal process hygiene limits (applicable during manufacturing) for three bacteria – coagulase-positive staphylococci, *E. coli* and Enterobacteriaceae – in certain foods. The FSAI therefore, requested the Scientific Committee's advice (Ref. BioI – see Annex I) on the appropriate microbiological guidelines to set for the bacteria/food combinations outlined below in points 1, 2 and 3.

For other ready-to-eat foods, the revised guideline limits produced by the HPA have already been agreed by the former Microbiology Sub-committee as appropriate to adopt in Ireland and will not be discussed in this Opinion.

I. Coagulase-positive staphylococci

Discrepancy: Guideline limits for ready-to-eat foods sampled at point-of-sale are stricter than the legal limits applicable during manufacturing for three categories of food (Table I).

²⁰ FSAI (2001) Guidelines for the Interpretation of Results of Microbiological Analysis of Some Ready-to-Eat Foods Sampled at the Point-of-Sale (Guidance Note No. 3)

²¹ Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs

²² HPA (2009) Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market

Table I: Coagulase-positive staphylococci – discrepancy between limits set at point-of-sale and during manufacturing

Food Category in Regulation 2073	Unsatisfactory Level (cfu/g) and Stage where Criterion applies stated in:		
	Guidance Note 3 (point-of-sale)	HPA limit (point-of-sale)	Regulation 2073/2005 (manufacturing)
2.2.3 Cheeses made from raw milk	10 ²	>10 ⁴	>10 ⁵
2.2.4 Cheeses made from milk that has undergone a lower heat treatment than pasteurisation (*) and ripened cheeses made from milk or whey that has undergone pasteurisation or a stronger heat treatment (*)	10 ²	>10 ⁴	>10 ³
2.4.1 Shelled and shucked products of cooked crustaceans and molluscan shellfish	10 ²	>10 ⁴	>10 ³

* Excluding cheeses where the manufacturer can demonstrate, to the satisfaction of the competent authorities, that the product does not pose a risk of staphylococcal enterotoxins

Question: Is it appropriate to set coagulase-positive staphylococci guidelines for the three foods listed in Table I and if yes, what should these guidelines be?

Proposed answer and rationale: Yes, but it is not appropriate that the limits set for these foods when sampled at point-of-sale are stricter than the limits set when sampled at manufacturing level. Numbers of coagulase-positive staphylococci decline during cheese maturation therefore numbers at point of sale may generally be expected to be lower than those which may occur during manufacturing. It is acknowledged that coagulase-positive staphylococci in cheese at retail level may reflect, in addition, the introduction of new coagulase-positive staphylococci into the food after manufacturing as a result of poor handling practices and/or proliferation due to temperature abuse. Although many cheeses present an unfavourable environment for growth of coagulase-positive staphylococci, growth in cheese is possible²³. However, if the number of coagulase-positive staphylococci in a cheese sample taken at retail is at or below a level that would have been acceptable during manufacturing, it is not possible in practical terms to determine if this reflects persistence of coagulase-positive staphylococci from the manufacturing process or poor handling/temperature abuse after manufacturing.

²³ For example in: surface smear ripened cheese where the growth of the bacterial smear raises the pH from acidic to towards neutral; whey cheeses as these have a high water activity and pH; and semi-hard and hard cheese of the lactic type. See Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health on Staphylococcal enterotoxins in milk products, particularly cheeses (2003). Available at: http://ec.europa.eu/food/fs/sc/scv/out61_en.pdf

In conclusion, the limit for “unsatisfactory” for coagulase-positive staphylococci (referred to as *Staphylococcus aureus* in Guidance Note 3) at point-of-sale should be revised upwards as per Table 2. Testing for enterotoxin is advised if numbers exceed 10^5 in order to confirm if enterotoxin has been produced in the food, because not all strains are capable of producing toxin and causing disease.

Table 2. Limits for coagulase-positive staphylococci at point-of-sale

Food Category in Regulation 2073	Unsatisfactory Level (cfu/g) and Stage where Criterion applies stated in:		
	Guidance Note 3 (point-of-sale)	HPA limit (point-of-sale)	Regulation 2073/2005 (manufacturing)
2.2.3 Cheeses made from raw milk ^(c)	<20	Satisfactory ^(d)	None
2.2.4 Cheeses made from milk that has undergone a lower heat treatment than pasteurisation ^(a) and ripened cheeses made from milk or whey that has undergone pasteurisation or a stronger heat treatment ^(a)	20 to 10^4	Acceptable ^(d)	Review: <ul style="list-style-type: none"> • Handling practices at retail level • Temperature control • Production hygiene • Selection of raw materials
2.4.1 Shelled and shucked products of cooked crustaceans and molluscan shellfish	$>10^4$ ^(c)	Unsatisfactory	<ul style="list-style-type: none"> • Review: <ul style="list-style-type: none"> – Handling practices at retail level – Temperature control – Production hygiene – Selection of raw materials • Test for enterotoxin if numbers $>10^5$, or where food has been suspected to have caused staphylococcal food poisoning • If enterotoxin detected, product withdrawal/recall required^(b)

^(a) Excluding cheeses where the manufacturer can demonstrate, to the satisfaction of the competent authorities, that the product does not pose a risk of staphylococcal enterotoxins

^(b) Under Regulation (EC) No 178/2002 (Article 14 Food safety requirements)

^(c) For cheeses made from raw milk, unsatisfactory is $>10^5$ in line with the process hygiene criterion in Commission Regulation (EC) No 2073

^(d) For cheese, acceptable/satisfactory levels of CPS do not guarantee that toxin is not present. In the early stages of cheese production, CPS may have grown to sufficient levels to produce enterotoxin and then died off

2. *E. coli*

Discrepancy: Guideline limits for ready-to-eat foods sampled at point-of-sale are stricter than the legal limits applicable during manufacturing for three categories of food (Table 3).

Table 3. *E. coli* – discrepancy between limits set at point-of-sale and during manufacturing

Food Category in Regulation 2073	Unsatisfactory Level (cfu/g) and Stage where Criterion applies stated in:		
	Guidance Note 3 (point-of-sale)	HPA (point-of-sale)	Regulation 2073/2005 (manufacturing)
2.2.2 Cheeses made from milk or whey that has undergone heat treatment	≥100	Refer to Regulation 2073	>10 ³
2.5.1 Pre-cut fruit and vegetables (ready-to-eat)	≥100	Refer to Regulation 2073	>10 ³
2.5.2 Unpasteurised fruit and vegetable juices (ready-to-eat)	≥100	Refer to Regulation 2073	>10 ³

Question: Is it appropriate to set *E. coli* guidelines for the three foods lists and if yes, what should these guidelines be?

Proposed answer and rationale: Yes. The guideline limits for point of sale for these three food categories should be revised upwards as per Table 4. Numbers of *E. coli* in a food product are unlikely to decrease dramatically from manufacturing level to point-of-sale. However, it is acknowledged that *E. coli* in these products at retail level may reflect, in addition, the introduction of *E. coli* into the food after manufacturing as a result of poor handling practices and/or proliferation due to temperature abuse. However, if the number of *E. coli* in a sample taken at retail is at or below a level that would have been acceptable during manufacturing it is not possible in practical terms to determine if this reflects persistence of *E. coli* from the manufacturing process or poor handling/temperature abuse after manufacturing. It therefore, is not appropriate that the limit set for “unsatisfactory” for these foods when sampled at point-of-sale are stricter than the limits set when sampled at manufacturing level.

Table 4. Limits for *E. coli* in ready-to-eat foods

Food Category	Count (cfu/g or ml)	Classification	Suggested Actions
2.2.2 Cheeses made from milk or whey that has undergone heat treatment	<100	Satisfactory	None
2.5.1 Pre-cut fruit and vegetables (ready-to-eat)	100 to 10 ³	Acceptable	<ul style="list-style-type: none"> • Review: <ul style="list-style-type: none"> – Hygiene procedures – Cleaning procedures • Consider taking investigative samples of food and the food preparation environment
2.5.2 Unpasteurised fruit and vegetable juices (ready-to-eat)	>10 ³	Unsatisfactory	<ul style="list-style-type: none"> • Review: <ul style="list-style-type: none"> – Hygiene procedures – Cleaning procedures • Take investigative samples of food • Undertake environmental monitoring of food preparation environment

3. Enterobacteriaceae

Discrepancy: Guideline limits for ready-to-eat foods sampled at point-of-sale are considerably more lenient than the legal limits applicable during manufacturing for six categories of food (Table 5).

Table 5. Enterobacteriaceae – discrepancy between limits set at point-of-sale and during manufacturing

Food Category in Regulation 2073	Unsatisfactory Level (cfu/g or ml) and Stage where Criterion applies stated in:		
	Guidance Note 3 (point-of-sale)	HPA (point-of-sale)	Regulation 2073/2005 (manufacturing)
2.2.1 Pasteurised milk and other pasteurised liquid dairy products	$\geq 10^4$	$> 10^4$	> 10
2.5.7 Milk powder and whey powder	$\geq 10^4$	$> 10^4$	> 10
2.2.8 Ice-cream (containing milk ingredients) and frozen dairy desserts	$\geq 10^4$	$> 10^4$	> 100
2.2.9 Dried infant formulae and dried dietary foods for special medical purposes intended for infants below six months of age	$\geq 10^4$	$> 10^4$	Presence in 10g
2.2.10 Dried follow-on formulae	$\geq 10^4$	$> 10^4$	Presence in 10g
2.3.1 Egg products	$\geq 10^4$	$> 10^4$	$> 10^2$

Question: Is it appropriate to set Enterobacteriaceae guidelines for the six food²⁴ lists and if yes, what should these guidelines be?

Proposed answer: No. Limits for Enterobacteriaceae at retail level should not be set for these six food categories. Enterobacteriaceae are a broad group which can be used to assess the general hygiene status of a food product. Unlike *E. coli*, Enterobacteriaceae are not a clear indication of faecal contamination and there is no level of Enterobacteriaceae which can be classified as hazardous.

²⁴ Note the Request for advice from the Scientific Committee (Bio1) lists five food categories – dried follow-on formula and dried dietary foods for special medical purposes intended for infants below six months of age was not included

ANNEX I. REQUEST FOR ADVICE FROM THE SCIENTIFIC COMMITTEE

Ref Number: Bio I

Topic: Advice on revision of the national microbiological guidelines for ready-to-eat (RTE) foods sampled at the point of sale (FSAI Guidance Note No. 3)

Date Requested: 10 June 2011

Origin of Request: FSAI

Date Accepted: 10 June 2011

Target Deadline for Advice: September/October 2011

Form of Advice required: A brief report which addresses the three questions posed

Proposed Sub-committee: Biohazards Sub-committee

Background/Context

National microbiological guidelines for ready-to-eat (RTE) foods sampled at the point of sale were published by the Food Safety Authority of Ireland in 2001 (FSAI Guidance Note No.3). While these guidelines do not have a legal basis, they provide a benchmark against which unacceptable microbial contamination of food can be identified. These guidelines are currently being revised by the FSAI to:

- Clarify the situation regarding guidelines for which there is an equivalent or related criterion laid down in Commission Regulation No 2073/2005; and
- Include current scientific advice from the recently revised UK guidelines upon which the Irish guidelines were originally based in 2001

Regulation 2073/2005 outlines two types of criteria:

- **Food safety criteria** which define the acceptability of a product or batch of foodstuffs and are applicable to products placed on the market
- **Process hygiene criteria** which indicate the acceptable functioning of the production process and are applicable to foodstuffs during their production

When the Regulation came into force, the FSAI produced an interim guidance document to identify the national microbiological guidelines which had been superseded by food safety criteria. In producing the interim guidance document, the FSAI identified three discrepancies between the guidelines (applicable to products sampled at the point-of-sale) and process hygiene criteria (applicable during manufacturing). In two cases (coagulase positive staphylococci and *E. coli*), the national guideline was stricter than the legal process hygiene criteria and in the 3rd case (Enterobacteriaceae), the national guideline was considerably more lenient.

Request Details

Coagulase positive staphylococci

The national guideline for coagulase positive staphylococci (applicable to products sampled at the point-of-sale) is stricter than the process hygiene criteria (applicable during manufacturing) laid down in the Regulation for:

- Cheese made from raw milk
- Cheeses made from milk that has undergone a lower heat treatment than pasteurisation and ripened cheeses made from milk or whey that has undergone pasteurisation or a stronger heat treatment
- Shelled and shucked products of cooked crustaceans and molluscan shellfish

Therefore, the current national guideline cannot be applied to these three categories of food.

In addition, when considering if revised national guidelines are appropriate for the two types of cheeses listed above, the Sub-committee should note that the:

- Regulation sets a food safety criterion for the staphylococcal enterotoxin in addition to the process hygiene criterion for coagulase positive staphylococci in these cheeses
- Report of the FSAI Scientific Committee on Review of the Sampling & Microbiological Examinations undertaken by the HSE, 2007 & 2008 recommends that “*Microbiological examination for coagulase positive staphylococci in cheese should generally be conducted early in the food chain (i.e. during the manufacturing process when the staphylococcal count is expected to be the highest) rather than at retail level*”; and
- 2003 Opinion of the scientific committee on veterinary measures relating to public health on staphylococcal enterotoxins in milk products, particularly cheeses

E. coli

The national guideline for *E. coli* (applicable to products sampled at the point-of-sale) is stricter than the process hygiene criteria (applicable during manufacturing) laid down in the Regulation for:

- Cheeses made from milk or whey that has undergone heat treatment
- Pre-cut fruit and vegetables (ready-to-eat)
- Unpasteurised fruit and vegetable (ready-to-eat)

Therefore, the current national guideline cannot be applied to these three categories of food.

Enterobacteriaceae

The national guideline for Enterobacteriaceae (applicable to products sampled at the point-of-sale) is very lenient compared to the process hygiene criteria (applicable during manufacturing) laid down in the Regulation for:

- Pasteurised milk and other pasteurised liquid dairy products
- Milk powder and whey powder
- Ice cream and frozen dairy desserts
- Dried infant formulae and dried dietary foods for special medical purposes intended for infants below six months of age
- Egg products

Therefore, the current national guideline may need to be revised for these foods.

Risk Management Questions

1. Is it appropriate to set coagulase positive staphylococci guidelines for the three foods lists and if yes, what should these guidelines be?
2. Is it appropriate to set *E. coli* guidelines for the three foods lists and if yes, what should these guidelines be?
3. Is it appropriate to set Enterobacteriaceae guidelines for the five foods lists and if yes, what should these guidelines be?

REFERENCES

Food Safety Authority of Ireland (2001) Guidance Note No.3 Guidelines for the interpretation of results of microbiological analysis of some ready-to-eat foods sampled at the point-of-sale

European Commission (2005) Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs

http://www.fsai.ie/uploadedFiles/Consol_Reg2073_2005.pdf

Food Safety Authority of Ireland (2007) Interim guidance document on the use of:

1) Food safety criteria specified in Commission Regulation (EC) No 2073/2005 on Microbiological Criteria for Foodstuffs and 2) Guidelines for the Interpretation of Results of Microbiological Analysis of Some Ready-to-Eat Foods Sampled at Point-of-Sale (FSAI GN No. 3)

Health Protection Agency (2009) Guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market

http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1259151921557

European Commission (2003) Opinion of the scientific committee on veterinary measures relating to public health on staphylococcal enterotoxins in milk products, particularly cheeses

http://ec.europa.eu/food/fs/sc/scv/out61_en.pdf

NOTES



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