LHAAC

LOCAL HEALTH AUTHORITIES ANALYTICAL COMMITTEE

COORDINATED SAMPLING PROJECT 26 -

Microbiological Quality of Food from Cold Wells

Conducted April to June 2019 with Local Government's across Western Australia

Local Health Authorities Analytical Committee

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Executive Summary

This survey aimed to assess the microbiological quality of ready-to-eat (RTE) food products sold to the public which were made using ingredients previously contained in 'Cold Well' storage. Typically, these foods included sandwiches, wraps, or kebabs.

Western Australian (WA) Environmental Health Officers (EHO) submitted samples for assessment to Agrifood Technology (AT) or Analytical Reference Laboratory (ARL) from April through June 2019. At the end of the sampling period, 37 Local Government Authorities (LGA) had submitted a total of 316 food samples to the laboratory for analysis. All food samples were tested for the presence of *Escherichia coli* (*E. coli*), coagulase-positive *Staphylococci*, *Bacillus cereus* (*B. cereus*), *Salmonella* species (spp.), *and Listeria monocytogenes* (*L. monocytogenes*). Samples which contained chicken underwent additional testing for the presence of *Campylobacter* spp., while the presence of *Vibrio parahaemolyticus* (*V. parahaemolyticus*) was tested for in samples which contained raw seafood.

Test results were assessed by the LGA against Food Standards Australia and New Zealand's (FSANZ) Compendium of Microbiological Criteria for Food. The results were categorised as satisfactory, marginal, unsatisfactory or potentially hazardous. Where necessary, further investigation or action by the appropriate LGA was undertaken.

E. coli test results indicated 98.4% of samples to be within the satisfactory range, while marginal levels were reported in 1.6% of samples. Coagulase-positive *Staphylococci* test results indicated 97.2% of samples to be within the satisfactory range, while marginal levels were reported in 2.5% of samples, and potentially hazardous levels were recorded in 0.3% of samples. *B. cereus* test results indicated 86% of samples to be within the satisfactory range, while marginal levels were reported in 12.4% of samples, and unsatisfactory levels were recorded in 1.6% of samples. *Salmonella* spp., as well as *L. monocytogenes*, test results indicated 100% of samples to be within the satisfactory range. *Campylobacter* spp. test results indicated 100% of samples to be within the satisfactory range. Similarly, *V. parahaemolyticus* test results indicated 100% of samples to be within the satisfactory range.

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Abbreviations

ARL	Analytical Reference Laboratory
AT	Agrifood Technology
CSP	Coordinated Sampling Project
EHO	Environmental Health Officer
FSANZ	Food Standards Australia and New Zealand
FSC	Food Standards Code
LGA	Local Government Authority
LHAAC	Local Health Authorities Analytical Committee
NATA	National Association of Testing Authorities
RTE	Ready-to-eat
Spp.	Species
WA	Western Australia

1.0 Introduction

1.1 Background

As the second microbiologically based Coordinated Sampling Project (CSP) conducted by the Local Health Authorities Analytical Committee (LHAAC), this project involved the microbial analysis of 'Cold Well' stored food products sold throughout WA. Cold well storage is commonly used by food businesses such as lunch bars and kebab outlets in the preparation and sale of RTE food products. Often the ingredients within the cold well can be stored for long periods of time before being sold for consumption or discarded. This survey aimed to assess the microbiological quality of RTE food products sold to the public which were made using ingredients previously contained in cold wells. Typically, these foods included sandwiches, wraps, or kebabs.

Microbiological guidelines can be used by regulatory agencies to check that food for sale is safe and suitable and the food handling controls and hygienic practices of a food business are adequate. The microbial quality of food products analysed for this project were assessed using FSANZ's Compendium of Microbiological Criteria for Food [1].

1.2 Bacterial Contamination Risk and Foodborne Illness

Cold well stored ingredients are synonymous with the production of RTE food products, often being subject to extensive handling prior to sale. In some cases, the food products prepared for consumption using ingredients from cold wells do not undergo a cooking process or other pathogen reduction step. The microbiological quality of food can be impacted by temperature control, food handler hygiene and food quality [2]. There is a risk of bacteria transferring to food ingredients at any stage including transport, processing, storage, and at the point of sale.

Food that is contaminated with pathogenic microorganisms can cause the consumer to suffer from foodborne illnesses. Bacteria that are commonly responsible for causing foodborne illnesses include *E. coli*, *Salmonella* spp., *L. monocytogenes*, and *Campylobacter* spp. [3]. In fact, the three microorganisms most commonly associated with microbial food recalls in Australia between 2008 and 2017 were *E. coli*, *Salmonella* spp., and *L. monocytogenes* [4].

1.3 Temperature Control

RTE food is not expected to undergo further cooking or processing prior to consumption. This can be considered potentially hazardous particularly if the food needs to be kept under temperature control to minimise the growth of pathogenic microorganisms that may be present in the food, or to prevent the formation of toxins in the food.

The production and sale of food in WA must comply with the requirements of the Australia New Zealand Food Standards Code (FSC). As per Standard 3.2.2 of the FSC, potentially hazardous food must be stored under temperature control which can be achieved by either refrigeration to below 5° Celsius or heating to above 60° Celsius [5]. In accordance with the FSC, a food business may maintain the food out of temperature control if it can be demonstrated that the alternative temperature does not adversely affect the microbiological safety of the food [5]. Food businesses often achieve this requirement with the application of the 2 hour – 4 hour rule (Appendix B), a process that requires documented procedures to ensure that potentially hazardous food is safe while stored out of temperature control for a limited time [6] [7].

2.0 Methodology

Sampling instructions were supplied to WA LGAs. Both metropolitan and non-metropolitan LGAs were encouraged to participate in this CSP if suitable products were available in their locality. The number of samples to be collected was determined by each LGA in consideration of their sampling allowance and other activity planned or anticipated for the financial year.

Samples of RTE foods from across WA, which used cold well ingredients, were submitted to either AT or ARL, the two appointed analysts to the LHAAC, between May 2019 and June 2019. The minimum sample size for submission to the analysts was 200 grams. Each laboratory conducted microbial analysis of the samples utilising National Association of Testing Authorities (NATA) accredited methods (Appendix A). All food samples were tested for the presence of *E. coli*, coagulase-positive *Staphylococci, B. cereus*, *Salmonella* spp., and *L. monocytogenes*. Samples which contained chicken underwent additional testing for the presence of *Campylobacter* spp., while the presence of *V. parahaemolyticus* was tested for in samples which contained raw seafood.

Upon completion, LGAs were requested to review the results by assessing them against the FSANZ's microbiological guidelines (Appendix C) [1]. Recommended follow-up actions were provided to each LGA within the sampling instructions.

3.0 Results

By the end of the sampling period, 37 LGAs had submitted a total 316 food samples of varying types to the laboratories for analysis (Figure 1.). Sample type distribution indicated that 60% (*n* = 191) of the submitted samples were sandwiches, 15% (*n* = 47) were wraps, 13% (*n* = 41) were kebabs, 3% (*n* = 10) were salad products, 2% (*n* = 5) were burgers, 1% (*n* = 3) were sushi products, and 6% (*n* = 19) were other samples which predominantly consisted of individual cold well ingredients.

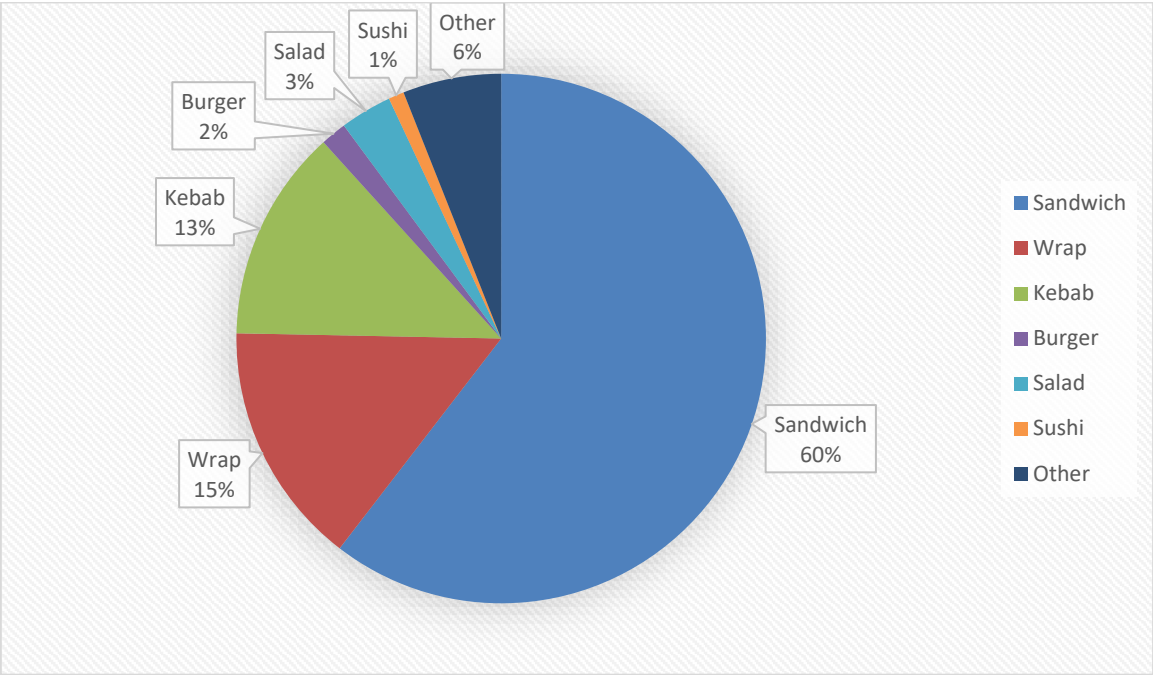

Figure 1. Distribution of sample types submitted for analysis

Overall, a total of 1,783 tests were carried out. All 316 samples were tested for the presence of *E. coli*, coagulase-positive *Staphylococci, B. cereus*, *Salmonella* spp., and *L. monocytogenes*. 201 samples, which contained chicken, underwent additional testing for the presence of *Campylobacter* spp., and 2 samples, which contained raw seafood, were tested for the presence of *V. parahaemolyticus*. All test results were compared against the FSANZ microbiological guidelines (Table 1).

Table 1. Guideline levels for determining the microbiological quality of RTE foods [1].

	Microbiological Quality (CFU/g)			
Indicator/Pathogen	**Satisfactory**	**Marginal**	**Unsatisfactory**	**Potentially Hazardous**
Escherichia coli	<3	3 - ≤10^2	>10^2	-
Coagulase-positive *Staphylococci*	<10^2	10^2 - <10^3	10^3 - ≤10^4	>10^4
Bacillus cereus	<10^2	10^2 - <10^3	10^3 - ≤10^5	>10^5
Vibrio parahaemolyticus	<3	3 - <10^2	10^2 - ≤10^4	>10^4
Listeria monocytogenes (RTE food in which growth is not supported)	Not detected in 25g	≤10^2	-	>10^2
Listeria monocytogenes (RTE food associated with high risk growth)	Not detected in 25g	-	-	Detected in 25g
Campylobacter spp.	Not detected in 25g	-	-	Detected in 25g
Salmonella spp.	Not detected in 25g	-	-	Detected in 25g

Upon analysis, *E.coli* test results indicated 98.4% (*n* = 311) of samples to be within the satisfactory range, while marginal levels were reported in 1.6% (*n* = 5) of samples. Coagulase-positive *Staphylococci* test results indicated 97.2% (*n* = 307) of samples to be within the satisfactory range, while marginal levels were reported in 2.5% (*n* = 8) of samples, and potentially hazardous levels were recorded in 0.3% (*n* = 1) of samples. *B. cereus* test results indicated 86% (*n* = 272) of samples to be within the satisfactory range, while marginal levels were reported in 12.4% (*n* = 39) of samples, and unsatisfactory levels were recorded in 1.6% (*n* = 5) of samples. *Salmonella* spp., as well as *L. monocytogenes*, test results indicated 100% (*n* = 316) of samples to be within the satisfactory range. *Campylobacter* spp. test results indicated 100% (*n* = 201) of samples to be within the satisfactory range. Similarly, *V. parahaemolyticus* test results indicated 100% (*n* = 2) of samples to be within the satisfactory range. Figure 2. represents the test results for each microbial contaminant and provides a visual distinction between levels.

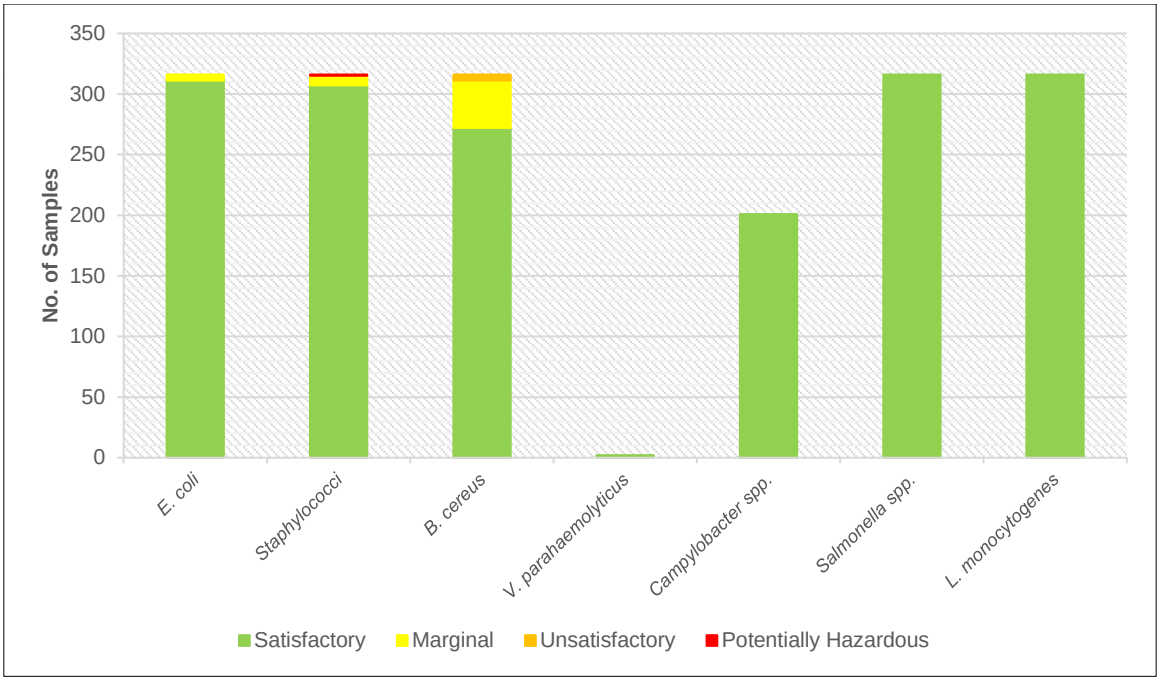

Figure 2. Test results for each microbial contaminant and distinction between levels

When compared against the FSANZ guideline levels for determining the microbiological quality of RTE foods, 96.7% of all test results indicated that the sample was within the satisfactory range, 2.9% indicated marginal levels of microbial contamination, 0.3% indicated unsatisfactory levels of microbial contamination, and 0.1% indicated potentially hazardous levels of microbial contamination (Table 2.).

Table 2. Test results by level of microbial contamination

Indicator/Pathogen	Satisfactory	Marginal	Unsatisfactory	Potentially Hazardous
Escherichia coli	311	5	0	0
Coagulase-positive *Staphylococci*	307	8	0	1
Bacillus cereus	272	39	5	0
Vibrio parahaemolyticus	2	0	0	0
Campylobacter spp.	201	N/A	N/A	0
Salmonella spp.	316	N/A	N/A	0
Listeria monocytogenes	316	0	N/A	0
Total	**1725**	**52**	**5**	**1**

4.0 Discussion

4.1 *Escherichia coli*

E. coli is a bacterium that is naturally found in human and animal intestines [9]. It is often spread to food via the faecal-oral route and can cause an infection when ingested [9]. Since the presence of *E. coli* suggests faecal contamination, microbiological tests are often conducted to provide a reference in order to evaluate the hygienic quality of food [10]. A previous Australian study, which analysed the microbial contamination of 851 sushi samples, found the presence of *E. coli* at marginal levels in 34 samples and unsatisfactory levels in 6 samples [6].

The results from this CSP indicated that 311 of 316 samples which were tested for the presence of *E. coli* fell within satisfactory levels when compared against the microbiological guidelines from FSANZ [1]. 5 samples indicated marginal levels. These samples included 2 sandwiches, 1 kebab, 1 sushi product, and 1 other ingredient. These results indicate that the samples tested had been handled hygienically.

4.2 Coagulase-positive *Staphylococci*

Staphylococcus is a genus of bacteria which can be further categorised by its ability to produce coagulase [11]. Coagulase-positive species are generally considered potentially pathogenic to humans [11]. *Staphylococcus aureus* (*S. aureus*) is a Coagulase-positive species that can cause food poisoning [12]. Some humans naturally carry *S. aureus* on their skin and in their nose. *S. aureus* can transmit to food as a result of poor food handling practices and temperature abuse can result in its multiplication [12].

The results from this CSP indicated that 307 of 316 samples which were tested for the presence of coagulase-positive *Staphylococcus* fell within satisfactory levels when compared against the microbiological guidelines from FSANZ [1]. 8 samples indicated marginal levels. These samples included 5 sandwiches, 2 kebabs, and 1 salad product. Additionally, 1 salad product sample presented with potentially hazardous levels. This result suggests that handling controls are not being implemented appropriately at the food premises where the affected samples were produced [1].

4.3 *Bacillus cereus*

B. cereus is a bacterium that is found in nature and is commonly detected in soil. It is usually found in raw ingredients and is commonly associated with rice dishes [1]. *B. cereus* illness is often related to improper cooling of food and temperature abuse [13]. There are two main types of foodborne illness that are caused by the bacterium, one is characterised by vomiting or nausea while the other causes diarrhoea [13]. A previous Australian study, which analysed 851 sushi samples, found unsatisfactory levels of *B. cereus* in two samples and potentially hazardous levels in 6 samples [6].

The results from this CSP indicated that 272 of 316 samples which were tested for the presence of *B. cereus* fell within satisfactory levels when compared against the microbiological guidelines from FSANZ [1]. 39 samples indicated marginal levels, while 5 samples indicated unsatisfactory levels. Of the 39 marginal samples, 21 were sandwiches, 9 were kebabs, 4 were wraps, 1 was a burger, 1 was a sushi product, and 3 were other ingredients. The 5 samples categorised as unsatisfactory were 3 sandwiches, 1 kebab, and 1 other ingredient.

4.4 *Vibrio parahaemolyticus*

V. parahaemolyticus is a bacterium found naturally in salt water [14]. It can cause gastroenteritis when consumed and is most often associated with raw or undercooked shellfish, fish or crustaceans [1]. Accordingly, this CSP only tested for the presence of *V. parahaemolyticus* in products containing raw seafood.

The results from this CSP indicated that 2 of 2 samples which were tested for the presence of *V. parahaemolyticus* fell within satisfactory levels when compared against the microbiological guidelines from FSANZ. This result suggests that the food outlets who supplied these samples have taken care in the preparation of the RTE meals which contained raw seafood.

4.5 *Campylobacter* spp.

Campylobacter is a species of bacteria that is found within the gastrointestinal system and faecal matter of animals and is most commonly in or on raw poultry [16]. A condition known as Campylobacteriosis is caused by ingesting undercooked or *Campylobacter* spp. contaminated meat, particularly chicken, which infects the digestive tract of humans [16]. Campylobacteriosis is considered to be the most common bacterial cause of human gastroenteritis worldwide, accounting for around half of all reported gastrointestinal infections in WA [16] [17]. Infections are generally mild, however, can turn fatal among young children, elderly, and immune-deficient individuals [17]. *Campylobacter* spp. infections can be minimised by following basic food hygiene practices when preparing food products [17].

The results from this CSP indicated that all 201 samples which were tested for the presence of *Campylobacter* spp. fell within satisfactory levels when compared against the microbiological guidelines from FSANZ. This result suggests that the food outlets who supplied these samples have taken care in both the preparation and in the cooking process of the RTE meals which contained poultry.

4.6 *Salmonella* spp.

Salmonella spp. are bacteria which are known to cause a disease called Salmonellosis which is characterised by abdominal pain, diarrhoea and occasionally vomiting [18]. *Salmonella* spp. can transmit from animals to contaminate food of animal origin (such as eggs, meat or dairy) or it can be transmitted by humans through the faecal-oral route [18].

The results from this CSP indicated that all 316 samples which were tested for the presence of *Salmonella* spp. fell within satisfactory levels when compared against the microbiological guidelines from FSANZ. This result suggests that the food outlets who supplied these samples have taken care in both the preparation and in the cooking process of the RTE meals included in this survey.

4.7 *Listeria monocytogenes*

L. monocytogenes is a bacterium responsible for causing a foodborne disease named Listeriosis [19]. Non-invasive Listeriosis can affect otherwise healthy individuals with symptoms including headache, muscle pain, fever and diarrhoea [19]. Invasive Listeriosis is a serious threat to high risk population groups including pregnant women, the immunocompromised, children and the elderly [20]. Symptoms of invasive Listeriosis can include septicaemia and bacterial meningitis, with symptoms capable of causing premature death [19]. Since *L. monocytogenes* cannot survive under intense heat, some RTE food products that use raw ingredients are at higher risk of contamination due to the lack of cooking process [20]. Previous foodborne outbreaks of Listeriosis have been linked to fish products [20]. As per the Compendium of Microbiological Criteria for Food, the presence of *L. monocytogenes* in RTE foods is an indication of inadequate processing or environmental cross contamination [1].

The results from this CSP indicated that all 316 samples which were tested for the presence of *L. monocytogenes* fell within satisfactory levels when compared against the microbiological guidelines from FSANZ. This result suggests that the food outlets who supplied these samples have taken care in both the preparation and in the cooking process of the RTE foods included in this survey. Continued education on safe food handling procedures will assist in preventing the spread of *L. monocytogenes* in food businesses [19].

5.0 Conclusion

This CSP looked at the microbiological quality of RTE food products made using ingredients from cold well storage. All samples were tested for the presence of *E. coli*, coagulase-positive *Staphylococci, B. cereus*, *Salmonella* spp., and *L. monocytogenes*. Samples which contained chicken underwent additional testing for the presence of *Campylobacter* spp., while the presence of *V. parahaemolyticus* was tested for in samples which contained raw seafood.

The results found that the vast majority (96.7%) of test results were within satisfactory levels of microbiological quality when assessed against FSANZ's microbiological guidelines for RTE food. These results indicate safe food practices were being implemented by the food businesses. Overall, continued management of food safety risks associated with microbial contamination of food stored in cold wells is required by food businesses and continued monitoring of food safety by LGAs is recommended.

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Appendix A

Raw Data

For further questions or inquiries about raw data, contact LHAAC Co-ordinator Trevor Chapman:

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Appendix B

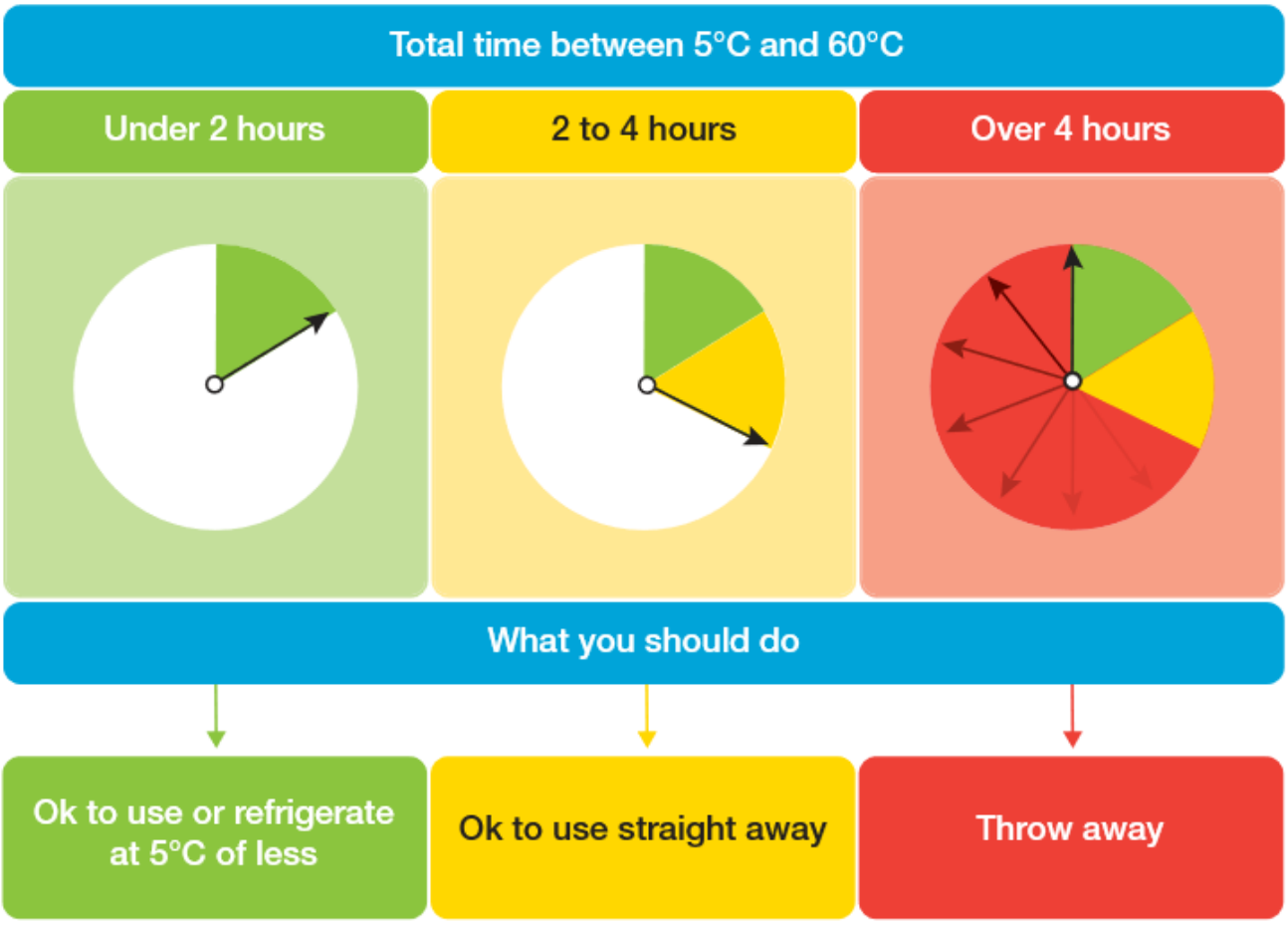

Figure 3. A visual representation of the 2 hour – 4 hour rule [7]

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Appendix C

Table 3. Guidelines for the determination of microbiological quality in RTE food products [1].

	Microbiological Quality (CFU/g)			
Indicator/Pathogen	**Satisfactory**	**Marginal**	**Unsatisfactory**	**Potentially Hazardous**
Escherichia coli	<3	3 - ≤10^2	>10^2	[a]
Coagulase-positive *Staphylococci*	<10^2	10^2 - <10^3	10^3 - ≤10^4	>10^4
Bacillus cereus	<10^2	10^2 - <10^3	10^3 - ≤10^5	>10^5
Vibrio parahaemolyticus [b]	<3	3 - <10^2	10^2 - ≤10^4	>10^4
Listeria monocytogenes (RTE food in which growth is not supported)	Not detected in 25g	≤10^2 [c]	-	>10^2 [d]
Listeria monocytogenes (RTE food associated with high risk growth)	Not detected in 25g	-	-	Detected in 25g
Campylobacter spp.	Not detected in 25g	-	-	Detected in 25g
Salmonella spp.	Not detected in 25g	-	-	Detected in 25g

[a] = Pathogenic strains of *E.coli* should be absent.

[b] = *V. parahaemolyticus* should not be present in seafood that has been cooked. For RTE seafood that is raw, a higher satisfactory level may be applied (<10^2 CFU/g). The potentially hazardous level of *V. parahaemolyticus* relates to Kanagawa-positive strains.

[c] = The detection of *L. monocytogenes* in RTE foods prepared specifically for 'at risk' population groups (the elderly, immunocompromised and infants) should also be considered as potentially hazardous.

[d] = Foods with a long shelf life stored under refrigeration should have no *L. monocytogenes* detected in 25 grams.