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Technical information note

Microbiological testing of finished dairy products

This technical information note outlines factors for dairy manufacturers to consider when developing a microbiological sampling and testing plan to verify the effectiveness of their food safety program, and for compliance with national microbiological standards and regulations.

The note outlines the licensee's minimum obligations for testing to satisfy Dairy Food Safety Victoria requirements. It also describes action to be taken when a pathogen is detected in a finished product.

The note highlights some of the challenges associated with microbiological testing, including the influence sampling plans can have on the validity of the final test result.

Reasons for testing finished dairy products

Dairy manufacturers licensed with Dairy Food Safety Victoria are required under the *Code of Practice for Dairy Food Safety 2002*[1] to have in place a testing program as part of their approved food safety program. Aspects that should be identified are broadly defined in the *Guidelines for Food Safety: Dairy Food Manufacturers*[2], and are explained in more detail in this note.

Testing of finished products was traditionally the main way food manufacturers confirmed the safety and acceptable quality of their finished products. The product would be tested and if the microbial count was below a documented maximum, the food would be released to the marketplace.

With the introduction of quality assurance methods involving adherence to good manufacturing practices (GMP) and the implementation of HACCP-based food safety programs, finished product testing became a means of verifying the effective implementation of food safety programs. By applying controls and interventions along the entire food chain these systems were designed to proactively reduce the likelihood of a food containing pathogens and presenting a risk to public health.

Hence the modern rationale for microbiological testing of finished products is to:

(a) verify that regulatory standards and guidelines have been met
(b) confirm process capability (validation)
(c) identify any issues and verify the remedial activity
(d) establish benchmarks and monitor trends
(e) meet customer specifications, including those of importing countries.

The outputs of microbiological testing need to be interpreted with an understanding of their limitations. If analyses are not properly planned and performed, microbiological testing can provide inaccurate information, and create false assurances or unwarranted concerns about the safety of the food being analysed.

Why is microbiological analysis so challenging?

Finished product testing provides information about the safety of foods, but it cannot be relied upon to guarantee the safety of a food. The number, size and type of the samples collected for analysis will all influence the results.

For liquid products such as milk that can be well mixed, it is possible for a sample to be truly representative of the 'lot' or batch being sampled. However, this is not always the case, and a lot may consist of individual units with wide differences in the distribution of undesirable microorganisms. Unless the entire batch is tested, it cannot be guaranteed that every unit in a lot is free from pathogens, or that it meets specified microbiological limits.

Typically, if microbiological hazards are present, the presence is at a low level, with variable prevalence and uneven distribution throughout a solid food matrix. Additionally, organisms may be injured or sub-lethally impaired. Therefore the finished product test results may or may not be representative of the actual risk.

Factors which impact on the reliability of test results include:

- product uniformity or heterogeneity *e.g.* liquids v solids
- the number of samples tested *e.g.* one sample v 60 samples per lot
- the presence of pathogenic organisms only at very low levels in a food
- pathogens that may be injured or sub-lethally impaired.

Microorganisms of interest

Pathogens

In dairy products, there are several important pathogens of concern. These include *Salmonella*, *Campylobacter* species, *Listeria monocytogenes*, enterohaemorhagic *E. coli*, *Staphylococcus aureus* and *Cronobacter sakazakii*.

The presence of these organisms in dairy products may pose severe hazards to the general population and/or vulnerable sub-populations, potentially resulting in life-threatening infections or long-term effects of illness.

Different contaminating organisms can die, survive or multiply under the varying compositional conditions they are exposed to in different products. For example, high moisture ripened cheese should be tested for the presence of *E. coli*, coagulase-positive staphylococci, and *L. monocytogenes*. These potentially pathogenic organisms are capable of surviving and multiplying in the relatively mild environment of this type of product. Milk powders on the other hand are more likely to be at risk of contamination by organisms such as *Salmonella* or *C. sakazakii*, both of which can readily survive in dry conditions.

All of these factors need to be considered when determining what specific organisms to test for in a licensee's microbial testing program, as a component of the food safety program.

Awareness of pathogens and the particular niches they occupy in the dairy industry is essential if the risk they present is to be addressed. The Australia New Zealand Food Standards Code specifies microbiological limits for some of these organisms.

Quality or indicator organisms

In addition to testing for the presence of pathogenic (disease-causing) organisms, testing for contamination by spoilage organisms can give an indication as to whether the product shelf life may be compromised, for example, by spore-formers in mature cheese or liquid milk products, yeast and moulds in yoghurts, or coliforms in mature cheese.

Indicator tests for coliforms, Standard Plate Count (SPC) or Enterobacteriaceae are often used in finished product testing to give a general indication of the levels of hygiene through production. In-line process or environmental sampling may also use these tests to proactively monitor for possible contamination in day-to-day operations.

Microbiological standards for dairy products

Standards established by Food Standards Australia New Zealand (FSANZ) in the *Food Standards Code*[3] define the microbiological content that a food must not exceed to be in compliance with the law. The Victorian *Code of Practice for Dairy Food Safety* mandates that pathogen levels specified in the *FSANZ User Guide, Microbiological Limits for Foods*[4] must also be complied with. Foods not meeting the standard are non-compliant, potentially unsafe, and must be removed from the market.

Product

Microorganism of concern

Minimum individual sample size for analysis

Minimum number of samples to be tested

Absolute limit. Counts above this number are unacceptable

Maximum number of defective units

Limit for defective units (2-class plan)

Limits for marginal quality (3-class plan)

Food	Microorganism	n	c	m	M
Butter made from unpasteurised milk and /or unpasteurised milk products	*Campylobacter*/25 g	5	0	0	
	Coagulase-positive staphylococci/g	5	1	10	10^2
	Coliforms/g	5	1	10	10^2
	Escherichia coli/g	5	1	3	9
	Listeria monocytogenes/25 g	5	0	0	
	Salmonella/25 g	5	0	0	
	SPC/g	5	0	$5x10^5$	
All cheese	*Escherichia coli*/g	5	1	10	10^2
Soft and semi-soft cheese (moisture content > 39%) with pH > 5.0	*Listeria monocytogenes*/25 g	5	0	0	
	Salmonella/25g	5	0	0	
All raw milk cheese (cheese made from milk not pasteurised or thermised)	*Listeria monocytogenes*/25 g	5	0	0	
	Salmonella/25 g	5	0	0	
Raw milk unripened cheeses (moisture content > 50% with pH > 5.0)	*Campylobacter*/25 g	5	0	0	
Dried milk	*Salmonella*/25 g	5	0	0	
Unpasteurised milk for retail sale	*Campylobacter*/25 g	5	0	0	
	Coliforms/ml	5	1	10^2	10^3
	Escherichia coli/ml	5	1	3	9
	Listeria monocytogenes/25 g	5	0	0	
	Salmonella/25 Ml	5	0	0	
	SPC/ml	5	1	$2.5x10^4$	$2.5x10^5$

Figure 1: *Microbiological limits for dairy products in Standard 1.6.1*

FSANZ has established microbiological standards for dairy products in Standard 1.6.1 (Figure 1). These microbiological criteria set limits for the acceptance or rejection of sample lots and address:

- food groups which must comply with the microbiological limits
- the microorganisms of concern
- the minimum number of sample units to be taken and tested
- the number of microorganisms considered acceptable, marginally acceptable or critical
- the number of samples that need to conform to these limits.

Understanding the way standards are written will assist manufacturers in using them as a means of verifying that their products meet requirements. The terms used in Figure 1 are described below:

n	The minimum number of sample units which must be examined from a lot of food to satisfy the sampling plan and assure compliance.
c	The maximum allowable number of defective sample units (2-class plan) or marginally acceptable sample units (3-class plan). When more than this number is found in a sample, the lot is rejected.
m	A microbiological limit in a 2-class plan that separates acceptable quality from defective quality, or separates good quality from marginal quality in a 3-class plan.
M	A microbiological limit in a 3-class sampling plan that separates marginal quality from defective quality. Values above M are unacceptable.

Lot – Is the quantity of food produced and handled under uniform conditions. An identifiable code is given to a batch (lot) of food produced over a period of time, such as a day or part of a day. A lot should be composed of food produced with as little variation as possible.

In Figure 1 for example, the microbiological criteria for *L. monocytogenes* in soft and semi-soft cheese is n=5, c=0, m=0. This is called a two-class plan. To demonstrate compliance with the Food Standards Code, five 25 gram samples of soft and semi-soft cheese must be tested, and no sample may exceed the limit of absence in 25 grams. Effectively 125 grams of cheese must be tested. It is a two-class plan because there are only two results: the product either meets the standard or fails the standard.

By comparison, the *E.coli* limit for all cheeses is n=5, c=1, m=10, M=100. This is a three-class plan and allows for up to one marginal quality sample, which may contain between 10 and 100 cells per gram. The other four samples must contain less than 10 *E.coli* per gram. The three results possible are acceptable quality, marginal quality, and defective (fail).

The stringency of the sampling plan reflects the degree of hazard associated with a food. Where the expected health hazard is low, a three-class sampling plan is appropriate. For moderate or severe hazards, a two-class sampling plan is normally used, such as for *Salmonella* and *L. monocytogenes*.

By increasing the number of samples tested, the manufacturer has greater assurance of the safety of the product. At the very least, manufacturers must test the number of units listed as n. A manufacturer can choose to test more samples from a lot than n, and this will provide a greater assurance of the safety of their products.

In the case where there has been a detection of *Listeria* species in product, a clearance program needs to be initiated and the number of samples tested in surrounding lots/batches must also be increased. Under the *Australian Manual for Control of Listeria in the Dairy Industry* ***(Listeria Manual)***[5] or the *National Guidelines-Pathogen Management* ***(Pathogen Manual)***[6] sampling would be at either 25 or 30 samples of 25 gram or ml of product. This increased level of sample testing will improve the likelihood of detecting any contaminated product.

Information which describes the probability of detecting defective lots of food using different sampling plans may be found in Annex 1.

Considerations when developing a testing program

When verifying the effectiveness of the food safety program, the dairy manufacturer needs to determine the appropriate microbiological tests to be performed on each product type, and the number of samples that should be submitted for analysis.

A representative sample should, as far as possible, reflect the composition of the lot from which it is drawn. The objective is to take the sample without bias and of sufficient number to be able to make a judgement about a lot. Factors to consider when sampling include the:

- compositional characteristics of each product to be tested
- frequency of testing (*e.g.* every lot, weekly)
- sample size and how the sample is to be collected (*e.g.* five 100 gram samples taken during a production run covering products from the beginning, middle and end of production)
- target organisms
- test methods to be used
- acceptance criteria (*e.g.* absence in 25 grams, <100/gram)
- actions to be taken where acceptance criteria are exceeded.

While FSANZ sets the microbiological limits, details on actual sampling and microbiological testing are contained

in various Australian Standards. Ensuring the correct sampling methods are being used, and that the testing conforms to requirements under these Australian Standards, also needs to be confirmed with the testing laboratory.

Note that some regulatory documents may stipulate a test methodology that has been superseded (such as the *Listeria Manual* which references AS1766.2.15-1988[7] as the test method, however this standard method has been replaced by AS 5013.24.1-2009[8]). In such circumstances, the *Pathogen Manual* allows provision for the laboratory to be able to adopt the latest version of the standard, as long as equivalence can be demonstrated as determined by the provisions of AS/NZS 4659[9].

Importantly, all external laboratories used by dairy licensees must be National Association of Testing Authorities (NATA) registered (or equivalent) and be accredited for the relevant testing methods, such as *Salmonella* testing, or enumeration of *E. coli*, as described in the *Minimum Sampling Guidelines for Dairy Products*[10].

Engaging the laboratory to test samples

It is important that dairy manufacturers work closely with their testing laboratory to ensure that the correct sampling and testing regime is performed. This includes providing advice and then confirming that the correct number of samples has been tested, using standard methods, and that the reporting conforms to requirements. It is advisable that manufacturers obtain written confirmation of the sampling and testing procedures used by their laboratory.

Under Standard 1.6.1, the sampling plan for *Salmonella* is n = 5, c = 0, m = 0. This means five samples of 25 grams need to be tested for *Salmonella*. Typically five samples of approximately 100 grams (or ml) are taken from each lot by the manufacturer and these are sent off to a laboratory for analysis.

In the laboratory, a 25 gram sub-sample is aseptically taken from each 100 gram sample and mixed with a diluent or enrichment solution at a rate of 1/10. That is, the mass or measure of the sample mixed with nine times its weight or volume. So a 25 gram sample of milk powder would be mixed with 225 ml of diluent. This is repeated for each of the five 100 gram samples provided by the manufacturer.

Under this 2-class sampling plan for *Salmonella*, all five samples must return a negative result to meet the standard. The laboratory should be providing the manufacturer with a report for each of the five samples tested. To meet the standard, all five samples must be reported as ***Salmonella not detected in 25 grams***. If the laboratory chooses to composite the five samples (see section below), they will then report the result as either ***Salmonella detected/not detected in 125 grams***.

Compositing test samples

It is possible when testing large numbers of samples from a single lot, to composite multiple samples, for example, under a Listeria clearance program. In this instance, a company may need to test 25, 30, or even 60 samples from a lot. Compositing allows for multiple samples to be combined, thereby increasing efficiency, and reducing the workload and cost of testing[11].

For example, if a manufacturer was undertaking a testing program of n = 30, the thirty test portions (each of ~100 grams) which are representative of the lot must be taken and tested either individually, or composited into six sets of five test portions, or two sets of 15. A sub-sample of 25 grams or 25 millilitres is taken from each test portion, combined, and then analysed together. Annex 2 shows an example of compositing for 30 samples.

Manufacturers need to ensure the laboratory is reporting the results for the combined total of product analysed. For example, if six sets of five test portions were analysed, the report would state absence in 125 grams for each of the six composites.

Where a three-class sampling plan is being used, for example, enumeration of *E. coli*, it is not appropriate to composite samples.

Small production runs

The requirement to test five samples from a lot or batch may be onerous for small manufacturers or for small production runs. If less than 30 units are produced in a batch, the manufacturer is advised to discuss an appropriate sampling plan with their food safety manager.

Records to support the testing protocols

Records on how a company's sampling and testing regime have been developed and validated for all products need to be maintained under food safety program requirements. Microbiological product testing results must be retained for a minimum three-year period.

Regular on-going analysis of test results can provide a useful tool for comparing operational trends over time, and may be used to support any reassessment of the food safety program.

Important requirements for Dairy Food Safety Victoria licensees:

- Samples should be taken and tested from a lot or batch at least every two weeks for high risk products[10].
- Five samples from the same product lot/batch must be tested for the microorganisms listed for that product in Standard 1.6.1 (Food Standards Code) and pathogens listed for that product in the User Guide to Standard 1.6.1 (FSANZ).
- For pathogens such as *L. monocytogenes* and *Salmonella*, ensure 25 gram samples are analysed.
- Work closely with the analytical laboratory to ensure the appropriate sampling and testing procedures are in place, and that the requirements of the Australia New Zealand Food Standards Code are being met.
- When pathogens are detected, alert Dairy Food Safety Victoria as a matter of urgency, and also check for the next steps as described in the relevant documentation (*Listeria Manual*[5], *Salmonella Manual*[12] or *Pathogen Manual*[6]). The manufacturer's approved food safety program should also document corrective action to be followed.

Key points to consider

- Victorian dairy manufacturers are required to have in place a microbiological testing program as part of their approved food safety program, as this serves a role in verifying that process control actions are working.
- Careful selection of the appropriate organisms to be tested, relative to the product matrix and testing frequency, is essential to ensure the results obtained are representative of the lot and provide meaningful data.
- Manufacturers are encouraged to work closely with their testing laboratory and seek guidance on the tests they require and how they are reported.

Annex 1: Sampling plans

Testing for microorganisms in foods is a difficult task. Even the most elaborate sampling and testing of finished-products cannot guarantee the safety of the entire lot or batch of product.

The limitations of sampling and testing finished products is demonstrated visually by an Operating Characteristic curve. These curves show the proportion of defective units (horiziontal scale) against the probability of a lot being accepted (vertical scale), based upon the number of samples tested. These curves clearly demonstrate the weaknesses associated with sampling batches of food.

The curve in Figure 2 shows acceptance probabilities for a two-class plan where n=5 sample units are tested and none (c=0) are permitted to be positive.

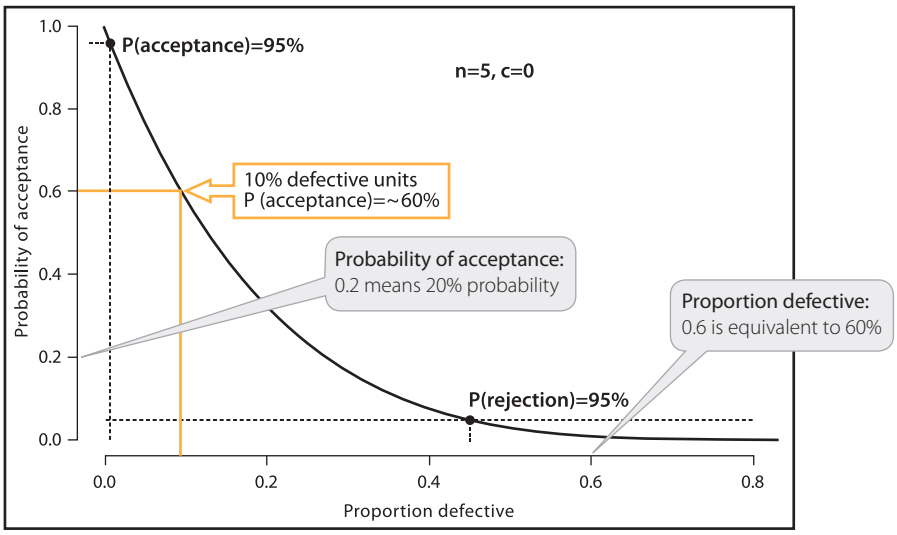

Figure 2: *Operating characteristic curve for n=5 sampling plan*

For a lot of food which contains 10% defective units (0.1 proportion defective), if five samples are tested there is approximately a 60% probability of accepting (0.6 probability) this lot where c=0. It is only where there are over 40% defective units that there is a 95% probability of correctly detecting this and rejecting the lot. Typically in dairy foods the challenge is dealing with very low levels of defective units, and uneven distribution of hazardous microorganisms.

The steeper the operating characteristic curve the greater the probability of rejecting defective units. Hence it is desirable that the curve would fall very steeply from 100% probability of acceptance to a 100% probability of rejection. Unfortunately no practical sampling plan can achieve this ideal, but it is improved by increasing the number of sample units tested per lot. The effect of increasing the number of samples tested is shown in Figure 3.

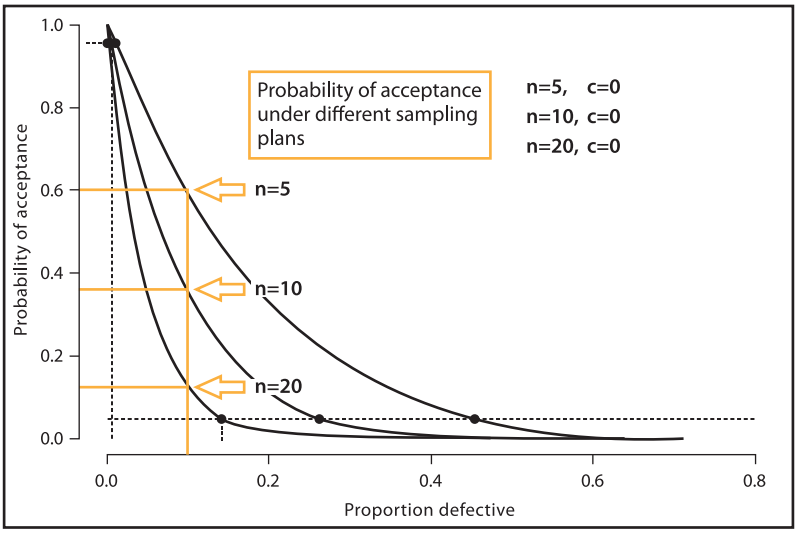

Figure 3: *Impact of increased sampling on the operating characteristic curve*

Increasing the number of samples to be tested does give greater confidence in the result, but often requires testing an impractical number of samples to detect low levels of contamination with any reasonable level of confidence as shown in Table 1.

Properties of the lot		Probability of accepting a defective lot (%)						
Acceptable (%)	Defective (%)	5 samples	10 samples	15 samples	20 samples	30 samples	60 samples	100 samples
98	2	90	82	74	67	55	30	13
95	5	77	60	46	36	21	5	1
90	10	59	35	21	12	4	<0.5	<0.5
80	20	33	11	4	1	<0.5		
70	30	17	3	<0.5				
60	49	8	1	<0.5				
50	50	3	<0.5					

Table 1: *Probability of accepting a contaminated lot based on properties of the lot and samples tested (ICMSF Volume 7).*

According to the table, if 10 samples are tested, there is less than a 0.5% chance of missing the contamination if the lot is 50% contaminated, but an 82% chance of missing the contamination if the lot is 2% defective. This shows statistically, that in instance of very low levels of contamination, virtually all product would need to be tested to detect it.

Annex 2: Compositing samples

The following graphics show the process for compositing samples of dairy products as outlined in the *Pathogen Manual*[6].

If a manufacturer was undertaking a testing program involving n = 30 samples, thirty representative test portions (each of ~100 grams) would be taken from the lot.

These thirty samples can be tested either individually or composited. Compositing can be as either six sets of five test portions or two sets of 15 test portions (See Figure 4 and 5 below).

For example, if a composite of six sets is analysed, each set would contain a sub-sample of five test portions of 25 grams or 25 millilitres (125 grams), which are combined, and dissolved or dispersed in 1.125 litres of diluent or enrichment medium by blending, stomaching or vortexing.

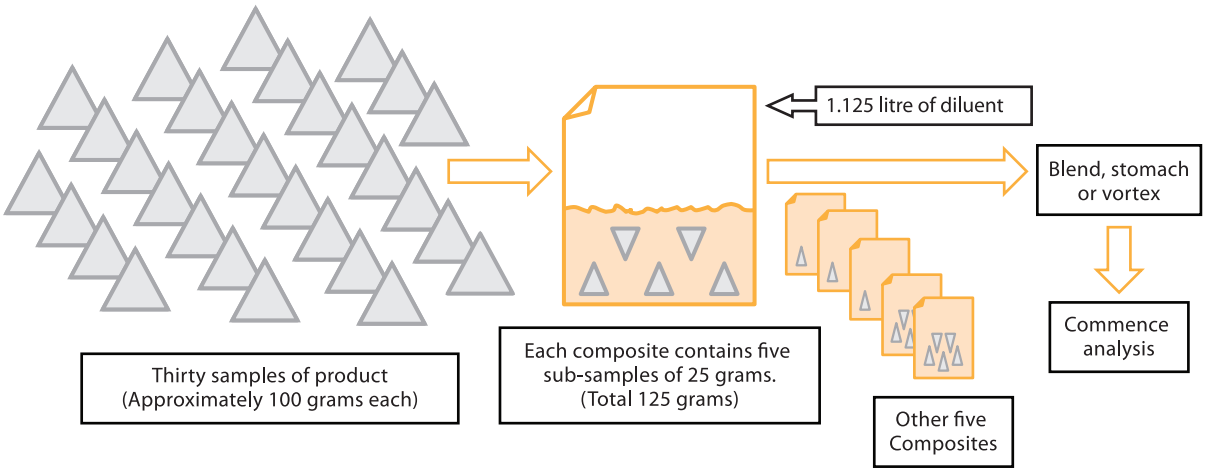

Figure 4: *Compositing of six sets of five test portions*

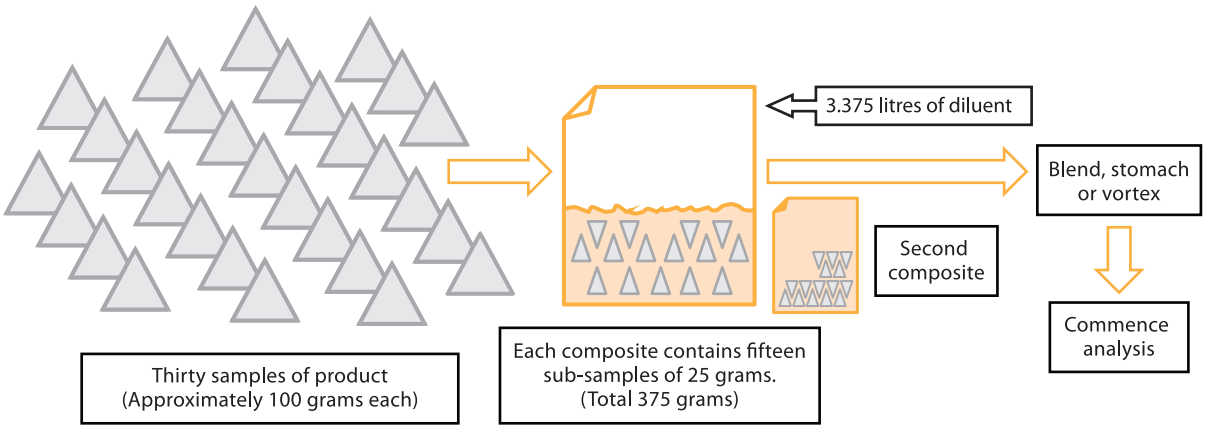

Figure 5: *Compositing of two sets of 15 test portions*

References

1. Dairy Food Safety Victoria, *Code of Practice for Dairy Food Safety, Section 5: Dairy Manufacturing Premises*, DFSV, Melbourne 2002.

2. Dairy Food Safety Victoria, *Guidelines for Food Safety: Dairy Food Manufacturers. Section 5.7: Testing Programs*, DFSV, Melbourne 2006.

3. Food Standards Australia New Zealand, Food Standards Code Standard 1.6.1 -Microbiological Limits for Food, Commonwealth of Australia, Canberra 2007.

4. Food Standards Australia New Zealand, User Guide to *Standard 1.6.1 - Microbiological Limits for Food*, FSANZ, Canberra 2001.

5. Australian Dairy Authorities Standards Committee, *Australian Manual for Control of Listeria in the Dairy Industry*, ANZDAC, Melbourne 1999.

6. Australian Dairy Authorities Standards Committee, *National Guidelines – Pathogen Management*, ANZDAC, Melbourne 2011.

7. Standards Australia AS/NZS 1766.2.15-1998, *Examination of specific organisms – Listeria monocytogenes in dairy products*.

8. Standards Australia, AS 5013.24.1-2009. *Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of Listeria monocytogenes - Detection method* (ISO 11290-1:1996, MOD).

9. Standards Australia AS 4659-1999 (Parts 1, 2 and 3), including Amendment 2002, *Guide to determining the equivalence of food microbiology test methods*.

10. Australian Dairy Authorities Standards Committee, *Minimum Sampling Guidelines for Dairy Products*, ANZDAC, Melbourne 2000.

11. ICMSF, *Microorganisms in Foods 7: Microbiological Testing in Food Safety Management*, Springer Science + Business Media, New York 2002. ISBN: 0306472627

12. Australian Dairy Authorities Standards Committee, *Australian Manual for Control of Salmonella in the Dairy Industry*, ANZDAC, Melbourne 1999.

Further information

Further food safety technical information is available at **www.dairysafe.vic.gov.au**

Or contact Dairy Food Safety Victoria on **(03) 9810 5900** or **info@dairysafe.vic.gov.au**