Effective Microbiological Testing: Dairy Products

Delivered By:

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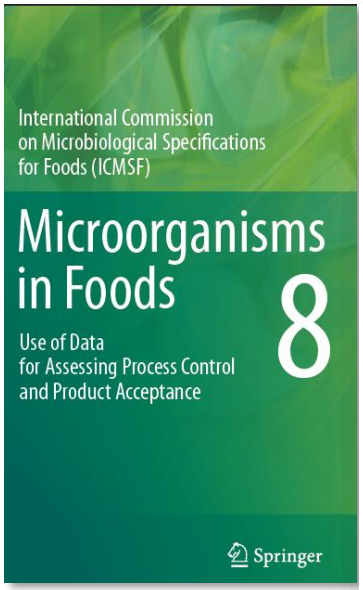
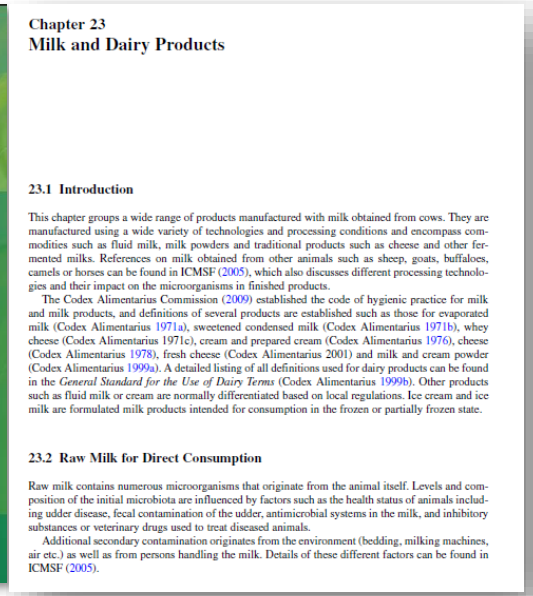
International Commission on Microbiological Specifications for Foods (ICMSF)





Chapter 23
Milk and Dairy Products

23.1 Introduction

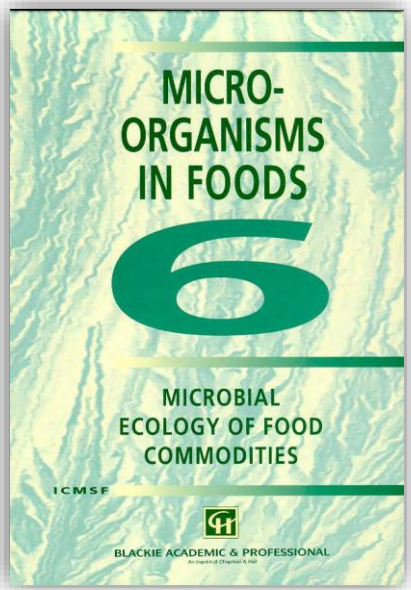
This chapter groups a wide range of products manufactured with milk obtained from cows. They are manufactured using a wide variety of technologies and processing conditions and encompass commodities such as fluid milk, milk powders and traditional products such as cheese and other fermented milks. References on milk obtained from other animals such as sheep, goats, buffaloes, camels or horses can be found in ICMSF (2005), which also discusses different processing technologies and their impact on the microorganisms in finished products.

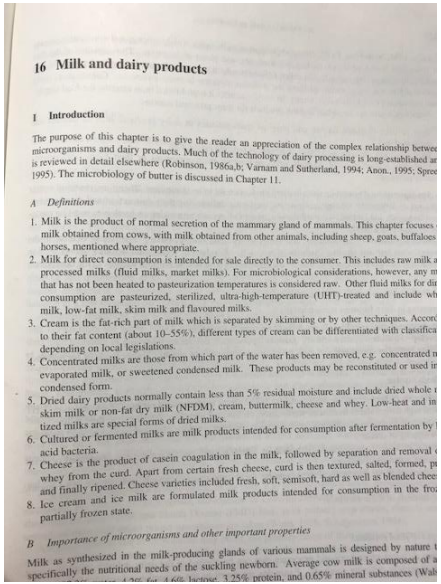
The Codex Alimentarius Commission (2009) established the code of hygienic practice for milk and milk products, and definitions of several products are established such as those for evaporated milk (Codex Alimentarius 1971a), sweetened condensed milk (Codex Alimentarius 1971b), whey cheese (Codex Alimentarius 1971c), cream and prepared cream (Codex Alimentarius 1976), cheese (Codex Alimentarius 1978), fresh cheese (Codex Alimentarius 2001) and milk and cream powder (Codex Alimentarius 1999a). A detailed listing of all definitions used for dairy products can be found in the *General Standard for the Use of Dairy Terms* (Codex Alimentarius 1999b). Other products such as fluid milk or cream are normally differentiated based on local regulations. Ice cream and ice milk are formulated milk products intended for consumption in the frozen or partially frozen state.

23.2 Raw Milk for Direct Consumption

Raw milk contains numerous microorganisms that originate from the animal itself. Levels and composition of the initial microbiota are influenced by factors such as the health status of animals including udder disease, fecal contamination of the udder, antimicrobial systems in the milk, and inhibitory substances or veterinary drugs used to treat diseased animals.

Additional secondary contamination originates from the environment (bedding, milking machines, air etc.) as well as from persons handling the milk. Details of these different factors can be found in ICMSF (2005).





16 Milk and dairy products

1 Introduction

The purpose of this chapter is to give the reader an appreciation of the complex relationship betwee microorganisms and dairy products. Much of the technology of dairy processing is long-established a is reviewed in detail elsewhere (Robinson, 1986a,b; Varnam and Sutherland, 1994; Anon., 1995; Spree 1995). The microbiology of butter is discussed in Chapter 11.

A Definitions

1. Milk is the product of normal secretion of the mammary gland of mammals. This chapter focuses milk obtained from cows, with milk obtained from other animals, including sheep, goats, buffaloes horses, mentioned where appropriate.
2. Milk for direct consumption is intended for sale directly to the consumer. This includes raw milk a processed milks (fluid milks, market milks). For microbiological considerations, however, any m that has not been heated to pasteurization temperatures is considered raw. Other fluid milks for di consumption are pasteurized, sterilized, ultra-high-temperature (UHT)-treated and include wh milk, low-fat milk, skim milk and flavoured milks.
3. Cream is the fat-rich part of milk which is separated by skimming or by other techniques. Accord to their fat content (about 10–55%), different types of cream can be differentiated with classifica depending on local legislations.
4. Concentrated milks are those from which part of the water has been removed, e.g. concentrated n evaporated milk, or sweetened condensed milk. These products may be reconstituted or used ir condensed form.
5. Dried dairy products normally contain less than 5% residual moisture and include dried whole skim milk or non-fat dry milk (NFDM); cream, buttermilk, cheese and whey. Low-heat and in tized milks are special forms of dried milks.
6. Cultured or fermented milks are milk products intended for consumption after fermentation by acid bacteria.
7. Cheese is the product of casein coagulation in the milk, followed by separation and removal whey from the curd. Apart from certain fresh cheese, curd is then textured, salted, formed, p and finally ripened. Cheese varieties included fresh, soft, semisoft, hard as well as blended chee
8. Ice cream and ice milk are formulated milk products intended for consumption in the fro partially frozen state.

B Importance of microorganisms and other important properties

Milk as synthesized in the milk-producing glands of various mammals is designed by nature specifically the nutritional needs of the suckling newborn. Average cow milk is composed of

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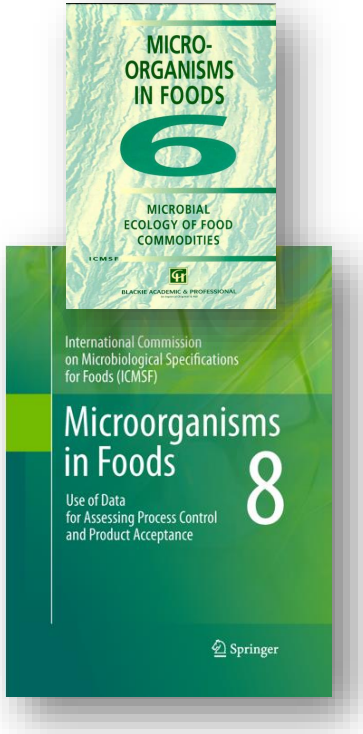
Overview

- Diversity In Dairy Products/Manufacturing
- Microbial ecology of milk and dairy products
- Dairy product-associated foodborne outbreaks
- On-farm controls
- Microbiological criteria by product type



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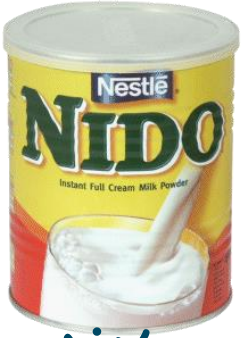
Diversity in Dairy Product Manufacture/Technology

UHT/Sterilization

Microbiology

Hurdles



Wet Mix Ingredients

Dry mix ingredients

Pasteurisation

Fermentation

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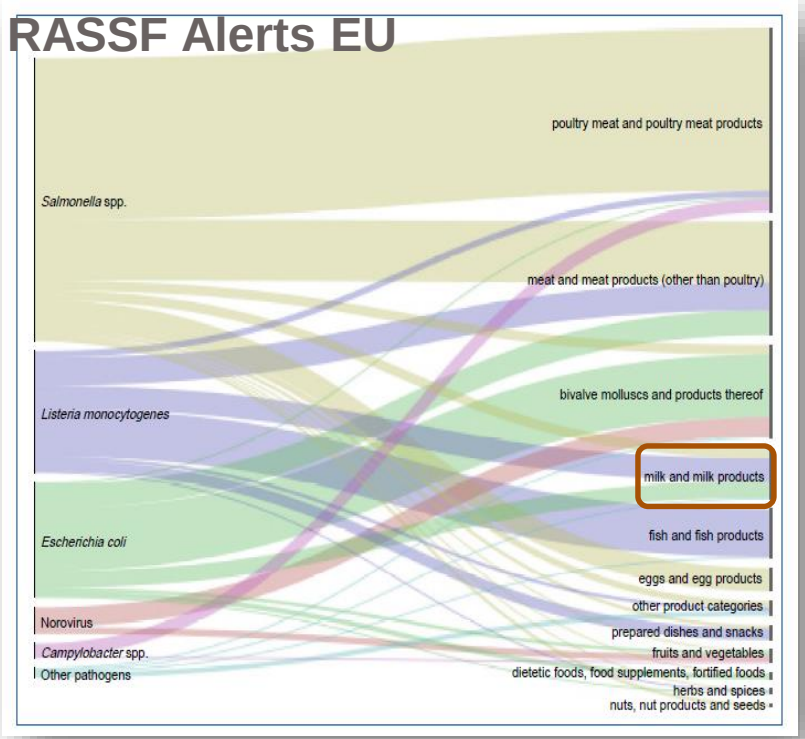
Epidemiology - Milk and dairy products

- Milk and milk products are not the highest contributor to foodborne illness.
- The major cause of milk-borne disease is consumption of raw milk/raw milk products, cheeses and recontaminated processed milk and mixed dairy products such as cream fillings



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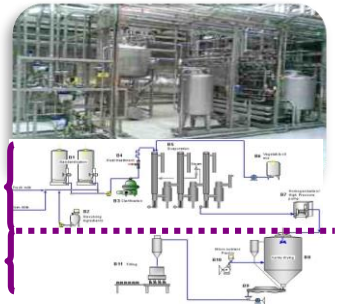
Food Safety Microbiology is CHALLENGING



Reducing the level of contamination of the raw material/I gradient (e.g., heat)

$$H_o - \Sigma R + \Sigma I \leq FSO$$

Reducing the concentration/prevalence of intrinsic contamination

Minimise the chance of contamination of reconstituted formula during preparation

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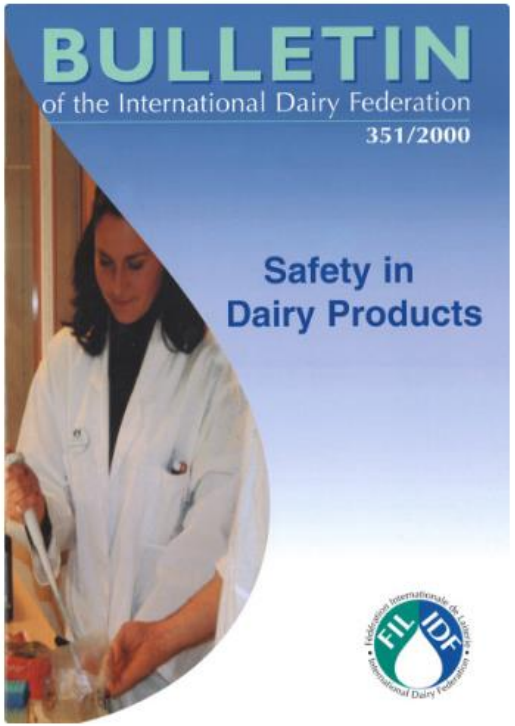
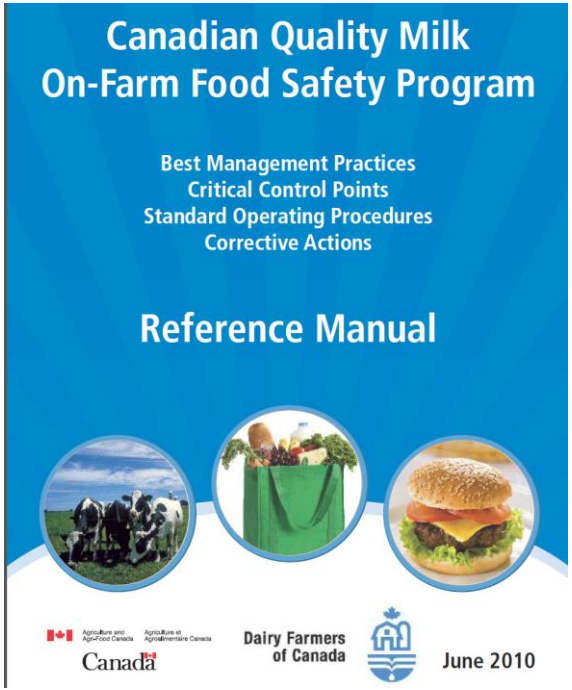
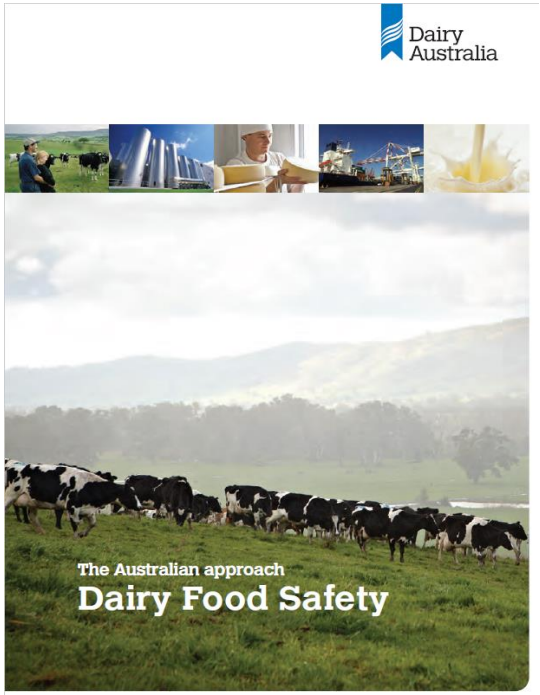
Upstream milk supply practices are vital for Dairy product safety and quality







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Pathogens in milk and dairy products

- Raw milk – numerous
- **Cheeses** – *L. monocytogenes*, Shiga-toxin E.Coli (STEC), *S. aureus*, *Salmonella* and *Campylobacter*
- Raw milk butter and cream - *L. monocytogenes*, STEC, *S. aureus*
- *Milk powders – Salmonella*, *S. aureus*, *B. cereus*, *Cronobacter spp.*
- In endemic areas, raw milk dairy products – *Brucella* spp. and *Mycobacterium bovis*
- Potential hazards – *Coxiella burnetii* and Mycobacterium paratuberculosis


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Key pathogenic bacteria associated with dairy foods: On-farm ecology and products associated with foodborne pathogen transmission

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Dairy-Related Illness/hospitalisations from US Outbreaks 2009-2014

Pathogen	Outbreaks associated with milk and cheese consumption, N = 87						
	Pasteurized				Unpasteurized		
	Outbreaks	Illnesses	Hospitalizations		**Outbreaks**	Illnesses	Hospitalizations
STEC	0	0	0		14	99	42
***Salmonella* spp.**	0	0	0		8	83	29
Listeria monocytogenes	10	100	87		1	1	1
***Campylobacter* spp**	1	2	0		53	465	56
Overall	11	102	87		76	648	128

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Listeriosis Outbreaks Linked to Cheese

Food	Year	# ill	# Hospitalized	# Deaths	Location
Soft cheese (Karoun)	2010/2015	30	28	3 (1 fetal)	USA (10 states)
Fresh curds/Hispanic style soft cheese	2014	5	4	1	USA (4 states)
Soft and semi-soft cheese (Crave Brothers)	2013	6	6	1 (1fetal)	USA (5 states)
Hispanic style soft cheese (Roos Foods)	2013	8	7	1	USA (2 states)
Jindi cheese	2013	25		3 (1 fetal)	Australia
Ricotta Salata cheese	2012	23	21	5	USA (14 states)
Latin-style fresh cheese	2012	2 (1 pregnant) and 1 baby			Spain
Blue-veined cheese (unpasteurized)	2011	15	1	1	USA
Hard cheese	2011	12	12		Belgium
Fresh cheese	2010/2011	40		13	Portugal
Camembert cheese	2010	17		3	Norway

Dairy Listeriosis Outbreaks - Causes

- Cross-contamination of cheeses at retail and consumer home an important control point
- Two of the cheese-related *Listeria* outbreaks identified knives and cutting boards as potential sources of contamination
- Risks of operating a dairy plant in a farm environment; potential for transfer of *Lm* from the farm environment to the plant **via shared toilet facilities**
- **Failure to protect water sources from wild birds;** multi-barrier failure in the water disinfection system
- Serving high-risk cheeses to patients in hospitals

Observations from Ice Cream Listeria Outbreak

10 cases, 3 deaths

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Salmonella, S. aureus, Cronobacter in heat-treated Dairy products

Powdered Infant Formula as a Source of *Salmonella* Infection in Infants

Sarah M. Cahill,[1] I. Kaye Wachsmuth,[2] Maria de Lourdes Costarrica,[1] and Peter Karim Ben Embarek[3]

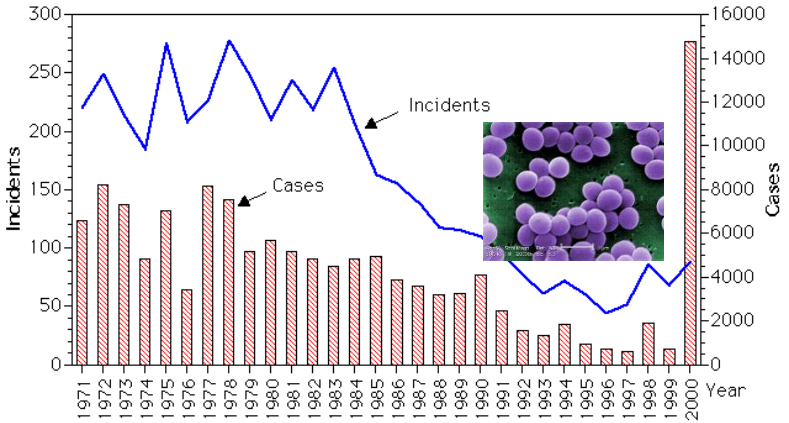
[1]Food and Agriculture Organization of the United Nations, Rome, Italy; [2]International Public Health Consultant, Private Practice, DeLand, Florida; and [3]World Health Organization, Geneva, Switzerland

Powdered infant formula is not sterile and may be intrinsically contaminated with pathogens, such as *Salmonella enterica*, that can cause serious illness in infants. In recent years, at least 6 outbreaks of *Salmonella* infection in infants that have been linked to the consumption of powdered infant formula have been reported. Many of these outbreaks were identified because the *Salmonella* strains were unique in some way (e.g., a rare serotype) and a well-established *Salmonella* surveillance network, supported by laboratories capable of serotyping isolates, was in place. Another common feature of the outbreaks was the low level of salmonellae detected in the implicated formula (salmonellae may be missed in routine testing). These outbreaks likely represent only a small proportion of the actual number of *Salmonella* infections in infants that have been linked to powdered infant formula. Managing this problem requires a multidimensional approach in which manufacturers, regulators, and caregivers to infants can all play a role.

Clinical Infect. Dis. 46: 2008

Milk/Milk Powder and S. aureus

Figure 1. *Staphylococcus* food poisoning in Japan, 1971-2000



(Statistics of Food Poisoning in Japan, Ministry of Health, Labour and Welfare)

IASR
Infectious Agents Surveillance Report

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Salmonella in powdered milk products

Table 2. Salmonellosis outbreaks during the period 1985–2005 that have been linked to powdered infant formula (PIF).

Salmonella serotype	No. of infants affected	Vehicle	Location	Year	Organism isolated from PIF	PIF implicated by epidemiologic study	Reference
Ealing	48	PIF	United Kingdom	1985	Yes	Yes	[19]
Tennessee	⩾3	PIF	United States, Canada	1993	Yes	Yes	[20]
Virchow	⩾48	PIF	Spain	1996	Yes	Yes	[21]
Anatum	17	PIF	United Kingdom, France	1996–1997	No	Yes	[22]
London	30	PIF	Korea	2000	Yes (open package only)	Yes	[23]
Agona	141	PIF	France	2004–2005	Yes	Yes	[24]
Total	⩾287	...	...	...	...	...	...

Cahill et al., 2008, Clinical Infectious Disease

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Salmonella outbreak in infant formula - Lactalis (France) 2017-18

2005

2006

2017

2017/18 - 40 babies ill in different Countries

Factory had same *S. agona* strain
In factory for 12 years

Whole Genome Sequencing proved link
to all isolates including
clinical (baby) isolates

Factory focus on end point testing?

French Authorities will now inspect all
Dairy powder/IF factories in France

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Cronobacter outbreaks linked to PIF

Korean J. Food Sci. An. 2018 April 38(2):376~390

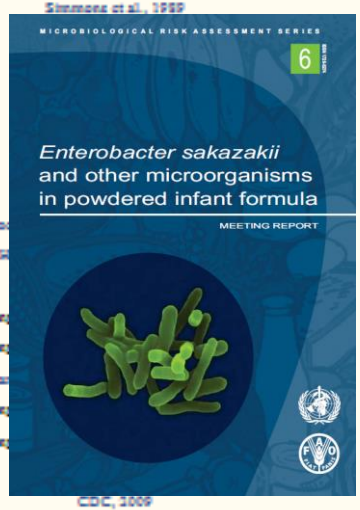
Codex Alimentarius CAC/RCP 21-1979

	n	c	m	M
Aerobic mesophilic counts	5	2	10^3	10^4
Coliforms	5	1	<3	20
Salmonella	60	0	0	

Codex Alimentarius CAC/RCP 66-2008

	n	c	m	M
Aerobic mesophilic counts	5	2	500	5000
Enterobacteriaceae (10g)	10	2	0	
E. sakazakii (10g)	30	0	0	
Salmonella (25g)	60	0	0	

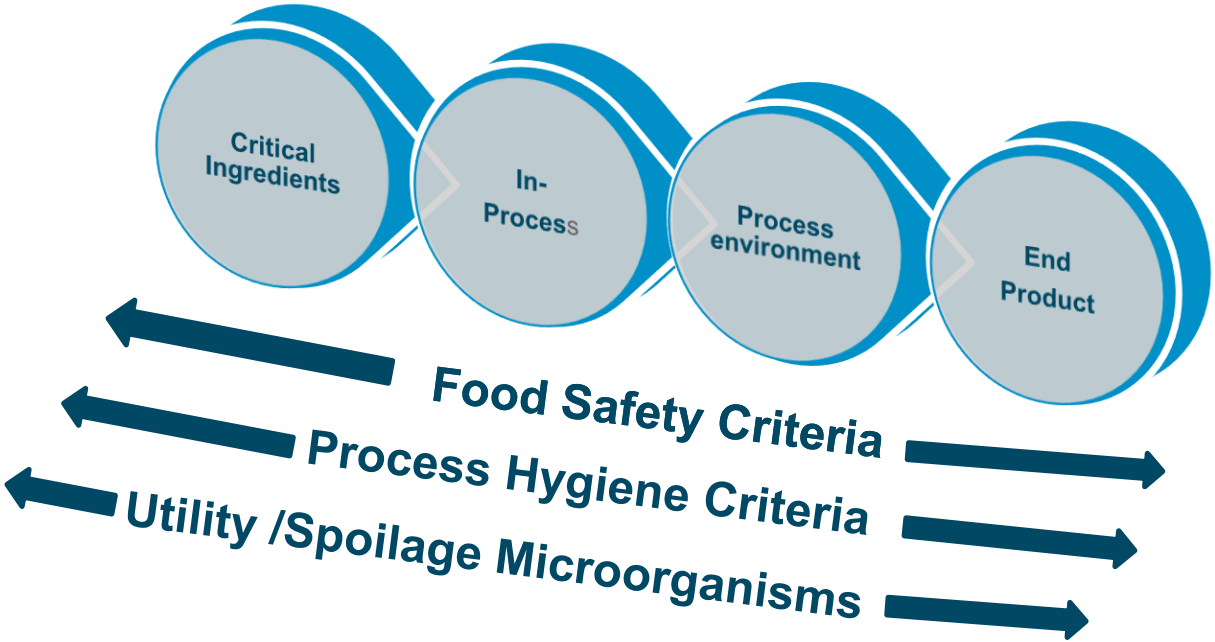
Location	Year	Cases/Death	References
St. Albans, England	1958	2/2	Urmenyi and Franklin, 1961
Denmark	1965	1/None	Jaker et al., 1965
Macon, GA, USA	1979	1/Not mentioned	Monroe and Tift, 1979
Indianapolis, IN, USA	1981	1/None	Kleiman et al., 1981
The Netherlands	1983	8/None	Muytjens et al., 1983; Muytjens, 1985; Muytjens and Kollee, 1990
Athens, Greece	1985	1/Not mentioned	Arseni et al., 1985
Reykjavik, Iceland	1986-1987	2/1	Skirrow et al., 1988; Clark et al., 1990
Boston, MA, and New Orleans, LA, USA	1987	2/Not mentioned	Willis and Robinson, 1988
Memphis, TN, USA	1988	4/Not mentioned	Simmons et al., 1989
Baltimore, MD, USA	1990	1/Not mentioned	
Cincinnati, OH, USA	1990	1/Not mentioned	
Israel	1992, 1995, 1997,1998	4/Not mentioned	
Boston, MA, USA	1995/1996	5/4	
Belgium	1998	12/2	
Winston Salem, NC, USA	2000	1/None	
Israel	1997-2000	5/None	Bla
Knoxville, TN, USA	2001	10/1	K
Tennessee, USA	2002	1/1	
Wisconsin, USA	2002	1/Not mentioned	CDC un
USA	2002	6/Not mentioned	CDC un
France	2004	2/Not mentioned	Caigna
USA	2004	2/Not mentioned	CDC un
USA	2005	2/Not mentioned	CDC un
Canada	2007	2/Not mentioned	
New Mexico, USA	2008	2/None	CDC, 2009
Queretaro, Mexico	2010	2/None	Flores et al., 2011
Florida, Illinois, Missouri, and Oklahoma, USA	2011	6/2	CDC, 2012
Not mentioned	2012	1/None	Braga and Lee, 2012
China	2017	2/1	Cui et al., 2017

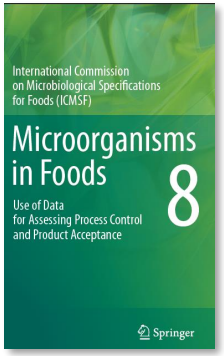


ICMSF – Book 8 Microbiological criteria

Chapter 23 - Milk and Dairy Products

- Use of data for assessing process control and product acceptance





1) Processed fluid milk

2) Ice cream

3) Dried dairy products

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ICMSF – Book 8 Processed Liquid Milk

Table 23.2 Testing of processed fluid milk products for microbiological safety and quality

Relative importance		Useful testing
Critical ingredients	Low	Testing for vegetative pathogens or indicators is only useful to verify that ingredients have been manufactured applying GHP.
	Medium	For sterilized or UHT products, testing for mesophilic and or thermophilic spore formers is useful for critical ingredients and in particular if the heat treatments applied are at the lower end of the scale. Typical industry standards are $10–10^2$ CFU/g.
In-process	Low	Routine in-process testing is not recommended. It is important for trouble-shooting to identify potential sources of contamination. Such investigative sampling should include critical steps of the processing line such as the plate heat exchangers, fillers, and intermediate storage tanks.
Processing environment	Low	Routine testing of the environment for vegetative and spore forming pathogens or spoilage microorganisms is not recommended. It can, however, be useful for trouble-shooting to identify potential sources of contamination (e.g., filter units, areas of the filling chamber or the fillers themselves).
Shelf life	Medium	For refrigerated products with extended shelf life (>17 days), shelf life testing may be useful to identify potential issues (see text)

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ICMSF – Book 8 Processed Liquid Milk

Table 23.2 Testing of processed fluid milk products for microbiological safety and quality

Relative importance	Useful testing
End product Low/High	Low for pasteurized products, high for sterilized or UHT products, for which testing and trend analyses to assess the performance of the line and detection of major deviations is recommended.

Product	Microorganism	Analytical method [a]	Sampling plan & limits/mL* Case	n	c	m	M
Pasteurized milk [b]	Enterobacteriaceae	ISO 21528	5	5	2	<1	5
Sterilized or UHT products	Presence/absence tests for spoilage microorganisms	Incubate at 30 and 55°C (if suitable) for 10-14 and 5-7 days, respectively. Destructive and non-destructive methods	Fixed numbers of samples up to 100% of batches depending on product type (see text).				

Some Regulatory Values APC?

Country	Micro	n	c	m	M
Aus	E. coli	5	1	1	10
Can	Coliform	5	2	1	10
EU	Eb	5	2	1	5
Ind	Coliform	5	0	Abs/0.1ml	

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ICMSF -Book 8 Ices/Ice Cream

	Relative importance	Useful testing
Critical ingredients	High	It is important to develop good supplier relationships for **critical dry mix ingredients** to ensure their safety. Requirements for such ingredients should be equivalent to those for finished products to ensure compliance. Depending on the confidence level of the supplier testing is performed either for acceptance or as monitoring.
In-process	High	Routine in-process testing is recommended at critical steps of the process. Testing for Enterobacteriaceae provides important information on the hygiene status of processing lines and levels exceeding those established for the finished product should trigger testing for *Salmonella*.
Processing environment	Low	In cases where regulatory requirements exist, testing of environmental samples for **L. mono** (absence in the samples taken) is recommended. ***Listeria* spp. can be used as a hygiene indicator** – while absence is certainly the target, low levels up 10 CFU/g may be acceptable, but need to be interpreted according to observed trends over time. **Testing for Enterobacteriaceae is not recommended with the exception of areas maintained dry** (suggested target values: 10^2–10^3 CFU/g).

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ICMSF - Book 8 Ices/Ice Cream

	Relative importance	Useful testing
End product	High	Testing for Enterobacteriaceae provides important information on the hygiene status of processing lines. High levels may then trigger investigative sampling for pathogens. Testing for *Salmonella* can be limited to verification, as long as in-process and environmental results show no deviations.

	Microorganism	Case	Sampling plan & limits/g/*25g			
			n	c	m	M
High	Enterobacteriaceae	2	5	2	10	100
Low	Salmonella	11	10	0	0	-

Some Regulatory Values

Country	Micro	n	c	m	M
EU	Eb	5	1	10	100
	APC	5	2	10^5	$5x10^5$
	Sal & L.mono	5	0	Abs & <100/g	
Ind	Coliform	5	3	10	100
	APC	5	3	10^5	$2x10^5$
	Sal & L. mono	5	0	Abs(25g) & Abs/g)	

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ICMSF – Book 8 Dairy Powders/Powdered Infant Formula

		Useful testing
Critical ingredients	High	Develop good supplier relationships for critical dry mix ingredients Requirements for such ingredients need to be equivalent to those for finished products to ensure its compliance..
In-process	High	Test product residues at critical operations and intermediate product for *Salmonella* and Enterobacteriaceae. Typical guidance levels: • Enterobacteriaceae – same requirements as finished products • *Salmonella* – absent in any of the samples
Processing environment	High	Test for *Salmonella* and Enterobacteriaceae in relevant areas. Typical guidance levels: • Enterobacteriaceae – 100 CFU/g or sample • *Salmonella* – absent
End product	High	Test for indicators for on-going process control and trend analysis. If APC's are consistently much lower, then internal limits should be adjusted accordingly.

				Sampling plan & limits/g			
	Product	Microorganism	Case	n	c	m	M
High	Dry milk powders	APC	2	5	2	10^4	10^5
High	Dry milk powders	Enterobacteriaceae	5	5	2	<3	9.8

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Example: Dried Dairy Products *(continued)*

	Relative importance	Useful testing
End product	Low to High	When in-process and environmental results show negative results, testing of smaller numbers of samples for verification is usually sufficient. However, testing end products for Salmonella for lot acceptance is relevant when environmental data indicate potential for contamination or when effectiveness of control measures seems impaired (e.g. construction, wet cleaning).

Country	Micro	n	c	m	M
EU	APC	5	2	$1x10^4$	$5x10^4$
	Eb	5	0	10	
	Salmonella	5	0	Abs (25g)	
	S. aureus	5	2	10	100
Ind (Processed)	APC	5	2	$2.5x10^4$	$5x10^4$
	Coliform	5	2	10	50
	Sal	5	0	Abs(25g)	
	S. aureus	5	2	10	100

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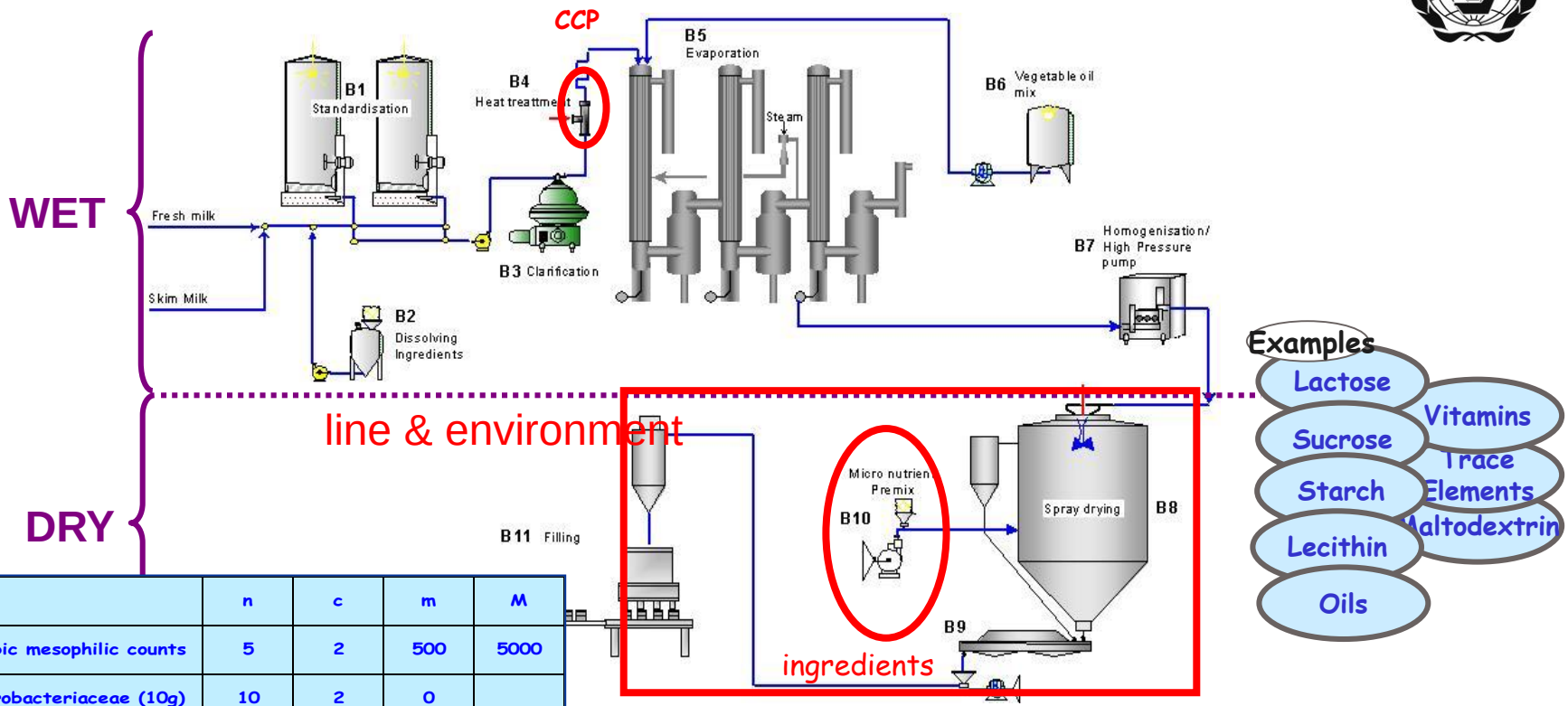
ICMSF - Book 8 Powdered Infant Formula



	n	c	m	M
Aerobic mesophilic counts	5	2	500	5000
Enterobacteriaceae (10g)	10	2	0	
E. sakazakii (10g)	30	0	0	
Salmonella (25g)	60	0	0	

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Microbial Sampling In Dairy Products: Summary

- Product Testing has severe limitations – Cannot test safety “into” products
- Focus on HACCP principles and associated PreRequisite Programmes (PRPs) (Zoning, sanitation, supplier management, training, etc)
- Focus on process control preferred
- Apply good Environmental monitoring at line and environmental samples
- Apply proper Root-cause analysis; eliminate biofilms/harbourage sites/poor hygienic design

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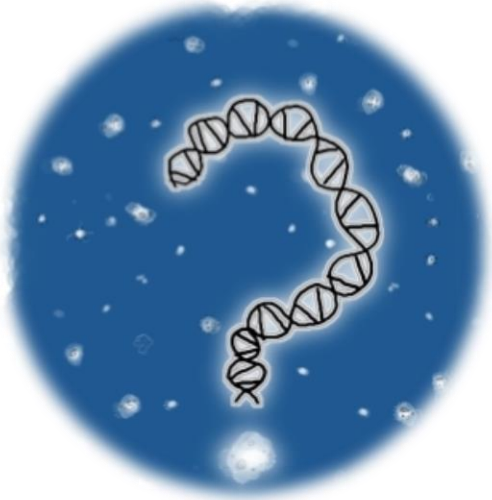
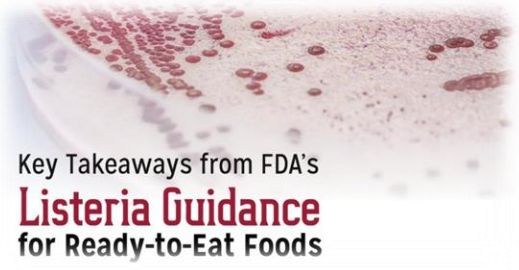
QUESTIONS



Preventing & Controlling *L. mono* in production environment



Seek and Destroy Approach

- **Environmental Monitoring Programs**
  (c.f. Pathogen & hygiene monitoring programmes)

- Personnel Practices
- Plant Design
- Equipment
- Cleaning/Sanitation
- Raw materials & ingredients

- Process control based on formulation
- Listericidal process control
- Storage Practices
- Time/Temperature controls

Nestlé Quality

ICMSF - Book 8 Cheeses

	Relative importance	Useful testing
Critical ingredients	High	**Raw milk cheese (RMC)** only: Good supplier relationship is important, targeting the absence of *Salmonella*, STEC and *L. monocytogenes* (Lm) or other pathogens that may survive cheese making.
In-process	High High to low	Monitor pH during acidification of the curd to detect slow fermentation. In-process testing for ***S. aureus* may be relevant if acidification does not proceed as anticipated.** For cheeses that support the growth of Lm and for RMC, testing residues and product contact surfaces may be important to verify the effectiveness of the preventive measures implemented. Pathogens of concern vary by cheese type. **Typical guidance levels: Lm & *Salmonella* – absent**
Processing environment	High to low	Significant hazards and routes of contamination vary by type of cheese, and testing the processing environment may be useful to assess the effectiveness of control measures taken. If appropriate, typical guidance levels are: *Lm & Salmonella* – absent
Shelf-life	Low	Testing may be conducted to determine the fate of pathogens during ripening and storage of cheese. Routine testing, however, is not recommended.

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	Relative importance	Useful testing
End product	High 	Testing for *E. coli* or *S. aureus* is useful to verify process control and hygiene conditions for certain cheese types. Upper limits (M) may vary de-pending on the extent of heat-treatment, but high levels may trigger investigative sampling for pathogens, including STEC, or staphylococcal enterotoxins.

Some Regulatory Values

Country	Micro	n	c	m	M
Health Canada (Unpast)	E. coli S. aureus Sal & L.mono	5 5 5	2 2 0	100 100 S- Abs & L.mono <100/g(or abs/25g)	1000 1000
Ind (Processed)	Coliform APC Sal & L. mono E. coli	5 5 5 5	0 2 0 0	10 $2.5x10^4$ Abs(25g) & Abs(25g) Abs/g	100 $5x10^4$

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Relative importance	Product	Microorganism	Case	Sampling plan & limits/g			
				n	c	m	M
High	Fresh cheese	S. aureus	8	5	1	10	10^2
High	Raw milk cheese	S. aureus	7	5	2	10^3	10^4
Low	Cheese from mildly heated milk or ripened	S. aureus	7	5	2	10^2	10^4
Medium	Cheese made from pasteurized milk	E. coli	4	5	3	10	10^2
Low	Cheese: No growth	L. monocytogenes	NA	5	0	10^2	-
				Sampling plan & limits/25g			
				n	c	m	M
High	Cheese: Growth supported	L. monocytogenes	NA	5	0	0	-
Medium or low	Cheese from raw or mildly heat-treated milk	Salmonella	10	5	0	0	-

Nestlé Quality

Cronobacter outbreaks linked to PIF

Location	Cases	Comments	Reference
Iceland	3; 1 death	2 normal term infants; 1 Down's	Biering et al., 1989
Tennessee	4; 3 sepsis, 1 bloody diarrhea	Cs; 8 cfu/100g	Simmons et al., 1989
Belgium	12 (all birth weights < 2000g)	6/12 with NEC positive for Cs	Van Acker et al., 2001
Tennessee	9	1 confirmed, 2 suspect, 6 colonized	Himelright et al., 2001
Israel	2	Cs isolated from stools of 3 asymptomatic infants	Bar-Oz et al., 2001