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The Management of *Cronobacter* in Powdered Infant Formula Manufacturing and/or Dairy Processing Plants in New Zealand

About this document

This document was developed by the New Zealand Food Safety Science & Research Centre as a partnership between researchers and NZ dairy industry partners. It is intended to assist powdered infant formula (PIF) manufacturers and dairy processors to develop, implement and review control measures for *Cronobacter* species in the context of a Risk Management Programme (RMP) or a Food Control Plan (FCP).

The information within this document is designed to complement rather than to replace any specific requirements or guidance for *Cronobacter* and/or other pathogens as described in New Zealand legislation, such as the Animal Products Act 1999, for the dairy industry.

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1. Scope

1.1. What is covered in this document?

This document outlines best management practice for controlling *Cronobacter* in the production or processing of PIF or powdered ingredients for use in PIF. This document should be used in conjunction with the Animal Products Notice: Manufacture of Dairy Based Infant Formula Products and Formulated Supplementary Foods for Young Children, June 2022 (Infant Formula Notice, 2022).

This document explains why control measures for *Cronobacter* should be put in place and how they should be applied, including the creation of a *Cronobacter* management programme (CMP).

Cronobacter species do not all pose the same threat to vulnerable consumers, however, owing to the similar ability of the various species to grow and/or survive in similar niches, the detection of any *Cronobacter* species is cause for significant concern and requires control measures to be escalated. Therefore, in this document the term '*Cronobacter*' includes all *Cronobacter* spp.

1.2. How will this document help enhance *Cronobacter* management?

After reading this document, you should have a better understanding of how to develop and implement a CMP appropriate to the risks associated with PIF production or processing. Importantly, this document is designed to provide insights into the control of *Cronobacter* from academic, regulatory, and dairy industry experts and practitioners. Note that controls for *Cronobacter* in PIF or PIF ingredients are similar to the controls for managing *Salmonella* spp. in low-moisture foods.

1.3. Glossary

In this document "The Management of *Cronobacter* in Powdered Infant Formula Manufacturing and/or Processing Plants in New Zealand", unless the context otherwise requires:

batch means a heterogeneous quantity of product manufactured during a discrete period of time as part of one continuous process.

biofilm means a population of microorganisms which are embedded in a matrix composed of extracellular polymers and are attached to a surface.

cleaning in place (CIP) means an automated system for cleaning without dismantling or opening the plant equipment.

cleaning out of place (COP) means a cleaning process that requires disassembly of equipment for cleaning, and/or its removal from the production area.

coliform means rod-shaped Gram-negative, non-spore forming and motile or nonmotile bacteria that can ferment lactose with the production of acid and gas when incubated at 35–37°C. They are a commonly used as indicators of the sanitary quality of foods and water. *Escherichia coli* and *Cronobacter* are coliforms.

colony forming unit (cfu) means a measure of the number of bacterial cells in a sample and is a measure of the level of contamination.

composite sample means a sample formed by combining and mixing all the primary samples taken from across a batch.

contaminated product means a batch of product, suspected or confirmed through testing to be contaminated with *Cronobacter.*

corrective actions mean the actions taken following the detection of *Cronobacter* species in an environmental or product sample to: restore control; identify any affected ingredient or food and ensure its safety and suitability; manage its disposal; or prevent recurrence of the loss of control.

critical control point (CCP) means a step at which processing control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

critical hygiene area means those processing areas which occur after a CCP for *Cronobacter* or final microbiological hurdle, and before the product is placed into the final packaging, including product contact and non-product contact surfaces. Examples include Zones 3 and 4 in a CMP; see also Appendix 1.

critical limit means a criterion that separates a level of acceptability from unacceptability.

***Cronobacter* control measure** means any action or activity that is applied to: control the initial level of *Cronobacter*; prevent an unacceptable increase in *Cronobacter*; and reduce or eliminate *Cronobacter*.

***Cronobacter* management programme (CMP)** means a documented programme that a food operator has in place to minimise the potential for product to be contaminated with *Cronobacter*.

Enterobacteriaceae means a large family of Gram-negative bacteria, including *Cronobacter, Klebsiella*, *Enterobacter*, *Citrobacter*, *Salmonella*, *Shigella*, *Proteus*, *Serratia*, *Escherichia coli* and other species, which are used as indicators of the sanitary quality of foods and water.

environmental samples mean material collected from a processing area or the external environment for the purpose of testing the surface or material for the presence of *Cronobacter*. This may include samples from surfaces which do not directly come into contact with the product but have the potential to harbour *Cronobacter*.

extrinsic contamination means the contamination of product after the packaging is opened by the user.

food control plan (FCP) means a plan designed for a particular food business to identify, control, manage, and eliminate or minimise hazards or other relevant factors for the purpose of achieving safe and suitable food (Food Act 2014).

good operating practice (GOP) (including good agricultural practice, good hygienic practice and good manufacturing practice) means documented procedures relating to practices that are required to ensure food is fit for intended purpose and are appropriate to the operating circumstances.

harbourage site/niche means a localised site (a nook or cranny, a crack, a crevice, a scratch, a ledge, etc.) in which food debris and moisture can accumulate, providing an area in which *Cronobacter* can become established and persist.

hazard analysis and critical control point (HACCP) means a system that identifies, evaluates, and controls hazards that are significant for food safety.

indirect product contact surface means surfaces which do not directly come into contact with the product but have the potential to introduce contamination directly to the product, such as equipment or ledges around the filling head.

input means any food material or product, additive, processing aid, ingredient, packaging or other associated thing where that associated thing is contained within, attached to, enclosed with, or in contact with, the food material or product.

intrinsic contamination means contamination of product during manufacture.

intrusive process means any activity that is carried out on a product contact surface that is normally inaccessible during the operation of the processing plant. Examples include opening the sifter or a bulk bin hatch for inspection or maintenance.

investigative sampling means the collection of samples (product or surface swabs) that are taken for microbiological tests which are not part of the normal microbiological testing regime but are carried out to locate a source of contamination. Investigative samples may be taken in response to an event (water spill, unclean site) or completely randomly. Such samples are also called **random samples, hunting samples, investigation samples or samples at the sampler's discretion**.

lot: refer to **batch**.

MPI means the Ministry for Primary Industries.

peracetic acid is a biocidal component of sanitisers, often used in formulations with hydrogen peroxide. It is an organic compound with the formula CH_3CO_3H and is also known as peroxyacetic acid and proxitane®.

potentially contaminated means all batches of infant formula that are potentially contaminated with *Cronobacter* as a result of being processed around the time that *Cronobacter* was detected in product or the environment.

product contact surface means the surfaces that exposed product touches prior to entering the final packaging.

quaternary ammonium compound means a group of biocidal chemicals which are positively-charged polyatomic ions of the structure $[NR_4]^+$, where R is an alkyl or aryl group. Commercially available formulations may comprise combinations of different quaternary ammonium compounds with different R-groups. The use of these chemicals in the NZ dairy industry has been phased out.

recall means to isolate and remove unsafe or unsuitable food from the market that is no longer under the manufacturer's direct control and has passed into the control of others in the storage, distribution, retail or consumer chain. (Note the definition of "**withdrawal**" for comparison).

redline means the hygiene barrier(s) in place to prevent direct access from the outside environment into the processing area. Often a double barrier entry system where external footwear is left on the outside of the first barrier, hands are washed and protective clothing donned, then internal footwear is changed into on the other side of the second barrier.

risk management programme (RMP) means a documented programme designed to identify and control hazards and other risk factors in relation to the production and processing of certain animal material and animal products, to ensure that the resulting animal product is fit for its intended purpose under the Animal Products Act 1999.

standard hygiene area means the processing area, a CCP for *Cronobacter* or a final microbiological hurdle where the raw ingredients, materials and intermediary products are handled. This includes the production/manufacturing area, raw ingredient storerooms, packaging storerooms, and chillers/refrigerators.

standard methods agar also called **standard plate count (SPC) agar** are microbiological growth media commonly used to estimate "total" or viable bacterial numbers, either in a sub-sample of product sample or recovered from a surface by a method such as swabbing.

standard plate count (SPC) or **aerobic plate count (APC)** means the number of mesophilic bacterial colonies growing under aerobic conditions on **standard methods agar**. This is an estimate of the number of bacteria that were present in the product or surface being tested.

test means to conduct a planned sequence of observations or measurements of control parameters to assess whether a process, procedure or CCP is under control.

water activity (a_w) means a measure of water available for the growth of microorganisms in food. Note that it differs from the total moisture as water may not be available if it is bound to components (such as salt and sugars) in the food.

whole genome sequencing means a laboratory method that is used to determine the entire genetic makeup of a specific bacterial isolate. This method can be used to find changes in

areas of the genome. These changes can help scientists to understand the relationship between different *Cronobacter* isolates and help in determining sources of contamination.

withdrawal (also known as a trade level recall) means the removal of an unsafe food from the distribution chain but does not extend to food sold to the consumer (Note the definition of "**recall**" for comparison).

zone refers to the division or a processing area based on the activities untaken and the risks they pose to safety of the product in its final form. The term zone and grading such as 1, 2 3 or 4 may be used as an alternative to the terms standard or critical hygiene areas (Appendix 1, Table 1).

1.4. Additional Resources

The documents listed below provide additional information on *Cronobacter* and/or pathogen management in PIF or dairy processing plants.

New Zealand Food Safety Authority. Dairy industry guidelines for risk organism preparedness and response, 2008.

Australia New Zealand Food Standards Code – Schedule 27 – Microbiological limits in food, 2021.

FSANZ (Food Standards Australia New Zealand). Approval report – Proposal P1039 Microbiological Criteria for Infant Formula, 2016.

MPI Food recall guidance for businesses.

CAC/RCP 66-2008. Codex Code of Hygienic Practice for Powdered Formulae for Infants and Young Children.

Lindsay D, Farber JM, Bright B, Shrubb O, Crowe D, Soboleva T. Controlling *Cronobacter* spp. in dairy manufacturing–Fundamental characteristics and practical guidance. Food Control. 2024;160:110299. doi: 10.1016/j.foodcont.2024.110299.

Summary of FDA's Strategy to Help Prevent *Cronobacter sakazakii* Illnesses Associated with Consumption of Powdered Infant Formula.

ICMSF (International Commission on Microbiological Specifications for Foods) Guidance on Microbiological Sampling and Testing for Key Commodities, 2018.

FAO (Food and Agriculture Organization) and WHO (World Health Organization) Joint Technical Meeting on *Enterobacter sakazakii* and *Salmonella* Powdered Infant Formula (2006: Rome, Italy), *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: meeting report. Microbiological risk assessment series no. 10, 2006. ISSN 1726-5274.

FDA (US Food and Drug Administration). Infant formula program—Import and domestic. In: Compliance Program Guidance Manual, Program #7321.006, 2018.

The Innovation Center for U.S. Dairy. Controlling Pathogens in Dairy Processing Environments – Guidance for the US Dairy Industry, 2019.

Grocery Manufacturers Association, USA. Control of Salmonella in Low Moisture Foods, 2009.

NZFS are preparing dairy pathogen management guidance, which will be available soon.

2. Information about *Cronobacter* spp

2.1. Why *Cronobacter* must be managed

Cronobacter is a micro-organism of increasing importance and concern for the dairy industry and, in particular, for infant formula processors. *Cronobacter* is associated with necrotising enterocolitis in preterm infants, and invasive infections in all infants, but especially neonates (infants under 28 days old). The invasive infections of greatest concern are meningitis, pneumonia and sepsis, and it is estimated that up to 40% of infants who become ill, die. *Cronobacter* infections in infants who have been fed PIF are almost always associated with contaminated PIF as the vehicle of transmission. Contaminated PIF is a clear risk factor for vulnerable infants, many of whom may require PIF if they are born prematurely or are unable to breastfeed. It is important to appreciate that PIF manufacturing is not designed to be a sterile process, as spores and some thermotolerant, non-pathogenic vegetative cells will not be killed by pasteurisation and spray drying. Hence it is important that PIF is prepared and stored in accordance with the manufacturers' instructions.

***Cronobacter* has a history of outbreaks associated with PIF and remains a problem.** Historically *Cronobacter* infections were outbreaks associated with PIF use in hospitals. A history of *Cronobacter* is described in Figure 1; Henry and Fouladkhah, 2019; and Strysko et al., 2020.

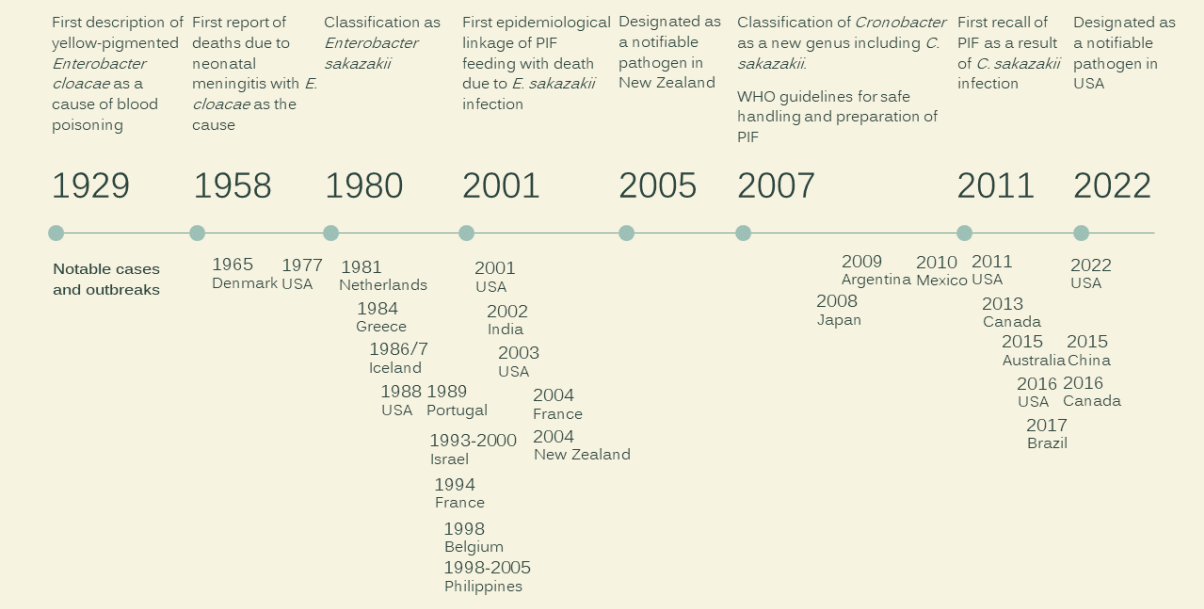

Figure 1. A timeline of key events in the history of *Cronobacter*.

In New Zealand, manufacturers of dairy-based infant formula products and formulated supplementary foods for young children must include *Cronobacter* spp testing of packaged infant formula (0-6 months) in their RMP sampling and testing plan (Infant Formula Notice, 2022). The detection of *Cronobacter* in the environment within or around PIF or dairy processing plants is not uncommon and on occasions its presence in PIF has resulted in product being withdrawn.

Infection caused by *C. sakazakii* became a notifiable disease in New Zealand in 2005 (NZFSA, 2009). In 2004, a premature baby at Waikato Hospital died of meningitis caused by *C.*

sakazakii infection, although authorities were unable to definitively link the case to contaminated PIF (NZFSA, 2009). In 1991, there was an unconfirmed report in New Zealand of *Cronobacter* infection of twins, with one twin recovering and the other later suffering brain damage and spastic quadriplegia (NZFSA, 2009).

Internationally, *Cronobacter* cases have been reported in neonates and young infants in numerous countries (Figure 1), however verified cases remain rare. A recent review of invasive *Cronobacter* infections during early infancy, 1961-2018 (Strysko et al., 2020) identified a total of 183 cases, with case numbers only reaching double figures in the Philippines, United Kingdom and the United States of America.

In the United States, in 2022, an outbreak associated with powdered formulas has been implicated in 2 infant deaths. This resulted in the shutdown of the implicated plant for 136 days, between 15 February and 1 July 2022 (Sealy and Hassan, 2022), the recall of millions of units of PIF and a shortage of infant formula across the USA that lasted months. The company involved reported an estimated revenue loss of approximately US$170 million (NZ$268 million) between the first quarter of 2021 and the first quarter of 2022 (Forbes, 2022), with analysts suggesting the total losses from the recall were in the order of US$325 million (NZ$513 million) (Block, 2022). A further consequence of this outbreak was that *Cronobacter* was added to the list of "Notifiable Pathogens" in the USA from 2024, with reporting required for infections of infants under 1 year old.

At the beginning of 2024, 675,000 cans of PIF products were recalled due to possible *Cronobacter sakazakii* contamination, following the detection of the bacteria in exported product at the Israel border (US FDA, 2024). No illness associated with the recall was reported and further testing of additional cans of the same batch, and at the implicated plant in the USA, failed to identify the presence of *Cronobacter*. The recall did not impact supply and the US FDA communicated with other manufacturers to ensure the supply of affected PIF products (US FDA, 2024).

Operators and other stakeholders in New Zealand should actively consider the security of their PIF supply to consumers, given the disruption seen in the USA in 2022, as part of a business continuity plan. In particular, consideration should be given to where the PIF product is sold, the market share, and how any disruption to supply due to *Cronobacter* would be managed.

2.2. The genus *Cronobacter*

Cronobacter spp. are Gram-negative bacteria, facultatively anaerobic, oxidase-negative, catalase-positive, rod-shaped members of the family Enterobacteriaceae. *Cronobacter* spp. are ubiquitous in the environment and most commonly associated with processing plants. The genus consists of seven identified species, including *C. sakazakii*, *C. condimenti*, *C. dublinensis*, *C. malonaticus*, *C. muytjensii*, *C. turicensis*, and *C. universalis* (Iversen et al., 2007). The predominant pathogen is *C. sakazakii*, with *C. malonaticus* and *C. turicensis* identified as the cause of infections in fewer cases. Note that prior to 2007, *Cronobacter sakazakii* was named *Enterobacter sakazakii*.

Cronobacter are desiccation-resistant and can survive conditions of low water activity (a_w = 0.25-0.5 for at least 1 year) which means that they can persist in dry products such as PIF (CDC, 2022). *Cronobacter* can grow between 6°C and 45°C, meaning that this bacterium has

the ability to increase in numbers at temperatures commonly found within PIF processing plants. When *Cronobacter* are present within a biofilm they can be difficult to eliminate as they have enhanced resistance to sanitisers. All *Cronobacter* spp, including heat-tolerant strains, are believed to be killed by high-temperature, short-time pasteurisation (70 °C is required for full inactivation).

The infectious dose of *Cronobacter* has proven difficult to estimate, with various factors including pathogenicity of the strain, and the health of the infected individual, being influential. Iversen and Forsythe (2004) proposed an infectious dose in the region of 1,000 colony forming units (CFU), and this number has been supported by subsequent studies (Mittal et. al., 2009, Richardson et al., 2009, Cruz et al., 2011, Joseph and Forsythe, 2011).

2.3. Microbiological limits for *Cronobacter*

In New Zealand, the Food Standards Code Chapter 1.6.1 (and associated schedule 27) and the Animal Products Act 1999 and associated regulations and notices specify microbiological limits for *Cronobacter* in PIF. In Australia, food safety requirements are set out in Chapters 3 and 4 of the Australia and New Zealand Food Standards Code.

2.3.1. *Cronobacter* in products

Cronobacter should be "not detected" in 30 × 10 g samples of PIF at the end of the manufacturing process. (see Schedule 27 in the Food Standards Code).

2.3.2. *Cronobacter in* the processing environment

Cronobacter should be "not detected" in swab samples taken from product contact surfaces or non-product contact surfaces in critical hygiene areas in PIF plants, or plants making ingredients for PIF.

3. Sources of *Cronobacter* contamination of PIF

Contamination of PIF can occur during production or use by the consumer. Intrinsic contamination refers to contamination of PIF during manufacture; extrinsic contamination refers to contamination occurring after the factory container is first opened by the user. A further challenge occurs if the manufacturers' instructions are not followed and reconstituted infant formula is not used directly. Unused formula should be discarded, as any *Cronobacter* present can increase in numbers and increase the risk of causing infection. This is a significant concern as it means that in theory a single *Cronobacter* bacterium, present as intrinsic contamination in the PIF could multiply to numbers above the infectious dose if the reconstituted PIF is not stored correctly (Parra-Flores et al., 2015).

In PIF manufacturing or processing plants, contamination can be present in the final product if there is a failure in pasteurisation processes leading to the survival of *Cronobacter* present in the ingredients. However, if appropriately validated and monitored controls are in place, any pasteurisation failures will be quickly noticed and the affected product quarantined and appropriately managed. Hence, in the processing environment, post-pasteurisation contamination is considered to represent the greatest risk of *Cronobacter* occurring in PIF. Post-pasteurisation contamination can occur through either exposure of the PIF to a contaminated surface (equipment, plant, PPE or worker), inadequate air filtration or control, or the introduction of contaminated ingredients post-pasteurisation.

Increases in the frequency of detection of *Cronobacter* spp. (or more generally numbers of Enterobacteriaceae which is used as a hygiene indicator), in processing environments can be due to the introduction of bacteria from outside of the plant due to poor operational practices, inadequate maintenance or, more commonly, changes in conditions (e.g. increase in water availability, reduced cleaning/sanitising effectiveness). This enables bacteria already present in the environment to increase in numbers or to be transferred from less critical to more critical hygiene zones (Bourdichon et al., 2021).

3.1. Sources of *Cronobacter* in PIF processing plants

Cronobacter is relatively widespread in the natural environment, hence its detection in the environs surrounding a PIF plant or on the exterior cladding (roof, walls) of a plant is not unexpected. Sources of *Cronobacter* in various parts of a manufacturing site are summarised in, but not limited to, Box 1.

Box 1: Examples of niches and other locations at risk of *Cronobacter* harbourage.

General facilities

- Warehouse bays
- Tanker bays
- Utility (workshops)
- Storerooms

PIF manufacturing plants

- Incomplete or poor welds
- Unsealed or poorly sealed joints between the floor and equipment, or between two pieces of processing equipment
- Cracks
- Drains
- Poor or damaged seals in walls, floors, around drains or in doors which enable moisture to enter and be retained
- Bearings or shafts on rollers, wheels or motors
- Horizontal runs of pipe which are partially occluded

Powder plants

- Surfaces in roller dryers or drying towers
- Spray-drying, roller drying, fluidized-bed or packing operations
- Horizontal surfaces
- Floors
- Open wire conduits
- Wall fittings (power boxes, light switches)
- BMF fittings (Blair Forres McPheat pressure-resistant connectors made from a clear, ether-based, thermoplastic polyurethane alloy with polyester scrim which are used to connect a vibrating section to a stationary section)

If the exterior cladding is not hygienically designed or if damage or inadequate maintenance (damage to seals when replacing air filters) leads to a breach in the exterior protective barriers, *Cronobacter* can enter a plant via either the passage of air or water, or an animal or insect vector. As discussed in later sections, *Cronobacter* can also enter a PIF plant if controls around the movement of people, product, ingredients, or equipment are poor or have been circumvented.

Cronobacter have been isolated from a wide variety of surfaces in the PIF processing environment. Due to its ability to form biofilms, *Cronobacter* can establish, grow and persist in niches/harbourage sites and become a source of contamination in the plant. As *Cronobacter* requires water to grow, the bacteria are not expected to be able to grow in critical hygiene zones (particularly Zones 3 and 4 dry Zones) in which the use of water is strictly controlled. Nevertheless, sites within Zones 3 and 4 can become sources of contamination if their design or operation facilitates the survival or persistence of *Cronobacter*, particularly if sites are routinely exposed to water (e.g. during cleaning and sanitising). Fire sprinklers in these hygiene zones are an example of fittings that should be monitored to ensure there are no leaks in the dry areas.

An important aspect in the hygienic design of manufacturing plants and processing equipment is preventing the occurrence of niches/harbourage sites, which due to their nature (e.g. occluded) or physical position are difficult to access, inspect or clean. Such sites are of concern because if sufficient water is present, they can become a source of persistent *Cronobacter* contamination.

In PIF manufacturing or processing plants drains can become a source of contamination if they become damaged, are inadequately cleaned, or if their cleaning implements are improperly cleaned and stored. To minimise the potential for cross-contamination, drains should be sealed and not open to the plant environment when product is being processed.

The interior of the processing environment (walls and floors) can act as reservoir of *Cronobacter* if surfaces are not fully sealed and impervious to water owing either to poor installation, damage, or wear and tear. It is the experience of NZ producers that the resurfacing / repair of floor surfaces and installation of impervious, hard, smooth, easier to clean floors is associated with a reduction in the occurrence of *Cronobacter*.

Cronobacter has been isolated from dry areas in powder plants, in particular areas where films of milk powder residues accumulate. It is important to appreciate that owing to the ability of *Cronobacter* to survive under conditions of low water activity, cells can persist in dry environments for long periods of time.

During operation the product itself (powder residues, reworked powders, fines return) has been reported to be a source of contamination by NZ producers.

In summary, there are many different potential sources of *Cronobacter* in a PIF plant, and controlling these sources can reduce the risk (which will be discussed in later parts of this document).

3.2. Transfer of *Cronobacter* within a PIF processing plant

The movement of *Cronobacter* within PIF processing plant, including across poorly controlled red line barriers, has been experienced by NZ manufacturers and scientifically investigated. Transfer of *Cronobacter* can occur due to movable fomites such as boots, trolley wheels or bins becoming contaminated with *Cronobacter* from floors, or from hands or gloves moving it from touch point to touch point, or through poorly designed or operated air flows and pressure differentials. It has been reported that from a starting inoculum of 10^6 CFU/mL, approximately 10^4 CFU/mL *Cronobacter* cells were transferred from each fomite onto each recipient surface during the initial transfer event and that if a movable fomite (boots or trolley

wheels), or gloves became contaminated, *Cronobacter* could be spread over a wide area within a manufacturing plant (Lindsay et al., 2019).

Within processing facilities, maintenance equipment (especially unanticipated items, rope, tools) and cleaning tools (e.g. vacuum cleaners) have also been suspected of as sources contributing to the transmission of contamination if they are moved across the red line.

3.2.1. Reduction of *Cronobacter* transfer

To reduce the possibility of boots being a source of contamination, boots put on within critical hygiene zones are recommended to be sprayed immediately before and after wearing with 60-70% ethanol or a sanitiser such as peroxyoctanoic acid. The spray treatments should be used with a validated drying time. Monthly scrubbing with a sodium hypochlorite sanitiser or a peracetic acid/peroxide sanitiser is also suggested.

To further reduce the possibility of boots transferring *Cronobacter* around the plant, disposable plastic shoe covers have been reported to be an effective means of preventing the movement of *Cronobacter* between rooms within Zone 4, or between floors accessed by a lift within Zone 3. To facilitate such a process, redline barriers may need to be installed at each floor entry and at the entry to the packaging room, despite its entry being off a Zone 4 corridor. In addition, sticky mats can be used to capture powder and prevent spread between rooms, but these need to be replaced daily or when the mat is no longer tacky and becomes unable to retain powder.

To reduce the possibility of trolley wheels, bins, maintenance equipment (especially unanticipated items such as rope or a specific tool) or cleaning tools (vacuum cleaners) becoming a source of contamination, it is recommended that such equipment is designated for use within a particular hygiene zone, and if equipment needs to be moved between zones, this can only occur after permission has been obtained from a person delegated to authorise such actions and then only after the equipment has been subjected to an approved validated cleaning / sanitising regime or risk assessment.

4. Control of *Cronobacter* in PIF Processing Plants

A CMP may be included as part of a food business's RMP.

The control of *Cronobacter* in PIF processing plants is achieved through the following processes:

- the establishment of controls to prevent *Cronobacter* entry and to limit the spread of any contamination that does occur
- strictly controlling the use of water in the manufacturing and packaging environments
- effective hygienic zoning, and controls between zones
- ensuring ingredients are of suitable microbiological quality
- ensuring segregation of ingredients from finished product
- the establishment and monitoring within a HACCP plan of validated pathogen reduction regimes, e.g. pasteurisation
- the establishment of effective cleaning and sanitation regimes, appropriate for the zone
- the implementation of environmental (hygienic) testing and monitoring regimes
- the implementation of environmental pathogen testing and monitoring regimes for *Cronobacter* spp
- the implementation of air monitoring regimes for *Cronobacter* spp and/or indicator organisms
- final product testing to verity that *Cronobacter* management in the plant is effective
- final product testing to ensure that *Cronobacter* is "not detected" and,
- pre-determined effective responses if *Cronobacter* is detected during environmental or product testing.

In general, the processes listed above are all critical components of pathogen GMP controls.

In addition to having audited plans and validated processes in place to control *Cronobacter* it is of vital importance that staff working within critical hygiene zones are fully aware of the unique challenges posed by *Cronobacter,* which can be summarised as:

- *Cronobacter* contamination in PIF can result in infants contracting a severe, life threatening illness
- *Cronobacter* is widespread in the natural environment
- in New Zealand and in many other countries there is a zero tolerance for *Cronobacter* in PIF
- *Cronobacter* can survive for extended periods of time in dry environments
- in moist environments *Cronobacter* can rapidly increase in numbers and form biofilms which can protect the cells from actions (sanitising, heat, drying) designed to kill it, and,
- *Cronobacter* can be readily transferred through a manufacturing / processing plant by a wide range of fomites, maintenance equipment, cleaning equipment, via workers, or through disruptions to airflows.

5. The establishment of controls to prevent entry and limit spread

In addition to implementing strict regimes to prevent the entry of *Cronobacter* into PIF processing plants, controls must also be put in place to limit the spread of any contamination that may enter the plant. The reader is referred to section 3.1 for potential source areas that should be a focus of hygienic design. Facilities and equipment should be designed, constructed, and operated so as to minimize the entry of *Cronobacter* species into high hygiene areas and to reduce the possibility of its establishment or growth in harbourage sites. An effective building maintenance system that proactively addresses building defects e.g. blocked gutters, damaged cladding, cracks etc., is an essential approach to prevent entry of *Cronobacter*. Dry processing areas should be maintained as high hygiene areas and kept as dry as possible. There must be effective segregation between wet and dry areas. The internal design and layout of plants manufacturing PIF must ensure the strict physical separation of wet from dry processing areas, particularly after the final pathogen reduction step. Further, where possible, wet areas should be eliminated from the manufacturing environment including in areas where wet processing occurs.

Good air filtration and well-designed air flows and pressure differentials are essential in the control of *Cronobacter.* Air filtration should encompass the use of insect screens (150 µm filter), primary (G4 dust) and secondary (F7, F9) filters and the use of HEPA (H13 or H14) filters. A 150 µm filter is an important addition to a filter bank as it can filter out small insects that could be vectors of *Cronobacter* and which can bypass traditional 500 µm insect screens, dust filters and F9 filters. Note that the G4, F7 and F9 Filter Classes are specified in EN 779, a standard that has been replaced by ISO 16890. G4 corresponds to ISO Filter Group Course with arrestance at ≥60% efficiency. F7 corresponds to ISO Filter Group ePM_1 with arrestance of particulate sizes ≤1 µm at ≥50% efficiency. F9 corresponds to ISO Filter Group ePM_1 with arrestance of particulate sizes ≤1 µm at ≥80% efficiency. HEPA filter classes >H13 in EN 1822 are specified by ISO 2943 as >35H.

The use of integral, and properly installed, filter banks is essential to preventing bypass of the filtration system. It is recommended that a) pressure differentials are in place between external environment, standard hygiene areas, critical hygiene areas, and different critical hygiene zones e.g. between Zone 3 and 4; b) best practice hygienic design includes separate HVAC systems for the most critical areas, that are separate to standard care areas to prevent cross contamination; and c) relative humidity is controlled to prevent cleaning challenges with the opportunity for pathogen establishment and growth.

In the production of PIF the major controls to prevent contamination of the processing environment include the establishment of hygienic zones (segregation of production areas, see Appendix 1) and control of the movement of people, product, equipment and air into or through the zones, and protection of internal product contact surfaces. It is also important to limit process intrusions by reducing the number of routine entry points into the process and the number of routine equipment/process checks. Where possible it is recommended that manufacturing equipment, tools and cleaning equipment are assigned to a specific area or room and should not be moved between different zones or areas.

As per the Infant Formula Notice 2022, hygienic zones (Appendix 1) must be clearly defined (physical site/schematics) and either active (controlled entry) and/or passive (redline barriers, training) systems must be in place to control movement between zones.

The Infant Formula Notice 2022 also specifies the design and construction of manufacturing areas; describes high hygiene areas; lays out the requirements of air pressure in high hygiene areas; outlines that entry into high hygiene areas must be via a buffer zone; states the requirements for people or items entering or exiting high hygiene areas.

6. Ensure ingredients and packaging are of suitable microbiological quality

Incoming ingredients and additives which are added after the pasteurisation step must be free of *Cronobacter* as verified by a validated HACCP program. Ideally, as a means of minimising risk, *Cronobacter* should not be detected in the ingredients which are added pre-pasteurisation. It is important to note that the detection of *Cronobacter* in a product pre-pasteurisation is not considered to increase the risk posed by the pasteurised product, as pasteurisation is designed to eliminate this risk.

Manufacturers should have robust raw material risk assessment procedures and a supplier quality programme to ensure that critical ingredients continue to meet required microbiological limits.

Packaging materials, in particular product contact materials (tins, lids, plastic liners) and outers which enter the critical hygiene zones, must be free of *Cronobacter* as verified using a validated HACCP program. To ensure that packaging material does not become contaminated it is important to ensure that the packaging is stored in a dry, covered area, with a protective outer wrap that is removed immediately prior to the packaging being transferred to the high care areas. As an additional safeguard, it is recommended that where possible, the packaging (e.g. cans) is subjected to a process(es), such as UV treatment or flushing with filtered or ozone-sterilised air, designed to kill or remove any potential contaminating micro-organisms, immediately prior to the addition of product.

7. Validated and verified pathogen reduction steps

A critical step in ensuring that *Cronobacter* is not detected in PIF is the implementation of an effective pathogen reduction regime (e.g. pasteurisation or equivalent) prior to spray drying, which is monitored within a validated HACCP plan.

For dry blending facilities that do not have a pathogen reduction step as part of their validated HACCP programme, raw material controls and GMP become critical parts of the CMP. Ingredient acceptance testing and robust final product testing regimes become critical to ensuring the ingredient and the final product are fit for purpose.

8. The establishment of effective sanitary regimes (cleaning or cleaning and sanitising)

8.1. Cleaning and sanitising in Wet Processing Zones (e.g. cleaning-in-place or CIP)

The aim is to ensure that the process equipment and the manufacturing environment in wet processing zones are clean and hygienic, with no harbourage sites for potential contaminants. In processing equipment, this is achieved by CIP or cleaning-out of place (COP) regimes using caustic and acid washes. Sanitisers can also be included in this CIP treatment. It is important to use a validated CIP circuit that includes a caustic step with temperatures greater than 70 °C for at least 10 minutes. If possible, CIP solutions should be hard piped to drains and not drain to the floor of the manufacturing facility.

The effectiveness of sanitisers against *Cronobacter* in broth, dried onto surfaces and as biofilms grown on surfaces, have been tested in the laboratory (Kim et al. 2007; Lindsay et al. 2022). Bacteria within biofilms are more resistant than free-living cells to a wide range of antimicrobial treatments, including exposure to sanitisers and represent the best model against which to judge sanitiser effectiveness. Some, but not all, formulations of quaternary ammonium sanitisers have been reported to be effective (≥ 3 log reduction in viable cells recovered after a 5-minute contact time) against *Cronobacter* biofilms on stainless steel surfaces (Kim et al. 2007). However, owing to the tightening of maximum residue level (MRL) in the EU for quaternary ammonium compounds, their use within the New Zealand dairy industry is now almost completely phased out. In a recent study, effective sanitisers (≥ 3 log reduction over 5-minute contact time) against *Cronobacter* biofilms on stainless steel and in a milk-matrix have been reported to include those containing hydrogen peroxide, hypochlorite, lactic acid, and peracetic acid/hydrogen peroxide combinations (Lindsay et al. 2022).

Commercially available sanitisers exhibit a variable efficacy against *Cronobacter* biofilms and the choice of which sanitiser to use should be based on a demonstrable activity measured as a ≥ 3 log reduction over 5-minute contact time, the sanitiser's potential human health and environmental impact, and regulatory requirements. The suitability of a sanitiser may change as new information on their safety and activity becomes available. Hence the suitability of a sanitiser should be regularly assessed.

8.2. Cleaning in Dry Processing Zones

Bacteria require water for growth, therefore a critical means of preventing the growth of *Cronobacter* within the critical hygiene zones is to ensure that they are kept as dry as possible.

To ensure that surfaces and in particular niches within Zone 4 are kept as dry as possible, dry cleaning strategies are generally used. Dry cleaning involves removing all visible powder residues using either a broom, brush, or vacuum appliance. Vacuum appliances can either be small portable units that are kept within the critical zone, or they can be fixed systems in which the power units and collection bags are outside of the critical zone with only a flexible hose and nozzle within the critical zone.

In dry cleaning GMP is critical, especially for intrusive processes such as maintenance. It is also important to ensure that there is suitable downtime between batches to complete a manual clean. After an intrusive dry clean, a validated flushing of powder not destined for infant formula, that has been through a validated heat treatment step (if available within the product range being processed) is recommended to be used to scour and clean the internal surfaces, prior to high-risk products being processed.

Some dry areas, such as the fluid bed, may need to be occasionally (fortnightly) cleaned and sanitised using a CIP process with a validated dry out step.

8.3. Cleaning and sanitising of manufacturing areas in response to detections or environmental breaches (e.g. wet cleaning with appropriate dry out and sanitiser use)

On occasions where *Cronobacter* is detected or suspected to be contaminating a surface within a Zone 4 area, it may be appropriate to use a "wet cleaning and sanitation" regime. This may also be appropriate in response to an environmental breach e.g., a building leak. The regime must include the use of a cleaning compound appropriate to remove the relevant soil (i.e. product or material), and a sanitiser that is effective against *Cronobacter* and biofilms. The sanitiser must be used on a clean surface to prevent biofilm formation under layers of product residue. Sanitisers should be used according to the manufacturers' instructions and any regulatory conditions. If a sanitiser requires a rinse step afterwards, water must be used in a controlled manner and a rapid dry out method applied afterwards.

In general, if using a wet clean in a 'dry zone' it is imperative to ensure that the cleaners and sanitisers are confined to the area being cleaned and do not increase residual moisture levels in non-targeted areas.

8.4. Microbiological status of cleaning equipment

Confidence in the cleaning equipment is important and care should be taken at procurement to purchase the correct equipment for the task. The equipment should have a specified cleaning protocol (e.g. controlled wet clean or dry clean); be regularly inspected and maintained, replacing the equipment or failing parts (e.g. frayed brushes, cracking or other integrity issues) as necessary; and be stored appropriately (e.g. away from walls). It is important to ensure that the equipment used to clean the plant is itself clean. A variety of approaches can be used to check the hygienic status of the cleaning equipment:

1. Testing for the presence of Enterobacteriaceae rather than *Cronobacter*. Enterobacteriaceae are considered to be a more effective process hygiene indicator, as discussed in more detail in Section 9. However, if a failure in hygiene is identified by these tests there will be a need for product segregation and retesting until the *Cronobacter* status is confirmed.
2. Testing for the presence of *Cronobacter*. However, this does not give a good indication of hygiene failures.
3. Testing for the presence of both Enterobacteriaceae and *Cronobacter*. This gives an indication of hygiene and the *Cronobacter* status but does contribute to a greater testing burden.

Regardless of tests undertaken the RMP needs to be clear on the actions (e.g. segregation of product, retesting required) to be taken if unfavourable Enterobacteriaceae results are obtained, with the default assumption that all of the detected organisms may be *Cronobacter*.

In general, the detection of *Cronobacter* or Enterobacteriaceae on cleaning equipment should trigger a similar response to the microorganism being found on a non-product contact surface within that Zone. If *Cronobacter* is not specifically tested for and indicator aerobic counts are elevated, or coliforms/Enterobacteriaceae are detected, tests specific for *Cronobacter* should be performed.

9. The establishment of an effective environmental pathogen monitoring plan

As per the Infant Formula Notice 2022, the RMP must contain a sampling and testing plan which includes environmental pathogen monitoring (noting that the sampling and testing plan may reference details held in the environmental pathogen monitoring plan).

Processing environment monitoring is recognized as a proactive approach to anticipate finished product contamination. In environmental testing regimes an indicator species may be looked for either in addition to, or in place of, *Cronobacter*. The International Dairy Federation (IDF, 2016) recommends the implementation of environmental monitoring programmes for Enterobacteriaceae (indicators for process hygiene) and *Cronobacter* spp., to minimize the risk associated with *Cronobacter* spp. in PIF.

Enterobacteriaceae are considered to be a useful indicator for hygiene standards in post heat-process areas of PIF manufacturing plants. Enterobacteriaceae is a family of Gram-negative bacteria, including *Salmonella* spp. and *Cronobacter*. In dry environments it has been reported that increases in Enterobacteriaceae numbers correlated well with increases in pathogen (*Salmonella* spp.) numbers where water had been introduced. Hence increases in the number of an indicator microorganism can provide an early alert to a change in environmental conditions that has potentially led to an increased risk of pathogens being present or being able to multiply and hence pose a greater threat to the safety of products manufactured in that environment. The setting of appropriate action limits can act as an early alert to such conditions and can enable a quicker response to be initiated, which may involve isolating the affected areas, identifying and eliminating the source(s) of water leaks and/or applying appropriate cleaning and sanitising regimes before more serious consequences can develop.

It is, however, important to appreciate that while the presence of Enterobacteriaceae or an increase in their number may indicate a higher likelihood of the presence of *Cronobacter* spp., somewhat controversially, their absence has been stated as not being sufficient evidence to conclude that *Cronobacter* is absent. The rationale behind this statement is based on research by Craven *et al.*, 2021, who, on investigating the correlation between the number of Enterobacteriaceae and coliforms with the occurrence of *Cronobacter* in milk powder manufacturing environments, found that the numbers for both of these indicators showed a better association with *Cronobacter* numbers than did the use of presence or absence tests for these organisms. For example, even when Enterobacteriaceae or coliform numbers were less than 1 CFU/cm^2, *Cronobacter* was still detected in 2 of 18 samples. While this result may sound contradictory as *Cronobacter* are a member of the Enterobacteriaceae and are coliforms, it reflects differences in sampling strategies and detection limits between test methods designed to determine the number of bacteria present, compared to presence/absence testing; for example, presence/absence testing involves a pre-incubation period in growth medium and so this is a better approach to isolate viable but not-culturable (VBNC) state bacteria than direct plating methods used for the enumeration of indicator species. Furthermore, it is important to recognise that bacterial recovery from swabs used in sampling and testing may only represent a fraction of the true numbers of organisms present.

Hence, it is important to set parameter values (microbial limits) for environmental monitoring and also to test, by sampling, if there has been an increase in the number of indicator microorganisms.

To this end, processing environment monitoring programmes should be designed to both identify critical sampling points to be routinely sampled and to search for niches harbouring *Cronobacter.*

In summary, while indicator organisms (Enterobacteriaceae and coliforms) can identify where contamination has occurred and despite an upward trend in the number of indicator microorganisms being detected indicates a loss of overall hygienic control and a greater risk of *Cronobacter* being present, the absence of indicators organisms does not guarantee the absence of *Cronobacter*. Where *Cronobacter* is not specifically tested for and indicator aerobic counts are elevated, or coliforms/Enterobacteriaceae are detected, tests specific for *Cronobacter* should be performed. Hence it is recommended that environmental monitoring programmes include a 50:50 mix of samples that test for either *Cronobacter* or the indicator organisms.

9.1. Sampling in Zone 1 and Zone 2

Zone 1 testing covers the environment outside the processing area(s) to determine possible sources of contamination so that they can be managed, and prevent the movement of *Cronobacter* into the processing area. Not all environmental testing programmes will include the sampling of Zone 1.

Zone 2 testing is to determine if *Cronobacter* is coming into the processing area, where it might represent a source of contamination of the Critical Hygiene Area. A high density of testing for *Cronobacter* in Zone 2 may not be deemed necessary and some manufacturers may use Enterobacteriaceae tests to monitor the effectiveness of GMP systems and/or use composite samples to reduce costs or justify more frequent sampling.

9.2. Sampling in Critical Hygiene (Zones 3 and 4) Area

The risk posed by contamination is greatest post-pasteurisation because if the product becomes contaminated there are no additional control steps that can ensure its safety. Critical areas include evaporation (Zone 3W) and spray drying (3D), dry blending (4D) and final product packing (4D). See Appendix 1**.** Within these zones, environmental testing is carried out for Enterobacteriaceae (or coliforms) as a marker for general hygiene, *Salmonella* (another pathogen that must be absent) and *Cronobacter*. In some situations, additional *Cronobacter* tests may be carried out if Enterobacteriaceae tests indicate a non-conformance. Two types of surfaces are included in environmental monitoring programmes:

i) non-food contact surfaces such as external parts of equipment, platforms, floors around the pipeline and high hygiene areas.

ii) food contact surfaces in high hygiene areas include the inside surfaces of equipment following the spray dryer and preceding packaging. Areas that should be targeted include those that are routinely exposed to the environment during manufacturing, or those which are routinely opened when product is not being processed. Other sites should be tested when microbiological data or observations such as powder clumping

in the sifter bed or tailing/covers, which may indicate that there could be a lack of control. The presence of either indicator organisms or *Cronobacter* on these food contact surfaces is an indication that there is a high risk that contamination of PIF has occurred.

It is well known that the swab type and surface condition (wet or dry) can affect swab efficiency, hence choosing the appropriate type of swab for the surface condition will increase recovery (Keeratipibul et al., 2017; Lindsay et al., 2010). Operator experience in New Zealand strongly suggests that wet swabbing followed by dry sampling is more effective than dry swabbing alone. For ease of use and potentially for better recovery of micro-organisms, sponge swabs are considered to be better than the use of gauze, although there does not appear to be any significant published evidence to support this. In addition, sponge swabs are more effective in sampling difficult to reach areas such as the interior surfaces of pipes. However, the ease of use and potentially better recovery associated with sponge swabs needs to be weighed against their greater cost compared to gauze. Hence for sampling large uniform areas such as floors, walls, and expanses of stainless-steel, gauze can be a cost-effective option, while for smaller more confined and complex (configuration, surface roughness) surfaces sponge swabs are recommended. For recommendations concerning swabbing please consult BS EN ISO 18593:2018 Microbiology of the food chain – Horizontal method for surface sampling.

When pre-moistened sponge swabs are used in critical hygiene areas (Zone 4D), it is important to ensure the removal of any residual recovery media and moisture. This is generally carried out by spraying the area which has just been swabbed (wetted) with 70% ethanol. Although 95% ethanol evaporates faster, the use of 70% (weight/volume) ethanol is recommended owing to its greater germicidal properties. Ethanol sprayed areas may subsequently be dried with the use of sterile cloths.

9.3. Air sampling

In high-risk areas, such as high hygiene or product release areas, air sampling should be completed to ensure safe handling as increases in the number of micro-organisms detected may indicate a potential loss of environmental control and an increased risk that *Cronobacter* could be present. Air can be sampled using a one stage air sampler at 200 L/min or using a settling plate technique, with exposures times specified in an accredited method.

Corrective actions and increased environmental sampling should be triggered where there is a failure of systems leading to conditions that favour the survival and growth of *Cronobacter*. Such failures include changes in the air pressure differential beyond its set limits if the HVAC is not working correctly, or if set limits for humidity or temperature are exceeded.

9.4. Designing a sampling plan

A review on the critical factors to consider when pathogen sampling in dry environments is provided in Bourdichon et al., 2021. These authors state:

"In summary, a risk-based processing environment monitoring (PEM) plan is an invaluable tool to verify control programs for the ingress and dissemination of pathogens in a food production facility. Specific sampling schemes, target organisms, methods, sampling areas, frequencies, and adequate corrective actions depend largely on the specific product hazard and local conditions, and so cannot be generically provided."

The development of microbiological sampling plans should involve a team comprising someone with microbiological knowledge or experience along with quality assurance and production staff. The following general principles should be applied:

- develop the sampling plan on a room-by-room basis
- consider the Hygienic Zone and risks as well as the flow of product, ingredients, manufacturing by-products, product waste, consumable packaging, samples for testing and equipment (e.g. trolleys)
- determine potential harbourage sites for bacteria based on the availability of water, nutrients and oxygen, the temperature and the ease of inspection and cleaning
- map high risk sites
- map multiple flow sites
- determine if a site or sites could provide good representation of a number of similar high-risk sites
- evaluate risks and sites based on historical sampling / incident records and team members' experience and expertise
- involve expert consultation
- determine the number of sites to be tested based on the results of the risk mapping exercise, not on a pre-mandated number
- justify sampling needs against the cost (budget)
- cover the risks, and
- provide a balance between product safety and cost benefit.

For each location / room the sampling plan should indicate the targeted number of samples to be obtained and their general location as shown on a map/schematic. For example, sampling a specified room may identify locations as two keyboard samples, three floor samples (by entry door, under locker, by through door) and the door handle.

It is considered that within a sampling plan, while the target area needs to be well defined, if the area of the zone allows it, the actual area sampled (e.g., portion of floor) within the general area should be varied. For example, if the target zone is a 1 m x 50 cm area by the entry doorway, it is suggested that within this target zone a random 30 x 30 cm (defined) area be tested, rather than the same 30 x 30 cm area each time.

9.5. Review of sampling plans

It is critical that sampling plans are reviewed on a bi-annual or annual basis. The triggers for a review of the sampling plans are varied. Usually, the entire pathogen management manual or documentation is reviewed regularly and so a review of the environmental pathogen monitoring plan is carried out as is part of this larger review so that a holistic approach is followed. A review of the environmental pathogen monitoring plan may also be triggered if there is a significant event. Reviews are also triggered by trends showing, for example, that certain areas do not have regular positive samples, so frequency of sampling may be updated.

The results from the sampling of non-routine sites will determine whether they should become routine testing sites.

10. Final product testing to ensure that *Cronobacter* is not detected

Product sampling of PIF is imperative to ensure the safety of released products.

As per the Infant Formula Notice 2022 (section 5.5) the RMP must contain a sampling and testing plan that includes (5.5 e) final product conformance testing.

A summary of international guidelines states that product must be tested indirectly for either general bacterial abundance using standard plate counts (SPC) and Enterobacteriaceae/coliform presence on selective agar, or directly for *Cronobacter* species using PCR and selective agar-plate testing.

Testing of PIF samples must be completed by a laboratory recognised by the local authorities and use test methods in the ISO/IEC 17025 scope of accreditation (acceptable, standardised, and quality testing) or methods specified under the legislation of the country. The current approved testing methods worldwide are labour-intensive and time-consuming. Culture based methods can typically take up to 6 days, whereas PCR protocols have turnaround times that can be between 2-3 days (Chen et al., 2018; Yan et al., 2012). In New Zealand, operators must use a laboratory recognised under the MPI Recognised Laboratory Programme and testing must be under the laboratories' scope of accreditation.

In New Zealand, the Production, Supply and Processing (PSP) notice (30 October 2023) and the FSANZ Food Standard Code (2021, Schedule 27 of standard 1.6.1) state that for PIF, the absence in the final product of both *Cronobacter* (n=30; c=0; not detected in each 10 g sample) and *Salmonella* (n=60; c=0; not detected in each 25 g sample) must be ensured. Testing is recommended to include indicator organisms such as Enterobacteriaceae (n=10; c=2; m=0/10g); coliforms (n=5; c=2; m=<3; M=10); and aerobic bacteria in Standard Plate Counts (SPC, n=5; c=2; m=1,000; M=10,000) where n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit which separates good results from marginal results; M = microbiological limit above which results are unacceptable or defective.

FSANZ Food Standards Code (FSANZ 2022) is comparable with international regulations and guidelines for Canada (CFIA, 2021); the European Union (European Commission, Regulations 852/2004; 853/2004 and 2073/2005); and USA (FDA, Title 21, Part 106, 2014). Indicator organisms may be quantified using Standard Plate Count (SPC) or coliform tests to detect possible hygiene issues and safety issues within the production process where the loss of control of a process suggests a higher probability for the presence of pathogens, including *Cronobacter* spp. Coliform tests across all countries must return no more than 10 coliform CFU in 3 of 5 samples of 20 g, or, if testing for Enterobacteriaceae, at least 8/10 samples of 10 g must be negative.

When testing PIF or PIF ingredients for *Cronobacter*, 30 samples each of 10 g are required and none of these samples must have any detectable *Cronobacter*. If unfavourable results are found using indirect indicators, additional sampling for direct testing of *Cronobacter* spp. must be completed (CAC/RCP66, 2008).

10.1. Microbiological testing for verification of the control of *Cronobacter*

For both product and environmental sampling where direct *Cronobacter* testing is required, pre-enrichment of samples may be necessary to increase the sensitivity (i.e. lower the detection limit) of the test. This is because good manufacturing practice usually results in very low levels of *Cronobacter* spp. in the environment and in final product powders. Most pre-enrichment protocols involve PIF being added into buffered peptone water and incubated between 34 °C and 38 °C for 18-24 h (Chen et al., 2018; Song et al., 2018).

To test for *Cronobacter* spp. two specific testing methods are acceptable internationally; ISO 22964 and the FDA's Bacteriological Analytical Manual (BAM) *Cronobacter* guidelines (ISO 22964, 2017; Chen et al., 2023). The BAM protocol initially uses PCR testing and then uses culture-based methods to confirm positive PCR results. Two chromogenic agars - Druggan-Forsythe-Iversen Agar (DFI Agar) and *Enterobacter sakazakii* chromogenic plating medium (ESPM) developed by R&F Laboratories (Restaino et al., 2006) - are used in this protocol. Chromogenic *Cronobacter* Isolation (CCI) agar and *Enterobacter sakazakii* Isolation Agar (ESIA) are optional commercially available agars that can be used in addition to DFI agar and ESPM agar in the most recent BAM protocol (Chen et al., 2023).

ISO 22964 uses only culture-based methods and uses ESPM chromogenic *Cronobacter* agar and tryptic soy agar (TSA) for confirmation and downstream biochemical testing. The various selective agents in ESPM give good neutralisation of competitors of *Cronobacter* and background organisms but may affect the growth of some *Cronobacter* spp. strains and lead to the possibility of false negatives. Furthermore, some *Cronobacter* spp do not appear yellow on TSA and so may lead to some false negatives (Sáez et al., 2012).

Presumptive *Cronobacter* colonies on DFI agar appear dark, weakly or brownish green. Whereas presumptive *Cronobacter* colonies on ESPM appear blue to black on a red background; if background microflora is present the red background may turn yellow with *Cronobacter* colonies appearing green to blue. Non-*Cronobacter* colonies may appear white, red, yellow, or white with a green centre.

Real-time molecular methods (e.g. PCR technologies) are available which allow for the rapid detection of *Cronobacter* spp. in foods and in environmental samples. Processors should look for accredited methods and third-party validations such as by the AOAC for its equivalence and acceptability to ISO 22964:2017 (Johnson et al., 2020). PCR-positive colonies can be further confirmed on selective agar and with additional testing such as using MALDI-ToF ID (Bastin et al., 2018) or whole genome sequencing.

11. Actions if *Cronobacter* has been detected

The CMP should include a response plan that identifies the actions that should be taken when *Cronobacter* has been detected. Ideally the plan should have been tested and reviewed using a mock exercise involving all relevant staff.

11.1. Notify the regulatory authority?

The Animals Product Act 1999 (APA) outlines requirements for notification of *Cronobacter* detections. Operators should refer to their RMP documents.

Cronobacter may be detected in a food, ingredient, or raw material or in a sample from the processing environment by:

- a testing laboratory in response to the dairy operator's own testing programme
- a regulator conducting a survey
- a regulator following investigation of a food complaint, illness, or other event
- a supplier of raw materials or ingredients, or
- a customer.

Note: The detection of *Cronobacter* from the processing area is more likely to be reported as part of the operator's environmental testing programme.

When the notification is received it is important to immediately gather as much information about the positive sample as possible.

For product positives, information required includes:

- type of sample
- batch number
- date of test
- what the laboratory has found
- how much product was produced
- where the product is now
- if a quantitative test, the number per gram of *Cronobacter* detected, and
- what other lots were produced at about the same time on the same line and might also need to be included in an investigation.

For a product contact surface, or a site that could act as a source of contamination for an exposed product (high care area / Zone 4) hygiene zone, information required includes:

- if a single site sample (recommended over composite samples), when was the site tested previously? What were the results?
- date of test
- what the laboratory has found
- if quantitative, the number per unit area detected, and
- which lots were produced about the same time and where are they now.

For a non-product contact site in the high care area / Zone 3 environmental sample site, information required includes:

- what the laboratory has found
- if this is a single site sample, what is the site sampled?
- if this is a composited sample, what are the sites sampled?
- are there any issues about the site to be aware of, e.g. equipment that has been recently serviced or repaired?
- if any unplanned events recently (e.g. within the last week) happened in the area e.g. a water leak or building breach?
- date of test
- previous microbiological testing results, e.g. trends of hygiene indicator organisms or *Cronobacter* spp. for the past month in the same area, including air quality trends, and
- if quantitative the number per unit area detected.

11.2. Notification of a 'presumptive positive' test result

When a laboratory notifies a 'presumptive positive' test result, this indicates that there may be *Cronobacter* in the sample tested. A presumptive positive notification generally means that colonies with the same morphology as *Cronobacter* have been detected on an agar plate, and that the presence of *Cronobacter* will need to be confirmed usually through further biochemical analysis. The laboratory will need several days to provide a confirmed test result. **The experience of people working in the New Zealand dairy industry is that most presumptive results are confirmed to be *Cronobacter*.**

Operators should begin to respond as soon as they receive notification of a presumptive result. Taking corrective action early has the potential to limit the financial cost and effort required in undertaking corrective actions, especially when product is involved.

New Zealand experience is that the molecular tests indicating the presence of *Cronobacter* are not unusual given its ubiquitous nature. Some manufacturers may choose to act on this presumptive positive result immediately (e.g. clean and sanitise) while waiting for a confirmed result, while some may choose to treat the presumptive result with less urgency than a positive result from the culture of live bacteria. The response chosen will be aligned to the risk posed to the product.

11.3. Confirmation that *Cronobacter* is present

The scale of the response should be proportional to the likelihood that the PIF could be contaminated with *Cronobacter*. The likelihood of *Cronobacter* being found in the product is low if the positive sample has come from the external areas (Zone 1) or the standard hygiene areas (Zone 2). The likelihood that PIF could be contaminated with *Cronobacter* increases if *Cronobacter* was found in Zones 3 or 4 (high care area/critical hygiene area).

11.4. Initial actions

All responses and actions made to finding *Cronobacter* should be based on robust and adequate information. Once the relevant information has been gathered, the appropriate response can be made. Boxes 2 and 3 include examples of the type of information and response required depending on where *Cronobacter* was found.

12. Responding to a *Cronobacter* detection in Zones 1-3

Immediately inform the designated person responsible for the CMP.

12.1. Outside the processing area - Zone 1

A Zone 1 detection is not a risk to the CMP as *Cronobacter* is expected to be detected in this zone. The action operators take will depend on their reason for sampling Zone 1 e.g. for information gathering purposes or verification of control measures.

Note: Not all environmental testing programmes will include the sampling of Zone 1.

12.2. Standard hygiene area - Zone 2

Immediately inform the designated person responsible for the co-ordination of the environment monitoring programme. Box 2 provides suggested actions that can be taken in response to the detection of *Cronobacter* in the Zone 2 environment (standard hygiene area).

12.3. Critical hygiene/high care area - Zone 3 (non-product contact surface)

Immediately inform the designated person responsible for *Cronobacter* management. See section 13.2.

The detection of *Cronobacter* in this zone is of concern as the *Cronobacter* could potentially make its way onto product contact surfaces (Zone 4) and then onto product. It is important to consider risk to the product and initiate segregation if appropriate. Box 3 provides examples of actions that can be taken in response to the detection of *Cronobacter* in Zone 3 (critical hygiene area/high care area – non-product contact surfaces).

Box 2: Suggested actions to take in response to the detection of *Cronobacter* in Zone 2 (Standard Hygiene Area)

Sampling

If the samples were analysed as a composite sample, take and analyse individual samples from the same areas and surrounding areas to determine the source of the contamination.

After corrective actions have been taken, repeat sampling in Zone 2 until **3** consecutive sampling days fail to detect *Cronobacter*.

Consider taking additional samples to determine whether the barriers between the standard and high care areas have been breached.

Review of results and trend analysis

Conduct a review of testing results and the trend analysis, taking account of Zone 2 results for *Cronobacter* and Enterobacteriaceae over a time period of 4-5 routine sampling regimes to gather enough data to determine patterns of contamination and potential sources. This would normally be over 3-6 months.

Review results from Zone 3. Look for increases in the number of indicator organisms and correlation with results between Zone 2 and Zone 3.

Note: If *Cronobacter* continues to be detected in Zone 2 it suggests persistent contamination which will require an increased level of vigilance. This may include taking additional product samples or increasing the number of environmental samples taken in Zones 3 and 4. That should occur if 3 consecutive sampling days of non-detections for *Cronobacter* cannot be achieved, or where the routine, e.g. 6-weekly (future trends analysis) records review suggests that there is recurring contamination.

***Cronobacter* controls review and corrective actions**

Isolate and inspect the contaminated area and equipment. Inspection should look for general cleanliness, potential sources of contamination or niches.

Review the cleaning and sanitation programme. Check sanitiser use and staff training.

Reassess access/entry restrictions into the standard hygiene area.

Assess whether any maintenance or environmental breaches, e.g. roof leaks, have occurred.

Review the results (if available) from outside the processing environment to identify any areas that may require a reassessment of controls to prevent the entry of any contamination.

Take corrective actions as appropriate to remove the contamination source and to prevent reoccurrence in future.

If there is evidence of recurring contamination in Zone 2, but no Zone 3/4/product positive samples, then manufacturers may choose to focus on robust cleaning and sanitation in Zone 2. Continued routine sampling in Zones 3/4, and associated trending of these results, can help to give confidence that isolates from Zone 2 have not spread to other zones.

Box 3: Suggested actions to take in response to the detection of *Cronobacter* in Zone 3

Sampling

If the samples were analysed as a single composite sample (not recommended), take and analyse individual samples from the same areas and surrounding areas to determine the source of the contamination.

Commence investigative sampling, e.g. sampling daily with a focus on finding and eliminating the source of the contamination.

Vector sampling (as described in section 14.4) is recommended.

After increased cleaning resample clean areas before processing recommences.

Maintain intensified sampling during processing until at least 5 consecutive sample days are clear for *Cronobacter*.

Review of results and trend analysis

Review testing results from Zone 4 and consider testing product as the critical hygiene zone is an area where product is exposed.

Review the trend analysis taking account of Zone 2 and Zone 3 results for *Cronobacter* and Enterobacteriaceae over a time period of 6 weeks to determine patterns of contamination and potential sources.

Review the testing results from the standard hygiene area (Zone 2).

Consider taking additional samples from Zone 2 to determine whether the barriers between the standard hygiene and high care areas have been breached.

***Cronobacter* controls review and corrective actions**

Review relevant prerequisite programme performance requirements, including for maintenance, equipment performance, cleaning, utilities, inwards goods.

Isolate and inspect the contaminated area and equipment.

Reassess processing and product handling procedures.

Carry out an aggressive cleaning and sanitising operation, using a sanitiser with proven efficacy against *Cronobacter*.

Take corrective actions as appropriate to remove the contamination source and to prevent reoccurrence in the future.

If corrective actions have not completely removed the source of the contamination and *Cronobacter* continues to be detected, PIF plant operators should be able to demonstrate that they are taking all reasonable steps to control the *Cronobacter* contamination and prevent the contamination of the high care area. This may include reinforcing to staff of the need to strictly adhere to red-line principles with regard to materials, equipment and workers; and checking that air filtration and ventilation systems are working as intended.

13. *Cronobacter* is found in Zone 4 (product contact surface) or product

This section describes recommended actions in response to:

- the detection of *Cronobacter* in product, or
- when *Cronobacter* is found in Zone 4 (product contact surfaces which come into contact with exposed product prior to packaging).

13.1. Detection on product contact surface

If *Cronobacter* is found in a Zone 4 product contact surface, product may also be contaminated. To ensure that no contaminated product is released, the response should be the same as when *Cronobacter* is found in product.

Immediate actions: isolate the batch and hold physically and electronically to prevent it being used, sold or distributed, including any flushings, samples or waste streams and any product received as a result of withdrawal or recall. Inform the designated person responsible for *Cronobacter* management.

Note that it is important to keep good records, e.g. a diary of events and to document the actions taken during the contamination event.

Maintaining records is an important way of demonstrating that you took the appropriate action. They will assist in post event reviews of the response plan, and they can be provided as a record of your actions if requested by a customer or regulatory body.

13.2. Detection of *Cronobacter* in product or a product contact surface

The contamination could have resulted from either contact with a contaminated surface or faulty processing. In the latter case, the contaminated process could then contaminate product contact surfaces.

The presence of *Cronobacter* indicates that there has been a failure in the pathogen reduction steps or post-pasteurisation hygiene that has resulted in contamination.

Immediate actions: isolate the batch and hold physically and electronically to prevent it being used, sold or distributed, including any flushings, samples or waste streams and any product received as a result of withdrawal or recall. Inform the designated person responsible for *Cronobacter* management.

Restrict access into the affected area(s) to control traffic and other activities.

13.3. Identify and hold contaminated and potentially contaminated product(s)

Potentially contaminated product includes:

- product processed on a line where *Cronobacter* has been detected on a product contact surface, and/or
- on a line used to process product in which *Cronobacter* was detected.

Actions include:

- prevent the affected product from coming into direct contact or having the potential to cross-contaminate other product(s), raw materials, packaging, equipment or surfaces
- clearly identify product to indicate status. For example, each carton or pallet could be marked with 'hold' labels, or it could be held using an electronic inventory control system to ensure product is not released
- conduct a full traceability exercise on potentially affected product. Determine which products, where and how much is affected. Other products may be contaminated as a result of being processed before or after the batch positive for *Cronobacter*. It is recommended that at least 3 batches prior to the contamination being detected be re-tested. Once processing has recommenced after traceback, clean and sanitising, it is recommended that the level (size of sample or number of samples taken) of testing be doubled for 3 - 5 subsequent batches.

It is important that all resources available are used to help narrow down the range of potentially contaminated product so that the response and testing can be targeted. This can be achieved by reviewing the results from routine testing and investigative sampling, as well as reviewing the process control and supporting system records.

13.4. Notify the regulatory authority

See section 11.1.

13.5. Withdraw or recall contaminated product

If contaminated product has left the processing premises but remains within the company's control it should be withdrawn from the distribution chain.

If contaminated product has left the company's control, a recall (trade or consumer level) will be required (see https://www.mpi.govt.nz/food-business/food-recalls/).

Any contaminated or potentially contaminated product that remains under control of the operator should be placed on hold.

For a recall the regulatory body is likely to require the following information:

- details about the contaminated and potentially contaminated product (shelf life, batch number(s) or other identification)
- current location, distribution and volume of affected product
- routine testing sampling plans, results and trend analysis of environmental results, and
- any decisions about the disposal of the food product.

14. Finding the source of the contamination

Finding the source or cause of the *Cronobacter* contamination can help to minimise any future reoccurrence. Note that the process below should be carried out with reference to sections **3.1 Sources of *Cronobacter* in PIF processing plants, 3.2 Transfer of *Cronobacter* within a PIF processing plant** and **4 Control of *Cronobacter* in PIF Processing Plants.** Note, that many of the actions outlined below should commence as soon as possible after notification of the positive detection. They do not necessarily need to be carried out in sequence.

14.1. Review supporting systems

Identify anything that may be a cause for concern and that should be targeted during the investigation.

Reviewing the supporting systems helps to identify the cause of the contamination (e.g. cleaning and sanitation programme, access restrictions, GOP, staff training).

Aim to check any records for the period around the date of detection, and if possible, back to the last non-detected result, to identify whether anything unusual or unexpected occurred.

Checklist for reviewing the support systems:

- were the cleaning and sanitation procedures followed correctly, including chemical concentrations and contact times, and environmental cleaning SOPs?
- did the CIP meet validated parameters, e.g. for time, temperature and flow?
- was there an equipment breakdown or maintenance work being carried out on or near the process line(s)?
- was a new ingredient used?
- did any planned or emergency modifications or repairs take place at or near the line(s) such as replacing flooring, repairing equipment or freeing a blockage?
- if modifications or repairs were carried out, what tools and equipment were brought into the area and how was their entry controlled?
- for the raw materials brought into the area, check the inward goods control records to see if they are complete and no inconsistencies are evident
- are there ongoing problems that could be linked to the hygienic design of the equipment or facilities?
- were there new or inexperienced personnel on the packaging line(s)?
- were the access/entry restrictions into high care areas being followed correctly, including any movement of equipment between areas?
- was there a breach of the hygiene requirements?
- was there potential for cross-contamination between the high care area, product contact surfaces and/or product? and
- was there a breach of the barriers into the manufacturing environment such as a roof leak or water ingress from the HVAC by-pass?

14.2. Review processing records

Reviewing the processing records helps to establish:

- the extent of possible contamination, and
- which lots could potentially be contaminated.

Checklist for reviewing the processing records:

- check the pathogen reduction step (e.g. pasteuriser) operation
- do records show that the critical limits were being met?
- was the equipment used for critical measurements calibrated (e.g. thermometers, gauges etc.)?
    - – are the readings accurate?
    - – where necessary, recalibrate the equipment
- check the competency and training of workers responsible for supervising CCPs. Observe them performing their tasks and assess their competency by questioning the actions that would be taken given certain scenarios
- was there a loss of process control at any particular step?
- was there an intrusive event before the affected batch was manufactured, e.g. sifter or powder bin opened for routine inspection?
- were there changes to product formulation, ingredient substitutions or were ingredients from a different supplier used?
- were the process and handling procedures followed correctly?
- were there frequent changes to the process line(s)? For example, several changes of ingredients, and
- how many Lots are possibly contaminated?

To assist in the identification of the source of the contamination, the inputs used should be reviewed. Test samples of any available inputs whose microbiological status cannot be confirmed by other means, e.g. through verified supplier guarantees etc., to determine if they could be the source of contamination. For example, using appropriate sampling plans, test:

- ingredients
- packaging (including inner and outer packaging, pallets and wrapping, etc.)

Identify and hold ingredients added to the product after pasteurisation to prevent accidental use until it is possible to test them for the presence of *Cronobacter*. Any *Cronobacter* obtained by the testing laboratory should be held and consideration given to having their genomes sequenced to help determine if the strain contaminating the ingredient was the source of product contamination. The advantages of whole genome sequencing (WGS) in determining the sources of microbial contamination have been discussed by Baert et al., 2021. If testing for Enterobacteriaceae, any colonies present should be analysed via MALDI-TOF or another species identification method to check whether they are *Cronobacter*.

14.3. Inspect process area(s) and equipment

Review the floor plan and process flow to identify areas that could be the most likely sources of contamination.

Carefully inspect the equipment and process area(s) to identify equipment and area(s) that may be the source and/or harbourage point of *Cronobacter*. Assess the state of equipment used, including the repairs and maintenance records to determine whether there are any hidden surfaces that may trap contaminants and allow them to build up.

The inspection is likely to involve dismantling some equipment and may require assistance from maintenance personnel. Isolation measures around the area and equipment should be maintained to minimise the spread of any contamination throughout the area. Note that caution should be taken if dismantling equipment for intrusive maintenance or swabbing as this could cause secondary contamination and contamination of further batches. Operators should consider the pros and cons of intrusive inspection or swabbing, and only undertake equipment dismantling after a thorough room clean. Post-inspection, a cleaning regimen determined by risk assessment and tailored to the specific area should be followed, prior to commencing inspection or swabbing.

If *Cronobacter* had been originally detected from a product contact surface and a composite sample was originally analysed, or the result is from a product, then the particular piece of equipment that contamination came from may not be identifiable. If so, review:

- the processing equipment together with the environmental testing results,
- the process records, and
- the supporting system records (GOP).

14.4. Conduct investigative environmental sampling to find the source of the contamination

14.4.1. In the case of the detection of *Cronobacter* on a non-product contact surface

Based on observations from the physical area inspection, past microbiological testing performance and relevant risk assessments (including environmental monitoring programme and HACCP), an investigative sampling plan should be created.

- Investigative environmental sampling will help to identify the contamination source(s) and rule out those areas that are not the source of contamination.
- Any investigative environmental sampling should commence as soon as possible, ideally within one working day after receiving the laboratory notification. If possible this should happen before any cleaning and sanitation occurs.
- Retaining *Cronobacter* isolates for whole genome sequencing (WGS) can be useful in identifying harbourage sources and niches, or for discounting other areas as potential sources of the contamination.

Investigative environmental sampling requires collecting a greater number of samples than those collected for routine testing from the processing environment. Where possible a thorough and systematic sampling plan should be applied to help identify the source of the contamination (i.e. the goal is to find the organism). It is important that the investigation is

thoroughly planned from the start and that sufficient samples are taken from well considered sample sites. Every effort should be taken to ensure that sampling does not need to be repeated due to mistakes or gaps in the initial sampling plan. The number of samples to be taken will depend on the complexity of the process and equipment, but generally the more samples that are taken from well considered sites, the better the chances of resolving the problem.

During the investigation, sampling immediately after cleaning is useful because any *Cronobacter* detected is more likely to be at or near the contamination source.

The person with responsibility for the CMP should be involved.

Getting positive results is good as this means that the sampling programme is effective and specific corrective actions can then be taken.

14.4.2. In the case of the detection of *Cronobacter* on a product contact surface:

- determine the site, date and time the positive swab(s) was taken. If the swabs were analysed as a composite sample, identify which sample sites were included in the composite, and
- review the environmental testing results from past testing to determine the sites with the greatest likelihood of being a source of contamination.

14.4.3. In the case of the detection of *Cronobacter* in product:

- determine the processing line, date and time when the product that tested positive for *Cronobacter* was processed
- determine the sample date of the last clear test for *Cronobacter* to help establish the time frame when potentially contaminated product may have been processed
- use any further product results to assist in expanding or reducing the scope of the search
- sample any likely product contact surface site(s) that have tested positive in the past
- select other product contact surfaces and other sites in the high care area to sample, particularly in hard to clean areas, and
- consider sampling indirect contact surfaces. These are sites that are not in direct contact with the product but may be an important source of contamination. For example, overhead surfaces from which accumulated powder may drop onto product or product contact surfaces.

14.4.4. When taking investigative environmental samples:

- include items such as powder from the floor or hidden ledges, and cleaning equipment, when selecting samples
- do not composite environmental swabs for microbiological analysis. The use of composite samples may delay identifying the source of contamination. The exception to this would be if all swabs in the composite come from the same piece of equipment or same surface
- the sampling method may differ during investigative sampling. For example, the area swabbed may be larger in an attempt to reach greater surface areas or nooks within a piece of equipment

- consider including pieces of equipment that move between the standard hygiene and high care areas, including the wheels
- explore all areas of possible contamination and not just on a limited / small part of the process as this can delay detecting the source and returning to full production
- if a positive is found on the floor retest using a vector sampling approach in which points around (360°) and up to 2 m away from where the positive was obtained are sampled
- the potential spread/scope of contamination needs to be considered based on traffic/product flow to determine if adjoining rooms need to be tested
- a positive on a touch point should generate additional sampling of that touch point and of similar touch points
- after sampling, clean and retest the sampling sites
- if any traceback sites were positive carry out vector sampling on the positive site and adapt the investigative sampling plan to account for new positives
- traceback swabbing is generally carried out until a negative result is obtained. It is recommended that this is
    - 3 consecutive days for Zone 2
    - 5 consecutive days for Zone 3
    - 7 consecutive days for Zone 4
- after sampling, the tested areas should be thoroughly sanitised (70% (w/v) ethanol) to ensure that any harbourage sites that may have been disturbed do not contaminate the processing areas or product.

Note: Based on a statistical sampling plan, include an additional number of composite product samples e.g. non-detect tests for 600 g. In general, in the experience of NZ operators, doubling the number of samples and/or sample weight is a reasonable approach. Statistical calculators and models are provided by the ICMSF and ISO, and others are freely available (e.g. https://pub-connect.foodsafetyrisk.org/sampling/simple-sample/).

Post traceback and once the incident has been deemed to be resolved it is recommended that operators maintain an increased frequency of testing for a period of time, which could be up to 2 months.

14.5. Clean and sanitise the affected area and equipment

Once investigative testing (swabbing) has been completed, the suspected contaminated area and equipment must be thoroughly cleaned and sanitised, and then resampled to determine whether corrective the actions have been successful.

15. Sampling and testing of product on hold

15.1. Intensive microbiological sampling requirements

If an operator intends to investigate the possibility of some of the product on hold being released, this product will need to be tested intensively (see section 15.3) to determine the extent and level of contamination between each batch. Intensive microbiological sampling differs from routine testing. Routine testing programmes are designed as an additional 'check' that a food safety control system is working properly over time rather a pass/fail for each batch of product.

Intensive sampling programmes are undertaken because a problem has been identified and there is an increased likelihood that product is contaminated. It cannot be assumed that the system is working as intended and therefore information on individual batches is required. Only a portion of each batch is likely to be affected because microbial contamination, unlike some other forms of contamination, tends to be unevenly distributed. As a result, higher sample numbers per batch are required so they can provide information on the acceptability of an individual batch.

15.2. Identify product to be tested

Each batch (see section 15.3) of product on hold will need to be sampled. Testing will help to:

- determine which product produced prior to, during and after the contamination event is contaminated, and
- allow decisions about the release or disposition of product to be made.

15.3. Sampling plans

When conducting intensive microbiological sampling, it is recommended that sampling plans that give a minimum of 95% confidence of detection are used.

When undertaking an intensive sampling programme the food operator should note that:

- As the number of samples taken decrease, the chance of accepting an unacceptable product batch increases. The costs involved in sampling and testing need to be weighed against the impact of making an incorrect decision
- Sampling using a sample size of 60, i.e. n = 60, per batch provides 95% confidence of detecting *Cronobacter* in at least one sample where 5% of the samples in a lot are contaminated. If the sample size was reduced to n= 5 the batch would need to be 45% contaminated for *Cronobacter* to be detected, and
- Consideration should be given to using a software sampling tool such as ICMSF tool.

Note that the minimum number of samples required to be tested is mandated by the regulator in the Infant Formula Notice 2022, as N = 30 x 10 g samples.

However, in practice companies may decide to take additional samples or customers will have stipulations/requirements increased testing that need to follow. In a general and statistical sense, increasing the sampling frequency will increase the chance of finding any contamination should it be present. However, this may not work if the contamination is at a

very low level (as is often the case for *Cronobacter*) or if it is sporadic (also often happens with *Cronobacter*). A formal risk assessment needs to be carried out to understand root cause, and show what corrective action has been taken (based on information in FSANZ 2022, and Food and Agriculture Organisation of the United Nations (FAO) and World Health Organisation (WHO) guidelines).

In addition to increasing the number of samples tested consideration should be given to increasing the sample size of product tested. Hence, while N = 30 x 10 g samples is mandated, it is recommended to take N = 60 x 20 g samples.

During traceback, and/or at other strategic times after the final pathogen reduction step, consideration could be given to testing samples obtained from the sifter and bulk bins as well as the packaging room, in order to more quickly pinpoint the potential sources of contamination, or to limit the scope of potentially affected process steps. In-process sampling should ideally be carried out at 30 x 10 g aliquots. During traceback testing, consideration should be given to restricting access to sites and stopping all regular QA testing or routine procedures, such as changing of magnets. Such an approach is believed to limit secondary contamination and make it easier to determine the primary cause of contamination.

On the resumption of production, testing for the presence of *Cronobacter* should be increased. The minimum number of batches subjected to additional testing since the last positive detection should be at least 3, but ideally 5.

15.4. False Positive Samples

When the initial finding of *Cronobacter* cannot be replicated by additional product or environmental testing there is a temptation to question if the original sample was a true positive or a false positive. It is however, important to appreciate that a positive result can only be considered a false positive if the laboratory who tested and reported the result withdraws the result and confirms the result was a false positive, through identification of control failures, testing failures, or sub-sampling and laboratory handling cross contamination, or if the company can link the detection to cross contamination in the sampling or sample handling process. There is a very high burden of proof that needs to be met for a false positive to be validated (e.g. WGS links).

15.5. Release of potentially contaminated product

Potentially contaminated product is product that was processed on or around the time that another batch was found to be positive or when a product contact surface was positive, on or around the time the product was processed. Note regardless of subsequent testing results, it is not recommended that product which has a confirmed positive *Cronobacter* detection be released. Potentially contaminated product may be released:

- after all the results from all the testing has been received, or
- on a case-by-case basis, or
- or another valid disposition method agreed by the verifier.

The particular approach taken to release product depends on:

- the particular contamination event, and
- the results from additional analysis of different batches or production days.

16. Managing product once processing resumes

There may be a break of days to weeks before processing resumes, as corrective actions such as investigations, cleaning and repairs and maintenance occur.

Once processing resumes, product and product contact surface samples should be taken to allow determination of whether the corrective actions have been successful, and the contamination source has been identified and addressed. Post contamination, the testing of product and surfaces should include an increased number of tests, and for product and increased size of sample (see earlier sections 14 and 15), until there are at least 3, but ideally 5 consecutive processing days of clear results.

17. Review of *Cronobacter* management controls after the event

It is strongly recommended to review the CMP after a contamination event. The event should be formally closed out and relevant staff debriefed.

As part of a review consider the:

- access/entry restrictions between areas, including compliance with personnel hygiene requirements and the movement of equipment between areas
- cross-contamination potential between the process areas and product contact surfaces
- cleaning and sanitation programme, including the chemical concentrations and contact times
- processing and product handling procedures
- validated controls (where identified as a cause of the contamination)
- sanitary design and condition of the facilities and equipment
- effectiveness of sampling programmes and testing methods (environmental and product)
- *Cronobacter* management programme to confirm that it is appropriate and complete
- procedures around the management of the contamination event, including the recall procedures; where used, and
- effectiveness of corrective actions and general points that have been learnt from the event. For example, what went well, what did not go well and what has been learnt that could be applied to similar events in the future.

18. Roles and Responsibilities

To enable an effective response to a *Cronobacter* contamination event, clear roles, responsibilities, and who has the appropriate authority needs to be outlined in the CMP. It is important that the person with overall responsibility is relatively senior, or is able to report directly to senior management. This is to ensure that, when an event occurs, senior management is immediately alerted and participates in the response process. The plan should include the name or position of the person responsible for:

- overall responsibility for the CMP
- documenting and reviewing the CMP
- maintaining specified *Cronobacter* control measures
- *Cronobacter* testing for GOP and CCPs, etc
- responding to the detection of *Cronobacter* and,
- identifying problems in association with GOP and/or HACCP application including CCP or CL failures; staff training and education; and HACCP application review.

19. Staff training

The person with overall responsibility for the CMP should have a good knowledge of *Cronobacter*:

- what it is
- the illness it causes
- measures to prevent contamination, and
- actions to take in the event of its detection.

It is important for all other staff to receive training and education on the risks to the operation and consumers from *Cronobacter* contamination and their role in minimising the potential for contamination to occur. Staff involved directly with the production PIF should have appropriate training in the following:

- the risks to consumers from contamination of product
- the nature of *Cronobacter* and how it may be carried into and move around processing areas
- common harbourage sites
- control measures that apply during processing, distribution, marketing, use and storage, and
- means for verifying effectiveness of pathogen management programmes (including the CMP).

In large operations, it may be useful to develop in-house training programmes or to use external providers for training.

The training of staff involved with the processing of PIF foods helps to reinforce the food safety and hygiene messages and to develop a food safety culture. Training of staff working in high-care areas, including process staff, cleaners and engineers should be tailored to the work performed and should provide an understanding of:

- general process controls and why they are in place
- *Cronobacter* and any unique characteristics
- specific control measures that should reduce the risk of *Cronobacter* contamination during processing, distribution, marketing, use and storage of PIF
- verification of the effectiveness of specific control programmes, including sampling and analytical techniques
- specific procedures needed by the food operator for each control measure
- specific documentation and record keeping including any measurements taken, training records, version control etc.
- specific roles and responsibilities in relation to a CMP, and
- what should happen if *Cronobacter* is detected.

HACCP training and competency should be essential for staff who develop, implement and review a CMP. Training may be provided internally or externally. The training programme records should include:

- frequency of training and retraining
- ongoing review, peer review and visual observation, and mentoring of new staff (induction process), and

- the name and date when each staff member attended so that refresher courses can be scheduled and, that staff who have received appropriate training are selected for specific tasks, for example:
    i) undertaking corrective actions
    ii) interpreting laboratory reports, and
    iii) training samplers.

Training records should be kept, and every effort made to familiarise new staff with the risks posed by *Cronobacter* at the earliest opportunity so that the CMP is not compromised by a lack of awareness.

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Appendix 1

Table 1 Hygienic Zone Description

Zone	***Name (s)***	***Characterised by***	***Examples***
4D	Critical / High	Dry zone. Post pasteurisation. Product in this zone will not receive any further heat or other treatments designed to eliminate pathogens Exposure of the product to the "environment" occurs	packing room, sifter room, powder bin room, pre-gasser section as well as the areas that directly service these rooms such as control rooms and adjacent corridors and stairwells.
3D	High / Medium	Dry zone. Post pasteurisation. Product in this zone will not receive any further heat or other treatments designed to eliminate pathogens Under normal conditions exposure of the product to the environment will not occur	dryer stages, dryer fan room, control rooms and adjacent corridors and stairwells.
2D	Medium / Basic	Dry zone. Exposure of the product to the "environment" occurs Product will undergo subsequent heat treatment designed to eliminate pathogens (pasteurisation)	ingredients (vitamins, powders) and ingredient bins, dispensing rooms and equipment (tippers)
2E	Medium / Basic	Wet processing zone. Exposure of the product to the "environment" occurs Product will undergo subsequent heat treatment designed to eliminate pathogens (pasteurisation)	vitamin mixing, Intermediate Bulk Container (IBC) room, evaporator room, concentrator room, wet process rooms, wet process mixing, raw milk room and associated support areas workshops, wash rooms, MCC room, control rooms, laboratory.
1E	*External*	Product is protected by primary and secondary packaging Product comes into contact with the external environment	external areas of the warehouse where loading/unloading occurs and the tanker bay