PART 5 COMMON USE IN FOOD BEFORE 1958

Bovine lactoferrin was not used as an added ingredient in food (including infant and toddler formulas) prior to 1958. The statutory basis for this GRAS Notice is based on scientific procedures as described under 21 CFR § 170.30 (b).

Although known to be present in bovine milk, it was not until 1960 that the isolation, and identification of bLf, was successful (Groves, 1960). Large scale commercial manufacture of bLf for use as a food ingredient, did not begin until 1985 (Tomita et al., 2009).

The exposure of human infants to bLf dates back to the attempted, but generally unsuccessful, use of animal milks (including cow's milk) to feed infants that were unable to be breastfed, which is recorded at least as early as the 2nd century (Obladen, 2014). By 1867 formula contain cow's milk, together with wheat flour, malt flour and potassium bicarbonate was commercially available in the USA (Committee on the Evaluation of the Addition of Ingredients New to Infant Formula, 2004; Fomon, 2001). Improvements in general sanitation, dairying practices, milk handling and food processing technologies, together with the general utility of cow's milk provided the necessary basis for the development of modern commercially prepared infant formula (Fomon, 1974, 1993; Fomon, 2001). In 1915 commercial formula contained cow's milk, lactose, oleo oils, and vegetable oils, and was available in powdered form (Committee on the Evaluation of the Addition of Ingredients New to Infant Formula, 2004). In 1962 protein modified formula were introduced, with an increased whey:casein ratio to better match that of human milk (Fomon, 2001). By 2000 whey-predominant formula had become the most widely consumed milk-based formula (Committee on the Evaluation of the Addition of Ingredients New to Infant Formula, 2004).

In summary, although infants and toddlers have been exposed to bLf in their diets through the consumption of cow's milk for centuries, isolated bLf was not available for use prior to 1958, and therefore common use in food does not form the basis for this GRAS notice.

PART 6 NARRATIVE

6.1 INTRODUCTION

The information provided in this Narrative (Part 6) is generally available. In this Part Synlait presents an extensive review of the effects and safety of bLf in relation to its consumption by infants (0 - 12 months) and toddlers (13-36 months) which together with the data and information confirming the food grade quality of bLf (Part 2), and the dietary exposure data (Part 3) forms the basis of its conclusion that bLf is GRAS under the conditions of its intended use.

6.2 INTENDED TECHNICAL EFFECT

The intended effect is to increase the intake of bLf from the consumption of cow's milk-based term infant formulas to make the consumed level of lactoferrin more similar to levels in human breast milk.

There are marked differences in the protein composition of cows' milk infant formulas and human milk, notably human milk contains significantly higher concentrations of lactoferrin (Figure 6-1). Increasing the lactoferrin content of formulas is one method of making the protein composition of formulas more similar to that of human milk.

Lactoferrin is a multifunctional protein that mediates a number of physiological processes that contribute to the advantages which breastfed infants have over their formula-fed peers (Donovan, 2016). In infants and young children, these include immunoregulation, antibacterial activity and antiviral activity (Lönnerdal, 2016) together with the potential to improve early neurodevelopment and cognition. At doses of between 0.5 and 1.0 g/day, the clinical benefits of bLf for infants <12 months of age include the likely decrease in burden of respiratory and gastrointestinal morbidity, and a reduction in the burden of colonization by some parasites in underdeveloped settings (Manzoni, 2016). Based on clinical evidence, Manzoni (2016) suggests bLf likely delivers the same clinical benefits as human lactoferrin.

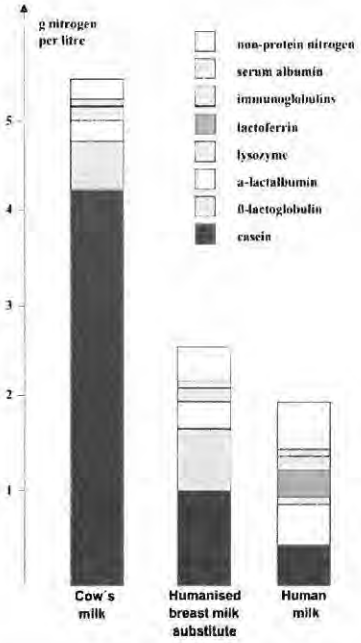

Figure 6-1: Protein composition of cows' milk, infant formula and human milk

(from Hambraeus et al. (1977), copied in Chatterton, Rasmussen, Heegaard, Sørensen, & Petersen, 2004)

6.3 SAFETY ASSESSMENT

6.3.1 Absorption, Distribution, Metabolism, and Excretion of bLf

By the 24th week of gestation, the human fetal gut is sufficiently developed to enable the digestion and absorption of nutrients; hence, even premature infants are able to digest and absorb macronutrients (Lentze, 2015). Endogenous levels of lactoferrin exist in numerous organs of the human fetus, and is understood to be associated with maturity of the immune system (Reitamo, Konttinen, Dodd, & Adinolfi, 1981). Breastfed infants are exposed to dietary lactoferrin that has the capacity to exert a number of physiological functions including immunomodulation, antiviral and antibacterial activities (Lönnerdal, 2016).

The ability to study mechanisms of digestion, distribution, and metabolism in human infants is relatively limited; hence, various animal models are used as a proxy, most commonly rodent models. However, for human infants, the piglet is a more suitable model; postnatal gastrointestinal development and the nutritional requirements of piglets better reflecting that of the human infant (Alizadeh et al., 2016; Donovan, 2016; Miller & Ullrey, 1987; Moughan, Birtles, Cranwell, Smith, & Pedraza, 1992). The digestion of lactoferrin has been extensively

studied in piglet models, enabling some understanding of its absorption, distribution, metabolism, and excretion.

The biological activities of dietary lactoferrin from breast milk, or bLf supplemented formula can occur as either local effects in the gut lumen; e.g., bacteriostatic or bactericidal effects; or systemically mediated by the lactoferrin receptors and transport into the systemic circulation, e.g., iron uptake, immunomodulatory effects, and epithelial growth and differentiation (Lönnerdal et al., 2011). A proportion of the lactoferrin ingested by infants persists throughout the gastrointestinal tract (Dallas, Underwood, Zivkovic, & German, 2012; Davidson & Lönnerdal, 1987; Spik, Brunet, Mazurier-Dehaine, Fontaine, & Montreuil, 1982).

Early *in vitro* digestion models suggested that lactoferrin was relatively resistant to digestion and intestinal degradation (Brock, Arzabe, Lampreave, & Pineiro, 1976). For lactoferrin to exert biological functions in the small intestines there is a requirement that it is, at least to some extent, resistant to digestion. Using radio-labeled proteins, Drescher et al. (1999) studied the prececal digestibility of lactoferrin in comparison to casein in both suckling and adult miniature pigs. The ^{15}N-digestibility of lactoferrin, both bovine (82.3 +/- 4.8%) and porcine (84.4 +/- 3.2%), was significantly lower than casein digestibility (97.6 +/- 0.5%) in the distal small intestine of suckling piglets ($P < 0.05$), with 4.5% of non- and partially digested lactoferrin found in the last third of the small intestine of piglets (Drescher et al., 1999). These results suggest lactoferrin has relatively low digestibility. In the adult pigs, no differences in the digestibility of lactoferrin and casein were observed, both being nearly completely digested (Drescher et al., 1999). Sampling the gastric digesta of infants, Britton and Koldovsky (1989) and Chatterton et al. (2004), determined dietary lactoferrin may be partially degraded by preterm infant gastric fluid. At the prevailing postprandial gastric pH, hydrolysis is minimal, hence, both intact and bioactive fragments of lactoferrin are available for subsequent biological action within the infant (Liao et al., 2012). Substantial amounts of bovine lactoferrin also survive the more challenging (low pH) gastric digestion in human adults (Troost, Steijns, Saris, & Brummer, 2001). Using proteomic techniques, Grosvenor, Haigh, and Dyer (2014) tracked the truncation and relative abundance of peptides released during time-course simulated gastric digestion of bLf, noting differences in the peptide patterns between pasteurized and unpasteurized samples. They concluded that the bioavailability of specific peptides may be influenced by thermal processing of the food prior to consumption, with some peptides becoming more available and others less available (Grosvenor et al., 2014). The nutritional or clinical implications of such effects is not currently understood. Recently Dallas et al. (2014) investigated the digestion of human milk in the infant stomach, analyzing gastric aspirates of 4 to 12 day old neonates, sampled 2 hours after feeding. Peptide analysis was completed for both the digested and an undigested sample of the milk. There was a remarkable difference on the peptides present between the intact milk and gastric samples; 64

peptides were common to both sample points, 135 peptides were present only in the intact milk and no thte digested sample; and, 586 peptides were present only in the gastric samples. The pattern of peptides suggested that degradation within the intact milk and stomach is protein selective (Dallas et al., 2014). Peptides released from lactoferrin were not present in the intact milk but were present in significantly higher concentrations in the gastric samples (Dallas et al., 2014). The authors concluded the increase in unique peptides from proteins in the stomach, including lactoferrin, has clinical relevance because the antibacterial, immunomodulatory, and other functions of these peptides are particularly relevant in the small bowel (Dallas et al., 2014).

A certain proportion of lactoferrin and its peptides is absorbed within the intestinal lumen, and able to exert a range of systemic effects. Lactoferrin receptors occur throughout the intestine in the brush border membrane enabling the absorption of lactoferrin and potentially some large fragments such as a "nicked" but otherwise intact form of lactoferrin (Hutchens, Henry, & Yip, 1991), or lactoferricin that result from any proteolysis in the gut (Gislason, Douglas, Hutchens, & Lönnerdal, 1995; Gislason, Iyer, Douglas, Hutchens, & Lönnerdal, 1994; Kawakami & Lönnerdal, 1991). Recognition of lactoferrin by its receptor does however appear to be somewhat species specific, but not entirely (Kawakami & Lönnerdal, 1991). More recently, (Lönnerdal et al., 2011) found that bLf could be taken up by the human lactoferrin receptor (hLfR).

In an investigation into the transport of lactoferrin from the intestinal lumen of piglets, Harada, Itoh, et al. (1999) found that following oral administration in neonatal pigs, bovine lactoferrin appeared in the blood circulation and reached a peak level after 2 h. It was confirmed immunohistochemically that lactoferrin was transported by endocytosis via the epithelial cells. Lactoferrin absorbed into the blood was also detected in the bile and reached a peak value 12 h after oral administration. Transport of lactoferrin from the intestinal lumen into the bile via the bloodstream was also observed in weaning piglets. Lactoferrin transported into plasma and bile was confirmed to be the same substance as administrated lactoferrin by electrophoresis and immunoblotting methods. Lactoferrin transported into bile was re-absorbed into the blood in neonatal pigs. This suggests that orally administered lactoferrin is transported, at least partially, from the intestinal epithelium into the peripheral circulation, excreted into the bile and re-absorbed into the bloodstream of neonatal pigs, suggesting the presence of entero-hepatic circulation of bLf in neonatal pigs (Harada, Itoh, et al., 1999). Feeding formula containing physiologic concentrations of added bLf increased hepatic protein synthesis in newborn pigs, suggesting lactoferrin may have an anabolic function in neonates (Burrin, Wang, Heath, & Dudley, 1996). Kitagawa et al. (2003) investigated the absorption and transport route of intestinally administered bLf in growing pigs and showed that the absorption of bLf was mediated by lactoferrin-binding factors on the epithelial cell membranes. Almost all of the

absorbed bLf was transported via the lymphatics and the portal vein into the systemic circulation (Kitagawa et al., 2003). The potential for lactoferrin to modify brain function was demonstrated by Harada, Sugiyama, et al. (1999) after orally and intestinal administered bLf in neonatal pigs was detected in cerebrospinal fluid and was matched to that appearing in the serum by electrophoretic and ELISA analysis. Using gene expression technology, together with a radial maze assay, Chen, Zheng, et al. (2015) showed that neonatal piglets fed 0.6 g/L bLf showed improved neural development (as demonstrated by upregulation of canonical pathways associated with neurodevelopment and cognition; influence on multiple genes involved with cell migration and differentiation, the growth and targeting of axons; and upregulation of transcription factors associated with key pathways and signaling in neurodevelopment), together with enhanced cognition as measured in a maze test. Using a piglet model, Mudd et al. (2016) determined that a novel combination of prebiotics, bovine-derived milk-fat-globule membrane phospholipid complex and bLf (0.3 g/100 g) administered between days 2 to 31, was well tolerated, supported normal growth (Berding et al., 2016), and positively influenced postnatal brain development in the piglet beyond that afforded by DHA and ARA.

More recently, preterm piglet models have been used to investigate the mechanism of how bLf may contribute to the protection of vulnerable infants from developing inflammation and necrotizing enterocolitis (NEC) (Nguyen et al., 2016; Nguyen et al., 2014), and how it regulates the homeostasis of the immature intestine. One hundred and twenty-three (123) different intestinal epithelial cell (IEC) proteins were altered by bLf. Low bLf doses (0.1-1 g/L) up-regulated 11 proteins associated with glycolysis, energy metabolism and protein synthesis, indicating support for cell survival. In contrast, a high bLf dose (10 g/L) up-regulated three apoptosis-inducing proteins, down-regulated five anti-apoptotic and proliferation-inducing proteins and 15 proteins related to energy and amino acid metabolism, and altered three proteins enhancing the hypoxia inducible factor-1 (HIF-1) pathway. In the preterm pig intestine, bLf at 10 g/L decreased villus height/crypt depth ratio and up-regulated the Bax/Bcl-2 ratio and HIF-1alpha, indicating several undesirable effects; elevated intestinal apoptosis and inflammation Figure 6-2. The authors concluded, given that bLf dose-dependently affects IECs via metabolic, apoptotic and inflammatory pathways, that it is important to select an appropriate dose when feeding neonates with bLf to avoid detrimental effects bought about by excessive doses (Nguyen et al., 2014). Beneficial effects (increased crypt proliferation (60%), crypt depth and area and increased β-catenin mRNZ expression) on IEC in neonatal piglets fed bLf up to 3.6 g /L were observed in a recent study by Reznikov, Comstock, Yi, Contractor, and Donovan (2014), suggesting that undigested bLf can potentially affect intestinal proliferation through direct contact with IEC's. The same study investigated the effect of bLf on mucosal and systemic immune development (Comstock, Reznikov, Contractor, & Donovan, 2014), showing that

dietary bLf can alter the capacity of the mesenteric lymph nodes (MLN) and spleen immune cells in response to stimulation. In piglets fed transgenic bovine milk containing recombinant human lactoferrin, a significantly reduced incidence of diarrhea, enhanced humoral immunity, T helper (Th1 and Th2) cell responses, an improvement in the structure of the intestinal mucosa, no observed induction of food allergy led Li et al. (2014) to conclude that in neonatal piglets lactoferrin could improve both systemic and intestinal immune responses. In a piglet trial investigating the potential of bLf to improve immune function to reduce mortality in piglets during the stressful phase of weaning, Shan, Wang, Wang, Liu, and Xu (2007) found significant beneficial changes in a number of immune markers, a reduction in incidence of diarrhea and improved growth and performance of the piglets fed bLf. Together these studies provide further evidence for a supporting role for lactoferrin in the initiation of protective immune responses in neonates. A recent study, (described in detail in Section C.2) confirms that infant formula fortified with bLf to 1.0 g/L is well tolerated and associated with normal growth and development of human infants up to 1 year (Johnston et al., 2015)

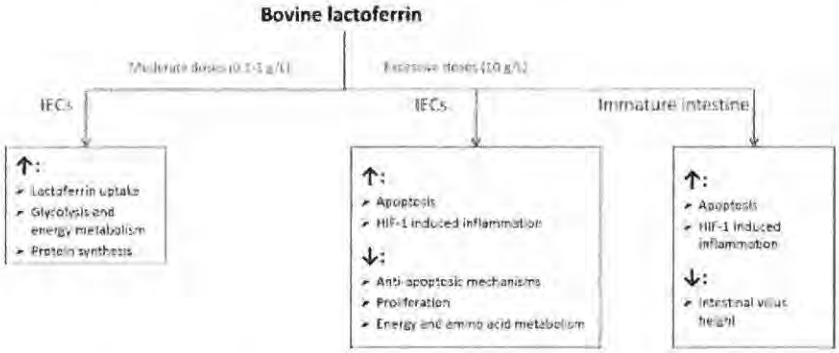

Figure 6-2. Effect of bLf on Intestinal epithelial cells

(From Nguyen et al., 2016)

The excretion of undigested lactoferrin and lactoferrin fragments is well documented. In an adult, and a growing pig model, Schmitz, Hagemeister, and Gortler (1988) observed that up to 20% of ingested lactoferrin was excreted undigested, in the feces. This supports the earlier findings of Spik et al. (1982), who monitored lactoferrin in fecal extracts of breastfed infants, concluding that lactoferrin (both human and bovine in origin) are not completely destroyed during digestion, retain their ability to bind iron, and hence may supplement the bacteriostatic effects of endogenous lactoferrin in the intestinal tract. Further support is provided by alternate measures of amino acid digestibility, where the true digestibility of a number of amino acids in

human milk protein were less digestible compared to others (Darragh & Moughan, 1998) . Those amino acids found to be less digestible are present in greater proportions in the immune proteins, including lactoferrin, than other proteins of human milk (Darragh & Moughan, 1998). Goldman, Garza, Schanler, and Goldblum (1990) identified similar fragments of lactoferrin in the stools and urine of very low birth weight infants fed human milk that appeared to be produced by *in vivo* proteolysis and originating in the gastrointestinal tract. Using isotope labeled human milk proteins, Hutchens, Henry, Yip, et al. (1991) confirmed that intact (78kDa) lactoferrin of maternal origin is absorbed by the gut and excreted intact in the urine of preterm infants.

Based on this information, Synlait concludes there is compelling evidence that a substantial proportion of both intact lactoferrin and its peptides resist gastric digestion, persists throughout the gastrointestinal tract and is excreted in the feces. To a lesser extent lactoferrin may also be it is absorbed in the intestinal lumen via LFR, exerting a range of systemic effects. This duplicity of fates affords it to play a range of different metabolic roles and manifest its bioactivity via a range of different mechanisms. On this basis the inclusion of bLf in milk-based infant and toddler formulas may support the clinical benefits associated with lactoferrin.

6.4 TOXICOLOGICAL STUDIES

6.4.1 Acute Toxicity Study in Rats

The acute toxicity of bLf was evaluated in rats by Nishimura and colleagues (1991), as cited in GRN 465;Yamauchi, Toida, Nishimura, et al. (2000); and EFSA Panel on Dietetic Products Nutrition and Allergies (NDA) (2012)). The study was the subject of a detailed review during the GRAS Notice of bLf for use in infant formula and other food uses (GRN 464, 2014, p. 84 (pdf); GRN 465, 2014, p. 81 (pdf)), and is therefore incorporated by reference to GRAS notices GRN 464 and GRN 465 into this GRAS Notice.

In summary, when rats were exposed to a single oral dose of 1,000 or 2,000 mg/kg bw of bLf or iron-saturated bLf, no adverse effects or deaths occurred in either the acute phase or over a 14-day follow-up period. Based on this study, the lethal dose of bLf exceeds 2000 mg/kg.

6.4.2 4- Week Sub-chronic Oral Toxicity in Rats

The safety of bLf was evaluated in rats by Nishimura and colleagues (1997 as cited in GRN 465, Yamauchi, Toida, Nishimura, et al. (2000) and EFSA Panel on Dietetic Products Nutrition and Allergies (NDA) (2012)). The study was the subject of a detailed review during the GRAS Notice of bLf for use in infant formula and other food uses (GRN 464, 2014 (p. 84 (pdf); GRN

465, 2014 p. 81 (pdf)), and is therefore incorporated by reference to GRAS notices GRN 464 and GRN 465 into this GRAS Notice.

In summary, male and female Sprague-Dawley rats were gavaged once daily with doses of 0 (water), 200, 600 or 2,000 mg/kg bw of bLf for 4 weeks. There were no adverse effects observed, no deaths or treatment-related changes in body weight, feed consumption, organ weight, ophthalmology, hematology, blood chemistry, urinalysis, gross pathology or histology. On this basis, the NOAEL (no-observed-adverse-effect-level) of bLf was estimated to be in excess of 2,000 mg/kg/day (GRN464, 2014 p. 82 (pdf)).

6.4.3 13-week Sub-chronic Oral Toxicity in Rats

In a 13-week oral repeated administration toxicity study of bLf in male (12/group) and female (12/group) Sprague-Dawley rats, once daily with doses of 0 (water), 200, 600 or 2,000 mg/kg bw of bLf bw of bLf were given by oral gavage (Yamauchi, Toida, Nishimura, et al., 2000). The study was the subject of a detailed review during the GRAS Notice of bLf for use in infant formula and other food uses (GRN 464, 2014 (p. 86 (pdf); GRN 465, 2014 p. 83 (pdf)), and is therefore incorporated by reference to GRAS notices GRN 464 and GRN 465.

In summary, no clinically relevant effects were observed in any of the 4 groups. There were no significant differences observed in body weight or feed consumption between the groups over the duration of the study. Furthermore, there were no changes in ophthalmological measures, blood chemistry or gross pathological examination outcomes that could be attributed to the consumption of bLf in any of the groups (Yamauchi, Toida, Nishimura, et al., 2000). No changes in organ weights of animals in the 200 or 600 mg/kg groups were observed; however, females only in the 2000 mg/kg group had a slight but significant reduction in thyroid weights. The changes were not considered related to the bLf as they were related only to females and not correlated to any morphological findings on histopathological examination (Yamauchi, Toida, Nishimura, et al., 2000).

Two animals died during the treatment period. On investigation, the deaths were not attributed to the consumption of the bLf. In week 10, one male in the 200 mg/kg group died as a result of an error in intubation. One female in the 2000 mg/kg group died as a result of a spontaneous lymphoma, which is not uncommon in Sprague-Dawley rats (Yamauchi, Toida, Nishimura, et al., 2000). Neither death was attributable to the administration of the bLf.

A slight, but significant reduction in urinary pH was observed for both males and females in the 2000 mg/kg bw of bLf group. Lactoferrin was not detected in the urine (detection limit 0.1 µg/ml), Yamauchi, Toida, Nishimura, et al. (2000) however, suggested that the potential

presence of undetected bLf fragments in the urine may influence urinary pH. Both intact lactoferrin and fragments of maternal lactoferrin have been detected in the feces and urine of breastfed infants (Hutchens, Henry, Yip, et al., 1991). Other observed urinalysis differences in male rats only included minor changes in urine volume and daily excretion of sodium, potassium and chloride ions. These differences were not related to bLf dose (Yamauchi, Toida, Nishimura, et al., 2000). Histological examination of the kidneys revealed no abnormalities. In short, minor changes in urinalysis were not considered to be of any toxicological significance.

Islet fibrosis in the pancreas was observed in male rats, with the incidence and severity (slight to mild- control- 3/12; 200 mg/kg- 7/12; 600 mg/kg- 6/12 and 2000 mg/kg- 6/12) of the finding in each bLf administration group being slightly higher than for the control group. Islet fibrosis in the pancreas is known to occur at relatively high frequency as a phenomenon associated with aging in the Sprague-Dawley rat. This effect is supported by Imaoka, Satoh, and Furuhama (2007), who reported the incidence of spontaneous pancreatic islet fibrosis in rats corresponding to the same age of rats used in the 13-week study of (Yamauchi, Toida, Nishimura, et al., 2000). The islet fibrosis was not considered to be a consequence of bLf administration.

The overall conclusion of the 13-week oral toxicity study was that none of the observed differences were due to the administration of bLf, and that the NOAEL of bLf was 2,000 mg/kg BW per day, the highest dose tested.

6.4.4 Chronic Oral Toxicity in Rats

Tamano et al. (2008) completed 2 chronic feeding studies in male and female F344/DuCrj (Fisher) rats to determine if bLf and related compounds have any toxic effects in long-term feeding studies. The study was the subject of detailed review during the GRAS Notice of bLf for use in infant formula and other food uses (GRN 464, 2014 (p. 88 (pdf); GRN 465, 2014 p. 85 (pdf)), and is therefore incorporated by reference to GRAS notices GRN 464 and GRN 465.

In summary, the studies were completed to determine if bLf and related compounds had any toxic effects when fed long term. In the first study, 15 male rats (starting age 6-weeks) were fed either a basal control diet containing no bLf, or the basal diet containing 0.2% bLf for 40 weeks. At the end of the 40 weeks, blood samples were analyzed for a range of biochemical markers, and gross examinations of organs and tissues were completed at necropsy. No adverse treatment related clinical indications, effects on body weight or macroscopic changes were reported (Tamano et al., 2008).

In the second experiment, both female and male F344/Crj rats (25/sex/group in control and high dose group; 10/sex/group in other groups) were fed a basal diet containing 0 (control), 0.02%,

0.2%, 2.0% or 5.0% bLf for 60 weeks (males) or 65 weeks (females). Gross examination was completed at necropsy and major organs weighed. Tissue samples of a number of organs, and any large lesions, were processed for histopathological examination. No reported significant treatment-related adverse effects on final body weight, organ weight, gross or histopathology, including carcinogenicity, were evident for either sex (Tamano et al., 2008). The authors concluded that the studies provided subjective support for the safety of clinical studies of bLf for supplement use. As the study report (Tamano et al., 2008) did not provide full data sets it could not be used to determine a NOAEL.

6.4.5 Genotoxicity

Yamauchi, Toida, Kawai, et al. (2000) evaluated the genotoxic potential of bLf using the Ames mutagenicity test (Ames, McCann, & Yamasaki, 1975). A total of 5 test strains including 3 base-pair substitution-type strains, *Salmonella typhimurium* TA100, TA1535 and *Escherichia coli* WP2uvrA, and 2 frameshift-type strains, TA98 and TA1537, were used in the test. The test was performed by both the direct method and the metabolic activation method (provided by an Aroclor-induced, rat liver microsome fraction (S9mix)), with pre-incubation applied in each instance. The test bLf solution was tested at 6 concentrations: 160, 320, 630, 1250, 2500, and 5000 μg/plate, based on the results of a preliminary study to evaluate potential growth inhibition of the selected bacterial strains and to determine the dose levels (Yamauchi, Toida, Kawai, et al., 2000). Physiological saline was the negative control, and was used to dissolve and dilute the bLf to the target concentrations. Testing was completed in duplicate.

Results from the positive and negative controls were used to establish whether the study was conducted appropriately – the number of revertant colonies induced by the positive control was more than twice (2x) that of the negative control for each test strain, and the number of colonies formed for each of the controls aligned with expected ranges based on other reverse mutation tests using the same controls (Yamauchi, Toida, Kawai, et al., 2000). At all concentrations of bLf tested, and across all bacterial strains both with and without activation, the number of revertant colonies was 1.4 times or less than that of the negative control. A factor of greater than 2 was required to denote a positive result.

Based on the results of this study the mutagenicity of bLf was judged negative. Bovine lactoferrin did not exhibit mutagenicity in the Ames test used (Yamauchi, Toida, Kawai, et al., 2000).

6.4.6 Summary of Toxicity and Genotoxicity Studies

Based on the results from the acute, sub-chronic and chronic animal toxicity studies, Synlait concludes that bLf is well tolerated with no significant adverse effects or toxicity at the concentrations tested. The NOAEL, based on these toxicity studies, is determined to be 2,000 mg/kg. The compound bLf is also non-genotoxic, as determined by the Ames mutagenicity test.

6.5 ALLERGENICITY

Cow's milk allergy (CMA) is a hypersensitivity reaction to milk initiated by specific immunologic mechanisms. The main allergens of cow's milk are distributed among the whey and casein protein fractions, the 4 whey allergens including alpha-lactalbumin, beta-lactoglobulin, bovine serum albumin and the bovine immunoglobulins (Fiocchi et al., 2010). Lactoferrin, present at approximately 0.09 g/L in cow's milk, is not listed as one of the milk allergens, and its clinical relevance as an allergen is unknown. Crittenden and Bennett (2005) reported the incidence of CMA is more prevalent in infants (2–6%) than in adults (0.1– 0.5%), and the dominant immunological mechanisms driving allergic reactions change with age.

In most children with CMA, the condition can be immunoglobulin E (IgE)-mediated and is thought to manifest as a phenotypical expression of atopy, together with (or in the absence of) atopic eczema, allergic rhinitis and/or asthma. A subset of patients, however, have non-IgE mediated (probably cell-mediated) allergy and present mainly with gastro-intestinal symptoms in reaction to the ingestion of cow's milk (Fiocchi et al., 2010).

The potential for IgE-mediated hypersensitivity and non-IgE-mediated hypersensitivity was extensively reviewed during the GRAS Notice of bLf for use in infant formula (GRN 465, 2014 p. 45- 73 (pdf)), and is therefore incorporated by reference to GRAS notice GRN 465. Since the bLf discussed in this document is essentially equivalent to the bLf discussed in GRN 465, the discussion in GRN 465 is applicable here as well. In summary, that review, consistent with other reports (Natale et al., 2004; Wal, 1998; Wal et al., 1995) concluded that, although infants and individuals with CMA have anti-bLf IgE antibodies, there is no evidence to support a role for bLf as a causative agent for CMA (Goodman et al., 2007). Importantly, given that oral administration reduces an antigen's immunoreactivity, providing small amounts of bLf may in fact contribute to the development of oral tolerance (GRN 465, 2014, p. 50 (pdf)). Gaudin et al. (2008) concluded that based on IgE binding affinity bLf could be classified as a strong allergen to young children with CMA, however that the caseins are the main allergens in milk and that α_{S1}-casein is more allergenic than α_{S2}-, β- and κ-caseins, which were recognized with almost a similar frequency by the sera of patients.

In the USA, the Food Allergen Labeling and Consumer Protection Act (FALCPA) of 2004 is an amendment to the Federal Food, Drug and Cosmetic Act and requires that the label of a food that contains an ingredient that is or contains a protein from a "major food allergen" declare the allergen in the manner prescribed by the law. The major food allergens identified in FALCPA include milk (including milk proteins). This necessitates a requirement for all milk based-formula to be labeled and clearly identified as containing milk. Infants with CMA should not be fed cow's milk-based formula.

The subject of this notification is the use of bLf at levels of up to 100 mg/100g of infant formula solids in cow's milk-based formula. As such, infants with known CMA should not be fed the intended infant formula. As bLf is not one of the major cow milk proteins linked to CMA (Fiocchi et al., 2010), in the event an infant with CMA is fed a bLf fortified cow's milk-based infant formula, it is unlikely the bLf would be the primary causative agent of any immunologically driven hypersensitivity (CMA) (Ahrens et al., 2012; Gaudin et al., 2008).

6.6 HUMAN STUDIES OF bLf FED TO INFANTS AND TODDLERS

6.6.1 bLf in Milk-based Formulas for Pre-term Infants and Very Low Birth Weight (VLBW) Infants

Preterm and low or very low birth weight infants represent one of the most vulnerable populations, at risk of developing neonatal sepsis, a major cause of neonatal deaths (Turin et al., 2014). Lactoferrin has been evaluated as a prophylactic for NEC and sepsis in preterm and very low birth weight (VLBW) infants for over 30 years (Table 6-1). In the studies, no adverse events or intolerance to bLf in these infants have been observed, and the safety of bLf administration was confirmed in all studies. (Ochoa, Pezo, Cruz, Chea-Woo, & Cleary, 2012). In a review of potential prophylactics for the prevention of common gastrointestinal complications in premature or VLBW infants Vongbhavit and Underwood (2016) concluded supplementation with bLf is a safe and potentially useful strategy in the prevention of the gastrointestinal infections typically associated with long-term morbidity and high mortality. There is ongoing research interest in bLf for reducing the risk of infection in VLBW infants. Turin et al. (2014) identified 10 registered clinical trials in progress at that time, involving more than 5,700 neonates, investigating lactoferrin for the prevention of neonatal sepsis. To date, the result of only one of these registered trials, the LACUNA study (Barrington, Assaad, & Janvier, 2016) in Canada have been published, and is discussed below. The antimicrobial and immunological functions of lactoferrin in human milk is well established (Trend et al., 2015; Trend et al., 2016), Manzoni (2016) suggests that bLf likely delivers the same clinical effects as human lactoferrin.

More recently, recombinant human lactoferrin (talactoferrin (TLf)) has also been of interest for its prophylactic potential. Sherman et al. (2016) evaluated the safety and efficacy of TLf to reduce infection in VLBW neonates (750-1500 g) (NCT00854633). Infants received enteral TLf (n = 60) or placebo (n = 60) on days 1 through 28 of life; the TLf dose was 150 mg/kg every 12 hours. The researchers found no clinical or laboratory toxicity and a trend toward less infectious morbidity in the infants treated with TLf.

The updated Cochrane Review (Pammi & Abrams, 2015) was designed to assess the safety and effectiveness of oral lactoferrin in the prevention of sepsis and necrotizing enterocolitis (NEC) in preterm neonates as the primary outcome. Secondary objectives were to determine the effects of oral lactoferrin used to prevent neonatal sepsis and/or NEC on duration of positive-pressure ventilation, development of chronic lung disease (CLD) or periventricular leukomalacia (PVL), length of hospital stay to discharge among survivors, and adverse neurological outcomes at two years of age or later, and to determine the adverse effects of oral lactoferrin in the prophylaxis of neonatal sepsis and/or NEC. In this review involving more than 1000 preterm neonates, no adverse effects due to oral lactoferrin were reported. Using the Cochrane Systematic Review quality of data criteria, the reviewers found moderate to low quality evidence to suggest that oral lactoferrin prophylaxis, with or without probiotics, decreases late-onset sepsis and NEC stage II or greater in preterm infants without adverse effects. The reviewers identified four (4) ongoing trials that will provide evidence from more than 6000 preterm neonates and may enhance the quality of the evidence in the future. The need for clarification regarding optimum dosing regimens, type of lactoferrin (human or bovine), and long-term outcomes were noted. Importantly, no adverse effects were reported from the use of bLf in the neonates.

Preterm or VLBW neonates are not the target population for bLf-supplemented formula that is the subject of this notification. The safe history of use of bLf in this vulnerable group of infants is however relevant support as to the safe use of bLf for infants, and young children.

Nine (9) studies have been identified evaluating the effects of bLf in preterm or VLBW neonates (Table 6-1). These are discussed below. Daily exposure of the neonates to bLf was either on a daily dose basis (100 mg/day to 200 mg/day) or on a body weight basis (9.75 mg/kg/day to 200 mg/kg/day). These levels exceed the EDI of bLf proposed in this notification for the youngest groups of term infants (0-4 months) (Table 6-1).

Barrington et al. (2016) (The Lacuna Trial) investigated the safety and efficacy of bLf in very preterm infants (<31 weeks gestational age), who were enrolled at <48 hours of age, if they had not yet been fed or had received milk for <24 hours. Seventy-nine (79) infants were randomized to receive either milk (human milk or preterm formula) without or with added bLf (100 mg/day administered in a single feed). The primary outcome of this trial was feeding tolerance (length

of time taken to achieve full feeds, defined for this study as 140 ml/kg/day). A range of secondary outcomes related to morbidity and mortality were determined. There was no difference between groups in the time to achieve full feeds (primary outcome), or other feeding related events and indices of intestinal tolerance. Mortality, late onset sepsis (LOS), NEC and other complications of prematurity did not differ between treatment groups; however, the authors noted that the trial was underpowered to detect such effects. The trial showed that bLf at 100 mg/day is well tolerated by the very preterm neonates (Barrington et al., 2016). No treatment related adverse events were reported.

Ochoa et al. (2015) investigated the potential of bLf for the prevention of the first episode of LOS in 190 neonates with a BW of 1591 ± 408 g and a gestational age of 32.1 ± 2.6 weeks. Infants enrolled into the study within the first 72 hours of life were randomized to receive bLf at 200 mg/kg/d (in 3 doses) or a placebo for 4 weeks. Pre-weighed doses of bLf or placebo were mixed with the feed the infants were receiving at the time (breast milk, formula or dextrose), immediately prior to feeding. Overall, 33 clinically defined first late-onset sepsis events occurred. The cumulative sepsis incidence in the bLf group was 12/95 (12.6%) versus 21/95 (22.1%) in the placebo group, and 20% (8/40) versus 37.5% (15/40) for infants less than or equal to 1500 g. The hazard ratio of Lf, after adjustment for BW, was 0.507 (95% CI: 0.249-1.034). There were 4 episodes of culture-proven sepsis in the Lf group versus 4 in the placebo group. In a secondary exploratory analysis using time since the start of the treatment as a variable in the model, the effect of bLf achieved significance. Ochoa et al. (2015) stated there were no serious adverse events attributable to the intervention.

Kaur and Gathwala (2015) investigated the efficacy of bLf in the prevention of the first episode of LOS in 130 low birth weight (2,000 g) neonates. Infants admitted to the NICU (neonatal intensive care unit) within the first 12 hours of life, with no maternal risk factors for sepsis were randomized to receive either bLf supplemented or a standard formula (placebo) from day 1 to 28 of life. The amount of bLf administered was based on weight. Outcome measures included the incidence of culture-proven sepsis and sepsis-attributable mortality after 72 h of life. A significantly lower incidence of first episode of culture-proven LOS was seen in the bLf group vs. placebo [2/63 (3.2%) vs. 9/67(13.4%); risk ratio, 0.211; 95% CI, 0.044-1.019; p = 0.036]. A statistically significant reduction in the sepsis-attributable mortality was also seen after use of prophylactic bLf [0/63 (0%) vs. 5/67 (7.5%); p = 0.027]. The authors concluded that bLf supplementation in LBW neonates reduces the incidence of first episode of LOS (Kaur & Gathwala, 2015). There were no treatment related adverse events noted.

Manzoni et al. (2014) investigated if bLf alone or in combination with a probiotic bacteria (*Lactobacillus rhamnosus* GG (LGG)) can reduce the incidence of NEC in VLMW neonates.

Seven hundred and forty-three infants were randomly assigned to receive orally either bLf (100 mg/day) alone (group LF; n = 247); or with LGG (at 6 x 10 (9) CFU/day; group BLF + LGG; n = 238); or placebo (Control group; n = 258) from birth until day 30 of life (45 days for neonates <1000 g at birth). The primary outcome measures were ≥ stage 2 NEC; death-and/or-≥ stage 2 NEC prior to discharge. Outcomes of the multi-center international trial showed the NEC incidence was significantly lower in groups BLF and BLF + LGG [5/247 (2.0%)] and [0/238 (0%)], respectively than in controls [14/258 (5.4%)] (RR = 0.37; 95% CI: 0.136-1.005; p = 0.055 for BLF vs. control; RR = 0.00; p < 0.001 for BLF + LGG vs. control). The incidence of death-and/or-NEC was significantly lower in both treatment groups (4.0% and 3.8% in BLF and BLF + LGG vs. 10.1% in control; RR = 0.39; 95% CI: 0.19-0.80; p = 0.008; RR = 0.37; 95% CI: 0.18-0.77; p = 0.006, respectively). NEC is a devastating bowel disease affecting approximately 7% of VLBW infants, and together with other gut-related sepsis complications can be responsible for 45% of late deaths in the NICU (Sherman, Bennett, Hwang, & Yu, 2004). NEC is associated with substantial morbidity and mortality, prolonged care in NICU's, high care costs, late and impaired neurodevelopment and decreased quality of life for survivors. The immunomodulatory, gut maturation and differentiation, and antibacterial properties of lactoferrin, together with its naturally high level in human colostrum and breast-milk mean that bLf is a prime candidate for evaluation in VLBW infants to prevent these diseases. In this study, the authors concluded that, compared with the placebo, bLf supplementation, alone or in combination with the probiotic LGG, reduced the incidence of ≥ stage 2 NEC and of death -and/or ≥ stage 2 NEC in VLBW neonates. Furthermore, bLf, for which the authors stated no adverse effects or intolerances, is a promising strategy in the NICU to prevent NEC (Manzoni et al., 2014).

Akin et al. (2014) investigated the potential for oral bLf to reduce nosocomial infections and NEC, together with possible effects on regulatory T cells (immune function) in VLBW infants and / or those born at <32 weeks gestation. During their hospitalization, infants received either 200 mg/kg BW/day bLf (n=25) or a placebo (n=25). A term infant groups (n=16) was enrolled as a comparator group. Fewer sepsis episodes were observed in the bLf-treated infants (4.4 vs. 17.3/1,000 patient days, p = 0.007). No participants in the bLf group developed NEC, however the result did not reach statistical significance. Regulatory T-cell (Treg) levels, that may indicate a potential modulation of the immune system, at birth and discharge were similar, while preterm infants showed significantly lower levels than term controls. Individual increases in Treg levels were higher in the LF group. Treg levels in preterm infants were lower than in term infants and an increase of Treg levels under bLf treatment was observed. An increase in Treg levels explain the protective effects of LF on nosocomial sepsis. No treatment related adverse events were recorded.

A prospective, multicenter, double-blind, placebo-controlled, randomized trial conducted in 11

Italian tertiary neonatal intensive care units, investigated the ability of bLf alone, or in combination with LGG, to reduce the incidence of LOS in VLBW neonates (Manzoni, 2009). Four hundred and seventy-two (472) VLBW infants enrolled into the study at <3 days of age and were randomly assigned to receive orally administered bLf (100 mg/d) alone (n = 153 Group A1), BLF plus LGG (6×10^9 colony-forming units/d) (n = 151, Group A2), or placebo (n = 168, Group B) from birth until day 30 of life (day 45 days for neonates <1000 g at birth). The incidence of LOS was significantly lower in the bLf and bLf plus LGG groups (9/153 [5.9%] and 7/151 [4.6%], respectively) than in the control group receiving the placebo (29/168 [17.3%]) (risk ratio, 0.34; 95% confidence interval, 0.17-0.70; P = 0.002 for bLf vs control and risk ratio, 0.27; 95% confidence interval, 0.12-0.60; P < 0.001 for bLf plus LGG vs control). The decrease occurred for both bacterial and fungal sepsis. No adverse effects or intolerances to treatment occurred.

Manzoni et al. (2014) undertook a secondary analysis of data collected during the 2009 study summarized above, with the objective of determining the rate of fungal colonization, invasive fungal infection (IFI), and rate of progression from colonization to infection in all groups. Overall, the incidence of fungal colonization was comparable (17.6%, 16.6%, and 18.5% in the bLf group (A1), bLf + LGG group (A2), and placebo group (B), respectively; P = 0.89 [A1] and 0.77 [A2]). In contrast, IFIs were significantly decreased in A1 and A2 (0.7% and 2.0%, respectively) compared with B (7.7%; P = 0.002 [A1] and 0.02 [A2]), and this was significant both in <1000 g (0.9% [A1] and 5.6% [A2], vs 15.0%) and in 1001 to 1500 g infants (0% and 0% vs 3.7%). The progression rate of colonization-infection was significantly lower in the bLf groups: 3.7% (A1) and 12% (A2), vs 41.9%; P < 0.001 (A1) and P = 0 .02 (A2). No IFI-attributable deaths occurred in the treatment groups, versus 2 in the placebo group. The authors concluded that the prophylactic oral administration of bLf reduces the incidence of IFI in preterm VLBW neonates. Whilst no effect is seen on colonization, the protective effect on IFI is likely due to limitation of the ability of fungal colonies to progress toward invasion and systemic disease in colonized infants (Manzoni et al., 2012). No adverse effects or intolerances occurred.

In a prospective intervention, Kawaguchi et al. (1989) supplemented the formula given to 9 premature, low birth weight infants (1454-2034g BW; 29-36 weeks gestational age) with 100 mg/100 ml bLf for2 weeks, to investigate changes in the microbial population of the feces. Stable bottle feeding was achieved at approximately 150 ml/kg/day, providing 150 mg bLf/kg BW/day. With the relatively high dose of bLf, 8-9 mg of lactoferrin/g feces was detected at the completion of the 2-week feeding period. Transient shifts in the bacterial species present in the feces were observed. Composition ratios of *Bifidobacterium* and *Veillonella* sp. increased relative to baseline while the ratio of *Enterobacteriaceae* and *Clostridium* sp. declined. Changes

were not apparent one week after completing the bLf intervention, and no other effects on fecal pH or organic acid content were observed. No treatment related adverse effects were reported.

Kawaguchi, Suzuki, and Okuyama (1986) studied the effects of bLf in 16 low birth weight infants (>1500g) who were fed a standard commercial formula until a stable feeding pattern was established (150 ml/kg BW/day). The standard formula was then replaced with a bLf supplemented formula (50 mg/100g powder (approximately 6.5 mg bLf /100 ml and 9.75 mg bLf/kg BW /day assuming a stable intake of formula at 150 ml/kg/day) and fed for 2 weeks, before resumption of the standard infant formula. Low levels (20-75 µg/g) of bLf were detected in feces during the bLf intervention period. There were a number of changes in fecal characteristics (stool softening, reduction in fecal pH (week 2), increased fecal lysozyme activity, increased fecal organic acid content), and an increased ratio of *Bifidobacteria* to total bacteria, while the ratio of Staphylococci tended to be lower. No treatment related adverse effects were reported.

In a recent review of the clinical relevance of lactoferrin supplementation in children, Manzoni (2016) identified 7 key findings related to lactoferrin use in neonates:

1. Start lactoferrin administration as soon as possible;
2. No efficacy in preventing LOS in larger neonates;
3. Better to use >100 mg/day dosages;
4. To prevent NEC, it is best to combine lactoferrin with probiotics;
5. Lactoferrin is likely more effective in preterm than term neonates;
6. Lactoferrin is effective for preventing infections, but not preventing enteric colonization; and,
7. Efficacy on gram-positive bacteria driven LOS may be limited.

In totality the conclusion of the preceding studies is that lactoferrin is clinically relevant in infant feeding and likely that bLf delivers the same clinical benefits as human lactoferrin (Manzoni, 2016). Furthermore, the studies confirm that even in a highly vulnerable population, bLf is safe and well tolerated.

Table 6-1: Clinical studies of bovine lactoferrin in pre-term and very low / low birth weight (VLBW / LBW) infants

Reference	Setting	Population	Objective	Intervention	Main results
Barrington et al. (2016)	Canada	Infants <31 weeks gestation Admitted to NICU within 24 hours of birth n= 40 bLf n=39 Control	To determine the tolerability of bLf in very preterm infants, and if the intervention can be masked Range of secondary outcomes associated with sepsis events	100 mg bLf per day administered in a single feed (breast or formula) (randomized) Duration: until infant 36 weeks post menstrual age or discharge Control: milk with no added bLf All infants also received probiotics. ISRCTN66482337	There was no effect of bLf on the primary outcome. In addition, mortality, late onset sepsis and other complications of prematurity were no different. Equal numbers of parents in both groups believed their infant received bLf. Study demonstrated that bLf is well tolerated, easy to administer and its presence in prepared milk is not evident.
Ochoa et al. (2015)	Peru	Infants with BW<2500 g 190 neonates with a BW of 1591 +/- 408 g and a gestational age of 32.1 +/- 2.6 weeks were enrolled	To determine the effect of bLf on the prevention of the first episode of LOS Randomized, placebo-controlled double blind trial	200 mg bLf/kg/d divided into 3 doses per day Placebo: maltodextrin Duration: 28 days	33 clinically defined first late-onset sepsis events occurred. The cumulative sepsis incidence in the LF group was 12/95 (12.6%) versus 21/95 (22.1%) in the placebo group, and 20% (8/40) versus 37.5% (15/40) for infants less than or equal to 1500 g. The hazard ratio of bLf, after adjustment by BW, was 0.507 (95% CI: 0.249-1.034). There were 4 episodes of culture-proven sepsis in the LF group versus 4 in the placebo group. In a secondary exploratory analysis using time since the start of the treatment as a variable, bLf achieved significance. There were no serious adverse events attributable to the intervention. Overall sepsis occurred less frequently in the LF group than in the control group.
(Kaur & Gathwala, 2015)	India	Infants with birth weight (BW) < 2000 g admitted to NICU in first 12 hours n= 63 bLf (apo-lactoferrin) n=67 control	To evaluate the efficacy of bLf in preventing first episode of late onset sepsis (LOS) in LBW infants Incidence of culture-proven sepsis and sepsis-attributable mortality after 72 h of life was recorded	mg bLf The amount of bLf varied with birth weight, but was not stated Duration: 28 days All infants also received probiotics.	Incidence of first episode of culture-proven LOS was significantly lower in the bLf group vs. placebo [2/63 (3.2%) vs. 9/67(13.4%); risk ratio, 0.211; 95% CI, 0.044-1.019; p = 0.036]. Statistically significant reduction in the sepsis-attributable mortality was also seen after use of prophylactic bLf [0/63 (0%) vs. 5/67 (7.5%); p = 0.027].

Table 6-1: Clinical studies of bovine lactoferrin in pre-term and very low / low birth weight (VLBW / LBW) infants

Reference	Setting	Population	Objective	Intervention	Main results
					bLf supplementation in LBW neonates reduced the incidence of first episode of LOS.
Manzoni et al. (2014)	Italy NZ	743 VLBW infants enrolled n=247 bLf n=238 bLf + probiotic LGG n=258 control (placebo)	To determine if bLf, alone or together with a probiotic, can reduce the incidence of NEC in VLBW infants An international, multicenter, randomized, double-blind, placebo-controlled trial	Treatment from birth until day 30 of life (or day 45 for infants <1000 g BW) bLf = 100 mg/day (both intervention groups) Infants assessed for NEC until discharge from NICU ISRCTN53107700	Demographics, clinical and management characteristics of the 3 groups were similar, including type of feeding and maternal milk intakes. NEC incidence was significantly lower in groups bLf and bLf + LGG [5/247 (2.0%)] and 0/238 (0%), respectively] than in controls [14/258 (5.4%)] (RR = 0.37; 95% CI: 0.136-1.005; p = 0.055 for bLf vs. control; RR = 0.00; p < 0.001 for bLf + LGG vs. control). The incidence of death-and/or-NEC was significantly lower in both treatment groups (4.0% and 3.8% in bLf and bLf + LGG vs. 10.1% in control; RR = 0.39; 95% CI: 0.19-0.80; p = 0.008. RR = 0.37; 95% CI: 0.18-0.77; p = 0.006, respectively). No adverse effects or intolerances to treatment occurred. Compared with placebo, bLf supplementation alone or in combination with LGG reduced the incidence of >/= stage 2 NEC and of death-and/or >/= stage 2 NEC in VLBW neonates.
Akin et al. (2014)	Turkey	VLBW infants or born ,32 weeks gestational age. n=25 bLf n=25 placebo Comparator healthy group of term neonates (n=16)	To determine whether oral bLf reduces nosocomial sepsis episodes and necrotizing enterocolitis (NEC) in VLBW infants and to evaluate the possible effects of LF on Treg levels. Episodes of culture proven nosocomial sepsis and NEC were recorded. The level of FOXP3 + CD4 + CD25hi lymphocytes measured at	200 mg/d bLf throughout hospitalization	Fewer sepsis episodes were observed in LF-treated infants (4.4 vs. 17.3/1,000 patient days, p = 0.007) with none developing NEC, without statistical significance. Treg levels at birth and discharge were similar, while preterm infants showed significantly lower levels than term controls. Individual increases in Treg levels were higher in the bLf group. bLf reduced nosocomial sepsis episodes.. Increase in Treg levels can be the mechanism for protective effects of LF on nosocomial sepsis.

Table 6-1: Clinical studies of bovine lactoferrin in pre-term and very low / low birth weight (VLBW / LBW) infants

Reference	Setting	Population	Objective	Intervention	Main results
			birth and discharge Randomized, placebo-controlled double blind trial		
Manzoni et al. (2012)	Italy	Preterm VLBW infants enrolled before 72 hours of life n=153 bLf (group A1) n=151bLf + probiotic LGG (group A2) n=168 control (placebo) Group B	To assess whether blf alone or in combination with a probiotic (LGG) is able to prevent fungal colonization and infection in preterm VLBW neonates . Secondary analysis of 2009 study A multicenter, randomized, double-blind, placebo-controlled trial	Treatment from birth until day 30 of life (or day 45 for infants <1000 g BW) bLf = 100 mg/day (both intervention groups) ISRCTN53107700	The incidence of fungal colonization was comparable between all groups. Invasive fungal infections (IFIs) were significantly decreased in A1 and A2 (0.7% and 2.0%, respectively) compared with B (7.7%; P = .002 [A1] and .02 [A2]), and this was significantly true both in <1000 g (0.9% [A1] and 5.6% [A2], vs 15.0%) and in 1001 to 1500 g infants (0% and 0% vs 3.7%). The progression rate colonization-infection was significantly lower in the bLf groups: 3.7% (A1) and 12% (A2), vs 41.9%; P<.001 (A1) and P = .02 (A2). No IFI-attributable deaths occurred in the treatment groups, versus 2 in placebo. No adverse effects or intolerances occurred.
Manzoni et al. (2009)	Italy	Preterm VLBW infants enrolled before 72 hours of life n=153 bLf (group A1) n=151bLf + probiotic LGG (group A2) n=168 control (placebo) Group B	To assess whether blf alone or in combination with a probiotic (LGG) is able to reduce the incidence of LOS in VLBW neonates A multicenter, randomized, double-blind, placebo-controlled trial	Treatment from birth until day 30 of life (or day 45 for infants <1000 g BW) bLf = 100mg/day (both intervention groups)	Incidence of late-onset sepsis was significantly lower in the BLF and BLF plus LGG groups (9/153 [5.9%] and 7/151 [4.6%], respectively) than in the control group receiving placebo (29/168 [17.3%]) (risk ratio, 0.34; 95% confidence interval, 0.17-0.70; P = .002 for BLF vs control and risk ratio, 0.27; 95% confidence interval, 0.12-0.60; P < .001 for BLF plus LGG vs control). The decrease occurred for both bacterial and fungal sepsis. Compared with placebo, BLF supplementation alone or in combination with LGG reduced the incidence of a first episode of late-onset sepsis in VLBW neonates No adverse effects or intolerances to treatment occurred.

Table 6-1: Clinical studies of bovine lactoferrin in pre-term and very low / low birth weight (VLBW / LBW) infants

Reference	Setting	Population	Objective	Intervention	Main results
Kawaguchi et al. (1989)	Japan	Low birth weight infants (1454-2-34g); 29-36 weeks gestational age Intervention occurred at least 10 days after stable feeding was reached n=9	To study the effect of bLf-enriched infant formula on premature LBW infants Intervention only	Formula supplemented with 100 mg bLf/100ml of formula powder. Duration: 2 weeks Calculated exposure to bLf = 150 mg/kg/d Follow-up 1 week post discontinuation of trial	Changes in the bacterial populations in the infants feces were detectable by week 2. Several changes persisted to end of 1 week follow up; increase in *Bifidobacterium* and *Veillonella* increased relative to baseline and the ratio of *Enterobacteriaceae* to *Clostridium* declined. A transient increase in *Bacteroidaceae* was observed at week 2. No significant effects on fecal pH and organic acid content were noted. bLf was detected in feces indicating its relative stability in the LBW infant gastrointestinal tract.
Kawaguchi et al. (1986)	Japan	Low birth weight infants >1500g who were being bottle fed Intervention occurred once stable feeding was reached. n=16	To study the effect of bLf-enriched infant formula on premature LBW infants Intervention only	Formula supplemented with 50 mg bLf/100g of formula powder. Duration: 2 weeks Calculated exposure to bLf = 9.75 mg/kg/d	During bLf supplementation fecal changes observed included; softening, pH reduction (week 2), fecal lysozyme activity increased, organic acid content of feces increased (week 2), Bifidobacteria population increased and Staphylococcus decreased bLf was detected in feces indicating its relative stability in the LBW infant gastrointestinal tract. No adverse or intolerance events related to the intervention observed

6.6.2 bLF in Milk-based Formulas for Term Infants

Ten studies in healthy term infants, evaluating the effects and safety of bLf in more than 1300 infants have been published (Balmer, Scott, & Wharton, 1989; Chen et al., 2016; Chen, Zhang, et al., 2015; Chierici, Sawatzki, Tamisari, Volpato, & Vigi, 1992; Fairweather-Tait, Balmer, Scott, & Minski, 1987; Hernell & Lönnerdal, 2002; Johnston et al., 2015; King et al., 2007; Liu et al., 2016; Lönnerdal & Hernell, 1994; Roberts et al., 1992; Schulz-Lell, Dorner, Oldigs, Sievers, & Schaub, 1991). Details of the protocols along with the main findings and including any adverse effects reported in these studies are summarized in Table 6-2. The EDI of bLf in those studies ranges from as low as 36 mg/day (Chen et al., 2016; Chen, Zhang, et al., 2015) to 2,300 mg/day (formula concentration 285 mg/100ml) (Balmer et al., 1989), with most studies estimated to have given an EDI of approximately 800-850 mg/day. The mean and 90th percentile EDI's for term infants subject of this notification (102 mg/day and 148 mg/day respectively) are supported by 8 of these publications (Balmer et al., 1989; Chierici et al., 1992; Fairweather-Tait et al., 1987; Hernell & Lönnerdal, 2002; Johnston et al., 2015; King et al., 2007; Lönnerdal & Hernell, 1994; Roberts et al., 1992; Schulz-Lell et al., 1991). Two of the studies (Chen et al., 2016; Chen, Zhang, et al., 2015; Liu et al., 2016) report EDI's of less than 100 mg/day. Both of these studies were conducted in China using commercial infant formula, which must necessarily conform to regulatory requirements within China, where a maximum bLf content of 1 g/kg formula powder, is equivalent to the maximum proposed level (100 mg/100 g of formula solids) of this notification.

A number of early studies investigated the role of bLf in supporting (Fairweather-Tait et al., 1987) iron absorption or as a source of dietary iron (Chierici & Vigi, 1994; Lönnerdal & Hernell, 1994; Schulz-Lell et al., 1991). However, the work of Davidsson, Kastenmayer, Yuen, Lönnerdal, and Hurrell (1994) suggested that, at least in human milk, lactoferrin did not have a direct role in the enhancement of iron absorption. Other studies investigated the role of bLf in influencing the microflora of infants, with early results drawing mixed conclusions (Balmer et al., 1989; Roberts et al., 1992; Wharton, Balmer, & Scott, 1994) compared to more recent work using more sophisticated molecular techniques to accurately determine bacterial species present in the feces (Liu et al., 2016).

Chen, Zhang, et al. (2015) reported on growth outcomes and changes in iron status associated with the consumption of bLf fortified infant formula, from a study that also investigated the effect of the formula on diarrhea and respiratory tract infections in previously weaned infants (Chen et al., 2016). A total of 316 infants (4 to 6 months) were enrolled in the study; 115 previously weaned infants received the study formula with bLf, and 98 received the control

formula; 103 were enrolled as a breastfed control group. The control formula was a commercial infant formula with an iron content of 4 mg/100g. The test formula is described as containing 38 mg/100 g bLf and iron at 4 mg/100g. This suggests a potential total iron content of the test formula being higher than that of the control. Assuming an iron saturation level of 17-18% (typical for commercial bLf), the added bLf may have resulted in an additional iron content of 6-7 mg/100g and may have contributed to the findings. This aspect is not clearly addressed in the publications. Nonetheless the results are relevant to the additional effecter of the added bLf. In the growth study, no comparative data are presented for the breastfed control group. Feed intake between the 2 formula groups was not significantly different, and the authors report no significant difference in "iron element" intake. This is interpreted as referring to the iron content of the basal formula. There were significant differences in several anthropometric measures (weight (8723± 245 g versus 8558 ± 214g); weight for age z-scores (1.02 ± 0.31 versus 0.44 ± 0.18) and weight for height z-scores (0.98 ± 0.31 versus 0.41 ± 0.12)) between the bLf and control groups, respectively. Measures of iron status were also significantly higher in infants receiving the bLf fortified formula (hemoglobin (Hb), 125.5 ± 15.4 g/L versus 116.9 ± 13.1 g/L; Serum ferritin (SF), 44.7 ± 17.2 μg/L versus 31.6 ± 18.4 μg/L; transferrin receptor (TFR-F) index, 1.88 ± 0.41 versus 1.26 ± 0.39; total body iron content (TBIC), 6.12 ±- 0.78 mg/kg versus 5.26 ± 0.55 mg/kg for the test group and control group respectively; $P < 0.05$). Following the intervention, the bLf group was significantly lower ($P < 0.05$) in the prevalence of anemia (4.1% versus 7.5%), iron deficiency (13.9% versus 24.4%), and iron-deficient anemia (1.7% versus 6.1%). Together these differences in growth and iron status may reflect the underlying difference in total iron content of the formula, the bLf contributing additional iron. There is insufficient data to assign the differences to any specific physiological advantage of bLf. It is well known that, at about six months, infants are at risk of developing iron deficiency (ID) because of the exhaustion of their iron stores needed for rapid growth (Atkins, McNaughton, Campbell, & Szymlek-Gay, 2016). In addition, the iron concentration in breast milk is relatively low (Qasem & Friel, 2015). Iron fortified formula can be a significant source of dietary iron in infants over 6 months of age who are not breastfed (Atkins et al., 2016). In the second part of the study investigating the incidence of diarrhea and respiratory tract infections, the 2 formula groups were compared to each other and to the breastfed control cohort. Respiratory related events were found to be not statistically significantly different between the breastfed control and the bLf test groups, but both were significantly ($p<0.05$) lower than for the control formula group, as were the incidence of associated symptoms (running nose, cough, wheezing). Whilst the incidence of vomiting, nausea and colic were not statistically significantly different between any of the 3 groups, the incidence of diarrhea and occurrence of diarrhea related illnesses were significantly ($p<0.05$) lower in both the breastfed

and bLf groups compared to the control formula group. The results of this trial show that bLf supplemented formula supports the growth and development of infants.

In a recently completed clinical trial (NCT02239588) evaluating the effects of an infant formula (0-6 months) manufactured by Synlait Milk Ltd., containing bLf at 60 mg/100g, normal growth and development was observed and the formula was well tolerated (Bright Dairy Ltd, personal communication (Appendix 5, pg. A5: 2), June 2016). Quantitative details of the growth and tolerability studies are not available at this time. Published aspects of the study, involving exclusive consumption of the formula, showed beneficial effects on the infants' fecal microbial profile, and the concentrations of fecal short chain fatty acids (Liu et al., 2016). At the time of the study, Synlait did not manufacture the bLf used to make the commercial formula. However, a comparison of the specifications of the bLf used in the formula with the specifications of Synlait's bLf indicate that they are essentially equivalent. Since 2014, the commercial formula has contained bLf manufactured by Synlait. The formula has been sold and consumed in China without any reported adverse effects attributable to the bLf.

In a large multi-center, double blind, parallel-designed, gender-stratified prospective study (Johnston et al., 2015) 480 infants were randomized to receive a commercial cow's milk-based formula (control, n=155) or one of 2 test formulas with bLf at 0.6 g/L (LF0.6, n=165) or bLf at 1.0 g/L (LF1.0, n=116). The concentrations of bLf in the test formulas are within the range of lactoferrin concentration in human milk. The test formula also contained a proprietary prebiotic mix of polydextrose and galactooligosaccharides, and adjusted arachidonic acid levels. The primary outcome for the study was growth rate from 14 to 120 days of age, with growth monitored over the duration of the study through to 1 year. No statistically significant differences were observed for growth rate from day 40-120. With the exception of one non-clinically significant difference in head circumference observed in females between the LF1.0 and control group (day 14-60), no other significant differences were observed for mean achieved weight, length or head circumference at any point up to day 365. Mean achieved weight for males and female were within the 25th and 75th percentiles of the WHO weight-for-age growth charts from days 14-365. Acceptance and tolerance of test formulas was good, with no significant differences detected in fussiness, gassiness, or mean stool frequency at all time points. This study provides support for the safety, tolerance and associated normal growth of healthy term infants consuming formula containing bLf at levels of up to 1.0 g/L.

King et al. (2007) examined the impact of long-term feeding of a bLf supplemented infant formula on growth, hematologic and immune parameters and the impact on childhood illnesses in term or near term healthy infant. Infants, who were strictly bottle-fed, and were enrolled between 0 and 4 weeks of age, were randomized to receive either control formula (Similac with

iron formula (3 mg/L elemental iron and background level of 102 mg/L bovine lactoferrin)) or test formula (Similac with iron with 850 mg/L added bLf), and followed to 1 year of age. Protein was not normalized across the two formulas, the bLf test formula containing approximately 5% additional protein. It is unknown if the additional protein received by the test formula group may have influenced the growth rate of those infants. No statistically significant difference in growth parameters was observed between the treatment and control groups. A non-statistically significant ($p>0.06$) trend of greater increase in weight for the test bLf formula group was observed up to 6 months, the trend not continuing after 6 months. Although a significant reduction in the frequency of LRTI's (lower respiratory tract infections) ($p<0.05$), particularly wheezing illnesses was observed in the bLf group, there were no differences in the other childhood illnesses. At 9 months of age, infants receiving the bLf formula had significantly higher hematocrit levels ($p<0.05$), with numerically but not significantly higher hemoglobin and MCV (mean corpuscular volume). No significant differences in hematological values were observed at 12 months. No tolerance issues were raised with almost equal numbers of dropouts in both treatment arms, and equal numbers of serious adverse events in each group. The results of this study support the safe use and tolerance of bLf in formula at up to 850 mg/L (0.085% w/v).

Hernell and Lönnerdal (2002) investigated the effects of added bLf on iron status and hematologic indices in healthy term infants. Infants who were exclusively breastfed were allocated to a breastfed group (n=16) or one of 4 formula groups (n=10-12), according to parental choice, for 6 months. The experimental formula varied in iron content (1.6, 1.8, 2.2, or 4.0 mg Fe / L) contributed by $FeSO_4$, other than the 1.8 mg/L formula in which bLf contributed 1.3 mg/L of the Fe, the remaining 0.5 mg/L coming from $FeSO_4$. The bLf used in this study was iron saturated (1.24 mg Fe/g protein) giving a bLf concentration in the test bLf formula of 104 mg bLf /100 ml (0.104% w/v). The 2.2 mg Fe/L formula also contained added nucleotides (40 mg/L). At 4 and 6 months, there were no significant differences in hematologic indices (hemoglobin, MC, serum iron, total iron binding capacity (TIBC), log serum ferritin, serum TfR) or in serum zinc and copper concentrations. Infants in the bLf group had significantly ($p<0.05$) greater weight gain than those in the nucleotide supplemented group at 6 months, but did not differ from other groups. All formulas were well tolerated. This evidence serves to support the use of bLf in infant formula.

Lönnerdal and Hernell (1994) investigated the iron, zinc, copper, and selenium status of both breastfed infants, and infants fed fortified milk-based formula from 1.5 to 6 months of age. The hematological status of infants receiving formula containing 4mg/L of iron delivered either as $FeSO_4$ or a combination of bLf and $FeSO_4$ (1.2 mg/L Fe from bLf and 2.6 mg/L as $FeSO_4$) was compared to that of the breastfed infant group, and a third formula group (iron content 7 mg/L

as $FeSO_4$). There were no significant differences in hematological status of all groups at 6 months, all infants having acceptable iron status. Serum transferrin receptor levels, a potential indicator of iron status, were highest in breast-fed infants, suggesting a cellular need for iron, and lowest in infants receiving formula with 7 mg of iron/l. Selenium status, as assessed by serum glutathione peroxidase activity, was similar at 6 months of age in breast-fed infants and infants fed formula fortified with selenium but lower in infants fed unfortified formula. The lowest levels of glutathione peroxidase activity were found in infants fed the highest concentration of iron (7 mg/l). Serum copper concentrations were similar in all groups, but the lowest levels were found in infants fed the highest concentration of iron (Lönnerdal & Hernell, 1994). The authors concluded that 4 mg of iron /L is adequate for infants up to 6 months, and that higher levels may have some negative effects. There were no significant effects in iron balance when bLf was a partial source of iron. No adverse effects relating to the consumption of bLf were reported.

In a study of 51 healthy term infants (Chierici et al., 1992; Roberts et al., 1992) evaluating the effects of bLf on the fecal flora (Part 1; Chierici et al. (1992)) and on blood iron and zinc parameters (Part 2; Roberts et al. (1992)), infants were assigned to one of 4 treatment groups: breastfed control group, n=12; control formula group, n=14; 10 mg bLf/100ml (0.01% w/v), formula, n=15; 100 mg bLf /100ml (0.1% w/v) formula, n=14. The infants were demand-fed from birth, to at least the end of the third month (day 90), exclusively on the assigned feed for Part 1 (Chierici et al., 1992), and continued to be fed the assigned feed to 5 months (150 days) for the iron and zinc study Part 2 (Roberts et al., 1992). The iron content of the formulas differed as a function of the bLf content (control formula 70 µg /100ml; 10 mg bLf formula 72.8 µg/100 ml; 100 mg bLf formula 98 µg/100 ml). Serum was sampled at days 0, 7, 30, 90 and 150 (from birth) and tested for hemoglobin, hematocrit, ferritin, iron and zinc. There were no statistically significant differences in serum zinc levels between groups at any time. No statistically significant differences in hematocrit or hemoglobin values were reported between the groups at any time; however, 2 breastfed infants had low hemoglobin levels at day 90. The high dose bLf formula group had significantly higher serum ferritin levels ($p=0.02$) compared to the control formula group at day 150, which may or may not be related to the higher iron intake from the supplemented formula. The serum iron levels of the breastfed infants were significantly lower than those of both the control formula group ($p=0.012$) and the high bLf formula group ($p=0.041$). At the completion of the 3-month study (Part 2), 50% of the breastfed group developed a fecal flora rich in *Bifidobacterium* species, and relatively low in obligate anaerobes such as *Clostridium* and *Bacteroides*. At 3 months, 57% of infants receiving the high bLf flora also produced a *Bifidobacterium* flora; the effect was not observed in the low bLf group. Both *Clostridium* and *Bacteroides* sp. were common isolates from both bLf -

enriched formula groups. There was no reported treatment related adverse effect in either part of the study.

Schulz-Lell et al. (1991) completed an iron balance study in 16 term infants between weeks 3 and 17 of life. Infants received either the control formula (no added bLf, n=9) or a bLf supplemented formula (100 mg bLf/100 ml, n=7). The bLf-supplemented group received 169 µg iron/kg BW/day and retained 63 µg/kg BW/ day. The mean iron intake of infants fed with the control formula without bLf was 118 µg/kg BW/ day, with an iron retention of 43 µg/kg BW/ day. There was no significant difference in the mean percentage retention of iron in the bLf supplemented group (36%), in the non-supplemented group (28%). No adverse effects of the bLf supplementation were reported.

The potential for the addition of bLf when added to formula to modulate the fecal microflora of formula fed infants was also studied by Balmer et al. (1989). Newborn infants whose mothers had chosen not to breastfeed were fed one of three formulas for 14 days (basic formula (0.4 mg/l Fe), n=20; bLf supplemented formula (0.8 mg/L Fe, 2.8 g/L bLf), n= 18; iron and bLf supplemented formula (9.16 mg/L Fe, 2.8 g/L bLf), n= 20). Unexpectedly, bLf was not associated with a shift of fecal microflora pattern towards that of the breastfed infant (more bifidogenic). *E. coli* and fewer staphylococci, versus the other groups, colonized more infants receiving the bLf plus iron formula. The level of lactoferrin excreted in the feces was significantly ($p<0.001$) higher in the 2 groups receiving the bLf-fortified formula than the control formula group. Overall the authors found no significant effects of bLf, suggesting the bLf was ineffective in this study. There were no adverse effects of the bLf intervention reported.

The potential for bLf to affect iron absorption in infants was studied by Fairweather-Tait et al. (1987), using isotope (^{58}Fe) labeled bLf. Thirty-six (36) healthy term infants received either a bLf supplemented formula (285 mg/bLf /100 ml, 86 µg Fe /100 ml) or a basic formula (Fe= 40 µg/100ml) for 14 days. On day 7 of life, half the infants in each formula group received a single orally administered dose of either ^{58}Fe -labeled bLf or $^{58}FeCl_3$ (plus ascorbic acid) in the basic formula. Fecal samples were collected for 3 days and analyzed for ^{58}Fe balance. No overall difference in iron retention (%) was found between the bLf and ferric chloride group, with a wide variation in retention observed. This study exposed infants to a high dose of bLf (285 mg/100 ml), compared to other studies. No adverse treatment related effects were reported.

Table 6-2: Clinical studies of bovine lactoferrin in term infants

Reference	Setting	Population	Objective	Intervention	Main results
Chen et al. (2016) Chen, Zhang, et al. (2015)	China	Term infants previously breastfed, weaned to formula Enrolled at 4 to 6 months of age n= 130 bLf formula (115 completed trial) n= 130 control formula (98 completed trial) n=130 breastfed reference group	To determine if formula fortified with bLf and iron significantly improves hematologic indices and iron status in term infants. To determine the incidence and duration of diarrhea and respiratory tract infections (RTI's). Growth study was included as measure, but not reported for breastfed group. Multicenter, randomized, blinded controlled trial,	Test formula: 38 mg/100 g bLf Control formula: no added bLf Both formula: 4 mg Fe/100g There were no significant differences in the average amount of daily intake of formula milk (94.3 +/- 9.8 g versus 88.2 +/- 8.7 g for FG and CG; P > 0.05) and iron element (3.8 +/- 0.4 mg versus 3.7 +/- 0.6 mg for FG and CG; P > 0.05). The average daily intake of bLf for infants in bLf formula group was 35.8 +/- 3.7 mg. Duration: 3 months	Measures of weight (8723 +/- 245 g vs. 8558 +/- 214g), WFA (1.02 +/- 0.31 vs. 0.44 +/- 0.18), WFH (0.98 +/- 0.31 vs. 0.41 +/- 0.12), together with a range of blood measures; Hb 125.5 +/- 15.4 g/L vs. 116.9 +/- 13.1 g/L), SF (44.7 +/- 17.2 mug/L vs. 31.6 +/- 18.4 mug/L), TFR-F index (1.88 +/- 0.41 vs. 1.26 +/- 0.39), and TBIC (6.12 +/- 0.78 mg/kg versus 5.26 +/- 0.55 mg/kg) of the bLf group were all significantly higher than those of infants in control group weight (P < 0.05), but significantly lower (P < 0.05) for the prevalence of anemia (4.1% versus 7.5%), iron deficiency (13.9% versus 24.4%), and iron-deficient anemia (1.7% versus 6.1%). When infants who were exclusively breastfed were subsequently supplemented with lactoferrin-fortified milk, significant increases in TBIC and iron absorption in the intestine were seen. There was a lower incidence rate of respiratory-related illnesses and fewer symptoms of running nose, cough, and wheezing for infants in the bLf group and breastfed groups compared with those in the control group (P < 0.05) No adverse events and formula well tolerated.
Liu et al. (2016)	China	Term infants between 1 week and 3 months of age enrolled n=40 bLf group (35 completed) n=40 control group (37 completed) N=40 breastfed group (38 completed)	To evaluate the influence of a bLf formula on the microbial population and its metabolism in infants fed a bLf formula. Randomized, double-blind,	Test formula: 60 mg bLf /100 g (9 mg/100 ml) bLf Control formula was not specified and was a range of commercially available formula chosen at parents discretion.	Fecal Bifidobacterium concentrations (mean log copy number +/- SEM) were higher (P = 0.003) in breastfed (BF) infants (8.17 +/- 0.3) and bLf-fed infants (8.29 +/- 0.3) compared with those fed other formula (6.94 +/- 0.3). Fecal acetic acid (mean +/- SEM) was also higher (P = 0.007) in the BF (5.5 +/- 0.2 mg/g) and bLf (5.3

Table 6-2: Clinical studies of bovine lactoferrin in term infants

Reference	Setting	Population	Objective	Intervention	Main results
			single center, controlled trial	Estimated daily intake of bLF* 74 mg/day Duration: 4 weeks NCT02239588	+/- 2.4 mg/g) groups compared with other formula-fed babies (4.3 +/- 0.2 mg/g).. CONCLUSIONS: Fecal bacterial composition and SCFA concentrations were similar in babies fed SPCF or HM. Normal growth and development of infants in the bLf group was observed and the formula well tolerated.
Johnston et al. (2015)	USA	Healthy 12-16 day old infants with BW>2500g 480 infants randomized to receive: LF0.6 BLf formula n= 165 (127 completed) LF1.0 bLf formula n=160 (116 completed) Control formula n=155 (110 completed)	To evaluate the growth and tolerance of infants fed a formula containing bLf within the range of mature human milk. Weight growth rate from 14-120 days was primary outcome measure. Growth tolerance, and adverse events assessed through to 1 year. Multi-center, double-blind, parallel-designed, gender-stratified prospective trial	LF 0.6 formula = bLf at 0.6 g/L LF1.0 formula bLf at 1.0 g/L Control formula: no added bLf Formulas were isocaloric. Test formula, but not control, contained 4 g/L polydextrose (PDX) and galactooligosaccharides (GOS) in a 1:1 ratio ARA in control formula =34 mg/100 kcal vs. 25mg/100 kcal in test formula. Estimated daily intake of bLf* 820 mg/day NCT01122654	No group differences in growth rate (g/day) from 14-120 days of age; 353 infants completed the study through 365 days of age (CONTROL: 110; LF-0.6: 127; LF-1.0: 116). Few differences in growth, formula intake, and infant fussiness or gassiness were observed through 365 day of age. Group discontinuation rates and the overall group incidence of medically-confirmed adverse events were not significantly different. From 30 through 180 days of age, group differences in stool consistency ($P < 0.005$) were detected with softer stools for infants in the LF-0.6 and LF-1.0 groups versus Study demonstrated routine infant formulas with bLf, (0.6-1.0g/L) a blend of PDX and GOS, and adjusted ARA were safe, well-tolerated, and associated with normal growth when fed to healthy term infants through 365 days of age.
King et al. (2007)	USA	Healthy strictly formula fed infants, ≥ 34 weeks gestation, ≥2000g BW 79 infants enrolled n= 39 bLf formula (26 completed) n=40 control formula (26 completed	To examine the impact of bovine lactoferrin supplementation in infants on growth, hematologic and immune parameters, and the assessment of common childhood illnesses in term or near term infants	bLf formula: Similac with iron and added bLf (850 mg/L) Control formula: Similac with iron (basal Lf content 102 mg/L) Estimated daily intake of bLf* 695 mg/day Duration: through to 1 year of age.	The bLf-enhanced formula was well tolerated. There were significantly fewer lower respiratory tract illnesses, primarily wheezing, in the 26 bLf-fed (0.15 episodes/y) compared with the 26 regular formula-fed (0.5 episodes/y) infants ($P < 0.05$). Significantly higher hematocrit levels at 9 months (37.1% vs 35.4%; $P < 0.05$) occurred in the bLf-supplemented group compared with the control formula group. Lactoferrin

Table 6-2: Clinical studies of bovine lactoferrin in term infants

Reference	Setting	Population	Objective	Intervention	Main results
			Randomized, placebo-controlled, double blind trial.		supplementation was associated with potentially beneficial outcomes such as significantly fewer lower respiratory tract illnesses and higher hematocrits.
Hernell and Lönnerdal (2002)	USA	Healthy term infants (4±2 weeks to 6months) n= 16 exclusively breast fed n = 10-12 exclusively formula fed according to parents choice.	To evaluate the hematologic indices and iron status at 6 months in infants either breastfed or fed formula with differing levels of iron, the addition of lactoferrin and nucleotides.	Formula groups: 1. 1.6 mg Fe/L (Fe as $FeSO_4$), n=12 2. 1.8 mg Fe/L (1.3mg Fe as bLf, 0.5 mg Fe as $FeSO_4$) n= 10 Calculated as 1.05g bLf/L 3. 2.2 mg Fe (as $FeSO_4$) + 40 mg nucleotides / L n=10 4. 4.0 mg Fe/L (as $FeSO_4$) n=11 Estimated daily intake of bLf* 860 mg/day Duration: formula fed for 6 months.	No significant differences in hematology or iron status were observed between groups at 4 and 6 months of age. No effect of bLf on hematological factors or iron status bLf fortification did not effect serum zinc or copper, or the fatty acid composition, except DHA, of the erythrocyte membrane at anytime point. All formula groups exhibited significantly lower levels of DHA in the erythrocyte membrane at 4 & 6 months, versus the breastfed group. At 6 months, no significant difference in mean body weight and height of bLf group vs. breastfed group, 1.6 mg Fe group, or 4.0mg Fe group. Weight and height significantly higher ($p<0.05$) vs. 2.2 mg Fe +nucleotide group. Fortification with bLf or nucleotides did not benefit either iron status or erythrocyte fatty acids No adverse effects of bLf supplementation observed.
Lönnerdal and Hernell (1994)	Sweden	50 healthy term infants, 6±2 weeks of age at enrollment 6 groups including breastfed control n=10 per group	To study the hematologic effects of iron, zinc, copper and selenium supplementation on infant growth, and iron and copper status. Only comparison between breastfed infants and those	Test formula: A: 4.3 mg/l FeSO4, 5 µg/L selenium (Se), 0.4 mg/L Copper (Cu) B: 4.4 mg/l FeSO4, 15.6 µg/L Se, 0.4 mg/L Cu C: 1.3mg Fe /L as bLf, 2.5 mg/L FeSO4, 15.6 µg/L Se, 0.7 mg/L Cu D:4.7 mg/L FeSO4, 3.9 µg/L Se,	There were no significant differences in hematological indices among the groups at 6 months of age; all infants had satisfactory iron status. No significant differences in weight or height of infants at 6 months

Table 6-2: Clinical studies of bovine lactoferrin in term infants

Reference	Setting	Population	Objective	Intervention	Main results
			receiving bLf supplemented formula (C) are relevant. Randomized, double blind trial	0.46 mg/L Cu E: 6.9 mg/L FeSO4, 4.0 µg/L Se, 0.4 mg/L Cu F: breast milk Estimated daily intake of bLf* 860 mg/day Duration: 6 months	No adverse events noted
Roberts et al. (1992) Chierici et al. (1992)	Italy	Healthy term infants. n=12 breastfed n=14 commercial formula n= 15 commercial formula + 10mg bLf/100ml n= 14 commercial formula + 100mg bLf/100ml	To investigate the development of fecal flora in breast- and formula-fed infants in the first 3 months of life, and evaluate the effect of bLf addition to the formula. To evaluate the effects of bLf supplemented formula on serum iron, ferritin and zinc levels. (Second part of study reported by (Roberts et al., 1992))	Feeding groups: A: breastfed B: Standard formula C: Standard formula + 100mg/L bLf D: Standard formula +1000mg/L bLf Estimated daily intake of bLf* 820 mg/day Duration: 3 months /150 days	Breast-fed infants developed a flora rich in Bifidobacterium sp. Facultative anaerobes were ubiquitous, but in relatively small numbers within the diet group. Other obligate anaerobes, such as Clostridium sp. and Bacteriodes sp. were rarely isolated. Standard formula produced a flora rich in Bifidobacteria, but the growth of facultative organisms was not suppressed by this diet. Clostridium sp. and Bacteroides sp. were more common in this feeding group. After the addition of lactoferrin at 10 mg/100 ml to the formula diet, a flora similar to that of the standard formula-fed babies was achieved. Lactoferrin at 100 mg/100 ml was able to establish a "bifidus flora" in half of the babies given this formula, but only at age three months. Clostridium sp. and Bacteroides sp. were common fecal isolates from babies receiving both the lactoferrin diets. Serum zinc levels were not altered by bLf supplementation. Ferritin levels of breast-fed infants were significantly higher than in non-supplemented formula-fed infants at day 30 and day 90. This difference was seen only at day 30, when

Table 6-2: Clinical studies of bovine lactoferrin in term infants

Reference	Setting	Population	Objective	Intervention	Main results
					comparing breast-fed infants to lactoferrin-supplemented formula-fed infants. Comparing the infants receiving formulae, the formula supplemented with the higher amount of bovine lactoferrin induced significantly higher serum ferritin levels compared to the unsupplemented formula at day 90 and day 150, providing putative evidence for a role of bLf in iron absorption. No adverse effects of the bLf were reported in either publication..
Schulz-Lell et al. (1991)	Germany	16 term infants, evaluated from 3rd week of life to 17th. n= 7 test formula n=9 standard formula	To perform iron balance studies in term infants to assess the role of bLf in iron absorption	Test formula= standard formula + 100 mg bLf /100ml Estimated daily intake of bLf* 820 mg/day	The bLf-supplemented group received 169µg Fe/kg BW/day, and the un-supplemented group 118µg Fe/kg BW/day. Iron retention in the bLf-supplemented group was 63µg/kg/d versus 43µg/kg/d in the standard formula group and 32µg/kg/d in breastfed infants. The mean % Fe retention was 36% in the bLf group and 28% in the standard group however there was no significant difference. No adverse effects of the bLf supplemented formula were observed.
Balmer et al. (1989)	UK	Healthy term infants Basic formula n=20 Test formula L n=18 Test formula LF n=20	To determine the effects on fecal flora of the addition of bLf to the diets of bottle fed infants.	Basic formula L: basic formula + bLf 2.8 g/L LF: basic formula + bLf 2.8 g/L + Fe (9.2 mg/L) Estimated mean intake of formula= 1031ml/d, bLf exposure estimated at 2.9 g/day (2900 mg/day) Fecal samples collected and analyzed for microflora and residual Lf	The addition of lactoferrin had little effect upon the fecal microflora and did not move the pattern of the fecal flora in the direction of the breast fed baby. The addition of iron to the formula had more effect on the fecal flora than did lactoferrin. At day 4 it encouraged Escherichia coli and discouraged staphylococcal fecal colonization. At day 14 the addition of iron to the formula discouraged Bifidobacteria. Fecal lactoferrin excretion was higher in the bLf supplemented infants versus those receiving the standard formula, but was less than predicted. No adverse health effects associated with bLf

Table 6-2: Clinical studies of bovine lactoferrin in term infants

Reference	Setting	Population	Objective	Intervention	Main results
					ingestion were observed in this intervention
Fairweather-Tait et al. (1987)	UK	36 healthy term infants (2060-3800g) 29 entered study, four groups n= 8 Basic formula + $^{58}FeCl_3$ n=8 Basic formula +^{58}Fe bLf n=8 High Fe formula +$^{58}FeCl_3$ n=5 High Fe formula +^{58}Fe bLf	To measure the effect of bLf on iron retention in infants.	Infants fed one of 2 formulas Basic Fe= 40 µg Fe/100ml High Fe = 86 µg Fe/100ml Infants fed formula ad libitum. On day 7 they were fed the formula + the ^{58}Fe labeled bLf or $FeCl_3$ Feces collected for 3 day for isotope analysis Estimated daily intake of bLf* 235 mg/day	No differences observed across groups for fecal iron concentration or total iron excreted during the 3-day collection period post administration of labeled Fe. There was no significant difference between the 2 Fe sources, nor did previously fed formula influence iron retention from either labeled source. This study confirmed earlier findings in animal models that Lf does not influence the availability of non-heme iron.
* Where daily total exposure to bLf not stated, this has been calculated using the formula intake levels based on the 2007-2008 NHANES data for infants 0-4 months (mean 818g formula /day)					

6.6.3 bLF in Milk-based Formulas for Toddlers and Children

Seven published studies investigating the potential effects of bLf in children (>12 months of age) are summarized in Table 6-3. The level of bLf intake ranged from 100 mg per day to 3000 mg/day. Two of the studies were in HIV-infected children (Zuccotti et al., 2006; Zuccotti et al., 2007), and one study used a combined bLf and curcumin supplement, the level of bLf and frequency of administration unknown (Zuccotti et al., 2009). Administration of the bLf in the studies conducted in toddlers (>1 year) and children, was typically in the form of oral supplements, versus in a milk-based formula. The daily exposure of children to bLf ranged from 100 mg/day through to 3000 mg/day. The high exposure studies were specifically in children with HIV. The daily amount of bLf consumed in studies with healthy children ranged from 100 mg/day to 1000 mg/day. The mean EDI for children consuming bLf supplemented milk-based formula subject to this notification is estimated to be 77 mg/ day (90^{th} percentile 137 mg/day), is below the daily exposure of children to bLf in these studies. None of the studies reported any adverse events related to administration of the bLf treatment.

A community-based randomized, double blind placebo controlled study in Peruvian children to investigate the effects of bLf on the prevention of diarrhea in children was reported by Ochoa et al. (2008) (NCT00560222). Community health workers visited the homes of participants twice daily (Monday –Saturday) for the duration of the trial (6 months) to administer the treatment (either 0.5 g bLf or 0.5 g maltodextrin placebo) in 25 ml of water, and to gather data. In total, 555 children were randomized: 277 to lactoferrin and 278 to placebo; 65 dropped out; 147,894 doses were administered (92% compliance). Overall there were 91,446 child-days of observation and 1,235 diarrhea episodes lasting 6,219 days. The main pathogens isolated during diarrheal episodes were norovirus (35.0%), enteropathogenic *E. coli* (11.4%), Campylobacter (10.6%), enteroaggregative E. coli (8.4%), enterotoxigenic *E. coli* (6.9%) and Shigella (6.6%). The diarrhea incidence was not different between groups: 5.4 vs. 5.2 episodes/child/year for lactoferrin and placebo, respectively (p=0.375). However, the diarrhea longitudinal prevalence was lower in the lactoferrin group (6.6% vs. 7.0%, p=0.017) as well as the median duration of episodes (4.8 vs. 5.3 days, p=0.046), and the proportion of episodes with moderate or severe dehydration (1.0% vs. 2.6%, p=0.045) as well as the liquid stools load (95.0 vs. 98.6) liquid stools/child/year, (p<0.001). Although there was no reduction in the incidence of diarrhea, longitudinal prevalence and severity decreased with lactoferrin administration. No adverse events related to the intervention occurred.

Zuccotti et al. (2009) investigated the potential of a bLf and curcumin oral supplement to modulate the immune markers in healthy children prone to recurrent respiratory tract infections. The amount and frequency of lactoferrin administered daily could not be determined from the abstract (full paper not available). Together with the safe administration and tolerance of the supplement, beneficial modulation of a number of immune markers (either up-regulated or down-regulated) were observed (Table 6-3), suggesting the oral supplement may be beneficial in supporting immune response in children prone to respiratory tract infections. No treatment related adverse effects were reported.

In a community-based study to determine the effect of bLf supplementation on the prevention of diarrhea, 52 previously weaned children aged 12-36 months were randomized to receive either 0.5 g bLf or, a maltodextrin placebo, twice daily, 6 days per week for 9 months (Ochoa et al., 2008). In addition to addressing the hypothesis that lactoferrin given to previously weaned children will decrease the prevalence of pathogen colonization and /or diarrheal illness, the study also intended to determine the safety and effects of bLf supplementation and collect data for sample size calculations for future larger prospective trials. Importantly, the study determined that bLf supplementation at 1 g/day was safe for children in the age group (12-36 months), with no serious adverse events related to the bLf intervention. Comparison of overall diarrhea incidence and prevalence rates found no statistically significant differences between the intervention and control groups. However, there was a lower prevalence of colonization by *Giardia* species in the lactoferrin group. Furthermore, height-for-age (HFA) scores were significantly greater in the bLf group when analyzed by group ($p=0.03$) and the interaction of group and month ($p=0.03$). There was no difference in weight-for-age scores. It is unknown whether the decrease in prevalence of *Giardia* colonization was related to the HFA z-scores. No treatment related adverse effects were recorded.

Zuccotti et al. (2007) investigated the modulation of innate and adaptive immunity by bLf in human immunodeficiency virus (HIV)-infected, antiretroviral therapy (ART)-naïve children aged 4-17 years. All participants received the treatment: a total of 3 g/day bLf administered in 1 g doses every 8 hours, for 4 weeks. Favorable changes in a number of immune parameters were associated with the bLf intervention; (phagocytosis ($p=0.01$) and killing ($p=0.009$), toll-like receptor 2 (TLR2) expression ($p=0.01$) at 4 weeks versus baseline). bLf also significantly increased the interleukin-12 (IL-12) to IL-10 ratio in LPS (lipopolysaccharide)stimulated CD14+ cells ($p=0.001$) at 4 weeks versus baseline. These immune modulations may be beneficial in HIV positive individuals. No adverse events related to bLf consumption were reported.

In a study designed to demonstrate the potential preventative effects of bLf on rotaviral gastroenteritis in children in a day-care setting, Egashira, Takayanagi, Moriuchi, and Moriuchi (2007) enrolled 298 children under 5 years old who were attending either nursery school or kindergarten. Children with cow's milk allergy or chronic illness were excluded from the trial. Children were allocated to either the bLf group (100 mg bLf per day either as tablet or in a yoghurt), or a placebo. The bLf was in combination with lactulose and bifidobacterium (concentrations not stated). Although the number of children with rotaviral gastroenteritis was similar between treatment groups, the frequency ($p=0.0106$) and duration ($p=0.0137$) of vomiting and frequency ($p=0.0446$) and duration ($p=0.0285$) of diarrhea were all significantly decreased in the bLf group versus the control group. No adverse event attributable to the bLf intervention occurred.

Zuccotti et al. (2006) aimed to evaluate the plasma viral load and CD4+ cell counts in HIV-1 vertically infected children before, during, and following a 6-month intervention with oral bLf supplementation. The study also investigated the response of HIV-infected children to bLf supplementation in relation to the ARV (antiretroviral therapy) they were receiving at the time of enrolment. Twenty-two (22) children between the ages of 3 and 18 years (mean age 9 years) were enrolled in the study and each received the bLf treatment (3 x 1 g doses 8 hourly, total 3 g/day) for the duration of the study. No significant changes were observed during the pre-treatment period. By 6 months, mean (± SD) plasma viral load (log_{10}) declined from 4.54 (± 0.65) to 4.28 (± 0.60); median percentage CD4+ cell count increased from 21.5% to 24.5%. Two months after treatment discontinuation, mean plasma viral load did not differ significantly from baseline or month 6 levels, but the percentage CD4+ cell count remained significantly higher than the baseline value. LF plus antiretroviral (ARV) therapy was more effective at increasing CD4+ cell count than LF alone. None of the patients showed any new HIV-1-related symptoms at follow-up. The researchers concluded bLf may be a useful addition to ARV therapy. No adverse events reported to bLf administration were reported.

In a study investigating the effects of bLf on *H. pylori* colonization in children and adults, Okuda et al. (2005) enrolled 25 healthy children and 34 healthy adults with a diagnosed *H. pylori* infection either with or without minimal upper gastrointestinal symptoms, and who were not undergoing treatment for the infection. *H. pylori* infection was diagnosed when the ^{13}C-urea breath test (UBT) and a serum–based or urine-based ELISA (enzyme-linked immunosorbent assay) were both positive. A decrease in UBT value of >50% of the baseline value between week 0 and week 12 was considered a positive response. Subjects were mostly randomized to receive either the bLf (two x 100 mg tablets twice per day, total 400 mg/day) or the matched placebo tablets (two x 100 mg tablets twice per day). Siblings and their parents were grouped to prevent mixing of treatments. In the combined (child and adult) cohort after 12 weeks

supplementation a positive response was observed in 10 of 31 bLf-treated subjects (32.3%) and 1 of 28 control subjects (3.6%) indicating that the rate of positive response in the bLf group was significantly higher than that in the control group (bLf vs. control $p<0.01$). The UBT levels of most responders in bLf group returned to baseline levels by 4 weeks after the end of the study. No adverse effects of bLf were reported.

Table 6-3: Clinical studies of bovine lactoferrin in toddlers (>1year) and children

Reference	Setting	Population	Objective	Intervention	Main results
Ochoa et al. (2013)	Peru	Children 12 -18 months at enrollment. n= 277 bLf n=278 placebo	To determine the effect of bovine lactoferrin on prevention of diarrhea in children Randomized, placebo-controlled double blind trial	2 x 0.5 g per day bLf (total 1 g/day) or placebo (maltodextrin) diluted in 25 mL water. Given under supervision, and data collected by health workers. Duration = 6 months NCT00560222	No impact on overall diarrhea incidence or pathogen specific diarrhea rates, however longitudinal prevalence and severity were reduced with lactoferrin. A clinically significant but small increase in HFA (height-for-age) ($p<0.01$) noted in bLf group over duration of study, compared to placebo group, however no differences in WFA (weight-for -age) . Interpretation / significance unknown. No adverse events related to intervention reported.
Zuccotti et al. (2009)	Italy	Healthy children with recurrent respiratory tract infections	To determine the immune modulation potential of an oral lactoferrin and curcumin supplement in children with recurrent respiratory tract infections	Mixed lactoferrin and curcumin (LC) oral supplement (concentration unknown) Control: unknown	Reduction of infection rates in children receiving the LC supplement. LC supplementation resulted in a significant skewing of CD8+T lymphocytes maturation. Additionally: 1) CD14+, toll like receptor (TLR) 2-expressing cells augmented ($p= 0.005$) whereas CD14+/TLR4+ diminished ($p= 0.004$); and 2) IL10 production by CD14+ cells was reduced in children receiving LC. LC supplementation results in immune modulation and could be clinically beneficial.
Ochoa et al. (2008)	Peru	52 previously weaned children n=26 bLf n=26 placebo	To determine the effect of bovine lactoferrin on prevention of diarrhea in children Randomized, placebo-controlled double blind trial	2 x 0.5 g per day bLf (total 1 g/day) or placebo (maltodextrin) for 6 days per week Duration = 9 months	No significant difference in incidence or prevalence rates of diarrhea in between groups observed There was a lower prevalence of colonization with *Giardia* species and better growth among the children in the lactoferrin group. No adverse events related to intervention reported.
Zuccotti et al. (2007)	Italy	11 HIV-infected, ART-naïve children aged 4-7 years n= 11 bLf (no control/ comparator)	To assess the effect of bLf supplementation on immunological parameters in HIV-infected, ART-naïve children.	Oral supplement of 1 g bLf every 8 hours (total 3 g/day) no control Duration: 4 weeks	A short course of LF results in immune modulation of the innate and adaptive immune responses. In particular, skewing of theCD8T-lymphocyte differentiation pathway towards the mature, lytic forms, and a significant increase in phagocytosis and killing by CD13+ phagocytes indicate that different immune cell populations, as well as diverse effector mechanisms, are stimulated by LF.
Egashira et al. (2007)	Japan	298 children under 5	To demonstrate *in vivo*	100 mg bLf per day as either	Frequency ($p=0.0106$) and duration ($p=0.0137$) of

Table 6-3: Clinical studies of bovine lactoferrin in toddlers (>1year) and children

Reference	Setting	Population	Objective	Intervention	Main results
		years attending either nursery school or kindergarten n=136 (final analysis) bLf n=98 (final analysis) placebo	effects of bovine lactoferrin on rotaviral gastroenteritis in children. in a day care setting Open-label, non-randomized study	tablet or in a yoghurt. Placebo not stated. Duration: 12 weeks	vomiting and frequency (p=0.0446) and duration (p=0.0285) of diarrhea were all significantly decreased in the bLf group versus control group The number of children with rotaviral gastroenteritis was similar between treatment groups There was no significant difference in the duration of fever between the treatment groups. No adverse events related to intervention reported.
Zuccotti et al. (2006)	Italy	22 HIV-1 vertically infected children 3-18 years (mean age 9 years) at enrollment.	To evaluate plasma viral load and CD4+ cell counts in HIV-1 vertically infected children before, during and after a 6-month period of oral bLF supplementation.	3 g bLf per day orally (1g administered 8 hourly) Given in addition to ARV (antiretroviral) treatments. No control Duration: 6 months	Significant reduction in plasma viral load during treatment however did not persist after discontinuation. Percentage CD4+ counts increased and remained significantly higher than baseline at 3 month follow-up . bLf plus ARV therapy was more effective at increasing CD4+ cell counts than bLf alone, suggesting bLf may be a useful addition to ARV therapy.
Okuda et al. (2005)	Japan	25 healthy children n= 14 bLf n=11 placebo (& 34 healthy adults) with *H.pylori* infection either without or with minor upper gastrointestinal symptoms who were not being treated.	To evaluate the efficacy of bLf on *H. pylori* colonization in humans *H. pylori* infection was diagnosed when both the ^{13}C-urea breath test (UBT) (positive response defined as >50% decrease of UBT value) and serum or urine-based ELISA (immunosorbent assay) were positive. Randomized, double-blind, placebo-controlled trial.	Children only 2 x 100 mg tablets bLf twice daily (400 mg bLf /day) or 2 x placebo tabs twice per day Duration: 12 weeks	Mean UBT values were significantly different at week 0 between the 2 child groups (p<0.01), which may have introduced greater tendency for change in UBT observed, versus adult group. In the combined (child and adult) cohort after 12 weeks supplementation a positive response was observed in 10 of 31 bLf-treated subjects (32.3%) and 1 of 28 control subjects (3.6%) indicating that the rate of positive response in the bLf group was significantly higher than that in the control group (bLf vs. control p<0.01) The UBT levels of most responders in bLf group returned to baseline levels by 4 weeks after the end of the study. Overall the results suggest bLf administration is effective to suppress *H. pylori* colonization.

6.6.4 Summary of Studies of the effects of bLf in Infant Formula and Toddler Formula

A significant body of evidence supports the safety of bLf for infants, and provides support for the safe consumption of bLf under the intended use in milk-based formula for term infants and toddlers. In the 36 clinical trials identified in infants (from preterm and term at birth – 12 months) and in children (>12 months) and involving approximately 4000 participants, no adverse events related to the administration of bLf have been reported. Studies consistently report that the use of bLf is well tolerated.

These studies include a wide range of exposure to bLf levels; (from 9.75 mg/kg BW/day (Kawaguchi et al., 1986) to 200 mg/kg BW/day (Ochoa et al., 2015) in preterm and VLBW infants; 36 mg/day (Chen et al., 2016) to 2,300 mg/day (Balmer et al., 1989) in term infants; and, 100 mg/day (Egashira et al., 2007) to 3,000 mg/day (Zuccotti et al., 2009) in children).

The range in amounts of bLf safely consumed and tolerated in these studies adequately addresses the maximum predicted EDI's of bLf subject to this notification (mean 102 mg/day, or 17.9 mg/kg BW/day, 90th percentile 148 mg/day or 27 mg/kg/BW /day) in term infants aged 0 - 4 months Table 3-1.

Most of the studies in preterm and/ or VLBW infants are typically targeted toward the reduction in incidence or duration of LOS, NEC or other infective conditions to which this highly vulnerable population is especially prone. The studies confirm the safe consumption and tolerance of bLf in these infants, and suggest that bLf affords a degree of protection from infection but not necessarily colonization by pathogenic species (Manzoni, 2016).

The safety and tolerance of bLf for term infants has recently been specifically addressed in the study by Johnston et al. (2015) who investigated the use of bLf in term formulas at levels of 0.6 g/L and 1.0 g/L, each of which are 4 – 5 times the level of bLf added to term formula that is the intended use of this notification. The study concluded that bLf, together with other functional ingredients, added to formula was safe, well tolerated and associated with normal infant growth. This is supported by a number of other studies, that have also looked at potential benefits of bLf added to term formula. Formula supplemented with bLf may decrease the burden of respiratory (King et al., 2007) and gastrointestinal (Chen et al., 2016; Ochoa et al., 2013) morbidity in term infants (Manzoni, 2016). A number of studies have considered the role of bLf as an iron source, however there is little evidence to suggest it provides a more bioavailable source than that of elemental iron sources (Lönnerdal & Hernell, 1994), although there is some evidence to suggest bLf is involved in iron transport (Chierici et al., 1992). Other studies suggest bLf may modulate

fecal flora (Liu et al., 2016; Roberts et al., 1992), and thereby provide indirectly a range of other developmental and health benefits to infants.

The potential of bLf to prevent or mitigate the severity of infections in toddlers and children has been investigated in a number of studies, all of which have not reported any adverse treatment related effects. In especially vulnerable immunocompromised children, high daily doses of bLf were well tolerated and no adverse treatment related effects were reported.

In conclusion, there are a substantial number of studies in infants and toddlers that provide convincing and consistent evidence for the safe consumption and tolerance of bLf for the intended use in milk-based formula for term infants and toddlers.

6.7 CONCLUSIONS FROM SAFETY AND HUMAN STUDIES WITH bLf

Having reviewed the available data and information, Synlait concludes the significant body of evidence, including both animal and human exposure and safety data, supports the safe consumption of bLf under the intended conditions of use in milk-based formula for term infants and toddlers. Specifically:

- Studies on the metabolic fate of lactoferrin show that it is only partially degraded in the gastric phase, and is, therefore, available in either intact or as large fragments for biological action post the gastric digestion phase.
- The biological activities of dietary lactoferrin occur as either local effects in the gut lumen, or as systemic effects.
- The piglet is an appropriate model for studying the metabolic fate of dietary components, such as lactoferrin, as it relates to infants due to the similar physiology of gastrointestinal development.
- In both piglets and human infants intact and large fragments of lactoferrin are excreted in the feces and urine.
- Based on studies in piglets, lactoferrin receptors in the brush border membrane of the intestinal lumen transport lactoferrin into the systemic circulation via the portal vein. Once in peripheral circulation, it can be excreted into the bile and reabsorbed into the blood-stream, suggesting the presence of entero-hepatic circulation of bLf.
- Evidence also suggests that bLf can be transported from the serum into cerebrospinal fluid, potentially signaling links to neurodevelopment.

- The direct interaction of lactoferrin with cells of the gastrointestinal tract and its ability to modulate cellular function and affect the regulatory functions of proteins is also understood to be a key mechanism of how lactoferrin is able to influence gut maturation, and inflammation, and regulate the homeostasis of the immature gut of infants.
- Both acute and sub-chronic (4-week and 13-week) oral toxicological studies in rats indicate that bLf is safe for consumption, with a no observed adverse effects level (NOAEL) of 2000 mg/kg, and does not result in treatment-related adverse effects or significant changes in clinical measures.
- Chronic oral toxicity was evaluated in 40 and 65-week feeding studies containing bLf at 0.2% of diet and up to 5% of the diet respectively. No significant treatment related effects were reported in either study, however the studies could not be used to establish a NOAEL.
- No potential mutagenicity of bLf was determined, based on the Ames test.
- Cow's milk allergy (CMA) is a hypersensitivity reaction initiated by immunologic mechanisms in response to bovine proteins. In most children it is IgE-mediated. Currently there is no evidence to support a role for lactoferrin as a causative agent in CMA. The intended uses of bLf are in milk-based formula, which by law require labeling for the major allergen, milk protein of which bLf is a minor fraction.
- Human tolerance and safety of bLf has been established in a large number (36) of intervention studies in infants (pre-term and VLBW, term) and young children. The studies consistently report that the addition of bLf to formula or as a supplement was well tolerated, or that no adverse treatment-related effects were observed. Furthermore, the range of bLf safely consumed and tolerated in these studies adequately addresses the maximum predicted total (background formula levels of bLf plus added bLf) EDI's of bLf subject to this notification (mean 269 mg/day, or 44 mg/kg BW/day, 90th percentile 395 mg/day or 69 mg/kg/BW /day) in term infants aged 0 - 6 months.

6.8 SUMMARY

Synlait has reviewed the available data and information and is not aware of any data and information that are, or may appear to be, inconsistent with its conclusion that bLf has been determined to be safe under the conditions of its intended use. This conclusion, that bLf under the intended conditions of use, bLf is GRAS, is consistent with that of suitably qualified independent experts (the GRAS Panel), as evidenced in Part 1 of this GRAS Notice

PART 7 SUPPORTING DATA AND INFORMATION

7.1 ABBREVIATIONS

Abbreviation	Description
AAP	American Academy of Pediatrics
AE	Adverse Events
AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
ARA	Arachidonic Acid
ARV	Antiretroviral
AS	American Standards
BAM	Bacterial Analytical Manual
BBMV	Brush Border Membrane Vesicles
bLf	Bovine Milk-derived Lactoferrin
BS	British Standards
BW	Body Weight
CAS	Chemical Abstracts Service
CCP	Critical Control Point
Co.	Company
CFR	Code of Federal Regulations
cfu	Colony Forming Units
Ch.	Chapter

Abbreviation	Description
CLD	Chronic Lung Disease
CMA	Cows Milk Allergy
CV	Coefficient of variation
Da	Daltons
DHA	Docosahexaenoic Acid
EC	European Commision
EDI	Estimated Daily Intake
ELISA	Enzyme-linked Immunosorbent Assay
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EU	Endotoxin Units
EU	European Union
FALCPA	Food Allergen Labeling and Consumer Protection Act
FCC	Food Chemical Codex
FDA	Food and Drug Administration
FeSO4	Ferrous Sulphate
FFDC	Federal Food, Drug, and Cosmetic Act

Abbreviation	Description
FOSHU	Foods for Specified Health Use
FR	Federal Register
FSAI	Food Safety Authority of Ireland
FSANZ	Food Standards Australia New Zealand
FSIS	Food Safety and Inspection Service
g	Gram
GI	Gastrointestinal
GRAS	Generally Recognized as Safe
GRN	The file number FDA assigns to a GRAS notice
HACCP	Hazard Analysis Critical Control Point
HFA	Height-for-age
HIF-1	Hydroxy Inducible Factor-1
HIV	Human Immunodeficiency Virus
hLfR	Human Lactoferrin Receptor
HM	Human Milk
HPLC	High Performance Liquid Chromatography
ICP	Inductively Coupled Plasma
IDF	International Dairy Federation
IEC	Intestinal Epithelial Cells

Abbreviation	Description
IgE	Immunoglobulin E
ISO	International Standards Organization
ITT	Intent-to-treat
kg	Kilogram
L	Liter
LBW	Low Birth Weight
LD_{50}	Median Lethal Dose
LC	Liquid Chromatography
LF	Lactoferrin
LOS	Late Onset Sepsis
LRTI	Lower Respiratory Tract Infections
MCV	Mean Corpuscular Volume
mg	Milligram
min.	Minimum
ml	milliliter
MLN	Mesenteric Lymph Nodes
MPI	Ministry of Primary Industries
MPN	Most Probabble Number
MS	Mass Spectrometry
N/A	Not Applicable
NaCl	Sodium Chloride
NCHS	National Center for Health Statistics
ND	Not Detected
NDA	Panel on Dietetic Products,

Abbreviation	Description
	Nutrition and Allergy
NEC	Necrotizing Enterocolitis
NCHS	National Center for Health Statistics
NHANES	National Health and Nutrition Examination Survey
NICU	Neonatal Intensive Care Unit
NOAEL	No Observed Adverse Effects Level
NZ	New Zealand
NZFSA	New Zealand Food Safety Authority
NZJDST	New Zealand Journal of Dairy Science and Technology
OES	Optical Emission Spectrometry
p. *or* pg.	Page
PDX	Polydextrose
PES	Polyethersulfone
ppb	Parts per billion
ppm	Parts per million
PSU	Primary Sampling Unit
PVL	Periventricular Leukomalacia
Reg. No.	Registration Number
SAE	Serious Adverse Events

Abbreviation	Description
RD	Rural Delivery
RH	Relative Humidity
RMP	Risk Management Programme
RO	Reverse Osmosis
RTD	Ready-to-drink
RTF	Ready-to-feed
SCFA	Short Chain Fatty Acids
SD	Standard Deviation
sp.	Species
TCH	Technical Manual
TfR	Transfer Receptor
TIBC	Total Iron Binding Capacity
TLf	Talactoferrin
Treg	Regulatory T-cell
UBT	Urea Breath Test
US	United States
USA	United States of America
USDA	United Sates Department of Agriculture
USFDA	United States Food and Drug Administration
VLBW	Very Low Birth Weight
WFA	Weight-for-age
°C	Degrees Centigrade
%m/m	Percentage mass/mass

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Zuccotti, G. V., Vigano, A., Borelli, M., Saresella, M., Giacomet, V., & Clerici, M. (2007). Modulation of innate and adaptive immunity by lactoferrin in human immunodeficiency virus (HIV)-infected, antiretroviral therapy-naive children. *Int J Antimicrob Agents, 29*(3), 353-355. doi:10.1016/j.ijantimicag.2006.11.017

PART 7:
APPENDIX 1: Raw Material And Packaging Specifications

The data and information presented within Appendix 1 is Confidential to Synlait Milk Ltd and is **not generally available**.

Document Information

Product Name	:	Lactoferrin Powder		
Prepared by	:	Arnab Sarkar	Status :	Approved
Supersedes	:	NA		

Product Identification

This product can be identified in various systems as the following:

System Name	System ID	Coding
Synlait ERP	M3	LFN05105

Product Attributes

Description	:	95% Protein, pasteurised, spray dried lactoferrin, pink to tan, free flowing powder.
Product descriptor	:	Lactoferrin (38)
Allergen(s)	:	Dairy Product
Traceability	:	Production lot record
Ingredients	:	Lactoferrin

General Composition

Parameter	Unit	Typical	Min	Max	Test Method
Protein as is	%m/m		95		ISO 8968-1 / IDF 20-1:2001, AOAC 991.20
Lactoferrin	% Protein		95		HPLC method (In House Method: TCH-05-0009)
Ash	%m/m			1.3	BS 1741:1988 (modified), BS 1743:1968 (modified)
Moisture	%m/m			4.5	IDF 26A: 1993
Iron	mg/kg			200	Acid Digest, ICP OES
Iron Saturation	%			20	In house method (TCH-05-0011)

Physical and Chemical Attributes

Parameter	Unit	Typical	Min	Max	Test Method
Sediment	/25g	A	A	A	ADMI Bull. 916 1990
Foreign matter	/25g	Absent		Absent	AS 2300.4.5:1994
pH		6.0	5.2	7.2	BS770:1986, ISO 7238 / IDF 104:2004, IDF 115A:1989, APHA (17th Edition) Ch 15
Solubility		Transparent			In house method (2% solution, 20°C) TCH-05-0010

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Sensory Attributes

Parameter	Description	Test method
Appearance	pink to tan, free flowing powder	Visual Observation

Microbiological Standards

Parameter	Unit	Max.	Test method
Aerobic Plate Count	cfu/g	1000	ISO 4833:2003
E.coli	/g	Not detected	ISO 11866 – 1:2005 (E)/IDF 170-1 :2005 (E) (mod)
Yeast and moulds	cfu/g	10	ISO 6611/IDF 94:2004
Salmonella	/250g	Not detected	ISO 6579:2002 (E)
Coagulase Positive Staphylococcus	/g	Not detected	ISO 6888-3:2003
Coliform	/g	Not detected	ISO 4832:2006
E.sakazaki	/300g	Not detected	ISO/TS 22964 / IDF/RM 210:2006
Enterobacteriaceae	/g	Not detected	ISO 21528-1:2004

Contaminants and Residues

Parameter	Unit	Limit	Test method
Nitrates	mg/kg	≤50	NZJDST 15, 83-90, 1980, ISO 14673-2, IDF 189-2, AOAC 968.07 (mod)
Nitrites	mg/kg	≤2	NZJDST 15, 83-90, 1980, ISO 14673-2, IDF 189-2, AOAC 968.07 (mod)
Heavy Metals	mg/kg	<10	Acid Digest ICPMS
Melamine [1]	ppm	<0.1	LC-MS/MS **(Detectable limit)**
Arsenic [1]	mg/kg	<0.02	Wet oxidation ICP MS **(Detectable Limit)**
Aluminium	mg/kg	<4.8	Wet oxidation ICP-MS
Cadmium	mg/kg	<0.1	Wet oxidation ICP-MS
Mercury	mg/kg	<0.1	Acid Digest ICPMS
Lead	mg/kg	<0.15	Wet oxidation ICP MS
Aflatoxin M1	µg/kg	<0.5	G Barbieri et al, J Food Sci, 59 (1994) p1313-

[1] to be reported as "Not Detected" on the COA

Product Statements

This product complies with the following requirements:

General spec	Spec descriptions
	HALAL
	GMO-free

This product is manufactured and packed according to Synlait RMP requirements

Synlait Milk Ltd
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Packaging

Packaging materials	Descriptions
Foil bag	5 kg – Laminated foil quad pouch (polyester 12 µm/foil 7 µm/PE 130 µm)
Carton	2 x 5 kg bags – RSC STC 510*380*160
Pallet detail	30 carton per pallet

Labelling Information

Each bag is pre-printed with	Synlait ™ Spray Dried Lactoferrin Net weight 5 kg Product of New Zealand Registration Number – 540 Address details Storage details Pasteurised product Fit for human consumption
Each bag is labelled with	Production date and best before date Bag Number Lot number
Each Carton is pre-printed with	Synlait ™ Spray Dried Lactoferrin Net weight 10 kg Product of New Zealand Registration Number – 540 Address details
Each Carton is labelled with	Store cool, dry, ventilated Production date Lot number Carton Number Units per Carton: 2 bags

Storage

Shelf life	:	36 months
Storage instructions	:	Temperature < 25 °C Relative humidity <65% Store in cool, dry, and well ventilated place Stored off the floor and away from walls once opened use within 1 month

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Revision History

Version	Nature of Change	Initiated by	Approved by	Date dd-mm-yyyy
1	New spec.	Arnab S.	Tom A.	04-03-2014

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FDA COMPLIANCE

All FSI polypropylene filtration media product lines are manufactured using FDA compliant materials under the Federal Food, Drug, and Cosmetic Act under regulations:

21 C.F.R. 177.1520 (c) 1.1
21 C.F.R. 177.2800
21 C.F.R. 178.3400

Provided that the end user is complying with FDA's good manufacturing practices under Title 21 C.F.R. 174.5.

(b) (6)

Quality Assurance Manager

5/30/13
date

KOCH MEMBRANE SYSTEMS

KMS HFK□ -131 FOOD □ DAIRY UF ELEMENTS

Ultrafiltration 4", 6" and 8" Spiral Element Series

PRODUCT DESCRIPTION

Membrane Chemistry:	Proprietary semi-permeable polyethersulfone (PES)
Membrane Type:	HFK™-131 with observed separation range of 10,000 Daltons
Construction:	Sanitary spiral wound element with net outer wrap
Regulatory Status:	Conform to USDA 3-A standards and FDA regulations (CFR Title 21)
Options:	Diameter: 3.8", 4.3", 6.3", 6.4", 8.0", or 8.3"
	Length: 33", 35.5", or 38"
	Feed Spacer: N (31 mil), V (46 mil), H (62 mil), or F (80 mil), D (100 mil)
	Outer wrap: Controlled (e.g. NYV) or trimmable (e.g. NYT)

SPECIFICATIONS

Model	Active Membrane Area									
	NYV/T Spacer (31 mil)		VYV/T Spacer (46 mil)		HYV/T Spacer (62 mil)		FYV/T Spacer (80 mil)		DYV/T Spacer (100 mil)	
	ft²	(m²)	ft²	(m²)	ft²	(m²)	ft²	(m²)	ft²	(m²)
3838 HFK-131	72	(6.7)	58	(5.4)	45	(4.2)	-	-	-	-
4333 HFK-131	93	(8.6)	73	(6.8)	55	(5.1)	44	(4.1)	-	-
4336 HFK-131	95	(8.8)	79	(7.3)	59	(5.5)	-	-	-	-
4338 HFK-131	102	(9.5)	81	(7.5)	-	-	-	-	-	-
6338 HFK-131	228	(21.2)	180	(16.7)	142	(13.2)	119	(11.1)	102	(9.5)
6438 HFK-131	228	(21.2)	180	(16.7)	142	(13.2)	119	(11.1)	-	-
8038 HFK-131	358	(33.2)	276	(25.6)	215	(20.0)	-	-	-	-
8338 HFK-131	-	-	308	(28.6)	241	(22.4)	194	(18.0)	-	-

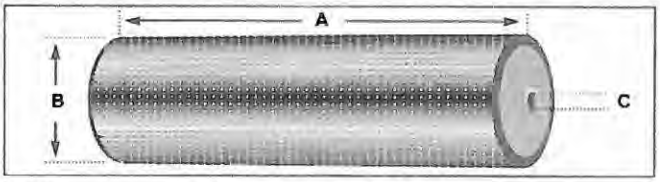
Not all combinations are available.
6438 elements are only available in controlled configuration. 6338 elements are only available in trimmable configuration.

OPERATING AND DESIGN INFORMATION*

Typical Operating Pressure:	30 - 120 psi (2.1 - 8.3 bar)
Maximum Operating Pressure:	140 psi (9.7 bar)
Operating Temperature Range:	41 - 131°F (5 - 55°C)
Cleaning Temperature Range:	105 - 122°F (40 - 50°C)
Allowable pH - Continuous Operation:	2.0 - 10.0
Allowable pH - Clean-In-Place (CIP):	1.8 - 11.0
Design Pressure Drop Per Element:	N spacer: 12-15 psi (0.8-1.0 bar)
	V spacer: 15-20 psi (1.0-1.4 bar)
	H or F spacer: 15-25 psi (1.0-1.7 bar)
Design Pressure Drop Per Vessel (3 in series):	N spacer: 36-45 psi (2.5-3.1 bar)
	V spacer: 45-60 psi (3.1-4.1 bar)
	H or F spacer: 45-75 psi (3.1-5.2 bar)
Design Pressure Drop Per Vessel (4 in series):	N spacer: 48-60 psi (3.3-4.1 bar)
	V spacer: 60-68 psi (4.1-4.7 bar)

* Consult KMS Process Technology Group for specific applications.

NOMINAL DIMENSIONS



Model	A	B	C
	inches (mm)	inches (mm)	inches (mm)
3838 HFK-131	38.0 (965)	3.8 (96)	0.831 (21.1)
4333 HFK-131	33.0 (838)	4.3 (109)	0.831 (21.1)
4336 HFK-131	35.5 (902)	4.3 (109)	0.831 (21.1)
4338 HFK-131	38.0 (965)	4.3 (109)	0.831 (21.1)
6338 HFK-131	38.0 (965)	6.3 (160)	1.138 (28.9)
6438 HFK-131	38.0 (965)	6.4 (162)	1.138 (28.9)
8038 HFK-131	38.0 (965)	7.9 (201)	1.138 (28.9)
8338 HFK-131	38.0 (965)	8.3 (211)	1.138 (28.9)

Note: Not all combinations are available.

Membrane Characteristics:

- The membrane used in these modules consists of a semipermeable polyethersulfone (PES) layer on a polyester backing material.
- Pure water flux of these PES HFK-131 membranes is 1.0-2.2 gfd/psi (24-53 l/m²/h/bar) at 77°F (25°C).
-

Operating Limits:

- **Operating Pressure:** Maximum operating pressure is 140 psi (9.7 bar).
- **Permeate Pressure:** Permeate pressure should not exceed baseline (concentrate) pressure at any time (including on-line, off-line and during transition). Reverse pressure will damage the membrane.
- **Differential Pressure:** The maximum differential pressures per element are listed on the front of this document, including design values for multi-element housings.
- **Temperature:** Maximum operating temperature is 131°F (55°C). Maximum cleaning temperature is 122°F (50°C).
- **pH:** Allowable range for continuous operation is 2.0 to 10.0. Allowable pH range for cleaning is 1.8 to 11.0.

Water Quality for Cleaning & Diafiltration:

- **Turbidity and SDI:** Maximum feed turbidity is 1 NTU. Maximum feed SDI is 5.0 (15-minute test).
- **Guidelines:** Please refer to the KMS "Water Quality Guidelines for CIP and Diafiltration" for more detailed information.

Chlorine and Chemical Exposure:

- Adherence to cleaning and sanitizing procedures including chemical concentrations, pH, temperature, and exposure time is necessary to achieve maximum useful element life. Accurate records should be maintained.
- KMS standard cleaning procedures for dairy applications should be followed. Recommended chlorine exposure time at the defined conditions is 30 minutes per day.
- Residual chlorine concentration during cleaning cycle (CIP) should be 150 ppm @ pH 10.5 or higher. Chlorine concentration should never exceed 200 ppm.
- Chlorine should only be added to the cleaning solution after the pH has been adjusted to 10.5 or higher.
- Iron or other catalyzing metals in the presence of free chlorine or hydrogen peroxide will accelerate membrane degradation.
- Sanitizing should be done only after a complete cleaning cycle and with water of acceptable quality. Refer to cleaning instructions and feedwater quality technical bulletins.

Cationic Polymers and Surfactants:

HFK-131 membranes may be irreversibly fouled if exposed to cationic (positively charged) polymers or surfactants. Exposure to these chemicals during operation or cleaning is not recommended and will void the warranty.

Lubricants:

For element installation, use only water or glycerin to lubricate seals. The use of petroleum or vegetable-based oils or solvents may damage the element and will void the warranty.

Supplemental Technical Bulletins:

- UF Element Cleaning Procedures
- Water Quality Guidelines for CIP and Diafiltration

Service and Ongoing Technical Support:

KMS has an experienced staff available to assist end-users and OEM's for optimization of existing systems and development of new applications. KMS also offers a complete line of KOCHKLEEN® membrane pretreatment, cleaning, and maintenance chemicals.

KMS Capability

KMS is the leader in crossflow membrane technology, manufacturing reverse osmosis, nanofiltration, microfiltration, and ultrafiltration membranes and membrane systems. The industries we serve include food, dairy and beverage, semiconductors, automotive, water and wastewater, chemical and general manufacturing. KMS adds value by providing top quality membrane products and by sharing our experience in the design and supply of thousands of crossflow membrane systems worldwide.

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• San Diego, California • Aachen, Germany • Lyon, France • Madrid, Spain • Milan, Italy • Wijnegem, Belgium • Beijing & Shanghai, China • Mumbai, India •
• Melbourne, Australia • Singapore • Sao Paulo, Brazil • Manama, Kingdom of Bahrain

Document Information

Material Name	:	Cheese Salt
Prepared by	:	Jo Steven
Supersedes	:	V3

Status :

Draft	Approved
	X

Material Identification

This product can be identified in various systems as the following:

System Name	Item name (as per M3)	Coding
Synlait ERP	Cheese Salt	RMIN00049
Dominion Salt	-	PDV Cheese Grade Salt

Material Attributes

Description	:	Pure dried vacuum (PDV) salt, with anticaking agent sodium ferrocyanide (E535). **Note: Anticaking agent not allowed for use for infant products**
Alternative name	:	Sodium Chloride, NaCl
Supplier	:	Dominion Salt, New Zealand; Production Site: Lake Grassmere (LG), or Mt Maunganui (MM)
Allergen(s)	:	None
Contains Dairy Material	:	No
Traceability	:	Production Batch
Grade	:	Food Grade
Ingredients	:	Salt, Sodium Ferrocyanide (E535)

Documentation Requirements

This product needs to comply with following requirements:

Documents Required	Frequency
Certificate of Analysis (CoA)	Every shipment
HALAL	On request
KOSHER	On request
GMO-free certificate/ declaration	On request
MSDS	On request
Allergen documentation	On request
Dairy material declaration as required (SOR / FIC & accompanying Health Cert.) Must contain the following attestations: Were derived only from animals and processed in countries which are recognised by the OIE World Organisation for Animal Health as free of foot and mouth disease, with or without vaccination; Were derived only from animals which meet OIE requirements for lumpy skin disease, sheep pox and goat pox freedom; The country of origin has controls in place to ensure that only healthy animals are used for milk production	N/A
Other technical documents	On request
Packing list	Every shipment

This product needs to be manufactured and packed according to HACCP regulations

General Composition

Parameter	Unit	Typical	Min	Max	Required on CoA	Comment	Testing plan (Synlait)*
Sodium Chloride	% DM		99.6		On Request	Monthly Monitoring	High + SL
Moisture	%			0.2	Yes	-	Low
Sodium Ferrocyanide	ppm			15	Yes	May be reported on CoA as Anticaking Agent $[Fe(CN)_6]^{4-}$	Low
Matter insoluble in water	ppm			300	On Request	-	Low

Physical and Chemical Attributes

Parameter	Unit	Typical	Min	Max	Required on CoA	Comment	Testing plan (Synlait)*
Scorched Particles (Black specks)	Disc/50g			A	Yes	ADMI Method. May be reported on CoA as visual foreign matter	Low
Other Foreign Matter	/50g		Absent		Yes	May be reported on CoA as unacceptable foreign matter absent	Low
Particle size passing 212µm	%			2	Yes	-	N/A
Particle size passing 850µm	%		100		Yes	-	N/A

Sensory Attributes

Parameter	Description	Required on CoA	Testing plan (Synlait)*
Appearance	White, relatively coarse uniformly sized crystals. No caking that does not break up under moderate pressure.	On Request	High (Internal Evaluation) + SL
Odour	Odourless - no foreign or off-odours	On Request	High (Internal Evaluation) + SL

Contaminants and Residues

Parameter	Unit	Limit (Max)	Required on CoA	Comment	Testing plan (Synlait)*
Cadmium (Cd)	mg/kg	0.2	Yes	Yearly Monitoring	Low
Arsenic (As)	mg/kg	0.5	Yes	Yearly Monitoring	Low
Copper (Cu)	mg/kg	2	On Request	Monthly Monitoring	N/A
Iron (Fe)	mg/kg	10	On Request	Monthly Monitoring	N/A
Lead (Pb)	mg/kg	1	Yes	Yearly Monitoring	Low
Mercury (Hg)	mg/kg	0.05	Yes	Yearly Monitoring	Low
Alkalinity (as Na_2CO_3)	mg/kg	300	On Request	Monthly Monitoring	N/A

*Test plan for Synlait RM test procedure: high = test every time; low = reduced test can be used when applicable; N/A: not tested (e.g. due to test method capability); +SL= tested when shelf-life extension is required.

Packaging

Pack Size	Descriptions
25 kg	Plastic (Polyethylene) Bag. Packaging must be suitable for food contact.

Labelling Information

This information is required on the label in accordance with the Australia New Zealand Food Standards Code:

- Product name
- Manufacturer's name and address
- Ingredient list (if applicable) – on the label or in accompanying documentation
- Date of manufacture
- Expiry or Best Before Date
- Weight or quantity
- Lot/batch number

Storage Requirements

Shelf life - unopened	:	60 months (5 years) from date of manufacture
Storage instructions	:	Store in dry, cool conditions, away from direct sunlight in original sealed packaging.
Shelf-life - opened	:	Shelf life = first opening date + 6 months OR original manufacturer shelf life, whichever is shortest. Must be stored in well-sealed foil pouch at recommended temperatures. Pre-weighed: max. 14 days when stored protected from light (in black plastic bag or similar) at recommended temperature.

Logistic Requirements

Method of shipping(s)	:	Road / Sea freight
Estimated lead time	:	2 - 4 weeks
Shipping requirement(s)	:	CoA and packing slip to accompany goods

Revision History

Version	Nature of Change	Initiated by	Approved by	Date dd-mm-yyyy
1	New Specification	KW	IH	07/09/12
2	Amend contaminant levels in accordance to GB update and customer requirement	KW	IH	08/02/13
3	Add new supplier. Ensure has both FCC and GB requirements	KW	TJ	17/04//15
4	Update information into new template and update suppliers. Add foreign matter requirements. Align units with current CoA	JS	TJ	23/11/15

PRODUCT SPECIFICATION

(Appendix 2 of the NZDI Salt Specification)

PURE DRIED VACUUM SALT (PDV)

DominionSalt

Head Office & N.I. Refinery

89 Totara Street, Mount Maunganui, New Zealand
PO Box 4249, Mount Maunganui South
Phone: 64 7 5756193 Fax: 64 7 575 3017
Email: sales@domsalt.co.nz
Website: www.domsalt.co.nz

Lake Grassmere & S.I. Refinery

Kaparu Road, Marlborough, New Zealand
PO Box 81, Seddon
Phone: 64 3 575 7021 Fax: 64 3 575 7002
Email: sales@domsalt.co.nz
Website: www.domsalt.co.nz

CHEESE SALT			
COMPONENTS	**NZ Dairy Salt Specification**	**TYPICAL**	**DSL Test Method** *(Reference Method)*
Sodium Chloride as NaCl - Minimum moisture free	Min 99.6 %	>99.8%	Calculated by difference
Moisture Content	Max 0.2%	0.02%	DSL Pt. 12 *(BS 7319:Part 2:1990)*
Matter Insoluble in water	Max 300 mg/kg	<10 mg/kg	DSL Pt. 11 *(BS 7319:Part 3:1990)*
Foreign matter[1]	ADMI - A	A	DSL Pt. 8 *(In-house)*
Sulphate as Na_2SO_4	Max 3000 mg/kg	<1500 mg/kg	DSL Pt. 14 *(BS 7319:Part 4:1990)*
Calcium as Ca	Max 100 mg/kg	<20 mg/kg	DSL Pt. 5 *(BS 7319:Part 5:1990)*
Magnesium as Mg	Max 100 mg/kg	<15 mg/kg	" "
Cadmium as Cd	Max 0.2 mg/kg	<0.01 mg/kg	DSL Pt. 4 *(BS 7319:Part 6:1990)*
Arsenic as As	Max 0.5 mg/kg	<0.01 mg/kg	DSL Pt. 2 *(BS 4404:1968)*
Copper as Cu	Max 2 mg/kg	<0.1 mg/kg	DSL Pt. 4 *(BS 7319:Part 7:1990)*
Lead as Pb	Max 1 mg/kg	<0.1 mg/kg	DSL Pt. 4 *(BS 7319:Part 8:1990)*
Mercury [2] as Hg	Max 0.05 mg/kg	<0.01 mg/kg	ICP *(BS 7319:Part 9:1990)*
Alkalinity as Na_2CO_3	Max 300 mg/kg	<100 mg/kg	DSL Pt. 1 *(BS 7319:Part 10:1990)*
Iron as Fe	Max 10 mg/kg	<1.0 mg/kg	DSL Pt. 4 *(BS 7319:Part 11:1990)*
Food Additives [3]:			
Additive 535 as $[Fe(CN)_6]^{4-}$	Max 15 mg/kg	4-6 mg/kg	DSL Pt. 9 *(BS 7319:Part 12:1990)*

Notes: < Less than > Greater than ppm = mg/kg = (% x 10,000)

1. "Foreign matter" is not defined in the FSANZ Code Volume 2, therefore reference "7CFR 2858.267 Scorched Particle Standards for Dry Milks" has been adopted to quantify the level of sediment. A photocopy of this reference is available on request to the Works Chemist.
2. Test performed on incoming bulk salt shipment before refining.
3. As specified in FSANZ Food Standards Code Volume 2, Part 1.3 schedule 1. (Available at website: www.foodstandards.govt.nz)

GRADE DESCRIPTION:

High purity certified vacuum salt especially prepared to be of relatively coarse crystals with a narrow grain size range. Strictly prepared in batch lots to optimise grain size uniformity. Suitable for salting in some mechanical cheese manufacturing plants using accurate pneumatic salt conveying equipment, which are sensitive to a wide or variable range of grain sizes.

Country of origin: Product of New Zealand

NUTRITIONAL INFORMATION

Component	Per 100g
Saturated Fat	Nil g
Mono Unsaturated Fat	Nil g
Poly Unsaturated Fat	Nil g
Trans Fatty Acids	Nil g
	Typically
Sodium	39.1g min
Chloride	60.5g min
Calcium	<0.4 - 4 mg
Potassium	2-4 mg
Iron	<1 mg
Cholesterol	Nil mg
Dietary Fibre – soluble	Nil mg
Dietary Fibre - Insoluble	Nil mg

GRAIN SIZE: 100% passing 850 microns
0 - 2% passing 212 microns

BULK DENSITY: Nominally: loose 1.25g/ml, compacted 1.43g/ml

COMPLIANCE:

- ***Certified to NZDI Salt Specification***
- Complies with BS998:1990 Vacuum Salt for Food Use
- Complies with FSANZ Food Standards Code Volume 2 Standard 2.10.2/Clause 2
- NOT a genetically modified food as defined under 1.5.2 of the FSANZ Standards Code Volume 2
- *Is Free from known Allergens*
- *Halal Certified*
- *Kosher Certified*
- *Dominion Salt is ISO 9001 certified*

PACK:

Bulk Bag Woven Polypropylene with Polyethylene liner (Weight by arrangement)
Bulk Bag Woven Polypropylene with Polyethylene barrier layer laminated to inside face of woven material.
25kg Polyethylene Bag (no outer)
Packaging material complies with US FDA regulations Title 21, parts 170-199
Print colour: Bulk Bag - Blue 072
25kg Bag - Spot Orange 021

Pallets:

Small packs: Standard pallet configuration is 48 x 25 kg bags (1.2 tonnes per pallet) The salt is stretch wrapped and capped on pallets with a pallet sheet between the pallet and the salt
Bulk Bags: Standard configuration is one bulk bag per pallet

Issue Date: 20.08.09
Issue No: 13

Raw Material Specification Synlait Skim Milk

	Month	Limits	September	March	June	September	March	June	Dec	Sept	Dec	Mar
	Year		2012	2013	2013	2013	2014	2014	2014	2015	2015	2016
Moisture	% m/m		90.69	90.34	90.98	90.67	90.02	90.84	90.58	90.82	90.53	90.63
Fat	% m/m	<0.15	<0.1	0.111	0.086	0.07	0.09	0.07	0.06	0.07	0.09	0.1
Protein	% m/m	>3.5	3.64	4.11	3.6	3.62	4.25	3.78	3.82	3.7	3.81	3.91
Lactose/carbo	% m/m		4.89	4.649	4.584	4.85	4.85	4.53	4.76	4.61	4.77	4.6
Ash	% m/m	<1.0	0.78	0.79	0.75	0.79	0.79	0.78	0.78	0.8	0.8	0.76
Total Solids (TS)	% m/m		9.31	9.66	9.02	9.33	9.98	9.16	9.42	9.18	9.47	9.37
MICRONUTRIENT												
Calcium	mg/100g	>100	130	140	130	140	140	130	140	120	130	130
Chloride	mg/100g	<200	96	102	106	90	100	107	95	89	94	102
Copper	ppm											<0.028
Copper	µg/100mL		7.8	5	7.5	4.1	3.2					
Iron	ppm		<0.025	0.027	<0.025	<0.025	0.023					<0.25
Iodine	ug/100g		7.5	4.7	15	5.2	4.8	10.0	6.2	0.09 mg/kg	3.5	3.6
Potassium	mg/100g		160	150	160	160	150	150	170	150	160	150
Manganese	mg/100g		<1.8	3.1	2.5	<1.75	3.3					<1.8 ug/100g
Magnesium	mg/100g		10	13	11	11	13	12	12	10	11	12
Sodium	mg/100g	<100	34	37	38	31	36	38	34	30	31	36
Phosphorus	mg/100g	<200	110	110	99	110	100	100	110	100	100	98
Selenium	mg/100g			1.5			1.5					1.3 ug/100g
Zinc	mg/100g		0.45	0.47	0.43	0.41	0.44	0.44	0.45	0.41	0.41	0.39
Vit B1 (Thiamine)	µg/100mL		<15.7	42.49	25.18	19.67	28.40	22.82	34.00	27.30	21.00	24.00
Vit B2 (Riboflavin)	µg/100mL		227	226	201	224	255	221	227	227	215	265
Vit B3 (Niacin)	µg/100mL			<150								
Vit B5 (Pantothenic Acid)	µg/100mL		351	200	400	500	400	500	500		0.42 mg/100	226
Vit B6 HCl	µg/100mL		29	33	33	28	32.0	30.5	39.0	29	35	32
Vit B12	µg/100mL		0.42	0.578	<0.2	0.51	0.529	0.656	0.537	0.5	0.587	0.558
Vit C	mg/100mL		<1	<1	<1	<1						
Biotin	µg/100mL		<8	<8		<8						
Total L-Carnitine	mg/100g		2.34	1.84	1.5	1.7	2.4	2.7	1.9	2.6	1.5	2.5
Choline	mg/100mL		10	13	11	11	5.7	15.0	11	9	10	9.25
Folic acid	µg/100mL			<8	<8							
Inositol	mg/100g		4.8	4.5	4.2	4.3	4.9	5.6	5	5.4	6.5	6.15
CONTAMINANT												
Total Heavy Metals	mg/kg	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Nitrate	mg/L	<1	0.1	<1	<1	<0.2	0.4	0.4	<0.2	<0.2	<0.2	<1
Nitrite	mg/L	<1	0.01	0.1	0.08	0.09	0.05	0.05	0.04	0.03	0.05	<0.03
Inhibitory substances	IU/mL	<0.0025	<0.0025	<0.0025	<0.0025	<0.0025	<0.0025	<0.0025		<0.0025		

Table 1 – Processing Aid Comparison Morinaga vs Synlait Bovine Lactoferrin

Table 2. Processing Aids and Chemicals Used in the Production of Cow's Milk-Derived Lactoferrin (cMDLf) - Page #10 (27 of 217) from GRAS 465.pdf			**Synlait Milk Ltd Spray Dried Bovine Lactoferrin**	
Processing Aid or Chemical	**Manufacturer**		**Processing Aid or Chemical**	**Manufacturer**
	At Milei for cMDLf-1, cMDLf-2	**At Riedlingen for cMDLf-2**		
Demineralized water	Milei	Riedlingen plant	Demineralized water	In-house RO water
Sodium chloride (NaCl)	Herkommer & Bangerte	Herkommer & Bangerte	Sodium chloride (NaCl)	Dominion Salt, New Zealand
Hydrochloric Acid (HCl)	Herkommer & Bangerte	Not used	Hydrochloric Acid (HCl)	Not applicable
CM Sephadex C-50 or SP Sepharose Big Beads	GE Healthcare	GE Healthcare	Resins for ion exchange	GE Healthcare
Filter cloth (1um)	Wolftechnik Filtersysteme	Wolftechnik Filtersysteme	Ultrafiltration	Koch Membranes
Filter cloth (5um)	Wolftechnik Filtersysteme	Not used	Microfiltration	Tami
GR61PP Membrane	Alfa Laval	Not used		

GE Healthcare

Certificate of Analysis

Product:

SP Sepharose™ Big Beads Food Grade

Code Numbers:
11-0008-29
11-0008-30
11-0008-31

Lot No: 10163437

Test/Characteristic:		Limits:	Results:
1	**Function** Elution volume; ml		
1.1	Wheat Germ Lectin - peak 1 - peak 2 - peak 3	 60 – 88 80 – 122 96 – 138	 71 98 110
1.2	β-Lactoglobulin	147 – 189	157
2	**Total capacity** mmol H^+ / ml packed gel	0.18 – 0.25	0.23
3	**Flow rate at 0.1 MPa**; cm/h	1200 – 1800	1450
4	**Particle size distribution** Volume share within 100 – 300 µm; %	min. 80	98
5	**Microbial contamination** Colony Forming Units / ml suspension	max. 100	0

Manufactured in compliance with our ISO 9001 certified quality management system.

Approval date (Year-Month-Day): 2013-06-03

Expiry date (Year-Month): 2018-05
Manufacturing date (Year-Month): 2013-05

Tests and limits according to AS 45-6015-84 Ed. AB

GE Healthcare Bio-Sciences AB
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Reg.No. SE 55 61 08 1919 01

Quality Assurance
Issued (Year-Month-Day) 2013-06-03 by Sten Pettersson

This document has been electronically produced and is valid without a signature.

28-9653-19 / AC
DOC1103901 / 1
Valid from 2012-02-24

GE Healthcare

SAFETY DATA SHEET

New Zealand

Section 1. Identification

Product name **SP Sepharose™ Big Beads, Food Grade, 10 L**

Catalogue Number 11-0008-30

Other means of identification Not available.

Product type Liquid.

Identified uses
Laboratory chemicals Liquid chromatography. Research and Development

Supplier

GE Healthcare UK Ltd
Amersham Place
Little Chalfont
Buckinghamshire HP7 9NA
England
+44 0870 606 1921

GE Healthcare Bio-Sciences
8 Tangihua Street
Auckland 1010

Person who prepared the MSDS :
msdslifesciences@ge.com

Emergency telephone number (with hours of operation)
0800 733 893
(10am - 7pm)

Section 2. Hazards identification

HSNO Classification 3.1 - FLAMMABLE LIQUIDS - Category C
6.4 - EYE IRRITATION - Category A (Irritant)

This material is classified as hazardous according to criteria in the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 and has been classified according to the Hazardous Substances (Classifications) Regulations 2001.

This material is classified as a dangerous good according to criteria in New Zealand Standard 5433:2007 Transport of Dangerous Goods on Land.

GHS label elements

Signal word Warning

Hazard statements Flammable liquid and vapor.
Causes serious eye irritation.

Precautionary statements

Prevention Wear protective gloves: 1-4 hours (breakthrough time): butyl rubber, neoprene. Wear eye or face protection: Recommended: safety glasses with side-shields. Keep away from ignition sources such as heat/sparks/open flame. - No smoking. Use explosion-proof electrical, ventilating, lighting and all material-handling equipment. Use only non-sparking tools. Take precautionary measures against static discharge. Keep container tightly closed.

Response IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists, get medical advice/attention. Wash hands after handling.

Storage Store in cool/well-ventilated place.

Disposal Dispose of contents and container in accordance with all local, regional, national and international regulations.

Symbol

Other hazards which do not result in classification Not available.

Section 3. Composition/information on ingredients

Substance/mixture	Mixture
Other means of identification	Not available.
CAS number/other identifiers	
CAS number	Not applicable.
EC number	Mixture.
Product code	11-0008-30

Ingredient name	%	CAS number
Ethanol	14 - 19	64-17-5

There are no additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

Occupational exposure limits, if available, are listed in Section 8.

Section 4. First aid measures

Description of necessary first aid measures

Inhalation	If inhaled, remove to fresh air. Get medical attention if symptoms appear.
Ingestion	Do not ingest. Get medical attention if symptoms appear.
Skin contact	Wash with soap and water. Get medical attention if irritation develops.
Eye contact	Immediately flush eyes with plenty of water, occasionally lifting the upper and lower eyelids. Check for and remove any contact lenses. Continue to rinse for at least 10 minutes. Get medical attention.

Most important symptoms/effects, acute and delayed

Potential acute health effects

Inhalation	No known significant effects or critical hazards.
Ingestion	Irritating to mouth, throat and stomach.
Skin contact	No known significant effects or critical hazards.
Eye contact	Causes serious eye irritation.

Over-exposure signs/symptoms

Inhalation	No specific data.
Ingestion	No specific data.
Skin	No specific data.
Eyes	Adverse symptoms may include the following: pain or irritation watering redness

Indication of immediate medical attention and special treatment needed, if necessary

Specific treatments	Not available.
Notes to physician	No specific treatment. Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.
Protection of first-aiders	No action shall be taken involving any personal risk or without suitable training. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation.

See toxicological information (section 11)

Section 5. Fire-fighting measures

Extinguishing media

Suitable	Use dry chemical, CO_2, water spray (fog) or foam.
Not suitable	Do not use water jet.
Specific hazards arising from the chemical	Flammable liquid and vapor. In a fire or if heated, a pressure increase will occur and the container may burst, with the risk of a subsequent explosion. Runoff to sewer may create fire or explosion hazard.
Hazardous thermal decomposition products	Decomposition products may include the following materials: carbon dioxide carbon monoxide
Hazchem code	Not available.

Special precautions for fire-fighters	Promptly isolate the scene by removing all persons from the vicinity of the incident if there is a fire. No action shall be taken involving any personal risk or without suitable training. Move containers from fire area if this can be done without risk. Use water spray to keep fire-exposed containers cool.
Special protective equipment for fire-fighters	Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

Section 6. Accidental release measures

Personal precautions, protective equipment and emergency procedures	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Shut off all ignition sources. No flares, smoking or flames in hazard area. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see Section 8).
Environmental precautions	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).

Methods and materials for containment and cleaning up

Small spill	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble. Alternatively, or if water-insoluble, absorb with an inert dry material and place in an appropriate waste disposal container. Use spark-proof tools and explosion-proof equipment. Dispose of via a licensed waste disposal contractor.
Large spill	Stop leak if without risk. Move containers from spill area. Approach release from upwind. Prevent entry into sewers, water courses, basements or confined areas. Wash spillages into an effluent treatment plant or proceed as follows. Contain and collect spillage with non-combustible, absorbent material e.g. sand, earth, vermiculite or diatomaceous earth and place in container for disposal according to local regulations (see section 13). Use spark-proof tools and explosion-proof equipment. Dispose of via a licensed waste disposal contractor. Contaminated absorbent material may pose the same hazard as the spilled product. Note: see section 1 for emergency contact information and section 13 for waste disposal.

Section 7. Handling and storage

Precautions for safe handling	Put on appropriate personal protective equipment (see Section 8). Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Remove contaminated clothing and protective equipment before entering eating areas. Do not ingest. Avoid contact with eyes, skin and clothing. Avoid breathing vapor or mist. Use only with adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Do not enter storage areas and confined spaces unless adequately ventilated. Keep in the original container or an approved alternative made from a compatible material, kept tightly closed when not in use. Store and use away from heat, sparks, open flame or any other ignition source. Use explosion-proof electrical (ventilating, lighting and material handling) equipment. Use only non-sparking tools. Take precautionary measures against electrostatic discharges. To avoid fire or explosion, dissipate static electricity during transfer by grounding and bonding containers and equipment before transferring material. Empty containers retain product residue and can be hazardous. Do not reuse container.
Conditions for safe storage, including any incompatibilities	Store between the following temperatures: 4 to 30°C (39.2 to 86°F). Store in accordance with local regulations. Store in a segregated and approved area. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10) and food and drink. Eliminate all ignition sources. Separate from oxidizing materials. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.

Section 8. Exposure controls/personal protection

Control parameters

Occupational exposure limits

Ingredient name	Exposure limits
Ethanol	**NZ OSH (New Zealand, 1/2002).** WES-TWA: 1880 mg/m³ 8 hour(s). WES-TWA: 1000 ppm 8 hour(s).

Recommended monitoring procedures	If this product contains ingredients with exposure limits, personal, workplace atmosphere or biological monitoring may be required to determine the effectiveness of the ventilation or other control measures and/or the necessity to use respiratory protective equipment.
Appropriate engineering controls	Use only with adequate ventilation. Use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits. The engineering controls also need to keep gas, vapor or dust concentrations below any lower explosive limits. Use explosion-proof ventilation equipment.
Environmental exposure controls	Emissions from ventilation or work process equipment should be checked to ensure they comply with the requirements of environmental protection legislation. In some cases, fume scrubbers, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable levels.

Individual protection measures

Hygiene measures	Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.
Respiratory protection	Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator. Recommended: A respirator is not needed under normal and intended conditions of product use.
Hand protection	1-4 hours (breakthrough time): butyl rubber, neoprene
Eye protection	Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts. Recommended: safety glasses with side-shields
Skin protection	Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product. Recommended: lab coat

Section 9. Physical and chemical properties

Appearance	
Physical state	Liquid. [and Suspension.]
Color	solution : Colorless. / Suspension. : White.
Odor	Sweetish. Alcohol-like. [Slight]
Odor threshold	180 ppm
pH	Not available.
Melting point	Not available.
Boiling point	Not available.
Flash point	Closed cup: 38 to 43°C (100.4 to 109.4°F)
Burning rate	Not applicable.
Burning time	Not applicable.
Evaporation rate	Not available.
Flammability (solid, gas)	Not available.
Lower and upper explosive (flammable) limits	Not available.
Vapor pressure	Not available.
Vapor density	Not available.
Relative density	Not available.
Solubility	Easily soluble in the following materials: cold water and hot water.
Partition coefficient: n-octanol/water	Not available.
Auto-ignition temperature	Not available.
Decomposition temperature	Not available.
SADT	Not available.
Viscosity	Not available.
Aerosol product	
Type of aerosol	Not applicable.
Heat of combustion	Not available.
Ignition distance	Not applicable.
Enclosed space ignition - Time equivalent	Not applicable.
Enclosed space ignition - Deflagration density	Not applicable.
Flame height	Not applicable.
Flame duration	Not applicable.

Section 10. Stability and reactivity

Chemical stability	The product is stable.
Possibility of hazardous reactions	Under normal conditions of storage and use, hazardous reactions will not occur.
Conditions to avoid	Avoid all possible sources of ignition (spark or flame). Do not pressurize, cut, weld, braze, solder, drill, grind or expose containers to heat or sources of ignition.
Incompatible materials	Reactive or incompatible with the following materials: oxidizing materials
Hazardous decomposition products	Under normal conditions of storage and use, hazardous decomposition products should not be produced.

Section 11. Toxicological information

Information on the likely routes of exposure

Inhalation	No known significant effects or critical hazards.
Ingestion	Irritating to mouth, throat and stomach.
Skin contact	No known significant effects or critical hazards.
Eye contact	Causes serious eye irritation.

Symptoms related to the physical, chemical and toxicological characteristics

Inhalation	No specific data.
Ingestion	No specific data.
Skin contact	No specific data.
Eye contact	Adverse symptoms may include the following: pain or irritation watering redness

Delayed and immediate effects and also chronic effects from short and long term exposure

Acute toxicity

Product/ingredient name	Result	Species	Dose	Exposure
Ethanol	LC50 Inhalation Vapor	Rat	124700 mg/m3	4 hours
	LD50 Oral	Rat	7 g/kg	-

Irritation/Corrosion

Product/ingredient name	Result	Species	Score	Exposure	Observation
Ethanol	Eyes - Mild irritant	Rabbit	-	-	-
	Eyes - Moderate irritant	Rabbit	-	-	-
	Eyes - Severe irritant	Rabbit	-	-	-
	Skin - Mild irritant	Rabbit	-	-	-
	Skin - Moderate irritant	Rabbit	-	-	-

Conclusion/Summary

Skin	Repeated exposure may cause skin dryness or cracking.

Sensitization

Not available.

Potential chronic health effects

General	No known significant effects or critical hazards.
Inhalation	No known significant effects or critical hazards.
Ingestion	No known significant effects or critical hazards.
Skin contact	No known significant effects or critical hazards.
Eye contact	No known significant effects or critical hazards.
Carcinogenicity	No known significant effects or critical hazards.
Mutagenicity	No known significant effects or critical hazards.
Teratogenicity	No known significant effects or critical hazards.
Developmental effects	No known significant effects or critical hazards.
Fertility effects	No known significant effects or critical hazards.

Chronic toxicity

Not available.

Carcinogenicity

Not available.

Mutagenicity

Not available.

Teratogenicity

Not available.

Reproductive toxicity

Not available.

Specific target organ toxicity

Not available.

Aspiration hazard

Not available.

Numerical measures of toxicity

Acute toxicity estimates

Not available.

Other information Adverse symptoms include the following: kidney abnormalities, liver abnormalities
Adverse symptoms may include the following: central nervous system depression

Section 12. Ecological information

Ecotoxicity No known significant effects or critical hazards.

Aquatic and terrestrial toxicity

Product/ingredient name	Result	Species	Exposure
Ethanol	Acute EC50 2000 ug/L Fresh water	Daphnia - Daphnia magna	48 hours
	Acute LC50 25500 ug/L Marine water	Crustaceans - Artemia franchiscana - LARVAE	48 hours
	Acute LC50 42000 ug/L Fresh water	Fish - Oncorhynchus mykiss	4 days
	Chronic NOEC <6.3 g/L Fresh water	Daphnia - Daphnia magna	48 hours

Persistence/degradability

Product/ingredient name	Test	Result	Dose	Inoculum
Ethanol	-	100 % - Readily - 20 days	-	-

Product/ingredient name	Aquatic half-life	Photolysis	Biodegradability
Ethanol	-	-	Readily

Bioaccumulative potential

Product/ingredient name	$LogP_{ow}$	BCF	Potential
Ethanol	-	0.66	low

Mobility in soil

Soil/water partition coefficient (K_{OC}) Not available.

Other adverse effects No known significant effects or critical hazards.

Section 13. Disposal considerations

Disposal methods

The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in a safe way. Significant quantities of waste product residues should not be disposed of via the foul sewer but processed in a suitable effluent treatment plant. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Section 14. Transport information

Regulatory information	UN number	Proper shipping name	Classes	PG*
New Zealand Class	Not regulated.	-	-	-
ADG Class	Not regulated.	-	-	-
UN Class	Not regulated.	-	-	-
ADR/RID Class	Not regulated.	-	-	-
IATA Class	Not regulated.	-	-	-
		Remarks IATA Special Provision A 58 - Aqueous solutions containing 24% or less alcohol by volume is not subject to these regulations.		
IMDG Class	Not regulated.	-	-	-

PG* : Packing group

Section 15. Regulatory information

New Zealand Inventory of Chemicals (NZIoC) All components are listed or exempted.

HSNO Approval Number HSR001144

HSNO Group Standard Not available.

HSNO Classification 3.1 - FLAMMABLE LIQUIDS - Category C
6.4 - EYE IRRITATION - Category A (Irritant)

Australia inventory (AICS) All components are listed or exempted.

Safety, health and environmental regulations specific for the product No known specific national and/or regional regulations applicable to this product (including its ingredients).

Section 16. Other information

History

Date of printing 12/16/2010.

Date of issue/ Date of revision 15 December 2010

Date of previous issue No previous validation.

Version 0.9

Key to abbreviations

ADN/ADNR = European Provisions concerning the International Carriage of Dangerous Goods by Inland Waterway
ADR = The European Agreement concerning the International Carriage of Dangerous Goods by Road
ATE = Acute Toxicity Estimate
BCF = Bioconcentration Factor
GHS = Globally Harmonized System of Classification and Labelling of Chemicals
IATA = International Air Transport Association
IBC = Intermediate Bulk Container
IMDG = International Maritime Dangerous Goods
LogPow = logarithm of the octanol/water partition coefficient
MARPOL 73/78 = International Convention for the Prevention of Pollution From Ships, 1973 as modified by the Protocol of 1978. ("Marpol" = marine pollution)
RID = The Regulations concerning the International Carriage of Dangerous Goods by Rail
UN = United Nations

References Not available.

Indicates information that has changed from previously issued version.

Notice to reader

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein.
Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

Part 7 Appendix 1

Customer Specification Sheet

Synlait Lactoferrin 5kg Reclosable Pouch PPRI01005

8 December 2015

Issue Number: 01

Amcor Item:	1044979
Customer Item Code:	PPRI01005

Product detail:

Customer	Synlait Milk Ltd
Description	SYN LACTOFERRIN 5KG
Material Structure description	Coated Polyester(14um)/ ink/adhesive/Foil(7um)/Nylon(15um) Polyethylene (90um)
Yield:	148.6gsm* Tolerance: +/-10gsm
Gauge:	133µm* Tolerance: +/-10µm
Estimated Oxygen Transmission Rate:	<0.3 cc/m^2/24hrs(100% O_2) 23°C/ 0% RH
Estimated Water Vapour Transmission Rate:	<0.3 g/m^2/24hrs 38°C 90% RH

* Excludes zipper

Amcor Flexibles Asia Pacific - ANZ
74 Branston Street; Hornby; Christchurch 8042; New Zealand
Ph: +64 3 349 1250 www.amcor.com

amcor

Customer Specification Sheet

Product and Packing Specifications:

Printing Process:	Flexographic.
Colour and Coatings:	To match customer approved standard.
Identification Labels:	Cartons: labels to state ID number, Item number, Description, Customer Code, Quantity, Carton number, Date and packer Pallet: Customer, product description, quantity, customer order number, customer stock number, pallet number, date, number of rolls, and Amcor job number.
Carton Handling:	Pouches should be kept out of direct natural light/sunlight and in a well-ventilated area. It is advantageous to condition the cartons to packing room temperature at least 24 hrs prior to use. At all times when not in use the carton should be sealed so performance is not impaired or contamination permitted.

Specification Data:

Customer Item Number	Amcor Item Number	Description		Length	Bags per Bundle	Bags per Carton
PPRI01005	1044979	SYN LACTOFERRIN 5KG	260X130X660 L81 RQPH	657	25	150

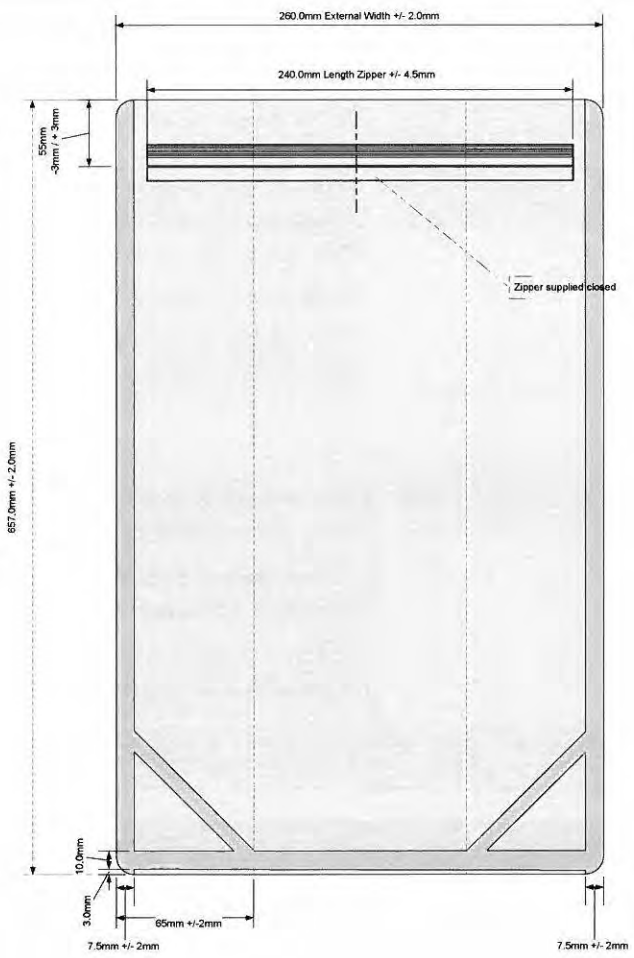
Reason for Revision:

Design change to 1 colour.

Amcor Flexibles Asia Pacific - ANZ
74 Branston Street; Hornby; Christchurch 8042; New Zealand
Ph: +64 3 349 1250 www.amcor.com

Customer Specification Sheet

Material Diagram: (not to scale)



Approved by (Amcor):	Approved by (Customer):
(b) (6)	
Position: Quality Manager	Position:
Date: 08/12/2015	Date:

Amcor Flexibles Asia Pacific - ANZ
74 Branston Street; Hornby; Christchurch 8042; New Zealand
Ph: +64 3 349 1250 www.amcor.com

PART 7:

APPENDIX 2: Synlait Manufacturing Certification And Registration Certificates

The data and information presented within Appendix 2 is Confidential to Synlait Milk Ltd and is **not generally available**.

NOTICE OF REGISTRATION

RISK MANAGEMENT PROGRAMME

Pursuant to section 22 of the Animal Products Act 1999, the Director-General has registered a risk management programme for:

Synlait Milk Limited

Located at:

1028 Heslerton Road, RD13 (Premises IDs S540,540)
RAKAIA

This risk management programme has been assigned the identifier:

SYNLAIT3/01

Risk management programmes manage hazards and other risk factors associated with animal products in order to ensure fitness for intended purpose, and are based on the principles of HACCP.

This registration is effective from 23/10/2015

Signed at Wellington on **19/01/2016**

(b) (6)

MINISTRY FOR PRIMARY INDUSTRIES
NEW ZEALAND

Maree Zinzley
Manager (Approvals Operations)
Acting under delegated authority
Ministry for Primary Industries

AsureQuality

This is to Certify

Synlait Milk Limited

1028 Heslerton Road, RD13, Rakaia, New Zealand

Has been assessed by AsureQuality Limited and found to comply with the standards based on:

Codex Alimentarius "Hazard Analysis and Critical Control Point (HACCP) System and Guidelines" Reference CAC/RCP 1 – 1969, Rev. 4 – 2003, Annex.

The scope of this certificate includes the following products:
Anhydrous Milk Fat, Colostrum Products, Milk Powders, Milk Proteins, Nutritional Powders and Specialty Powders.

Manufacturer Identification Numbers: 540, S540

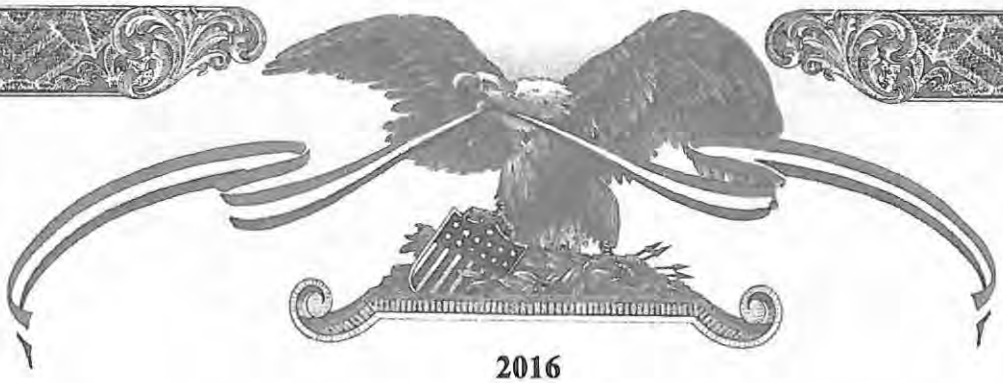
Certificate No:	DHACCP 059
Date of Issue:	2 February 2016
Valid Until:	1 February 2017

(b) (6)

John McKay
Chief Executive

Disclaimer: This certificate has been issued for commercial purposes only and is not intended to be supplied to competent authorities as a means of demonstrating compliance with New Zealand or importing country requirements.

This certificate remains the property of AsureQuality Ltd
7a Pacific Rise | Mt Wellington | Auckland 1741 | New Zealand
+64 9 573 8000 | www.asurequality.com | info@asurequality.com



2016

CERTIFICATE OF REGISTRATION

This certifies that:

Synlait Milk Ltd.
1028 Heslerton Road
RD 13, Rakaia, Canterbury 7783
New Zealand

is registered with the U.S. Food and Drug Administration pursuant to the Federal Food Drug and Cosmetic Act, as amended by the Bioterrorism Act of 2002 and the FDA Food Safety Modernization Act, such registration having been verified as currently effective on the date hereof by Registrar Corp:

U.S. FDA Registration No.:	**15930127872**
U.S. Agent for FDA Communications:	**Registrar Corp** 144 Research Drive, Hampton, Virginia, 23666, USA Telephone: +1-757-224-0177 • Fax: +1-757-224-0179

This certificate affirms that the above stated facility is registered with the U.S. Food and Drug Administration pursuant to the Federal Food Drug and Cosmetic Act, as amended by the Bioterrorism Act of 2002 and the FDA Food Safety Modernization Act, such registration having been verified as effective by Registrar Corp as of the date hereof, and Registrar Corp will confirm that such registration remains effective upon request and presentation of this certificate until December 31, 2016, unless such registration has been terminated after issuance of this certificate. Registrar Corp makes no other representations or warranties, nor does this certificate make any representations or warranties to any person or entity other than the named certificate holder, for whose sole benefit it is issued. Registrar Corp assumes no liability to any person or entity in connection with the foregoing. The U.S. Food and Drug Administration does not issue a certificate of registration, nor does the U.S. Food and Drug Administration recognize a certificate of registration. Registrar Corp is not affiliated with the U.S. Food and Drug Administration.

(b) (6)

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info@registrarcorp.com • www.registrarcorp.com

Russell K. Statman
Executive Director
Registrar Corp
Dated: September 9, 2015

PART 7:
APPENDIX 3: Analytical Methodology, Specifications And Results

The data and information presented within Appendix 3 (pages A3:10 - A3:32 is **generally available**.

Pages A3:2- A3: 9 and A3: 33 to A3:34 .... are Confidential to Synlait Milk Ltd and are **not generally available**

Standard Operating Procedure for Lactoferrin (LF) Analysis by RP-HPLC

-Applicable to products manufactured by Synlait Milk Limited

Initiated by: Jagan M Billakanti
Approved By: (b) (6)

Date effective: 25-02-2014

1. Purpose

To determine the purity of the lactoferrin content of liquid and powder lactoferrin products produced by cation exchange chromatography of milk.

2. Principle

HPLC analysis of bovine lactoferrin (LF) is carried out on a HPLC system equipped with a temperature controlled column oven and UV-Vis detector recording at 220 nm. Samples are diluted with deionized water, filtered through a 0.2 micron filter and injected onto a selected reversed-phase (RP)-HPLC column. Peaks present in the chromatogram recorded at 220 nm are integrated (3 – 9 minutes interval) and used for determination of lactoferrin purity. The LF content of the product is expressed as %LF. Identification of peaks is based on their retention times and absorption spectra at 220 nm when compared with a commercial lactoferrin protein standard.

3. Materials

The following materials are required to carry out the analysis.

3.1 Standards

Lactoferrin from bovine milk [L9507] - a purified protein standard with approximately 98% purity by HPLC is purchased from Sigma-Aldrich, Auckland, New Zealand.

3.2 Reagents

Water must be deionised (DI) and filtered through a 0.2 μm filter unit or of equivalent quality. Trifluoroacetic acid (TFA) with purity of ≥99% is used. Acetonitrile (CH_3CN) must be of HPLC or equivalent grade

3.3 Apparatus

- Analytical balance capable of weighing any sample mass to an accuracy of 0.0001g (four decimal places)
- HPLC/UPLC system equipped with a temperature controlled column oven, gradient system with an automatic sampler and UV-Vis detector recording at 220 nm
- Aeris™ 3.6 micron WIDEPORE XB-C8 200Å, LC Column 250 x 4.6 mm
- Cellulose acetate filters, 25 mm, 0.2 μm
- Micro-spin centrifugal filter units, 0.5 mL, 0.2 μm
- Amber HPLC vials

3.4 Method safety equipment

- Lab coats
- Nitrile free gloves
- Safety glasses
- Fume hood
- Breathing apparatus, if required

3.5 Mobile phase solvents

Solvent A: Deionised water containing 0.1% (v/v) TFA, dilute 1 mL of TFA in 999 mL DI water and filter through a 0.2 µm cellulose acetate filter unit

Solvent B: Acetonitrile containing 0.1% TFA (v/v), dilute 1 mL of TFA in 999 mL of HPLC grade acetonitrile

3.6 Lactoferrin standard preparation

A commercial LF protein standard stock is prepared as follows. An appropriate volume of phosphate buffer saline (PBS) is directly added to the LF vial of commercial protein to yield a final protein concentration of 10 mg/mL and mixed slowly for an hour at RT until the protein is completely dissolved. Protein stocks are filtered through a 0.2 micron centrifugal filter unit, divided into 50 µL aliquots (in low protein binding tubes), and stored at -20°C until the preparation of working concentrations. The LF protein standard stock is further diluted (10-fold) in HPLC solvent A to yield a final protein concentration of 1 mg/mL and serial dilutions (0 – 300 ng/µL) are prepared in the same solvent for generating calibration curves using HPLC system.

3.7 Liquid sample preparation

Liquid lactoferrin samples provided by the Client are prepared as follows. A stock LF solution is prepared by mixing 100 µL of liquid LF sample with 900 µL of DI water (10-fold dilution) and filtering the stock using a 0.2 micron centrifugal filter unit. A working concentration of LF for HPLC analysis is prepared by addition of 25 µL of the above stock to 975 µL of solvent A (400-fold final dilution, assuming that the protein content of liquid test samples are expected to be approximately 50 – 100 µg/mL). All prepared stocks (10-fold dilutions) are stored at -20°C for further use, if required.

3.8 Powder sample preparation

Powder lactoferrin samples provided by the Client are prepared as follows. A stock LF solution is prepared by accurately weighing approximately 50 mg of powdered sample into a 15 mL 'Falcon' tube, 4.95 mL of DI water is added to dissolve the

protein (10 mg/mL final). Sample tubes are kept on a horizontal shaker for an hour at RT to dissolve the protein completely.1 mL of the above stock solution above is transferred into a 1.5 mL microcentrifuge tube and spun-down for 5 minutes at 10000 rpm using a bench-top centrifuge to remove any undissolved particulate material in the sample. The supernatant from the above is filtered through a 0.2 micron centrifugal filter unit. A working stock of LF for HPLC analysis is prepared by addition of 25 µL of the above stock to 975 µL of solvent A (40-fold dilution of 10 mg/mL preparation). All stock preparations (10 mg/mL) are stored at -20°C for further use, if required.

4. References

Billakanti, J.M (2014). RP-HPLC method development for the estimation of lactoferrin purity. Callaghan Innovation reports – CIR-95.

5. Procedure applicability

This method is suitable for the determination of the LF content in both liquid and powder protein products prepared by cation exchange chromatography and containing various other basic milk proteins which commonly bind to cation exchange chromatography resins.

6. Instrument operation

Ensure the following operating conditions are set (See chromatography profile in the Appendix A and B)

Column: Aeris™ 3.6 micron WIDEPORE XB-C8 200Å, LC Column 250 x 4.6 mm (Phenomenex, New Zealand)

Detection wavelength: UV 220 nm

Mobile phases: Solvent A and Solvent B

Retention Time: Lactoferrin – 7.07±0.01 minutes

Injection volume: 25 µL

Flow rate: 1 mL/min

Column temperature: 30°C

Run time: 15 minutes

Mobile phase gradient: Table 1

Table 1: Mobile phase gradient profile for HPLC analysis of LF

Time (minutes)	% Solvent B
0.0	25
1.0	25
3.0	40
4.0	50
5.0	50
8.0	95
11.0	95
11.1	25
15.0	25

7. Determination of lactoferrin

- Program the mobile phase, set up the sequence table with sample details (minimum of triplicate injections for calibration standards with 25 µL of each injection) and save the method
- Prime the system and then equilibrate the column for 20 minutes
- Inject a blank sample with no protein (solvent A only)
- Inject samples (triplicate) containing known concentration of LF for comparison along with test samples
- When the sample run is complete (ensure the Shut Down program of the project is complete), wash the column with 65% acetonitrile (20 minutes) and store the column with 65% acetonitrile solvent system

Calculation of Lactoferrin, $\%LF = \frac{LF\ peak}{Sum\ of\ all\ peaks} \times 100$

Where, LF peak = Area of LF peak (peak at 7.07 minutes); Sum of all peaks = sum of all the areas of peaks in the chromatogram from 3 – 9 minutes

8. Quality control

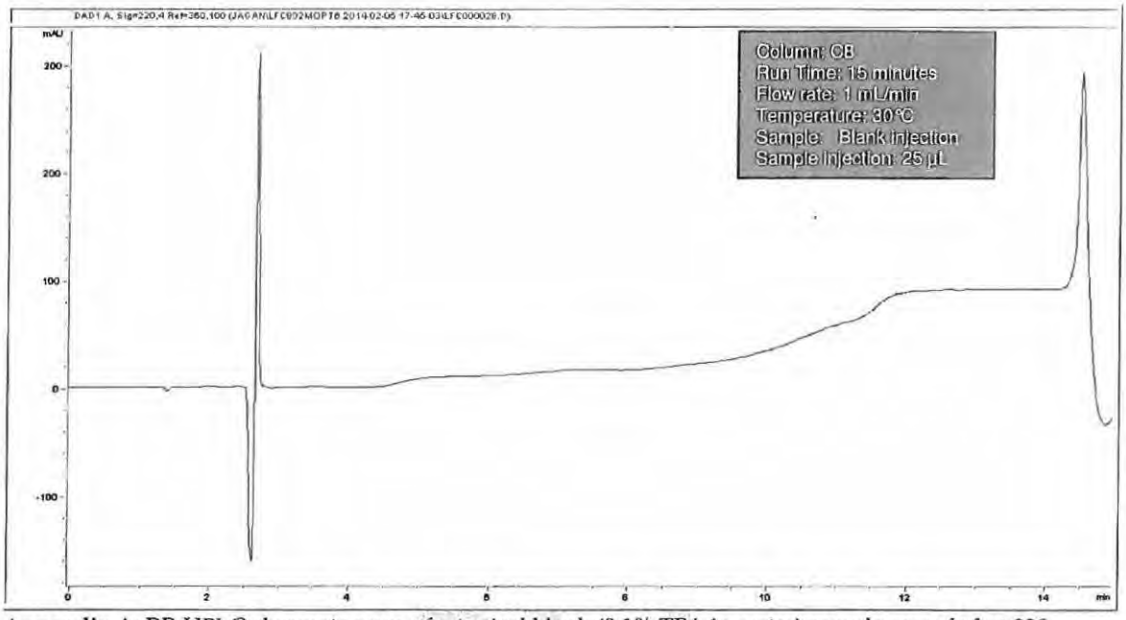
For each batch analysed, determine the purity of a commercial lactoferrin standard with known concentration and purity as a reference standard material. The percentage of recovery results shall be within the expected range.

9. Test report

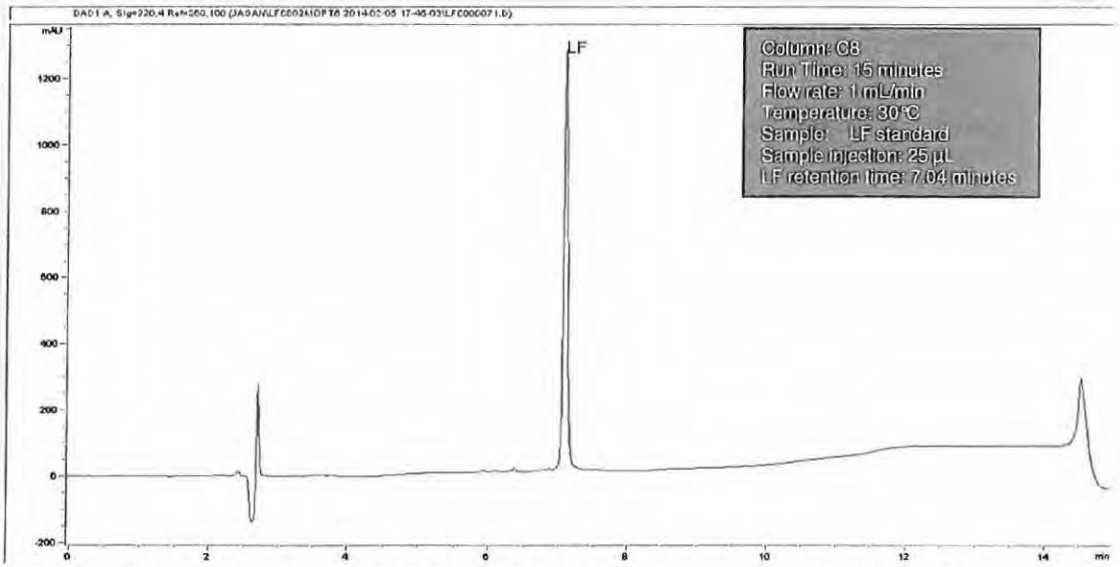
Report all results of lactoferrin (percentage of LF) to the nearest value (LF content in terms of %of protein) of one decimal place. As test method, mention "HPLC method" in the test reports.

10. Document control

Appendix



Appendix A: RP-HPLC chromatogram of a typical blank (0.1% TFA in water) sample recorded at 220 nm.



Appendix B: RP-HPLC chromatogram of a typical lactoferrin calibration standard (200 ng/µL) recorded at 220 nm.

SYNLAIT MILK THE COMMON SEAL OF LIMITED

Initiated by: Jagan M Billakanti
Approved By:

Date effective: 25-02-2014

Safety summary

See the relevant Material Safety Data Sheets (MSDS) for comprehensive information on the hazardous materials and the Laboratory Manual for spills and waste disposal procedures.

Chemical Hazards:

Substance	Hazardous	Potential Hazards and Dangers	Recommended Precautions
Acetonitrile	Yes	Highly flammable. Toxic by inhalation or swallowed. May cause irritation by contact to skin or eyes.	Avoid all ignition sources. Avoid inhaling and contact with skin or eyes. Use in a fume hood. Wear gloves when handling undiluted or concentrated solutions.
Trifluoroacetic acid	Yes	Corrosive. The substance is toxic to lungs, mucous membranes. May cause irritation by contact to skin or eyes.	Avoid inhaling and contact with skin or eyes. Use in a fume hood. Wear gloves when handling and preparing solvents.
Lactoferrin	No	None	

Process and Equipment Hazards:

Equipment	Potential Hazards and Dangers	Recommended Precautions
Centrifuge	Uncontrollable vibration	Ensure that the sample tubes are balanced before they placed in the centrifuge. Do not open the centrifuge cover until machine stops completely

Special First Aid Procedures: Record and report all incidents to management and seek immediate medical attention, if required.

Material	Recommended First Aid Procedures
Acetonitrile	Immediately flush eyes and skin with plenty of running water (cold water) for at least 15 minutes. Ingestion: If swallowed, do not induce vomiting unless directed to do so. Seek medical attention. (See MSDS for more details)
Trifluoroacetic acid	Immediately flush eyes and skin with plenty of running water (cold water) for at least 15 minutes. Do not use an eye ointment. Seek medical attention. Ingestion: If swallowed, do not induce vomiting unless directed to do so. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek immediate medical attention. (See MSDS for more details)

MASTERSIZER 2000

Result Analysis Report

Sample Name:
LFN05210 #1610004027 - Average

Sample Source & type:
Synlait

Sample bulk lot ref:
test in ethanol

SOP Name:
SMP 1.52 (in ethanol)

Measured by:
cehall

Result Source:
Averaged

Measured:
Tuesday, 26 April 2016 11:58:27 a.m.

Analysed:
Tuesday, 26 April 2016 11:58:28 a.m.

Particle Name:
SMP powder

Particle RI:
1.520

Dispersant Name:
Ethanol

Accessory Name:
Hydro 2000S (A)

Absorption:
0.001

Dispersant RI:
1.360

Analysis model:
General purpose (spherical)

Size range:
0.020 to 2000.000 um

Weighted Residual:
0.409 %

Sensitivity:
Enhanced

Obscuration:
10.90 %

Result Emulation:
Off

Concentration:
0.0511 %Vol

Specific Surface Area:
0.18 m²/g

Span :
1.781

Surface Weighted Mean D[3,2]:
33.250 um

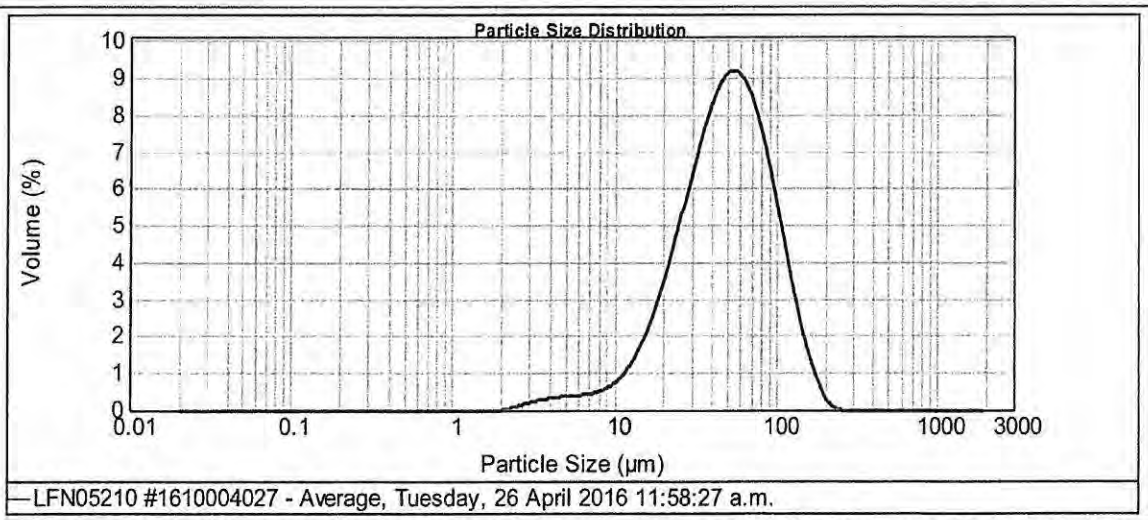
Uniformity:
0.553

Vol. Weighted Mean D[4,3]:
57.153 um

Result units:
Volume

---

d(0.1): 18.823 um **d(0.5):** 49.360 um **d(0.9):** 106.756 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.123	0.00	1.520	0.00	18.738	4.99	231.013	0.02	2848.036	0.00
0.012	0.00	0.152	0.00	1.874	0.02	23.101	7.06	284.804	0.00	3511.192	0.00
0.015	0.00	0.187	0.00	2.310	0.15	28.480	9.24	351.119	0.00	4328.761	0.00
0.019	0.00	0.231	0.00	2.848	0.31	35.112	11.14	432.876	0.00	5336.699	0.00
0.023	0.00	0.285	0.00	3.511	0.42	43.288	12.34	533.670	0.00	6579.332	0.00
0.028	0.00	0.351	0.00	4.329	0.49	53.367	12.46	657.933	0.00	8111.308	0.00
0.035	0.00	0.433	0.00	5.337	0.54	65.793	11.36	811.131	0.00	10000.000	0.00
0.043	0.00	0.534	0.00	6.579	0.62	81.113	9.19	1000.000	0.00		
0.053	0.00	0.658	0.00	8.111	0.81	100.000	6.45	1232.847	0.00		
0.066	0.00	0.811	0.00	10.000	1.24	123.285	3.77	1519.911	0.00		
0.081	0.00	1.000	0.00	12.328	2.03	151.991	1.69	1873.817	0.00		
0.100	0.00	1.233	0.00	15.199	3.27	187.382	0.39	2310.130	0.00		
0.123		1.520		18.738		231.013		2848.036			

Operator notes:

Morinaga Milk Industry Co. Ltd

Lactoferrin Specification as submitted in GRN 465 (2014)

MORINAGA MILK INDUSTRY CO., LTD.
33-1, SHIBA 5-CHOME, MINATO-KU, TOKYO 108-8384, JAPAN
TEL : 81-3-3798-0152
FAX : 81-3-3798-0107
E-mail: interntl@morinagamilk.co.jp

Free translation (summary) of specification for "Lactoferrin Concentration" in the existing food additives list in Japan

Definition :

Substance whose major content is lactoferrin derived from mammal milk.

Contents :

On dry matter basis, it should contain 14.0 – 16.5% of nitrogen (N=14.01). And in protein, more than 85% of lactoferrin should be contained.

Appearance :

Pink salmon color powder, no odor.

Confirmation test :

(1) When 1ml of sodium hydroxide solution and a drop of copper sulfate solution are added into 10 ml of lactoferrin solution and shaken, it brings about blue precipitation and color of solution turns to purple.

(2) When 1 ml of diluted hydrochloric acid is added into lactoferrin solution, the red color in the solution disappears.

Purity test :

(1) pH : 5.2 – 7.2 (1.0g, water 50ml)

(2) Iron content : not more than 0.050% as Fe. (Atomic absorption analysis)

(3) Heavy metals : not more than 20 μ g / g as Pb.

(4) Arsenic : not more than 4.0 μ g/ g as As_2O_3

Loss on drying : not more than 6.0% (105℃, 5 hours)

Residue on ignition : not more than 2.5%

Quantitative determination method :

(1) Nitrogen : Determines quantity of nitrogen Semimicro Kjeldahl method

(2) Lactoferrin in protein : HPLC

Make 50ml of test solution by dissolving 0.1g of lactoferrin into sodium chloride solution.

Measure 25 μ l test solution and do the HPLC test and determine lactoferrin contents by the following formula.

000175

MORINAGA MILK INDUSTRY CO., LTD.
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E-mail: interntl@morinagamilk.co.jp

- Lactoferrin (%) = ALF / APK x 100
  - ALF : Main peak area (lactoferrin)
  - APK : Total peak area

- Operating condition
  - Detector : Ultra-Violet Absorbance Detector (Detection wavelength : 280nm)
  - Column packing material : Polyvinyl alcohol gel made by chemical binding of 5 μ g of butyl group.
  - Column : Stainless column of 4.6mm inner diameter and 15cm length.
  - Column temperature : 30 – 40 ℃
  - Mobile phase A : Acetonitrile / NaCl solution (1:9)
  - Mobile phase B : Acetonitrile / NaCl solution (1:1)
  - Concentration gradient : 30 minutes of linear gradient from A:B (50:50) to A:B (0:100)
  - Flow rate : Adjust so that the retention time of main peak would be about 10 minutes.

000176

Standard and Specification > Natural Additives > Lactoferrin Concentrates

Lactoferrin Concentrates

Definition This is obtained by concentrating milk that is previously defatted and purified by separation. The major component is lactoferrin. It also contains whey protein.

[Compositional Specifications of Lactoferrin Concentrates]

Content Lactoferrin Concentrates should contain not less than 90.0% of lactoferrin.

Description Lactoferrin Concentrates is scentless pale orange red~pale reddish brown powder.

Identification When Lactoferrin Concentrates is quantitatively analyzed, a lactoferrin peak is observed at 280 nm.

Purity

(1) Arsenic : 0.5 g of Lactoferrin Concentrates is placed in a platinum, quartz, or porcelain crucible. 10 ml of magnesium nitrate in ethyl alcohol (1→50) is added to the crucible and then alcohol is ignited. It is then reduced to ash by heating at 450~550°. If carbonaceous substance persists, it is wetted with minute amount of nitric acid, which is further heat treated at 450~550°. After cooling, 3 ml of hydrochloric acid is added to the residue, which is then dissolved by heating in a water bath. When test for arsenic is carried out with this test solution, it should not be more than 2ppm.

(2) Heavy Metals : 2 g of Lactoferrin Concentrates are carbonized by heating mildly in a quartz or porcelain crucible. After cooling, add 2 ml of nitric acid and 5 drops of sulfuric acid, it is heated until white smoke disappears, which is then reduced to ash by further heating at 450~550°. After cooling, 2 ml of hydrochloric acid is added, which is then evaporated to dryness in a water bath. 3 drops of hydrochloric acid and 10 ml of hot water are added to the resulting residue, which is then heated for 2 minutes. After cooling, 1 drop of phenolphthalein indicator solution is added, then ammonia solution is added until the color of the solution becomes pale red. The resulting solution is transferred into a Nestler cylinder by rinsing with water. 50 ml of test solution is prepared by adding 2 ml of diluted acetic acid (1→20) and water. When this solution tested for heavy metals, the content should not be more than 10ppm. Color standard solution is prepared by the following procedure. 2 ml of nitric acid, 5 drops of sulfuric acid, and 2 ml of hydrochloric acid are added and evaporated to dryness in a crucible that is made of the same material used for test solution preparation. 3 drops of hydrochloric acid are added to the residue, which is then transferred into another Nestler cylinder as described above. Finally, 2 ml of lead standard solution, 2 ml of diluted acetic acid (1→20), and water are added to bring the total volume to 50 ml.

(3) pH : pH of this solution (2→100) should be 5.2~7.2.

(4) Coliform Group : Lactoferrin Concentrates is tested by Microbe Test Methods for [Coliform Group] in General Test Methods in Food Code. It should contain 30 or less per 1 g of this product.

Residue on Ignition When thermogravimetric analysis is done with 1 g of Lactoferrin Concentrates, the amount of residue should not be more than 1.3%.

Assay

Approximately 20 mg of Lactoferrin Concentrates is accurately weighed and dissolved in 0.5 M of sodium chloride solution (total volume 10 ml). The solution is filtered through a 0.45 μm Millipore filter (Test Solution). Separately, a Standard Solution is prepared with 20 mg of lactoferrin standard following the same procedure. 20 μl each of Standard Solution and Test Solution is injected into liquid chromatograph and the content of lactoferrin is obtained from the following equation.

$$\text{Content (\%)} = \frac{Au \times Ws}{As \times Wu} \times 100$$

Au : Peak area of Test Solution
As : Peak area of Standard Solution
Ws : amount of standard material (mg)
Wu : amount of sample (mg)

[Operation Conditions]
- Detector : UV 280 nm
- Column : Ashaipak C4P 50(4.6 mm × 150 mm) or its equivalent
- Column Temperature : Room temperature
- Mobile Phase : Solution A: Solution B (30: 70)
  Solution A : acetonitrile: 0.5M sodium chloride solution (1: 9)
  Solution B : acetonitrile: 0.5M sodium chloride solution (5: 5)
  Solutions A, B contains 0.03% of Trifluoroacetic acid.
- Flow rate: 0.8 ml/min

TECHNICAL SPECIFICATION

ISO/TS 22964

IDF/RM 210

First edition
2006-02-01

Milk and milk products — Detection of *Enterobacter sakazakii*

*Lait et produits laitiers — Détection de l'*Enterobacter sakazakii

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IDF/RM 210:2006(E)

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Published in Switzerland

Contents

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TS 22964|IDF/RM 210 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of IDF National Committees casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a Standing Committee may decide to publish another type of normative document which is called by IDF: *Reviewed method*. Such a method represents an agreement between the members of a Standing Committee and is accepted for publication if it is approved by at least 50 % of the committee members casting a vote. A *Reviewed method* is equal to an ISO/PAS or ISO/TS and will, therefore, also be published jointly under ISO conditions.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights.

ISO/TS 22964|IDF/RM 210 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by the Joint ISO-IDF Action Team on *Harmonization*, of the Standing Committee on *Microbiological methods of analysis*, under the aegis of its project leaders, Mr D.J.C. van den Berg (NL) and Mr H. Joosten (CH).

Milk and milk products — Detection of *Enterobacter sakazakii*

1 Scope

This Technical Specification specifies a method for the detection of *Enterobacter sakazakii* in milk powder and powdered infant formula.

The method is also applicable to environmental samples collected from milk powder or infant formula factories.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8261|IDF 122, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
presumptive *Enterobacter sakazakii*
microorganisms which form typical colonies on a chromogenic isolation agar, when tests are carried out in accordance with this Technical Specification

3.2
Enterobacter sakazakii
microorganisms which form typical colonies on a chromogenic isolation agar, form yellow colonies on tryptone soya agar and display biochemical characteristics as described, when tests are carried out in accordance with this Technical Specification

4 Principle (see also annex A)

4.1 Pre-enrichment in non-selective liquid medium

The pre-enrichment medium is inoculated with the test portion and incubated at 37 °C ± 1 °C for 16 h to 20 h.

4.2 Enrichment in selective liquid medium

The selective enrichment medium is inoculated with the culture obtained in 4.1 and incubated at 44 °C ± 0,5 °C for 22 h to 26 h.

4.3 Plating out and identification

A chromogenic agar is inoculated with the enrichment culture obtained in 4.2 and incubated at 44 °C ± 1 °C for 22 h to 26 h.

4.4 Confirmation

Typical colonies are selected from the chromogenic agar, and isolates producing a yellow pigment on tryptone soya agar are biochemically characterized.

5 Culture media and reagents

5.1 General

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity. The water shall be free from substances that might inhibit the growth of microorganisms under the test conditions specified in this Technical Specification. See also ISO 6887-1 and ISO 8261|IDF 122.

In order to improve the reproducibility of the results, it is recommended that, for the preparation of culture media, dehydrated basic components or dehydrated complete media be used. In that case, follow the manufacturer's instructions rigorously. See also ISO 6887-1.

The pH values given refer to a temperature of 25 °C. Adjustments, if necessary, are made by adding either hydrochloric acid [c(HCl) = 1 mol/l] or sodium hydroxide solution [c(NaOH) = 1 mol/l].

If not used immediately, store the prepared culture media and reagents under conditions that do not produce any change in their composition, in the dark at a temperature between 0 °C and 5 °C, for no longer than 1 month, unless otherwise stated.

5.2 Culture media

5.2.1 Buffered peptone water (BPW)

5.2.1.1 Composition

Enzymatic digest of casein	10,0 g
Sodium chloride (NaCl)	5,0 g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12\ H_2O$)	9,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,5 g
Water	1 000 ml

5.2.1.2 Preparation

Dissolve each of the components in the water, by heating if necessary. Adjust the pH, if necessary, to 7,0 ± 0,2 at 25 °C. Distribute the BPW in flasks or tubes according to the analytical needs. Sterilize at 121 °C for 15 min.

5.2.2 Modified lauryl sulfate tryptose broth (mLST)/vancomycin medium

5.2.2.1 Modified lauryl sulfate tryptose broth (mLST)

5.2.2.1.1 Composition

Sodium chloride (NaCl)	34,0 g
Enzymatic digest of animal and plant tissue	20,0 g
Lactose ($C_{12}H_{22}O_{11}$)	5,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	2,75 g
Dipotassium hydrogen phosphate (K_2HPO_4)	2,75 g
Sodium lauryl sulfate ($C_{12}H_{25}NaO_5S$)	0,1 g
Water	1 000 ml

5.2.2.1.2 Preparation

Dissolve each of the components in the water, by heating if necessary.

Adjust the pH, if necessary, to $6,8 \pm 0,2$ at 25 °C. Dispense 10 ml of mLST into tubes of dimensions 18 mm × 160 mm.

Sterilize the tubes at 121 °C for 15 min.

5.2.2.2 Vancomycin solution

5.2.2.2.1 Composition

Vancomycin	10 mg
Water	10 ml

5.2.2.2.2 Preparation

Dissolve the vancomycin in the distilled water. Mix and sterilize by filtration.

The vancomycin solution may be kept at 0 °C to 5 °C for 15 days.

5.2.2.3 mLST/vancomycin medium

Add 0,1 ml of vancomycin solution (5.2.2.2.2) to 10 ml of mLST solution (5.2.2.1.2) so as to obtain a final vancomycin concentration of 10 µg per millilitre of mLST.

The complete mLST/vancomycin medium may be kept at 0 °C to 5 °C for 1 day.

5.2.3 *Enterobacter sakazakii* isolation agar (ESIA™)[1)]

5.2.3.1 Composition

Pancreatic peptone of casein	7,0 g
Yeast extract	3,0 g
Sodium choride (NaCl)	5,0 g
Sodium desoxycholate	0,6 g
5-Bromo-4-chloro-3-indolyl α-D-glucopyranoside ($C_{14}H_{15}BrClNO_6$)	0,15 g
Crystal violet	2 mg
Agar	12,0 g to18,0 g[a]
Water	1 000 ml
[a] Depending on the gel strength of the agar.	

5.2.3.2 Preparation

Dissolve each of the components in the water by boiling. Adjust the pH, if necessary, to 7,0 ± 0,2 at 25 °C. Sterilize at 121 °C for 15 min.

Cool to between 44 °C and 47 °C. Pour about 15 ml of ESIA™ medium into sterile empty Petri dishes and allow to solidify on a cool even surface.

The medium may be kept at 0 °C to 5 °C for up to 14 days.

5.2.4 Tryptone soya agar (TSA)

5.2.4.1 Composition

Enzymatic digest of casein	15,0 g
Enzymatic digest of soya	5,0 g
Sodium chloride (NaCl)	5,0 g
Agar	9,0 g to 18,0 g[a]
Water	1 000 ml
[a] Depending on the gel strength of the agar.	

5.2.4.2 Preparation

Dissolve each of the components in the water by boiling. Adjust the pH, if necessary, to 7,3 ± 0,2 at 25 °C. Sterilize at 121 °C for 15 min. Cool to between 44 °C and 47 °C. Pour about 15 ml of TSA into sterile empty Petri dishes and allow to solidify on a cool even surface.

1) ESIA™ is the trade name of a product supplied by AES Laboratoire, Rue Maryse Bastié, Ker Lann, F-35172 Bruz (FR) . This information is given for the convenience of users of this Technical Specification|IDF Reviewed Method and does not constitute an endorsement by either ISO or IDF of the product named. Equivalent products may be used if they can be shown to lead to the same results.

5.2.5 Media and reagents for biochemical characterization

5.2.5.1 Reagent for detection of oxidase

5.2.5.1.1 Composition

N,N,N',N'-Tetramethyl-*p*-phenylenediamine dihydrochloride ($C_{10}H_{16}N_2 \cdot 2HCl$)	1,0 g
Water	100 ml

5.2.5.1.2 Preparation

Dissolve the component in the water immediately before use.

5.2.5.2 L-Lysine decarboxylation medium

5.2.5.2.1 Composition

L-Lysine monohydrochloride ($C_6H_{14}N_2O_2 \cdot HCl$)	5,0 g
Yeast extract	3,0 g
Glucose ($C_6H_{12}O_6$)	1,0 g
Bromocresol purple	0,015 g
Water	1 000 ml

5.2.5.2.2 Preparation

Dissolve each of the components in the water, by heating if necessary. Adjust the pH, if necessary, so that after sterilization it is 6,8 ± 0,2 at 25 °C. Dispense 5 ml of L-lysine decarboxylation medium into tubes of dimensions 18 mm × 160 mm.

Sterilize the tubes at 121 °C for 15 min.

5.2.5.3 L-Ornithine decarboxylation medium

5.2.5.3.1 Composition

L-Ornithine monohydrochloride ($C_5H_{12}N_2O_2 \cdot HCl$)	5,0 g
Yeast extract	3,0 g
Glucose ($C_6H_{12}O_6$)	1,0 g
Bromocresol purple	0,015 g
Water	1 000 ml

5.2.5.3.2 Preparation

Dissolve each of the components in the water, by heating if necessary. Adjust the pH, if necessary, so that after sterilization it is 6,8 ± 0,2 at 25 °C.

Dispense 5 ml of L-ornithine decarboxylation medium into tubes of dimensions 18 mm × 160 mm. Sterilize the tubes at 121 °C for 15 min.

5.2.5.4 L-Arginine dihydrolation medium

5.2.5.4.1 Composition

L-Arginine monohydrochloride ($C_6H_{14}N_4O_2 \cdot HCl$)	5,0 g
Yeast extract	3,0 g
Glucose ($C_6H_{12}O_6$)	1,0 g
Bromocresol purple	0,015 g
Water	1 000 ml

5.2.5.4.2 Preparation

Dissolve each of the components in the water, by heating if necessary. Adjust the pH, if necessary, so that after sterilization it is 6,8 ± 0,2 at 25 °C.

Dispense 5 ml of L-arginine dihydrolation medium into tubes of dimensions 18 mm × 160 mm. Sterilize the tubes at 121 °C for 15 min.

5.2.5.5 Media for fermentation of carbohydrates (peptone water with phenol red, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose and amygdaline)

5.2.5.5.1 Basic medium

5.2.5.5.1.1 Composition

Enzymatic digest of casein	10 g
Sodium chloride (NaCl)	5 g
Phenol red	0,02 g
Water	1 000 ml

5.2.5.5.1.2 Preparation

Dissolve each of the components in the water, by heating if needed. Adjust the pH, if necessary, so that after sterilization it is 6,8 ± 0,2 at 25 °C.

Dispense the basic medium into flasks of suitable capacity. Sterilize at 121 °C for 15 min.

5.2.5.5.2 Carbohydrate solutions (D-sorbitol, L-rhamnose, D-sucrose, D-melibiose or amygdaline), 80 mg/ml

5.2.5.5.2.1 Composition

Carbohydrate	8 g
Water	100 ml

5.2.5.5.2.2 Preparation

Dissolve separately each of the four carbohydrate components in the water so as to obtain four carbohydrate solutions. Sterilize all by filtration.

5.2.5.5.3 Complete carbohydrate fermentation mediums

5.2.5.5.3.1 Composition

Basic medium (5.2.5.5.1)	875 ml
Carbohydrate solution (5.2.5.5.2)	125 ml

5.2.5.5.3.2 Preparation

For each carbohydrate, add the prepared carbohydrate solution (5.2.5.5.2) aseptically to basic medium (5.2.5.5.1) and mix. Dispense 10 ml of complete medium of each carbohydrate aseptically into tubes of dimensions 18 mm × 160 mm.

5.2.5.6 Simmons citrate medium

5.2.5.6.1 Composition

Sodium citrate ($Na_3C_6H_5O_7$)	2,0 g
Sodium chloride (NaCl)	5,0 g
Dipotassium hydrogen phosphate (K_2HPO_4)	1,0 g
Ammonium dihydrogen phosphate ($NH_4H_2PO_4$)	1,0 g
Magnesium sulfate ($MgSO_4$)	0,2 g
Bromothymol blue	0,08 g
Agar	8,0 g to 18,0 g[a]
Water	1 000 ml

[a] Depending on the gel strength of the agar.

5.2.5.6.2 Preparation

Dissolve each of the components or the dehydrated complete medium in the water by boiling. Adjust the pH, if necessary, so that after sterilization it is 6,8 ± 0,2 at 25 °C.

Dispense 10 ml of Simmons citrate medium into tubes (6.7) of dimensions 18 mm × 160 mm. Sterilize the tubes at 121 °C for 15 min.

Let the tubes stand in a tilted position so as to obtain a butt 2,5 cm deep.

6 Apparatus and glassware

Disposable glassware is an acceptable alternative to reusable glassware, provided that it has suitable specifications.

Usual microbiological laboratory equipment and, in particular, the following:

6.1 Apparatus for dry sterilization (oven) or **wet sterilization** (autoclave)

See ISO 7218.

6.2 Total delivery pipettes, having a nominal capacity of 1 ml.

6.3 Water bath, capable of being maintained at 44 °C ± 0,5 °C.

6.4 Petri dishes, made of glass or plastic, of diameter 90 mm to 100 mm.

6.5 Incubators, capable of operating at 25 °C ± 1 °C, 30 °C ± 1 °C and 44 °C ± 1 °C, respectively.

6.6 Loop, made of platinum-iridium or nickel chromium, of diameter approximately 3 mm, or disposable loops.

6.7 Test tubes, of diameter 18 mm and length 160 mm (plugged or with screw caps).

6.8 pH meter, accurate to 0,1 pH unit at 25 °C ± 1 °C.

7 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this Technical Specification. A recommended sampling method is given in ISO 707|IDF 50.

8 Preparation of test sample

Prepare test samples in accordance with ISO 8261|IDF 122.

9 Procedure (see the scheme in Annex A)

9.1 Test portion

To prepare the primary dilution, add x g of the test sample (Clause 8) to 9 times x ml of pre-enrichment medium (5.2), which is the ratio of test sample to pre-enrichment medium specified in this method.

Allow dry samples to disperse in the liquid without stirring. If a sample has not been dissolved completely after 30 min, than mix it gently with the medium.

9.2 Pre-enrichment

Incubate the inoculated pre-enrichment medium (9.1) at 37 °C ± 1 °C for 18 h ± 2 h.

9.3 Selective enrichment

After incubation of the inoculated pre-enrichment medium, transfer 0,1 ml of the obtained culture (9.2) into 10 ml of mLST/vancomycin medium (5.2.2.3). Incubate at 44 °C ± 0,5 °C for 24 h ± 2 h.

It is recommended to use either a water bath (6.3) or a forced-air incubator to ensure that the maximum temperature (44,5 °C) is not exceeded.

9.4 Isolation of presumptive *Enterobacter sakazakii*

After incubation of the inoculated mLST/vancomycin medium (9.3), streak a loopful (*ca.* 10 µl) onto the surface of the *Enterobacter sakazakii* isolation agar plate (5.2.3.2). Incubate the plate at 44 °C ± 1 °C for 24 h ± 2 h.

After incubation, examine the chromogenic plate for the presence of typical colonies of presumptive *Enterobacter sakazakii*.

NOTE Typical colonies are small to medium sized (1 mm to 3 mm) green to blue-green colonies. Non-typical colonies are often slightly transparent and violet coloured.

9.5 Confirmation

9.5.1 Production of a yellow pigment

9.5.1.1 Selection of colonies

Select one to five of the typical colonies of presumptive *Enterobacter sakazakii* examined on the incubated chromogenic plate (9.4).

9.5.1.2 Incubation

Streak the selected colonies (9.5.1.1) onto the surface of the TSA plate (5.2.4.2) so that after incubation separate colonies can be observed. Incubate the plate at 25 °C ± 1 °C for 44 h to 48 h. After incubation, examine the TSA plates for the presence of yellow-pigmented colonies.

When only one colony is selected (9.5.1.1) and transferred to the TSA plate and after incubation no yellow-pigmented colonies can be seen, select four more typical colonies (9.5.1.1) and proceed according to 9.5.1.2. If there are fewer than five typical colonies, select all of them.

CAUTION — Some exceptional strains of *Enterobacter sakazakii* might not form a yellow pigment under the test conditions specified in this Technical Specification, or the pigment is lost due to sub-culturing. In such cases using this method might, therefore, overlook such strains.

9.5.2 Biochemical confirmation

9.5.2.1 General

Miniaturized biochemical identification kits, currently available commercially and permitting the identification of *Enterobacter sakazakii*, may be used.

9.5.2.2 Selection of colonies

Select one yellow pigmented colony from each tryptone soya agar plate (9.5.1.2) for further biochemical characterization according to 9.5.2.3 to 9.5.2.8.

9.5.2.3 Oxidase

Using a glass rod or disposable inoculation needle, take a portion of each selected characteristic colony (9.5.2.2).

Streak the taken portion on a filter paper moistened with the oxidase reagent (5.2.5.1) or on a commercially available disc. Do not use a nickel/chromium loop or wire.

Consider the test to be negative when the colour of the filter paper has not changed to mauve, violet or deep blue within 10 s.

9.5.2.4 L-Lysine decarboxylase

Using a loop, wire or glass rod, inoculate the L-lysine decarboxylation medium (5.2.5.2) with each of the selected colonies (9.5.2.2) just below the surface of the liquid medium. Incubate the tubes at 30 °C ± 1 °C for 24 h ± 2 h.

A violet colour after incubation indicates a positive reaction. A yellow colour indicates a negative reaction.

9.5.2.5 L-Ornithine decarboxylase

Using a loop, wire or glass rod, inoculate the L-ornithine decarboxylation medium (5.2.5.3) with each of the selected colonies (9.5.2.2) just below the surface of the liquid medium. Incubate the tubes at 30 °C ± 1 °C for 24 h ± 2 h.

A violet colour after incubation indicates a positive reaction. A yellow colour indicates a negative reaction.

9.5.2.6 L-Arginine dihydrolase

Using a loop, wire or glass rod, inoculate the L-arginine dihydrolation medium (5.2.5.4) with each of the selected colonies (9.5.2.2) just below the surface of the liquid medium. Incubate the tubes at 30 °C ± 1 °C for 24 h ± 2 h.

A violet colour after incubation indicates a positive reaction. A yellow colour indicates a negative reaction

9.5.2.7 Fermentation of various sugars

Using a loop, wire or glass rod, inoculate each carbohydrate fermentation medium (5.2.5.5.3) with each of the selected colonies (9.5.2.2) just below the surface of the liquid medium. Incubate the tubes at 30 °C ± 1 °C for 24 h ± 2 h.

A yellow colour after incubation indicates a positive reaction. A red colour indicates a negative reaction.

9.5.2.8 Utilization of citrate

Using a loop, wire or glass rod, streak the selected colonies (9.5.2.2) onto the slant surface of Simmons citrate medium (5.2.5.6). Incubate the tubes at 30 °C ± 1 °C for 24 h ± 2 h.

The reaction is positive if the medium turns blue.

9.6 Interpretation of the results of the confirmation tests

Interpret the results according to Table 1.

Table 1 – Interpretation of results

Confirmatory test	Positive or negative reaction	Percent of *Enterobacter sakazakii* strains showing the reaction
Production of a yellow pigment	+	>99
Oxidase	–	>99
L-Lysine decarboxylase	–	>99
L-Ornithine decarboxylase	+	±90
L-Arginine dihydrolase	+	>99
Acid from		
— fermentation of D-sorbitol	–	±95
— fermentation of L-rhamnose	+	>99
— fermentation of D-sucrose	+	>99
— fermentation of D-melibiose	+	>99
— fermentation of amygdaline	+	>99
— hydrolysis of citrate	+	>95

10 Control cultures

In order to check the ability of the enrichment and isolation media to support the growth of *Enterobacter sakazakii*, introduce a low level inoculum of a reference culture of a recently isolated *Enterobacter sakazakii* strain, or of a reference strain from a recognized culture collection centre, into control flasks of the pre-enrichment medium (9.2). Proceed with this control flask as for the test cultures to demonstrate that the positive control culture is recovered.

11 Expression of results

In accordance with the interpretation of the test results (9.4), report the presence or absence of presumptive *Enterobacter sakazakii* in the test portion. In this case, no confirmation of the presumptive *Enterobacter sakazakii* found on the chromogenic plate has been carried out.

After confirmation by the procedure described in 9.5, of one or more of the presumptive *Enterobacter sakazakii* obtained in 9.4, report the presence or absence of *Enterobacter sakazakii* in the test portion.

Specify the final test result per mass (in grams) or per volume (in millilitres) of the analysed test sample.

12 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

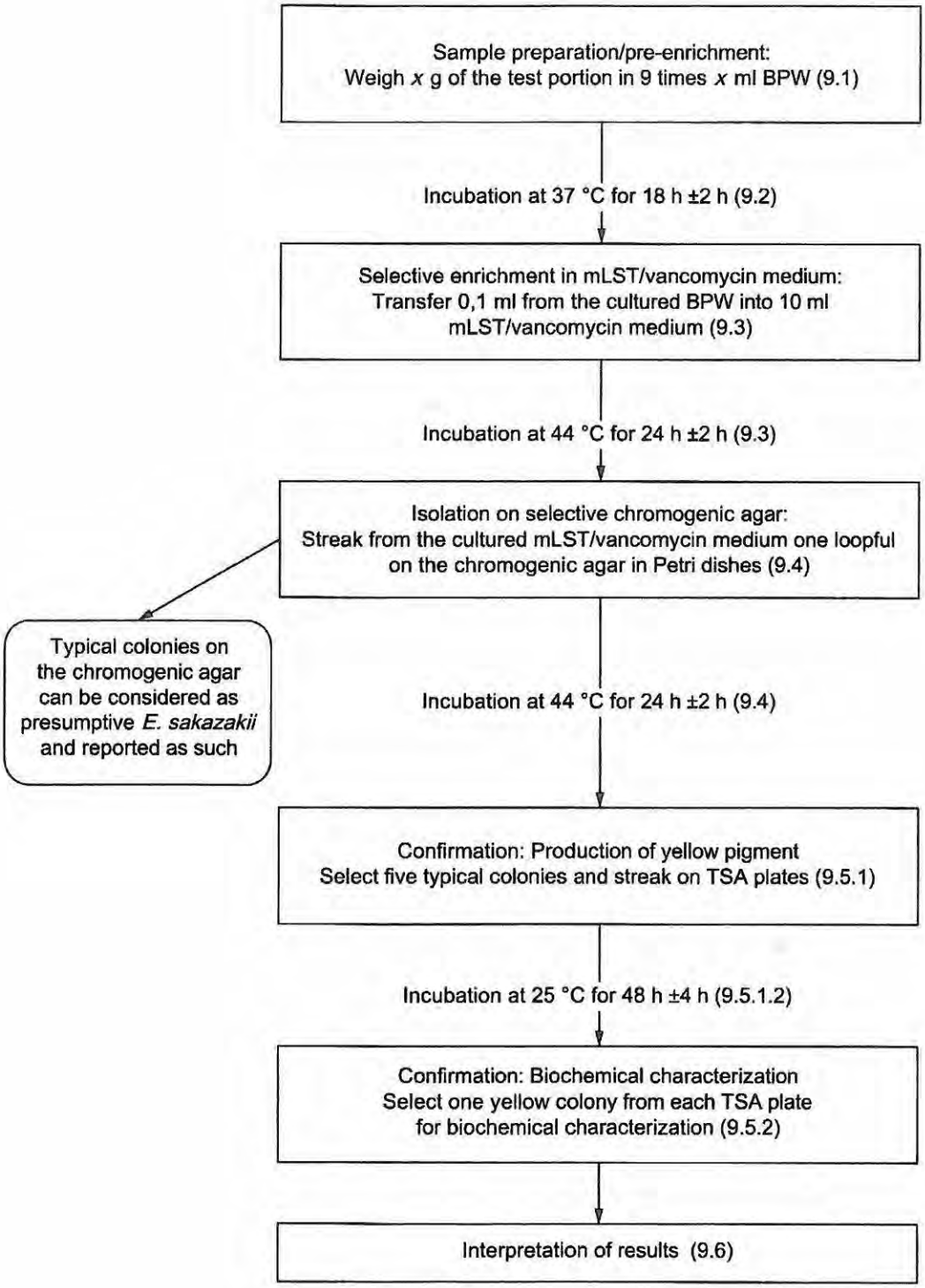
c) the test method used, with reference to this Technical Specification;

d) all operating details not specified in this Technical Specification, or regarded as optional, together with details of all incidents which may have influenced the result(s);

e) the test result(s) obtained.

Annex A
(informative)

Method flow scheme



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[1] ISO 707|IDF 50, *Milk and milk products — Guidance on sampling*

[2] ISO 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

[3] ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

[4] GUILLAUME-GENTIL, O., SONNARD, V., KANDHAI, M.C., MARUGG, J.D. and JOOSTEN, H. A Simple and Rapid Cultural Method for Detection of *Enterobacter sakazakii* in Environmental Samples. *Journal of Food Protection*, **68**(1), 2005, pp. 64-69

APPENDIX 3, MONTHLY WATER SAMPLING

Document No: QUA-20192
Version: 18
Date: 25 March 2015
Page: 1 of 2

Month of _____

Week	Test Site	Comments	Date/Sampler
Week 1	Bore1 & Bore 2, x 3 samples each Bore Pump House x 2 Samples Domestic water UF water x2 Cow waterx3 B&C 1 & 2 D3 #1-4 TBC		
Week 2	Chilled water Hose HS21 Hose USHO0343 Hose U2HO0345 UF water D3 #1-4 TBC		
Week 3	Bore1 & Bore 2, x 3 samples each Bore Pump House x 2 Samples Domestic water UF water x2 Cow water x3 D3 #1-4 TBC		
Week 4	Hose USHO0348 Hose HS1 UF water Bore 1 & 2 turbidity (1 each) D3 #1-4 TBC		

Prepared by:	(b) (6)	Date:	23/3/2015
Authorised by:		Date:	25/03/2015
Quality:		Date:	25/3/2015

Test Site	Test Frequency	Test For	Standard	Testing By	Responsibilty
Bore water from main feed lines into storage tanks (Bore 1, Bore 2 & Bore 3)	Annual	Chemicals and heavy metals	Refer table 2.2 NZDWS	ELS Ltd	Quality
	Fortnightly	E.coli	<1/100ml	External lab	Quality
	Fornightly	Total viable count	Record only	External lab	Quality
	Fortnightly	Nitrate/ Nitrite	Record only	External lab	Quality
	Monthly	Turbidity	≤1 NTU	External lab	Quality
	Daily	Turbidity	≤1 NTU	Energy Centre operators	Energy Centre
Treated water from bore pump house after chlorination	Fortnighly	E.coli	<1/100ml	External lab	Quality
	Fortnighly	Nitrate/ Nitrite	Record only	External lab	Quality
	Daily	Chlorine	<5ppm	Process staff	Production staff
Hose HS21	Monthly	E.coli	<1/100ml	External lab	Quality
Hose HS1	Monthly	E.coli	<1/100ml	External lab	Quality
Hose USHO0343	Monthly	E.coli	<1/100ml	External lab	Quality
Hose U2HO0345	Monthly	E.coli	<1/100ml	External lab	Quality
Hose USHO0348	Monthly	E.coli	<1/100ml	External lab	Quality
SMD treated water (TW)	6 Monthly	Bacterial Endotoxin	<0.25EU/ml	ELS Ltd	Quality
Condensate ex clean-steam generator	Monthly	E.coli	<1/100ml	External lab	Quality
B&C 1	Monthly	E.coli	<1/100ml	External lab	Quality
B&C 2	Monthly	E.coli	<1/100ml	External lab	Quality
Chilled water	Monthly	E.coli	<1/100ml	External lab	Quality
UF Water (batch UF or MPD UF)	Fortnightly (take samples only when UF plant is in operation)	E.coli	<1/100ml	External lab	Quality
	Weekly (take samples only when UF plant is in operation)	UV Transmittance	> 80 percent cm^{-1}	External lab	Quality
	6 Monthly	Bacterial Endotoxin	<0.25EU/ml	ELS Ltd	Quality
Domestic water	Fortnightly	E.coli	<1/100ml	External lab	Quality
Steam condensate	Annual	FeO	Record only	External lab	Quality
	Annual	Fe2O3	Record only	External lab	Quality
	Annual	NaOH	Record only	External lab	Quality
	Annual	HNO3	Record only	External lab	Quality
	Annual	Taste	Record only	Quality	Quality
Cow Water	Fortnightly	E.coli	<1/100ml	External lab	Quality
	Fornightly	Total viable count	Record only	External lab	Quality
	Fortnightly	Nitrate/ Nitrite	Record only	External lab	Quality

Treated water - Point of Use:

Code (as per map)	Location
USHO0343	SMD Wet process
U2HO0345	Dryer 2 Wet process RL32
USHO0348	Dryer 2 Goss room
HS-1 A1HO8306	AMF Wet process
HS -21	Dryer 1 Wet process
B&C 1	Ground floor Wet Wash Room
B&C 2	Level 1 critical change room
TW SMD	Aseptic Storage Hose

Code (as per map)	Location
Domestic water	Energy centre café/main office café
Steam condensate	Boiler house
Cow water	D1 wet process/ tanker bay silos
D3 #1 TBC	Dryer 3 Concentrate Room
D3 #2 TBC	Dryer 3 Wet Wash Room
D3 #3 TBC	Dryer 3 Evap Hall
D3 #4 TBC	Dryer 3 Lactose Almix

PART 7:
APPENDIX 4: International Regulations

The information presented within Appendix 4 is **generally available** other than :
The Certified Translation of the Draft Chinese Standard for Lactoferrin (pages A4: 10 to A4: 19
and,
The Certified Translation of the Preparation Notes for the Draft Chinese Standard for Lactoferrin (pages A4: 20 to A4: 26)
which are **not generally available**.

http://www.usp.org/food-ingredients/development-process/priority-new-food-ingredient-monographs

Pages 000199-000255 have been removed in accordance with copyright laws. The removed reference citations are:

Commission Implementing Decision (EU) 2015/568 of 7 April 2015 amending Annex I to Implementing Decision 2012/725/EU as regards the definition of bovine lactoferrin (notified under document C(2015) 2173)
OJ L 93, 9.4.2015, p. 71–71 (BG, ES, CS, DA, DE, ET, EL, EN, FR, HR, IT, LV, LT, HU, MT, NL, PL, PT, RO, SK, SL, FI, SV)
ELI: http://data.europa.eu/eli/dec_impl/2015/568/oj

National Standard of the People's Republic Of China, GB 14880-2012 National Food Safety Standard Standards for Uses of Nutritional Fortification Substances in Foods, https://chemlinked.com/regulatory-database/gb-14880-2012-national-food-safety-standard-standards-uses-nutritional-fortification-substances-foods

KFDA - Korea Food Additives Code 6/05/16, 3:27 PM, Standards for Manufacturing and Preparation >General Standards for Food Additive use in Foods,
http://fa.kfda.go.kr/standard/egongjeon_ilbansayong.jsp

SINGAPORE

CONSULTATION ON DRAFT FOOD (AMENDMENT) REGULATIONS 2015
(Pages 1 and 2 only)

Aim

The Agri-Food and Veterinary Authority (AVA) is seeking feedback from the food industry (local food manufacturers and importers) on the draft Food (Amendment) Regulations 2015.

Summary of amendments

The draft Food (Amendment) Regulations 2015 contains trade facilitating measures such as the provision for the use of advantame, a new sweetening agent, in foods under good manufacturing practice, as well as allowing bovine lactoferrin, a new ingredient, in infant formulas, at levels up to 100 mg/100 ml.

The amendments include a requirement that food products labelled as "organic" (or similar terms) must be certified as organic under an inspection and certification system that complies with the Codex Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods, GL 32-1999; or equivalent.

"Veterinary drugs" will be included under the definition for "Incidental constituents" under Regulation 29. In conjunction with this amendment, a definition for "veterinary drugs" (based on Codex definition) will be included in the Food Regulations.

Other changes include the prohibition of the import, sale and advertisement of raw milk for direct human consumption; and provision for the use of the generic term "Modified Starches" for labelling purposes. Editorial amendments will be made to Regulations 9, 12, 30(3) and 38, to update the terms used, as well as to spell out the provisions in a clearer manner.

A detailed description on the proposed changes can be found in ANNEX I.

Request for comments

AVA invites views and comments on the draft Food (Amendment) Regulations 2015. All submissions should be clearly and concisely written, and should provide a reasoned explanation for any proposed revisions.

Submissions should reach AVA no later than 12:00 p.m., 21 December 2015, through mail, or email, to the following addresses:

Mail:
Regulatory Programmes Department
Agri-Food and Veterinary Authority
52 Jurong Gateway Road #14-01
Singapore 608550
Tel: +(65) 6805 2910
Fax: +(65) 6334 1831

(Attention: Mr Cheng Chee Seng)

Email:
cheng_chee_seng@ava.gov.sg

ANNEX - PROPOSED AMENDMENTS TO THE FOOD REGULATIONS

The Agri-Food and Veterinary Authority of Singapore (AVA) has completed a review of the Food Regulations and proposes the following amendments:

(A) TO ALLOW THE USE OF NEW FOOD ADDITIVE AND INGREDIENT

Advantame, a sweetening agent, will be permitted for use in food under good manufacturing practice. The safety of advantame has been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and it is currently permitted for use as a sweetening agent in Australia, New Zealand, the European Union, Japan and the United States.

Due to advantame's intense sweetness (20,000 – 37,000 times sweeter than sucrose), use levels in food are low and self-limiting. Hence, there will not be a need to specify maximum use levels for advantame, and its usage will be governed by good manufacturing practice.

Bovine lactoferrin will be permitted for use in infant formula, at levels not exceeding 100 mg/100ml. Lactoferrin is a naturally occurring glycoprotein (complex oligosaccharide chains attached to polypeptide side chains) in milk. Because cow's milk contains approximately 10 times less lactoferrin as compared to human milk, addition of bovine lactoferrin to infant formula aims to emulate levels present in human breast milk.

Bovine lactoferrin has been allowed for use in infant formula in the EU, Japan, and the US. The proposed maximum level (100mg/100ml) is consistent with the level reported in the relevant EU legislation, as well as levels known to be used in the US.

(B) REQUIREMENT FOR CERTIFICATION FOR ORGANIC FOOD

In order to ensure that food products marketed as "organic" are indeed produced in a manner consistent with internationally accepted practice, AVA has been advising the food industry that they have to ensure that the food is certified as organically produced by the official certifying body for organic certification, which adopts the Codex Alimentarius Commission standards (or other similar standards) for organic food.

In this set of amendments, AVA proposes to include our advice to the industry in the Food Regulations, by incorporating a new provision that "organic food" must be certified under an inspection and certification system that complies with the Codex Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999), or equivalent.

(C) INCLUSION OF A DEFINITION FOR "VETERINARY DRUGS" IN REGULATION 29

SUBSTANTIAL EQUIVALENCE OPINION

Bovine Lactoferrin (Bioferrin®)

The Food Safety Authority of Ireland (FSAI) received an application in June of 2013 from Glanbia in Ireland for an opinion on the substantial equivalence of its bovine lactoferrin (Bioferrin®) to bovine lactoferrin previously authorised to Morinaga Milk Industry Co. Ltd. through Commission Implementing Decision 2012/725/EU. The source of Glanbia's lactoferrin is cow's milk whey, a by-product of the cheese manufacturing industry and also a source of the authorised lactoferrin. The production process for Bioferrin® is very similar to that for the authorised lactoferrin, yielding products with very similar specifications. Bioferrin® will be designated as "Lactoferrin from cow's milk" in line with Commission Implementing Decision 2012/725/EU, while it will be used only in the food groups set out in Annex II of that Implementing Decision. The applicant considers the ingredient to be novel and fall within the category of "food and food ingredients consisting of, or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practices and having a history of safe food use" as set out in *Article 1.2(e)* of the novel food Regulation EC No. 258/97.

Composition

Bioferrin® and the authorised lactoferrin are derived from cow's milk or its derivatives using very similar production and purification processes. A compositional comparison demonstrates the close similarity between Bioferrin® and the authorised bovine lactoferrin in terms of the level of protein, moisture, arsenic, ash etc, as specified in Annex I of the Implementing Decision. The applicant demonstrates batch consistency with respect to the composition of Bioferrin® along with a product stability of greater than 30 months.

Nutritional Value and Metabolism

Bioferrin® and the authorised lactoferrin are derived from cow's milk using very similar processes with the result that the composition of both products is practically

identical. Therefore the nutritional value and metabolism of Bioferrin® is not expected to be any different to the authorised lactoferrin.

Intended Uses

The applicant intends placing the Bioferrin® on the EU market in general foods and foods for particular nutritional (PARNUTS), including foods for special medical purposes (FSMPs) as well as infant and follow-on formulae. The permitted uses and maximum use levels set out in Annex II of Commission Implementing Decision 2012/725/EU that pertains to the authorised bovine lactoferrin will also apply to Bioferrin®.

Level of Undesirable Substances

Bioferrin® and the authorised lactoferrin are produced from the same raw material using a largely similar process and therefore it can be assumed that there will not be any significant differences in the levels of undesirable substances. The applicant demonstrates satisfactory results for lead and arsenic analysis in Bioferrin® along with a microbiological profile similar to that for the authorised lactoferrin.

Conclusions

The FSAI is satisfied from the information provided by the applicant that Glanbia's Bioferrin® is substantially equivalent to bovine lactoferrin authorised to Morinaga Milk Industry Co. Ltd. through Commission Implementing Decision 2012/725/EU. Bioferrin® will be designated as "Lactoferrin from cow's milk" in line with Commission Implementing Decision 2012/725/EU. Bioferrin® will only be used in the food categories and to the maximum use levels set out in Annex II of that Implementing Decision and without prejudice to the provisions of Regulation (EC) No 1925/2006 of the European Parliament and of the Council and Directive 2009/39 of the Parliament and the Council.